PROSPECTS FOR PRIMARY AND SECONDARY PREVENTION OF CERVICAL ADENOCARCINOMA

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ABSTRACT

This thesis comprises a portfolio of reports examining various aspects of the aetiology , diagnosis, treatment and pathogenesis of Cervical Intraepithelial Glandular Neoplasia. (CIGN) , a putative precursor of cervical adenocarcinoma.

The first is a multicentre case control study of risk factors for CIGN . This employed a standard questionnaire concerning lifestyle, reproductive , sexual and contraceptive factors and a serum assay for the presence of neutralising antibodies to herpes virus . The risk factor profile obtained for CIGN indicates that it has characteristics of a sexually transmitted disease, manifestation of the disorder possibly being dependent on an altered reproductive/endocrine milieu , as indicated by associations with late onset of menarche and low parity. There was no evidence in support of an association between CIGN and oral contraceptives . With respect to identification of a possible infective agent , the study fails to provide clear evidence of an association between either HSV-1 or HSV-2 and CIGN.

The second study is a multicentre prospective assessment of the effectiveness of cone biopsy as primary therapy for CIGN . Regular cytological examination was employed as a means of follow-up. Preliminary results indicate that following cone biopsy , further surgery is unnecessary when the margins of the specimen are free of atypical epithelium. To date there are no cases of residual CIGN or invasive disease in subjects so managed .

The final study employs the AgNOR technique to assess the possible presence of zones of poorly recognised epithelial atypia adjacent to adenocarcinoma-in-situ/high grade CIGN. The results give no support to the presence of such areas, and thus provide further experimental support for the validity of conisation as adequate primary treatment of CIGN.

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INTRODUCTION

This work attempts to address the question: 'Is cervical adenocarcinoma a preventable disease?' Much clinical and laboratory effort is devoted to the prevention of squamous cervical cancer, largely through the secondary preventive approach through mass cytological screening. Squamous cancer has an identifiable pre-invasive phase called cervical intra-epithelial neoplasia, (CIN) formerly known as dysplasia , or carcinoma-in-situ , which is easily detected by examination of cells exfoliated from the cervix. With respect to primary prevention, epidemiological studies have identified a risk factor profile which incriminates sexual activity in the aetiology of this cancer , and for the last 2 decades , researchers have been attempting to identify causal infective agents. However, with respect to cervical adenocarcinoma , there has been very little research into primary and secondary prevention , and this thesis attempts to systematically address these issues.

The problem outlined may be conveniently divided into 2 questions ;

a) Can we identify those at high risk of adenocarcinoma , and if so , could behaviour be modified such that their risk of disease was reduced? :

b) Can we identify and successfully treat the earliest stages of adenocarcinoma?

The first question is addressed in chapter 2, and the second in chapters 1 , 3 and 4 of this thesis. This work cannot provide a comprehensive answer to the primary inquiry , but provides some elucidation of the factors which may influence the development of cervical adenocarcinoma , and describes experience of management of pre-malignant lesions .

Most of our current insight concerning presumed precursor lesions

lesions of adenocarcinoma derives from small clinico-pathological series which focus mainly on the histological aspects of cervical adenocarcinoma-in-situ. This lesion constitutes the most cytologically atypical end of a spectrum of epithelial abnormality called cervical intraepithelial glandular neoplasia (CIGN) . An extremely limited clinical experience of these uncommon lesions means that management protocols are unclear , and aetiological factors are undefined. There have been no published analytical epidemiological studies of CIGN.

In an attempt to put the size of this problem into perspective , cancer of the cervix caused 2,170 deaths in the United Kingdom in 1988. The majority of cervical carcinomas (90-95%) are traditionally believed to be of squamous cell origin,the remaining 5-10% being adenocarcinomas . However, it is believed by many that the incidence of the latter is increasing. (Peters et al,1986 Schwartz and Weiss, 1986 Vesterinen et al ,1989). We are therefore concerned with a disease which causes approximately 100-300 deaths per year.

In my thesis, therefore , I have attempted to investigate ;

a) *Primary prevention of adenocarcinoma* :

the risk factors for the development of CIGN.

b) *Secondary prevention of adenocarcinoma*: :

i) the optimal clinical management of CIGN.

ii) The pathogenesis of CIGN , and its bearing on clinical management.

This work has been in progress since June 1987, and has been carried out in my capacity as research fellow , based at the Women's Hospital , Birmingham and the Department of Social Medicine in the University of Birmingham Medical School.

Statement of work carried out ; Claims to originality ; Acknowledgements

In order to mount an effective study of both the aetiology and clinical management of CIGN , it was necessary that recruitment take place from several centres across the country , and therefore there was a requirement for a national register of cases .

National Register of cases of CIGN

This register was set up in the Cancer Research Campaign Clinical Trials Unit at the Queen Elizabeth Hospital , Birmingham , in order to carry out a prospective study of clinical management of patients with CIGN diagnosed on a cone biopsy. The British Society for Colposcopy and Cervical Pathology gave its formal approval to this register and encouraged its members to register cases. Although this register was established (July 1986) by the time I commenced my research post , (June 1987) there were few registrations (20). Therefore my first task was to publicise the study in order to encourage recruitment . This was achieved by sending a copy of the study protocol to at least one consultant in every gynaecology unit in the country , these data being obtained from the Hospital's Yearbook. In this correspondence , I offered to speak about the study at departmental and postgraduate meetings , and as a result I spoke in Liverpool , Stafford , Coventry , Bristol , Hereford , Leicester, Oxford , Birmingham and Manchester. This led to a marked improvement in recruitment , to the extent that by the time my research fellowship ended , 130 patients had been registered in the study. In addition to obtaining recruits for the prospective clinical study of conisation , I adapted the register to provide subjects for a case

control study of risk factors for CIGN.

Study 1

A Case Control study of Cervical Intraepithelial Glandular Neoplasia

This chapter describes a multicentre case-control study which aims to investigate the aetiology of cervical intraepithelial glandular neoplasia (CIGN). I formulated the design of the study in conjunction with my thesis supervisors (see below) . I designed the study questionnaire , performed a pilot study of its use , and subsequently recruited and interviewed all subjects, either in the hospitals where treatment was carried out , or , for normal population controls , in the practice surgery of the case patient. I stored data in the computers of the Department of Social Medicine at Birmingham University Medical School , collected , spun down and stored all serum samples , and subsequently performed approximately 20% of the assays for neutralising antibodies to herpes viruses types HSV-1 and HSV-2 . I performed all the statistical analysis of the data , and I am the sole author of this work.

Originality

This is the first ever analytical epidemiological study of risk factors for CIGN.

Acknowledgements ; Study 1

I gratefully acknowledge the advice and guidance of Dr . Ciaran Woodman , MD , MFCM, MRCOG , Senior Lecturer in Epidemiology and Community Health , Birmingham University, and Professor Michael Marmot , Professor of Epidemiology and Community Health , University College Hospital , London .

My thanks extend also to all the general practitioners and gynaecologists who gave consent for their patients to be included in the study. I would specifically like to thank Mr . Tim Marshall for his statistical advice on both this study and study 3 ; Dr. Robert Lancashire , computer programmer in the Department of Social Medicine at Birmingham University , for the basic instruction in the use of the EGRET and DBase 111 packages , without which it would not have been possible to analyse the data. For her help with the analysis of serum samples , and for instruction in basic laboratory technique , I thank Sharon Randall , research assistant in the department of Medical Microbiology , Birmingham University Medical School. In addition , Dr. Gordon Skinner MRCPath gave valuable advice in the course of my work concerning herpes virus antibodies.

Study 2

A prospective study of conisation in the management of CIGN

Having obtained recruits , I liased with gynaecologists , cytopathologists and histopathologists in order to ascertain :

1) full clinical information on the management of the case (a standard questionnaire devised by the author was sent to the clinician at registration , and subsequently every 6 months)

11) relevant cytopathological and histopathological material .

In addition, I was responsible for the circulation of the pathology material between the members of the pathology review panels. I collected , stored and analysed all the data on subjects following retrieval of questionnaires and pathologist's reports. All authorship of this work is my own.

Originality

The study described in this thesis is the first prospective study of management of CIGN , and comprises the largest available series of management of this disorder.

Acknowledgements : Study 2

I acknowledge the assistance of David Luesley , M.D. MRCOG ,Senior Lecturer in Obstetrics and Gynaecology , University of Birmingham.

Also, Dr. Malcolm Anderson FRCPath, Dr. Hilary Buckley , FRCPath , Dr. Terence Rollason, MRCPath, Dr. Christine Waddell , M.B. B.S. and Dr Elizabeth Hudson MD MRCPath . These individuals comprise the pathology review panel . I am grateful for their co-operation and for the time they have unselfishly dedicated to this project.

Study 3

A study of nucleolar organiser regions in adenocarcinoma-in-situ of the endocervix

The silver impregnation method has been used to assess 'nuclear activity' within the endocervix of cases of adenocarcinoma in situ (AIS) and normal controls. I personally carried out all of the counting procedures .

Originality

This is the first study which uses the AgNOR technique to investigate the existence of a postulated zone of epithelial alteration adjacent to adenocarcinoma - in- situ in the absence of any histological abnormality on routine microscopy

Acknowledgements : Study 3

I acknowledge the help of Dr. T. Rollason , who chose the case material , and his Medical Laboratory Scientific Officer, Deepa Gill , who prepared and administered the silver stain.

General acknowledgements

I would like to thank the Heath Endowment Fund of the University of Birmingham for funding my 2 years of research, and special thanks to Mr . Joseph Jordan FRCOG for the constant encouragement he gave in the execution of this study.

PUBLICATIONS AND PRESENTATIONS

Some parts of this thesis have been published or have been accepted for publication , and some of the work has been presented at learned societies.

PUBLICATIONS

Cullimore , J.E. Rollason,TP Marshall,T (1989) Nucleolar organiser regions in adenocarcinoma-in-situ of the endocervix. Journal of Clinical Pathology , 42 , 1276 - 1280

Cullimore ,JE and Rollason,TP (1990) Cervical intraepithelial glandular neoplasia - a review ; accepted for publication in Contemporary reviews in Obstetrics and Gynaecology eds Chamberlain,GVP and Drife,JO (By Invitation) Butterworths. Scheduled for April 1990

Further Publications arising from , but not directly related to the work presented in this thesis

Cullimore , J.E. Rollason,TPR Luesley, DM Waddell,C Williams,D (1989) A case of glandular intraepithelial neoplasia of the cervix and vagina . Gynaecologic Oncology 34: (2) , 249 - 252

Rollason , TPR Cullimore, JE Bradgate , M (1989) A suggested columnar cell morphological equivalent of squamous carcinoma-in-situ with early stromal invasion . Int J Gynae Path. 8 :3 ,230 - 236

PRESENTATIONS TO LEARNED SOCIETIES

At the 7th World Congress of Colposcopy and Cervical Pathology , Rome , May 1990 .(Abstract published in the Journal of Clinical and Experimental Cancer research, Volume 9,1: Supplement, FC-168) and also at the British Society for Colposcopy and cervical pathology , Birmingham ,April 1988 (oral presentation) and Sheffield March ,1990 (poster presentation)

" A prospective study of conisation in the management of adenocarcinoma in situ and glandular atypia of the cervix"

At a meeting of the UK Co-ordinating committee on cancer research entitled : " Priorities for cervical cancer and screening research" Invited presentation entitled 'Adenocarcinoma - in - situ' Glasgow, March 1990

CHAPTER 1

HISTORICAL REVIEW

Endocervical adenocarcinoma

Introduction

The endocervical canal is lined by a flat single layer of columnar epithelium which covers a system of stromal ridges, giving rise to the classical appearance of endocervical villi or clefts. There is growing interest in the biology of malignant disease arising from this epithelium, stimulated by recent observations consisting of ;

a) claims that the disease is increasing in incidence, and that this parallels changes in the behaviour of the at risk population , notably with respect to consumption of the oral contraceptive pill;

b) evidence that this histological variant of cancer has a more aggressive clinical course than its squamous counterpart.

c) the description of a putative non-invasive precursor lesion has been described , which is amenable to cytological diagnosis.

These factors need to be taken into account when considering the prevention of adenocarcinoma , and are considered in more detail in the ensuing paragraphs.

1) An increasing incidence of cervical adenocarcinoma?

On reviewing the literature it is apparent that many authors believe the incidence of cervical adenocarcinoma to be increasing. (Weiss and Lucas 1986, Gallup and Abell 1977 , Shingleton et al 1981,Davis and Moon 1975 , Peters et al 1986 Schwartz and Weiss,1986 Vesterinen et al ,1989)

However , on reviewing the evidence in support of this belief it transpires that most authors, with some notable exceptions (Peters et al,1986 Schwartz and Weiss,1986 Chilvers et al ,1987) do not quote the absolute incidence of adenocarcinoma .They merely report the proportion of all cervical cancer which is believed to be adenocarcinomatous. In

some countries, cervical cancer screening has influenced cancer incidence rates and mortality figures from predominantly squamous cancers. (Hakama and Louhivuori 1988) Hence,the quoted increases in adenocarcinoma may be more likely to be relative than absolute .(Devesa et al 1984)

Peters et al,1986 , have specifically looked at the incidence rates of cervical adenocarcinoma by obtaining cervical cancer incidence data from a population based tumour registry over an 11 year period, 1972-82 . During this time there was a significant increase in the frequency of invasive adenocarcinoma , but only in women under 35 years of age. Adenocarcinomas made up 10.6% of all cervical carcinomas in this series. The increased incidence in the under 35 group occurred only in the high social class groups.This led the authors to hypothesise that the observed change in incidence may be related to oral contraceptive use,which was heaviest in the higher social groups. Schwartz and Weiss , 1986 reported a similar experience with reference to 10 years of cancer registry data from 9 regions of the USA. In women under 35 years, there was a more than 2 fold increase in incidence rates from 1973 - 1982 . Again there was no change in rates for those over 35 years. It was notable that the incidence of other variants of cervical cancer declined over this period , especially 'unspecified' carcinomas, and the decrease in these lesions was of similar magnitude to the increase in adenocarcinomas . It is therefore possible that the apparent rise in adenocarcinomas is due to better ascertainment of this diagnosis. Similarly , Peters et al , 1986 reported a reduction in the incidence of unclassified epithelial tumours in the under 35's but this was not significant. However , for all ages above 35 there was a significant reduction in the incidence of this category , and the size of this decline in incidence exceeded the decline in incidence for squamous cancer suggesting that there was some improvement in histological

categorisation. Further support for the view that histological ascertainment has improved comes from a recent analysis of cervical cancer registry data from Norway (Eide , 1987) for the period 1970 - 84. This study revealed an annual rate of increase of adenocarcinoma registrations of 38%,and this increase in adenocarcinoma was restricted largely to young women (<35 years) , yet simultaneously there was a fall in the incidence rates of squamous and undifferentiated tumours.
Chilvers et al ,1987 analysed data from 3 British cancer registries over a 10 year period . This revealed a 2-3 fold increase in incidence of adenocarcinoma in the under 35's. However , the incidence of squamous and unspecified cancers increased , though not to such a large extent, explaining the proportionate increase in adenocarcinoma from 5.7% to 7.6%. Nevertheless, there was no significant difference in the differential increase in rates for both squamous and adenocarcinoma. This contrasts sharply with the American data and suggests that the increase in adenocarcinoma is part of a general increase in cancer rates.

The cancer registry data provide strong evidence that cervical adenocarcinoma is increasing in incidence irrespective of what is occuring with other histological subtypes. However , as there is little data on clinical presentation and clinical stage of disease in these reports, it is impossible to judge the extent to which the increase in young women is associated with increased screening. The observation in 2 recent clinical surveys of adenocarcinoma (Vesterinen et al ,1989 Saigo et al , 1986) that approximately 2/3 of these cases were of early clinical stage may support this contention.

There are caveats to be aware of when considering data on the incidence of cervical adenocarcinoma as reported to cancer registries, particularly because of the possibility of unrepresentative histological diagnoses . For instance, in some centres, radiotherapy may be used as the predominant mode of treatment for cervical cancer. In this situation ,

diagnosis is often based on a small, potentially unrepresentative biopsy, which , nevertheless, determines registration. Similarly , a surgical bias in treatment will give the opportunity for thorough histopathological examination of larger quantities of tissue , which may lead to increased reporting of glandular lesions. Surgical specimens are also usually biased towards young patients with early stage disease,which may not be representative of all cervical cancer .Webb and Sheehan , 1989 observed an increase in proportionate incidence of adenocarcinoma from 10% to 25% in women under 35 by comparing the periods 1959 - 1980 with 1982 - 1986 . It was observed that in the latter period there was a more marked emphasis in surgical treatment with 80% of the latter group having surgery in comparison with 24% of the former. Likewise , the reporting of an increased proportionate incidence of adenocarcinoma by Vesterinen et al,1989 derived from a clinical background where 62.9% of subjects had radical surgery as part or whole of their therapy.

The situation is further confused by the effect of inconsistency in classification amongst reported series of adenocarcinoma.Table 1 illustrates the findings of recent clinical series of cervical adenocarcinoma. Reference to this table shows that there is inconsistency between authors in their definition of adenocarcinoma. Some authors include only "pure" adenocarcinomas in their series ,whereas others define adenocarcinoma as a cervical cancer which shows any evidence of glandular elements, e.g. adenosquamous tumours . In these latter cases,the effect will be to increase the observed proportionate incidence of glandular carcinoma.

Variations in techniques of histopathological assessment may also bias the reporting of adenocarcinoma. Some authors utilize mucin histochemistry in the routine assessment of pathological material.This will lead to increased reporting of glandular carcinoma. Benda et al,1985, and Buckley et al , 1988 have re-classified the histological diagnoses of

their series of cervical carcinomas, following the use of stains for intracellular mucin. Mucin production is generally considered to be an indicator of adenocarcinomatous differentiation. In Benda's series , 39% of tumours contained intracellular mucin. Prior to mucin staining , traditional classification methods indicated that only 16% of this series showed glandular differentiation.

Using mucin histochemistry, Buckley et al , 1988 have likewise demonstrated that some tumours originally classified as squamous, are, infact poorly differentiated solid adenocarcinomas. Using such techniques indicates that 14% of cervix cancer is mistakenly classified as squamous. If there is general agreement that there has been under recognition of malignant glandular elements in cervical carcinoma , then this might have major implications for epidemiological data derived from previous series of cervical carcinomas not subjected to such histopathological review.

If the incidence of cervical adenocarcinoma is increasing , one would expect some parallel increase in the incidence of precursor lesions,i.e. CIGN. Review of the literature shows that such findings are not readily apparent. However, the consensus view is that there is an undiagnosed reservoir of pre-invasive disease. (Christopherson et al,1979, Boon et al 1981a, Brown and Wells,1986, Luesley et al 1987) This appears to be a plausible explanation given that :

a) these lesions were first descibed relatively recently (Friedell and Mackay, 1953) and are uncommon, hence lack of awareness may be a factor.

b) there are problems in the detection of these lesions by routine exfoliative cytology due to their situation in the endocervical canal.

c) there are no distinguishing colposcopic features. (Teshima et al , 1985)

In conclusion, most of the available data indicate that cervical adenocarcinoma is increasing in incidence. However , it is not possible to exclude the possibility that *better ascertainment* of the diagnosis of adenocarcinoma accounts for the observed changes .

2) Is the prognosis for adenocarcinoma worse than its squamous counterpart?

Some authors have claimed a poorer prognosis for patients with adenocarcinoma as compared to squamous cancer. (Berek et al,1981 Hurt et al,1977 Milsom and Friberg,1983 Weiss and Lucas, 1986) However, these opinions usually result from retrospective series , often spanning many years of observation , where the treatment modalities employed varied , both between and within studies. These studies lack the benefit of suitable controls with squamous disease , and as noted earlier , there is often inconsistency between studies in the classification of adenocarcinoma. Adenocarcinoma typically develops within the endocervical canal and may be less readily detected than early stage squamous disease which develops on the ectocervical portio. Hence it seems plausible that adenocarcinoma may be detected at a later stage of the neoplastic process than squamous cancer. This might explain any differences in crude survival for the 2 tumour types.

Differences in survival?

More recently , some authors have used squamous cancer controls, again retrospectively , in an attempt to determine the comparitive prognoses of these tumours, (Shingleton et al ,1981 Ireland et al , 1985 Kleine et al 1989) .Tumours of similar clinical stage and histological grade, which have been similarly treated , have been compared. Although the studies

of Shingleton et al, 1981 and Ireland et al ,1985 were small, and the period of follow up was limited , they showed no significant difference in prognosis for these malignancies. Kleine et al 1989 assessed the prognosis of both adenocarcinoma and squamous cancer patients , the subjects being comparable for age , stage and treatment modality. Both 5 and 10 year survivals for adenocarcinoma were significantly lower than squamous cancers , although this was largely due to the fact that there was a significant difference in prognosis between stage 1 adenocarcinoma treated by radiotherapy (5 year survival 58.6%) , compared with stage 1 squamous cancer treated similarly (5 year survival 85%) . Surgically managed patients showed no significant differences in 5 and 10 year survival. Grigsby et al ,1989 failed to find any evidence of a poorer prognosis for adenocarcinoma patients compared to squamous cancers when stratified for clinical stage and treatment modality and similarly Kilgore et al, 1988 could find no significant differences in survival between their groups of adeno- and squamous carcinomas .

Differences in behaviour?

Buckley et al , 1988 maintain that the presence of mucin secreting cells even in small quantities in a squamous tumour influences prognosis adversely . Metastasis was found to be more common in women with mucus secreting carcinoma,and these neoplasms were encountered more frequently in women less than 40 years old. In addition, Benda et al ,1985 reported that mucin positive tumours were significantly more likely to metastasize than mucin negative ones. While these observations are of great interest from the point of view of squamous tumours, it may be inappropriate to extrapolate these findings to histologically overt

adenocarcinoma. Other authors have noted a relationship between youth and cervical carcinoma which metastasises more frequently (Ward et al 1985) and which has a poor prognosis (Stanhope et al , 1980) and it is not clear to what extent this observation may confound the relationship between mucin positivity and behaviour. In Buckley's series ,the highest incidence of metastasis occured in young patients with adenocarcinoma, where vascular permeation was present on histological section.

Drescher et al,1989 have observed that subjects dying from adenocarcinoma were significantly more likely to have para aortic nodal metastasis compared to squamous controls who had also died of their disease . While this may indicate a higher propensity for adenocarcinomas to disseminate widely ,it is notable that all except one of these subjects were managed by radiotherapy, and hence this may merely indicate that radiotherapy is less effective in the eradication of adenocarcinoma than squamous disease. Berek et al , 1985 reported that the presence of regional lymph node metastasis in association with adenocarcinoma indicated a grave prognosis. However, this conclusion was based on only 10 patients with positive regional lymph nodes , of whom nine died of distant metastases or abdominal carcinomatosis. Similarly , Tamimi and Figge , 1982 reported a significantly higher rate of recurrence after treatment of adenocarcinoma with nodal metastases compared to a similar group of subjects with squamous cancer. However , the adenocarcinomas studied consisted of a variety of histological subtypes and treatment regimens varied between the groups. Kjorstad and Bond , 1984 reported that for stage 1b adenocarcinoma , 5 year survival rates , and the frequency of pelvic and distant metastases did not differ significantly from stage 1b squamous tumours which had been managed in an identical manner.

It is therefore not clear whether the presence of lymph node metastasis in adenocarcinoma signifies a high likelihood of systemic disease.

Conclusion

While some series have provided support for the view that patients with adenocarcinoma have a worse prognosis than those with squamous disease , the few studies which have specifically addressed this question differ in their conclusions, and in the absence of results from large studies which control for variables such as : histological subtype , clinical stage / size of tumour , and treatment modality , this question will remain open.

TABLE 1

PUBLISHED SERIES OF CERVICAL ADENOCARCINOMA(1956-89)

1st author	Date	Tumour type	incidence
Rombeaut	1966	Adeno only	3%
Hurt	1977	Adeno only	3%
Berek**	1981	Adeno only	4.9%
Weiner	1975	Adeno only	5%
Kjorstad	1977	Adeno only	5.1%
Milsom	1983	Adeno only	6.2%
Silcocks	1987	Adeno only	13%
Vesterinen	1989	Adeno only	18.3%
Rutledge	1975	Adeno+mixed	5.9%
Mikuta	1969	Adeno +mixed	6.1%
Ireland	1985	Adeno+mixed	8.1%
Shingleton	1981	Adeno+mixed	9.3%
Gallup*	1977	Adeno+mixed	9.6%
Weiss*	1986	Adeno + mixed	12.8%
Glucksmann	1956	Adeno+mixed	13%
Reagan	1973	Adeno+mixed	16%
Julian	1977	Adeno+mixed	28%
Davis	1975	Adeno+mixed	34%

Series of Pure Adenocarcinoma only; incidence = 3 -18.3%

Adenocarcinoma including mixed lesions;incidence = 5.9-34%

* Rise in incidence noted in same institution

**No rise in incidence over 25 years

3) PRECURSOR LESIONS OF CERVICAL ADENOCARCINOMA

Cervical intraepithelial glandular neoplasia (CIGN) -A review

Histological characteristics

The first case of Adenocarcinoma-in-situ (AIS), also called high grade cervical intraepithelial glandular neoplasia (CIGN),was described in 1953 by Friedell and Mackay , and this case also demonstrated concurrent squamous carcinoma in situ. The diagnosis of adenocarcinoma-in-situ is made histologically, the main features being ;

1) Affected cells show characteristic malignant features. In AIS , neoplastic cells line the endocervical crypts and / or surface epithelium.These cells demonstrate nuclear hyperchromasia,an increase in nuclear-cytoplasmic ratio , mitotic activity and cellular crowding giving rise to pseudostratification. (See also Figure 1- 1)

2)There are usually abrupt transitions between abnormal and normal areas within the same crypt ,and affected crypts can be found immediately adjacent to normal ones. (Figure 1-2)

3)Abnormal crypts do not usually extend below the deepest normal cervical crypt.

4)The lesion is described as being focal, or diffuse and continuous, (Ostor et al,1984) or multicentric within the endocervix, (Brown and Wells,1986) There is however , little objective evidence for the latter being a common occurrence . Indeed there is a growing body of opinion which states that AIS is in the great majority of cases , situated in close

relation to the squamocolumnar junction. (Bertrand et al,1987 Teschima et al,1985)

5) Brown and Wells,1985 and Gloor and Hurlimann,1986 have postulated that there is a spectrum of pre-invasive endocervical disease, with AIS representing the most cytologically and architecturally advanced form of CIGN. Lesions of lesser histological grade than AIS were first described by Brown and Wells. To add to the terminological confusion , they called these lesions 'Glandular atypia' (GA) and postulated low grade and high grade lesions based on morphological criteria. By reviewing 100 cases of CIN111 diagnosed on conisation specimens,they reported a 16% incidence of associated 'glandular atypia' by critical review of glandular morphology. These lesions were mainly of low grade and are analagous to low grade CIGN. However,there have been no other reports of such a high prevalence of such abnormalities. Figure 1-3 represents an example of a 'moderate' grade glandular epithelial atypia . (TP Rollason, personal communication)

The disadvantage of this terminology is that it implies that adenocarcinoma in situ is a separate lesion from "glandular atypia" when infact there is no good evidence for this belief. Indeed it would appear that there is little, if any , difference between the definition of high grade glandular atypia (Brown and Wells,1986) and the definition of AIS. Indeed the above authors have stated that the distinction may be artificial in terms of biological behaviour .

Gloor and Hurlimann 1986, coined the term Cervical intraepithelial glandular neoplasia.(CIGN) .By direct analogy with Richart's classification of CIN (Richart,1967) ,they proposed a uniform terminology for glandular dysplasias and AIS. In my opinion , the advantage of the CIGN terminology , is that it recognises that the cellular abnormalities are qualitatively similar, while it is appreciated that the degree of abnormality

may differ from lesion to lesion .

Gloor and Hurlimann applied mucin histochemistry and lectin binding procedures to 23 cases of CIGN.The main histologic features of CIGN were observed to be ; nuclear abnormalities , mitoses , and reduction or complete absence of intracellular mucin . Grades of disease from 1-3 were described. Grade 3 represented the most atypical histological picture , and is believed to correspond to AIS.

CIGN was further subdivided into types A and B, on the basis of mucin histochemistry. Type A contained reduced quantities of mucin , the pattern of mucins being similar to normal endocervical tissue, with the presence of neutral mucins and sulphomucins in excess of sialomucins . In CIGN type B , the shape of the columnar cells was different , resembling intestinal goblet cells.There was virtual absence of sulphomucins in these cells .Absence of sulphomucins is believed by a variety of authors to indicate a functional disturbance of already pre - neoplastic cells prior to the appearance of morphological atypia (Ehsanullah et al 1985 , Montero and Segura 1980). Histological subtypes of AIS had been previously described by Gloor and Ruzicka,1982 ; types 1 and 2 corresponding to CIGN types A and B respectively. (Figure 1- 4) It has been postulated that these different varieties of AIS could conceivably represent forerunners of differing histologic types of adenocarcinoma.

Problems with histologic diagnosis

Difficulties can arise in the distinction between early invasive adenocarcinoma and AIS . This problem is usually encountered when AIS is florid , occupying most of the glandular field (Figure 5) , and when the specimen is difficult to orientate. (Pickel,1990 Brand et al , 1988) Invasive disease can never be excluded on a punch biopsy , which is insufficiently

representative for accurate diagnosis. The signs of the beginnings of stromal invasion are more difficult to recognise in atypical glandular epithelium. Unlike squamous carcinoma , there is no well established histological equivalent of early stromal invasion(ESI), and where the term microinvasive adenocarcinoma has been used , the criteria used for arriving at this diagnosis have been unclear (Gloor and Ruzicka , 1982). Standard teaching is to classify these lesions as either definitely in - situ or definitely invasive. However, we have observed features suggesting that there is an uncommon possible morphological equivalent of squamous ESI in glandular epithelium (Figure 1-6) (Rollason et al , 1989a) though it seems unlikely that this morphological alteration precedes the development of most cases of invasive adenocarcinoma.

Associated pathology

Squamous intraepithelial neoplasia

High grade CIGN coexists with CIN in approximately 70% of cases (Luesley et al,1987 Ostor et al,1984) . It has been postulated that both these lesions have a common cell of origin, the sub-columnar reserve cell.(Boon et al , 1981b) and this may be one factor which explains their association . However, why only a minority of intraepithelial neoplasias of the cervix show purely glandular differentiation is unknown. This thesis explores this question further in Chapter 2.

Predisposing factors

The aetiology of CIGN is unknown . Its frequent occurrence with CIN suggests that there are shared aetiological factors. However there are few data which provide any real insight into this problem. The following areas have been explored.

a) The oral contraceptive pill (OCP)

CIGN and invasive adenocarcinoma have been noted to occur in patients taking the OCP.(Valente and Hanjani 1986, Dallenbach Hellweg 1984) However the hypothesis that the OCP is causally related to the development of these lesions has not been formally tested. A small matched case control study has been carried out which compares women with both in-situ and invasive adenocarcinoma with CIN111 and invasive squamous cancer controls .A history of OCP use was less frequently found in patients with adenocarcinoma although the differences were not significant. (Jones et al , 1989) There is a need for further analytical studies of the possible relationship between pill use and adenocarcinoma, and Chapter 2 explores the relationship between CIGN and the OCP.

b) Human papillomavirus

Recent research has indicated that this virus may have a role as a carcinogen or as a co-carcinogen in the female lower genital tract. (See also page 48) Okagaki et al,1989 have studied the presence of HPV subtypes in AIS , low grade CIGN and early invasive adenocarcinoma by in-situ DNA hybridisation techniques. Two thirds of the AIS cases contained HPV-DNA and a similar proportion of invasive adenocarcinomas stained positive for HPV. Tase et al , 1989 reported similar results using in situ hybridisation with respect to the frequency of occurrence of HPV DNA in AIS. Using probes for HPV messenger RNA production, Farnsworth et al ,1989 demonstrated that 88.6% of their AIS lesions expressed HPV m-RNA and the majority of cases were positive for HPV-18. In this study, 4 cases of minor glandular atypia were negative for HPV expression. Similar findings were reported by Tase et al,1989 and they implied that these lesions therefore might have less malignant potential. In a further comparison with squamous intraepithelial neoplasia , Tase et al , 1988

found HPV DNA in 70% of AIS and 64% of CIN111 controls . HPV-18 was the preponderant type of DNA found in AIS and also in cases of microinvasive adenocarcinoma similarly studied. CIN which co-existed with AIS or early invasion contained the same type of HPV DNA as the associated glandular lesion , whereas CIN111 controls all contained HPV - 16 DNA.

All the above data on the prevalence of putatively oncogenic HPV subtypes can be criticised on the grounds that normal controls have not been used in any of these studies , making the validity of the observed association between disease and HPV difficult to evaluate.

Cervical Intraepithelial Glandular Neoplasia ; a precursor of cervical adenocarcinoma?

The evidence for the concept of a pre-invasive phase of cervical adenocarcinoma is circumstantial and somewhat limited . However ,the available evidence points to this lesion being a precursor of invasion.

Evidence :

1) There is histological similarity between cells of invasive cancer,and those of pre-invasive lesions. The cells exfoliated by AIS lesions appear indistinguishable from the cells of an invasive lesion .(see later)

2) In-situ lesions are often found adjacent to frankly invasive cancer (Delgidisch et al, 1984) In one series,this lesion was found in 43.9% of a series of adenocarcinomas (Abell and Gosling 1962) and as highlighted earlier , there are difficulties in the differentiation of early invasive disease from AIS .

3) Progression from an in-situ lesion to invasive cancer has been suggested by some workers. However, the evidence that this occurs is suspect, and some of this evidence is anecdotal (Nguyen and Jeannot 1984) . Boon et al 1981b, described 18 patients with adenocarcinoma who had endocervical biopsies taken some time prior to the diagnosis of invasion . In 5 of them,there was AIS which had been overlooked. Obata et al , 1987 reviewed cervical biopsies taken 1-3 years prior to the clinical presentation with adenocarcinoma, and found evidence of glandular dysplasias and/or AIS in a minority. However , biopsies such as those described give a very incomplete sample (Fu et al , 1987) and a conisation specimen is required to determine whether the disease is still infact in-situ. Even then, this can be stated with conviction only if the disease appears fully excised. Teshima et al , 1985 have hypothesised that adenocarcinoma may develop directly from normal epithelium , without the necessity for premalignant changes , although the data they present on the location of these tumours and their frequent asssociation with AIS seems only to underline the relevance of their association.

4)The age prevalence of these apparently pre-malignant lesions has been cited as supporting the concept of progression towards malignancy, although the evidence is weak . In the series of Qizilbash, 1975 the mean age of patients with AIS (n=7) was 35.8 years,whereas those with early invasion (n=7) had a mean age of 40.2 years , suggesting that the in-situ form precedes invasion by some years. Brown and Wells ,1986 suggested a "possible progression" from glandular atypia to AIS in 1.5-3 years , although this figure was arrived at by comparing the mean age of their patients with GA with the mean age of subjects with AIS in other studies.

Further evidence of the invasive potential of lesions of lesser severity than AIS is limited , being restricted to immunohistochemical studies linking glandular atypia to invasive adenocarcinoma (Brown et al 1987).A

monoclonal antibody to HMFG1,(Human milk fat globule antigen),was used to stain specimens of normal and abnormal cervices.The staining pattern found with cases of glandular atypia was similar to invasive adenocarcinoma , both of these staining patterns differing from normal cervical epithelium and microglandular hyperplasia of the cervix.(MGH)
It has been proposed by one author (Dallenbach Hellweg , 1984) that MGH is a precursor of cervical adenocarcinoma. However , there is no support for this view in the literature (Brown et al,1987 Okagaki et al,1989 Jones et al, 1989 Tase et al ,1989). There were no significant differences in the observed frequency of (MGH) between cases of adenocarcinoma and squamous cancer controls (Jones et al, 1989) and HPV was found to be notably absent from MGH (Okagaki et al,1989 Tase et al ,1989). MGH is generally considered to be benign, although it can be confused with CIGN by an inexperienced observer .

Problems establishing the natural history of CIGN

Given the anatomical situation of CIGN , and the difficulties outlined in diagnosis, (especially the differentiation between AIS and early invasive malignancy), it seems highly unlikely given the scope of present technology , that we will be able to gain more insight into the natural history of CIGN. The very process of diagnosis seems to be an effective treatment for these lesions, and it would be unethical to withold treatment from a patient with cytological findings suggestive of AIS because of the possibility of missing an invasive lesion. (See section on cytologic diagnosis)

Incidence

Unlike its squamous counterpart, (CIN) , we know comparitively little about the incidence of CIGN. However , it is an uncommon condition.An incidence figure has not been calculated for this lesion . In the series of

Christopherson et al,1979 the ratio of AIS to squamous carcinoma in situ was estimated as 1: 239. Based on the experience of a single hospital laboratory with an interest in the condition , over the period 1983-87 inclusive , the ratio of CIGN to CIN grades 2 and 3 was 1 : 88. (CH Buckley,personal communication,1987) This somewhat lower ratio may be partly explained by the inclusion of lesser grades of glandular atypia than AIS.

Age Distribution

The mean age of patients with this condition is illustrated in Figure 1.It can be seen that the overall mean age at diagnosis in these studies was 38.8 years.

Detection of CIGN

Exfoliative cervical cytology

In the vast majority of cases described in the literature,the finding of AIS was an unexpected one following the treatment of women who had squamous dyskaryotic changes on cervical smears. Subsequent review of smears usually revealed some additional cellular abnormalities thought to represent abnormal endocervical cells.The concept of cytologic screening for cervical adenocarcinoma was first proposed by Boddington et al,1976 who performed a retrospective examination of the cervical smears of 13 women who had adenocarcinoma of the cervix.With the benefit of hindsight,it appeared that 6/13 of these women had had abnormal smears at an interval of 2-8 years prior to diagnosis of invasion.This was assumed to provide evidence of a pre-invasive lesion which is amenable to cytological detection.

Cytology appears to be the sole means of detecting this lesion , as it gives rise to no symptoms, and there are no accepted diagnostic colposcopic

criteria (Luesley et al 1987). Given the situation of CIGN within the endocervical canal(albeit close to the squamocolumnar junction) and the fact that many cervical smears fail to sample endocervical cells (Gondos et al,1972), it appears that this lesion will be more difficult to detect than CIN using routine cervical cytology. It is generally agreed that if a cervical smear lacks the presence of endocervical or metaplastic cells,then the transformation zone has not been adequately sampled. (Gondos et al 1972). Accordingly , attention has been focussed on endocervical columnar cells in cytologic smears . It is possible , though not proven, that a by-product of this awareness may be the detection of a higher rate of cytological abnormalities in glandular cells (Boon et al 1986). In Birmingham, between 1971-78, there were 23 patients with a smear showing abnormal columnar cells of endocervical origin . Between 1980-84, 119 such smears were encountered(C Waddell , personal communication) .The consensus view from the literature is that there is an undiagnosed reservoir of pre invasive endocervical disease.(Christopherson et al,1979 Boon et al 1981a, Brown and Wells,1986 Luesley et al 1987) which may be amenable to cytologic detection.

Krumins et al,1977 described cytological criteria for the diagnosis of AIS in 6 cases. Bousfield et al 1980, from the same centre, described a larger series and modified the cytologic criteria for the diagnosis of AIS and 'early invasive' adenocarcinoma.These criteria were formulated on an experience of 19 cases of AIS, 3 cases of endocervical dysplasia, 19 cases of 'microadenocarcinoma' , and 11 cases of overtly invasive adenocarcinoma.

To date, these specific diagnoses have been rarely made on cervical cytology in day to day practice , and although there is some published work which supports such categories of cytological abnormality , the identification of such specific lesions by exfoliative cytology is not generally accepted .The exponents of cytodiagnosis (Ayer et al,1987) found

one example of early endocervical adenocarcinoma or its precursors for every 12,000 smears examined. Just under half of these were from histologically confirmed AIS ; i.e. approximately 1 case of AIS for every 25,000 smears examined.The rarity of this lesion explains why most of the work supporting cytological diagnosis of glandular lesions is derived from retrospective study, and there is a dearth of published work which apply these cytologic criteria in a prospective fashion.

The primary cytologic criteria for the diagnosis of AIS are: (See also Figure 1-8)

1) the presence of short ribbons of tissue or clusters of abnormal cells, some showing glandular openings.

2) Crowded nuclei ,with a pseudostratified appearance .

3) Stripping of cytoplasm giving rise to an irregular edge to these sheets of cells.

Using these criteria , 16/19 cases of AIS were correctly diagnosed (Bousfield et al 1980). In a later publication from the same centre, (Ayer et al ,1987) it was stated that cytology made possible the distinction between well differentiated and poorly differentiated types of AIS on the basis of nuclear differences, and variant patterns of AIS were described, i.e. ; endocervical , endometrioid and intestinal. Cytology was said to correspond closely with histopathological findings. To date , no other group has reproduced these findings.

The Sydney group also believe that it is possible to distinguish cytologically between AIS and the conditions of endocervical dysplasia,early invasive and deeply invasive adenocarcinoma (Bousfield et al,1980;Ayer et al ,1988).However, in the series of Bousfield et al , 1980, by taking all 30 invasive lesions into consideration, only 43% were predicted correctly. An updated series (Ayer et al 1988) revealed an almost identical predictive value for diagnosis of a series of 'microinvasive' adenocarcinomas. These authors acknowledged that there was a tendency

to 'overcall' poorly differentiated AIS as invasive disease , and likewise in very inflammatory smears, distinguishing neoplasia from reactive change was a problem.These findings tend to support the alternative opinion (Betsill and Clark ,1986 Nguyen and Jeannot ,1984) that the exfoliative cytology of AIS and invasive glandular lesions does not differ in any major way.

Some doubts about the specificity of diagnosis of glandular lesions have been expressed (Lee,1988) and experience of false positive cytological diagnoses has been highlighted.The Sydney group described their 3 false positive diagnoses in a separate publication, (Pacey et al,1988) representing 2% of all their cytological diagnoses of CIGN or early invasive adenocarcinoma. Some retrospective data is available from other centres ; In the series of Nguyen and Jeannot ,1984 , 65% had a smear showing abnormal glandular cells. In a retrospective series from Birmingham,(Luesley et al 1987) the sensitivity of cytology was 71% for AIS and glandular atypia. However , figures such as these fail to take account of false positive cytological diagnosis of these lesion, making the true accuracy of the technique impossible to gauge.

The accuracy of a cytological prediction of AIS was assessed by Laverty et al ,1988 . Fifty four predictions of AIS were made from 290,000 smears (1 case of AIS for every 5,370 smears examined.) and of these , 47 patients were fully investigated . Twenty cases of AIS were correctly predicted (positive predictive value 42.5%) However of the remaining 27 subjects , 14 had invasive adenocarcinoma , 3 adenosquamous carcinomas , 1 endometrial carcinoma and 8 CIN111 alone.

There is an obvious need for prospective studies of the cytological diagnosis of CIGN from more centres. Where attempts have been made at routine sampling of the endocervical canal with a brush smear in high risk patients , the effect has been to reduce the rate of inadequate and false negative smears (Van Erp et al ,1988). In order to show an effect on the rate

of diagnosis of CIGN much larger studies would be required.

Colposcopy

In a recent retrospective series of AIS and glandular atypia (Luesley et al,1987) , 27/31 patients underwent colposcopy . In 16 , CIN only was suspected, in 4 colposcopy was thought to be normal ; in 5 , invasion was suspected, and in 2 , the appearances were thought to be consistent with a glandular lesion. However, it could be concluded that colposcopic diagnosis in these 2 cases was biased by the cytological findings of glandular abnormality. On review of the literature , there are no established recognition criteria for these lesions (Andersen and Arffmann , 1989 Luesley et al 1987) and it is likely that any associated colposcopic abnormalities are due to concomitant squamous CIN. In a study of 30 cases of early adenocarcinoma , only 6/30 showed colposcopic features suggestive of invasion , while the remainder , including 8 cases of AIS , showed mild non-specific changes. (Teshima et al,1985)

Endocervical curettage

Some authors have employed endocervical curettage as a supplement to colposcopic examination.The diagnosis of AIS was rarely made on endocervical curettage (ECC) in any of the reports cited inTable 1-2 . Even a positive ECC result does not mean that all the affected tissue has been removed , and does not exclude the possibility of an invasive lesion.

Management

The majority of early studies advocated hysterectomy as definitive management of high grade CIGN/AIS . This recommendation is based on the belief that multifocal disease (i.e. skip lesions) can occur (Wells and Brown, 1986), or that the proximal (uterine) portion of the endocervix can

be a site for disease. (Brown and Wells , 1986) In addition , some concern has been voiced that lesions which were classified as *in-situ* disease have in fact been invasive. (Buscema and Woodruff , 1984)

Only two groups of authors have argued that cervical AIS may be malignant despite its benign histological appearances whereas , the consensus view amongst pathologists is that AIS is a benign lesion. Buscema and Woodruff , 1984 and Hopkins et al , 1988 recommended that pelvic lymph node sampling be carried out in the assessment of these lesions, quoting case histories of two patients who developed pelvic malignancy some years after hysterectomy which revealed only cervical AIS . For these reasons it was hypothesised that occult lymphatic spread may have occurred despite apparently benign histology . Such recommendations for radical therapy based on anecdotal evidence should be viewed critically .These authors failed to consider the possibility that:

a) there may have been an error in histological diagnosis - a recognised problem, and further justification for standardised histological assessment.

b) the patients in question may have had vaginal adenocarcinoma in situ in association with the cervical lesion (Cullimore et al ,1989). It is theoretically possible that failure to recognise this lesion could lead to pelvic 'recurrence' arising from failure to eradicate concomitant vaginal AIS , which subsequently progressed to invasion.

With respect to clinico-pathological studies of cervical AIS, Qizilbash,1975 reported that 8 patients had disease-free margins at conisation, and no residual disease was found after hysterectomy . (1 of these was a 'microinvasive' adenocarcinoma).Twelve patients in the series of Christopherson et al ,1979 had conization prior to hysterectomy , and 8 of these had residual disease in the hysterectomy specimen. However,these results failed to take account of the status of excision margins or the length of the cone specimen . Ostor et al , 1984 reported 9 patients with AIS who had hysterectomy after conisation. In 6 of these

patients the margins of the cone were involved by disease , and 4 of these 6 had residual disease at hysterectomy. In 3 cases where conisation demonstrated clear excision margins , there was no residual disease at hysterectomy. On the basis of these data, Ostor et al ,1984 proposed that conisation with disease free margins may be adequate therapy for AIS . Luesley et al,1987 reported 10 cases of hysterectomy following conisation for AIS. In 2 cases, excision of disease at conisation was thought to be complete on the basis of disease-free margins, and 1 specimen showed residual disease in the cervix . In 8 patients there was incomplete excision of disease at conisation , and 4 instances of residual disease were found at hysterectomy. In this series , conisation alone was associated with restoration of cytological normality in 12 cases of CIGN after a median follow-up of 2-3 years. Hopkins et al ,1988 recognised that when cone biopsy margins were uninvolved , then residual disease was the exception. However,given that one of 7 patients with disease free margins on cone had residual disease in the subsequent hysterectomy specimen ,they advocated abdominal hysterectomy.

The majority of the studies which advise on clinical management involve less than 20 subjects , and indeed the largest report is of 36. (Andersen et al,1989)

Recent evidence from histo - morphometric studies indicates that CIGN is usually distributed in close relation to the cervical squamocolumnar junction (Matsukama et al , 1989 Jaworski et al ,1988 Tobon and Dave,1988 Teshima et al , 1985) and 'skip ' lesions are uncommon in the upper endocervical canal (Teshima et al ,1985) : indeed multicentric disease is the exception . (Ostor et al ,1984 Bertrand et al , 1987) These recent insights into the distribution of CIGN support the hypothesis that cone biopsy may be sufficient to eradicate the lesion in the majority of cases . There is to date , no prospective data on the management of CIGN by conisation.

Conclusions

Cervical adenocarcinoma may be assuming more importance because of postulated increases in incidence of this disease . The potential exists for diagnosis at the pre-invasive stage . Given these observations , are we justified in screening the population for this disorder? Any invasive cancer poses an important health problem . The natural history of CIGN is by no means certain , and the evidence that high grade intraepithelial lesions are pre-malignant is circumstantial . Nevertheless , given this evidence, it would be impossible at the present time to envisage a situation in which high grade CIGN lesions were merely observed , in order to obtain further insight into their natural history.

Cytological recognition of CIGN is possible but more research is needed in order to validate and perhaps improve cytological recognition of this disorder, and it is clear that the outcome of this research will determine the answer to the question of whether we can successfully screen for the disease. Clinical management of high grade CIGN has not been researched in any detail . The available reports are divided in their opinion as to whether conisation should be recommended as primary therapy. This policy has been evaluated in a multicentre cohort study described in this thesis .

Table 1-2 Adenocarcinoma-in-situ ; Principle findings and recommendations for therapy

First Author	Number	Mean age	Margins	Residualdisease (Cone biopsy)	Recommendation (Hysterectomy)
Weisbrot, 1972	5	46.7	5 free	1	Hysterectomy
Qizilbash , 1975	7	35.8	0	0	Hysterectomy
Christopherson, 1979	16	42	Not reported	8/12	Hysterectomy
Ostor , 1984	21	38	6/9 involved 3/9 free	4/6 0/3	Cone biopsy (Hysterectomy if cone margins involved)
Luesley, 1987	31	36	8/10 involved 2/10 free	4/8 1/2	Cone ?>=25mm length
Bertrand,1987	5	-	1 involved 4 uninvolved	0/1 0/4	Cone biopsy >=25mm
Hopkins, 1988	18	37	5 involved 7 not involved	4/5 1/7	Hysterectomy, +lymph node sampling
Andersen ,1989	36	36	4 involved	0/4	Cone Biopsy

Epidemiological characteristics of invasive cervical adenocarcinoma

The epidemiological profile of cervical adenocarcinoma has been less extensively researched than its squamous counterpart. Most information derives from clinical case series , or comparisons with squamous carcinoma. There have been very few analytical case control studies . As already mentioned , precise information is hindered by the fact that adenocarcinoma is rare , it consists of a variety of histological subtypes,possibly with differing aetiologies , and there is widespread diversity in classification of adenocarcinoma in general. The available evidence indicates that the risk factor profile of cervical adenocarcinoma differs from squamous cancer. However , firm conclusions are difficult to arrive at given that most studies involve small numbers .

Potential risk factors

AGE

Most series report a higher mean age at diagnosis for patients with adenocarcinoma compared to squamous cancer (Hurt et al ,1977 Menczer et al,1981 Rutledge et al ,1975 Silcocks et al,1987) . However , the studies of Brinton et al , 1987b , Horowitz et al , 1988 and Milsom and Friberg , 1983 detected no significant age differences between squamous and glandular neoplasms. There is some evidence that adenocarcinoma is less likely to be detected as a result of the screening process and perhaps as a consequence of this , presentation occurs at a later stage than squamous cancer (Silcocks et al ,1987). This could explain the observed differences in age at diagnosis.

MARITAL STATUS

Women with adenocarcinoma were significantly less likely to :

i) be married than those with squamous cancer (Korhonen 1980 , Kvale et al, 1988) . and

ii) have been divorced or widowed (Milsom and Friberg , 1983)

PARITY AND OTHER REPRODUCTIVE FACTORS

Some authors have noted that patients with cervical adenocarcinoma are less likely to be parous than those with squamous cancer. (Menczer et al , 1978 Korhonen 1980, Milsom and Friberg , 1983 Silcocks et al ,1987) The last mentioned authors reported an elevated relative risk of 2.1 for nulliparous subjects, and in this series 44% of those with adenocarcinoma were nulliparous. In contrast with the above findings , Parazzini et al ,1988 reported that the risk of cervical adenocarcinoma increased with the number of births and was significant for >=3 births. This significance persisted despite controlling for age and age at first sexual intercourse. These authors also reported a significant excess of abortions , both spontaneous and induced , in subjects with adenocarcinoma . However there is no other report of an association between high parity and squamous disease . Parazzini et al ,1988 also reported that increasing age at first birth was a significant risk factor for adenocarcinoma and similar findings were reported by Kvale et al , 1988 in a comparison of adenocarcinoma with squamous cancer.

Some authors have noted no relationship between parity and cervical adenocarcinoma (Brinton et al ,1987b Horowitz et al ,1988).

GEOGRAPHICAL VARIATION

Menczer et al ,1978 , in a study of Jewish women, noted that adenocarcinoma was limited to those groups who were at lowest risk for squamous cancer , notably Asian and European born Jews. Patients with

adenocarcinoma were more likely to be Rural dwellers than city inhabitants. (Korhonen 1980 Milsom and Friberg , 1983)

SOCIAL STATUS

Unemployment , low income , cigarette smoking and less educational attainment were significantly less common characteristics of adenocarcinoma compared to squamous controls (Horowitz et al , 1988). Silcocks et al ,1987 reported that in women of known social class, (only 47% of subjects studied) there was a smaller proportion of cases of adenocarcinoma in the lower income groups, compared with squamous cancer.

ASSOCIATED PATHOLOGY

Adenocarcinoma is associated with CIN in 43% of cases. (Abell and Gosling,1962) , and is also found in association with squamous invasive disease . (Choo and Naylor , 1984) This suggests that these two lesions may have a common cell of origin , and it is conceivable that there may be shared risk factors. Teshima et al,1985 reported that early adenocarcinomas, including AIS/ high grade CIGN ,were situated near the region of the squamocolumnar junction,and were frequently associated with CIN. (Figure 1 - 7)

Two studies have reported that arterial hypertension is significantly more common in adenocarcinoma compared with squamous cancer. (Silcocks et al , 1987 Kohronen , 1980)

Milsom and Friberg , 1983 observed that patients with AC were significantly more likely to be diabetic than squamous controls.

SEXUAL BEHAVIOUR

Sexual behaviour in relation to adenocarcinoma has been explored in only 3 studies. An elevated risk of disease has been noted in association with young age at first intercourse (Parazzini et al ,1988) and increase in numbers of sexual partners . (Brinton et al ,1987b) Horowitz et al , 1988 reported that intercourse at <18 years occurred significantly less commonly amongst subjects with adenocarcinoma compared with squamous controls. Hence , in terms of the degree of sexual activity , subjects with adenocarcinoma appear to adopt an intermediate rank between normal subjects and those with squamous cancer . Some indirect evidence for the apparently less important role of sexual behaviour in AC compared to SC was provided by Menczer et al,1978 who reported that in Israelis , the ethnic groups with the lowest incidence of squamous disease, i.e. the most orthodox in terms of monogamous sexual behaviour , had the highest incidence of adenocarcinoma.

OBESITY

A relationship between excess weight and AC has been observed . (Parazzini et al , 1988 Brinton et al , 1987) These findings may indirectly support an endocrine hypothesis for the pathogenesis of AC . Obese subjects have also been observed to have a higher incidence of endometrial carcinoma (Gusberg et al ,1988) . The association of obesity with both diabetes mellitus and hypertension adds further weight to the hypothesis that AC has an hormonal basis.The opinion has been expressed that AC has more in common with the epidemiological profile of endometrial cancer than squamous cervical neoplasia. (Kohronen 1980 Milsom and Friberg , 1983)

ENDOCRINE FACTORS

Adenocarcinoma has been noted to occur in conditions where the patient's hormonal background differs from the normal. i.e. , in pregnancy and in those taking the oral contraceptive pill.

a) PREGNANCY

Certain tumours have been observed to be more common in pregnant patients or those recently delivered,notably adenosquamous tumours (Steiner and Friedell,1965)

b)THE ORAL CONTRACEPTIVE PILL (OCP)

The OCP and squamous cancer

It has been postulated that OCP use is linked to the development of cervical carcinoma.The association has been generally found to be weak and to disappear after controlling for sexual behaviour. It would appear that such a relationship would be biologically feasible.The process of cervical squamous metaplasia ,which involves active phagocytosis by the cells undergoing this change, takes place at a high rate in the teenage years.(Coppleson,1977)This process may render the cervix especially susceptible to any environmental carcinogen met at this time. Hence , early pill usage could provide a carcinogenic stimulus, or modify the effect of any sexually transmitted carcinogen.

Two significant studies highlighted increased risk associated with long term use of the OCP . (Vessey et al ,1983a WHO,1985) In the former study all 13 cases of invasive disease which developed did so in OCP users, 9 of whom gave a history of more than 6 years OCP use. Although this study was criticised for failure to control for sexual variables , a later study (Vessey et al ,1983b) revealed no appreciable difference in the sexual

histories of the OCP and IUCD users . In the WHO study, there was an adjusted relative risk value of 1.2 for ever use of the OCP. After 5+ years of use, the risk was 1.5 . However, it was uncertain whether this association was confounded by sexual and other variables. Other studies fail to show a positive relationship with OCP usage. (Thomas,1973 Clarke et al,1985)

The OCP and adenocarcinoma

Valente and Hanjani, 1986 and Dallenbach Hellweg, 1984 , noted an association between AIS /invasive adenocarcinoma and OCP use. Peters et al ,1986 found an increased incidence of adenocarcinoma during the course of the period 1972-1982, in women under 35 years of age from higher socioeconomic backgrounds. It has been postulated that this increased incidence in young women is linked to pill use. Contraceptive pills have been available since the mid 60's, i.e. at precisely the time when these women were teenagers embarking upon sexual activity. There is little objective evidence for an increase in the incidence of pre-malignant glandular lesions in women on the pill . (Mingeot and Fievez , 1974). However , it was noted that women on the pill had swollen epithelium , stromal oedema , congested capillaries , increased epithelial height , increased mucus production , and an increased rate of metaplasia. .The degree of these changes was greater than those induced by the pregnant state. Nevertheless , there was no increase in the incidence of atypical epithelium . Maqueo et al ,1966 reported that progestins (ingested in the OCP and on their own) caused hypersecretion , stromal oedema , and squamous metaplasia , but no increase in epithelial atypia. Dallenbach Hellweg ,1984 described 28 cases of adenocarcinoma of the cervix. Long term use of gestagens was noted in 82% , compared with only 40% of squamous carcinomas encountered by this author. In addition , out of 12 cases of AIS , 11 were on the OCP at some stage. The above author proposed that both microglandular hyperplasia (MGH) and atypical

adenomatous hyperplasia are pre-malignant conditions . She claimed that these lesions were present in endocervical biopsies prior to diagnosis of invasion. Reservations concerning the representativeness of small endocervical samples have been outlined earlier, (page 28) and it is possible that these malignancies may have been present at the time of the endocervical sampling . It was also claimed that specific histological subtypes were associated with pill use ; notably , adenosquamous and microalveolar tumours . However, other workers in this field regard MGH as a lesion which is benign (see page 29) ,and the consensus is that the most likely precursor lesion of invasion is adenocarcinoma in situ/high grade CIGN . A recent case control study of OCP ingestion in adenocarcinoma patients compared to squamous controls showed no significant differences in frequency of or duration of use of the OCP . (Persson et al , 1987)

INFECTION

Herpes Simplex(HSV)

A review of the relationship between Herpes virus and cervical cancer

These remarks relate almost exclusively to studies of squamous cancer, but are worthy of consideration at this stage. There was much circumstantial evidence in support of herpes simplex virus (HSV) being the infectious agent of cervical cancer. Both strains of HSV , types 1 and 2, cause well recognised genital lesions. HSV replicates in the human cervix, and is sexually transmitted. Patients with type 2 infection share certain epidemiological characteristics with cervical cancer patients (Adam,1972) . A higher than expected frequency of cervical carcinoma and carcinoma in situ occurred in women infected with HSV-2 (Naib et al ,1969). Viruses of the herpes genus have an established oncogenic potential in both animal

and human hosts (See Macnab,1987) . In addition , partially inactivated HSV can transform rodent cells in culture (Duff and Rapp 1971) , and both malignant tumours and dysplastic lesions have been produced in *in vivo* experiments with chronic exposure to HSV in mice . (Wentz et al,1981) . The plausability of HSV being oncogenic in the cervix is increased by the observation that the virus may become latent , with persitence in ganglionic sites .This would enable repeated infection to occur, and potentially, *in vivo* cellular transformation.

The acceptance of herpesvirus as the infectious agent of cervical cancer was hampered by an inability to detect HSV-2 DNA in anything but a small proportion of cancer tissues (Maitland,1988) Virtually all early studies failed to detect any evidence of a virus 'footprint'. Nevertheless ,these observations do not rule out a role for herpes virus as an initiator of oncogenesis under certain conditions (see later) .

Sero-epidemiological studies provided strong circumstantial evidence for the involvement of HSV-2 in cervical cancer. Such evidence derived exclusively from retrospective case control studies of the prevalence of circulating antibodies to HSV-2. These studies have been well summarised (See Melnick and Adam,1978) and with few exceptions (Priden and Lilienfield,1971) they revealed a higher prevalence of antibody to HSV-2 in cases of invasive carcinoma, carcinoma in situ and cervical dysplasia than normal controls.

The findings from these studies suggested the need for prospective studies of the relationship between infection and carcinoma . Initially healthy women were followed up for the development of cervical neoplasia and simultaneously tested for antibodies to HSV-2 (Vonka et al,1984 Adam et al ,1985). These studies failed to support an aetiological relationship between HSV-2 infection and cervical neoplasia, no appreciable risk of cervical neoplasia being observed in association with previous HSV infection. In the former study, only 21 cases of invasive

disease were identified, yet only 3 of these subjects were seropositive. Likewise only 2 out of 70 patients developing intraepithelial disease experienced seroconversion to HSV-2 positivity. In order to explain the results from retrospective studies ,the consensus view was that the apparent relationship between cancer of the cervix and HSV-2 infection existed as both diseases were independent co-variables of sexual promiscuity.

A recent hypothesis of the causation of cervical cancer accepts that cancer is the result of a multistage process.(zur Hausen,1982) and HSV may still have a role in this sequence. [although increasing attention has been focussed on the relationship between human papilloma virus (HPV) and carcinoma.]

A variety of cancers have been observed to occur in association with HPV but in order for malignant conversion to occur, a carcinogenic stimulus is required to operate against the background of a 'promoting' HPV infection. It is proposed that HSV may act as a potent mutagen in the HPV infected cervix , and thus initiate the development of neoplasia. This view is analogous to the 'hit and run' theory (Skinner 1976) , and provides a convenient explanation for the frequent observation that viral DNA is often undetectable in tumour biopsies. Furthermore ,such a model would allow for there to be other initiating events , e.g. cigarette smoking.

In conclusion,while such open ended theories are attractive ,the fact remains that within the scope of present technology,there is no proof of whether HSV-2 is a human carcinogen.

Herpes virus and Adenocarcinoma

Menczer et al,1981 have investigated the sera of patients with adenocarcinoma for neutralising antibodies against HSV1 and HSV2. Patients had already undergone surgery or radiotherapy when serum was collected.Age matched controls were used , consisting of gynaecology in-patients. Higher titres of neutralising antibody to HSV 1 & 2 were found in those with adenocarcinoma compared with normal controls . The results were highly significant for HSV-1, and of borderline significance for HSV2.

Wentz et al , 1975 and 1981 produced a variety of pre-invasive and invasive cervical and endometrial tumours in mice exposed to inactivated HSV types 1 and 2. In the first study , invasive cancer was induced in 30.2% of mice and all these tumours were adenocarcinomas. Squamous dysplasia developed in 58% , and 9% also had early stromal invasion arising from dysplasia. The later study (1981) , employed different strains of mice and of HSV-2 , as well as HSV-1. Following application of formalin inactivated HSV-1 to the mouse cervix, 55.5% developed epithelial dysplasia and 28.9% of mice developed invasive carcinoma , although the majority of these were squamous cancers. Following exposure to similarly inactivated HSV-2 , a similar proportion of mice developed dysplasia and invasive tumours , yet of the invasive cancers 75% were adeno- or adenosquamous carcinomas. These authors failed to elaborate whether the dysplastic epithelia noted were squamous or glandular. These findings raise the possibility that the phenotypic expression of this tumour may vary , in relation to altered presentations of a common aetiological agent , or in relation to genetically mediated factors in the host.

Human PapillomaVirus

Human papillomavirus (HPV) is a small double-stranded DNA virus with recognised oncogenic potential. There are a number of subtypes of HPV which are sexually transmitted , which most commonly produce genital warts or 'condyloma acuminata'. There has been a marked increase in the prevalence of these lesions , which more than doubled between the years 1971 - 1981 (Report of Communicable disease surveillance centre , 1983) . There were concomitant shifts in age-specific registration rates and mortality rates for carcinoma of the cervix in the under 35 age group, implicating HPV in the aetiology of cervical carcinoma . Because HPV cannot be propogated in vitro , it is impossible to raise specific antibodies against its genital subtypes , hence molecular biological techniques employing DNA technology have contributed the bulk of the evidence incriminating the virus as a carcinogen.

Smotkin et al 1986, found 6/9 cases of adenocarcinoma to be HPV positive. Tase et al, 1988 found HPV subtypes 16 and 18 in adenocarcinomas, the predominant type being HPV-18 in 45% of adenocarcinomas. However normal controls were not assessed in either of these studies making the relvance of these results unclear. (Munoz et al , 1988) Wilczynski et al ,1988 detected HPV in 7 of 11 adenocarcinomas , and again there was preponderance of HPV-18 DNA . Controls failed to demonstrate any HPV - DNA. However there was an association between age and HPV positivity which may have confounded this putatively aetiological relationship.

Epstein Barr Virus (EBV)

Singh et al,1990 reporting from Singapore , noted the association of cervical adenocarcinoma in 4 patients who also developed nasopharyngeal carcinoma. A close temporal association between diagnosis of both tumours was reported for 3 of the 4 patients. While nasopharyngeal

carcinoma is relatively common in this racial group , cervical adenocarcinoma constitutes only 8% of all cervical tumours. A large body of evidence incriminates EBV in the aetiology of nasopharyngeal cancer . Hence further exploration of the relationship between EBV and cervical adenocarcinoma may be worthwile.

Adenocarcinoma and adenosquamous tumours

Understandably few attempts have been made to distinguish the epidemiological characteristics of these 2 varieties of carcinoma of the cervix. On the basis of a small case control comparison , Brinton et al , 1987b commented that adenosquamous tumours appeared to be more closely related to squamous cancer than to AC, because of similarities in risk factors . However , Horowitz et al, 1988 found no significant differences in the profiles of these 2 tumours . Given the sparseness of these data, any conclusions must be guarded.

CONCLUSIONS- Epidemiology of AC

There has been very limited study of the risk factor profile of cervical AC. Most studies have involved comparisons with squamous cancer and these studies confirm that the risk factor profile of AC is appreciably different to SC. However , the precise nature of risk factors for AC is poorly defined due to limited comparisons with the normal population. Nevertheless , it would appear that sexual behaviour (increased number of sexual partners) and the reproductive environment (number of pregnancies , obesity) may be important in the pathogenesis of this carcinoma. The available data may support the hypothesis that a viral oncogenic stimulus (related primarily to risk factors involving sexual behaviour) operating against a background of an altered reproductive / hormonal environment may favour the development of glandular neoplasia . Analytical epidemiological studies are required to refute or confirm this hypothesis.

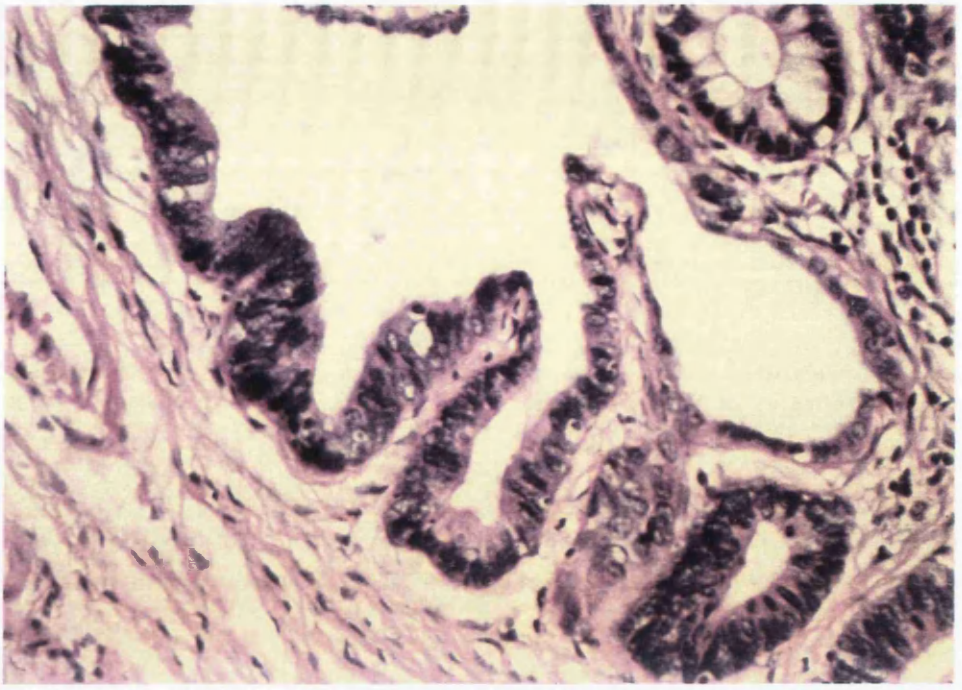


Figure 1-1 AIS/High grade CIGN is present in the crypts . An area of type 11 AIS is seen at 1 o'clock

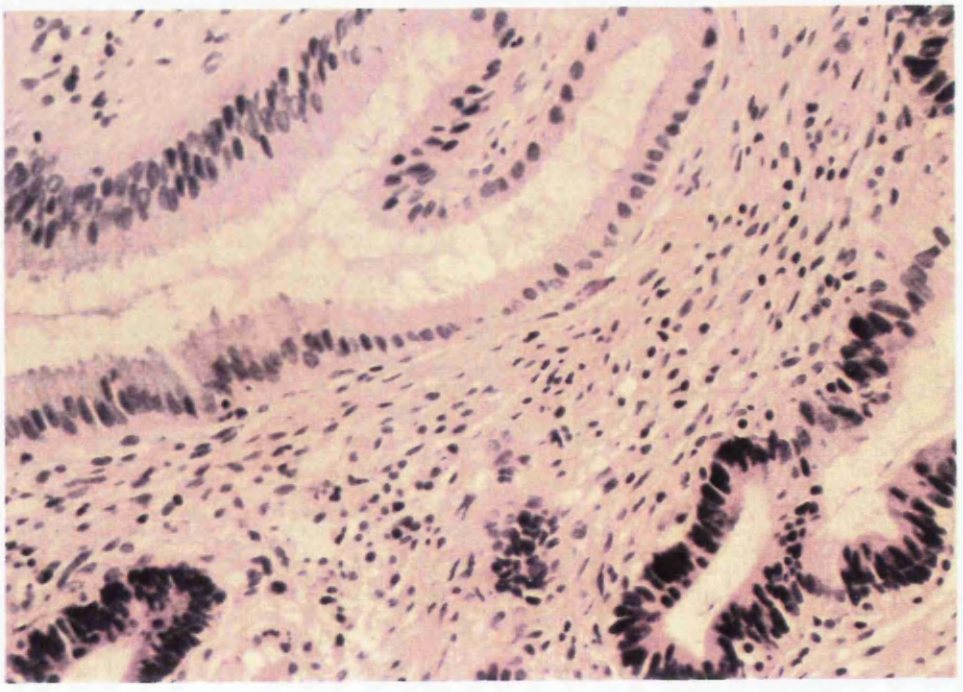


Figure 1-2 Crypts showing high grade CIGN are seen adjacent to a normal crypt.

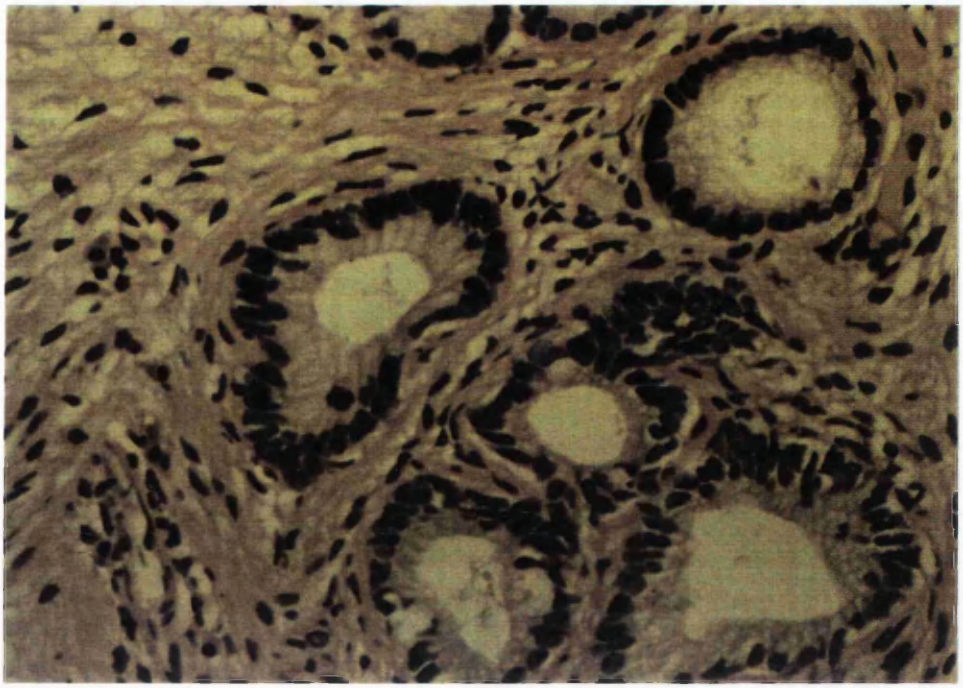


Figure 1-3 A case of intermediate grade CIGN

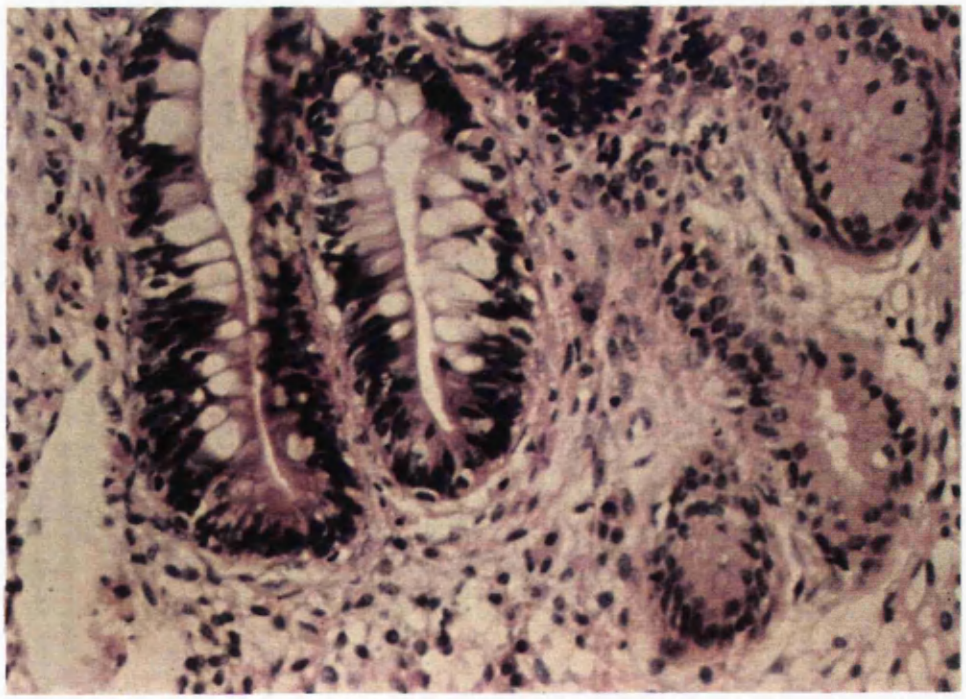


Figure 1-4 Type 11 (Goblet cell) AIS

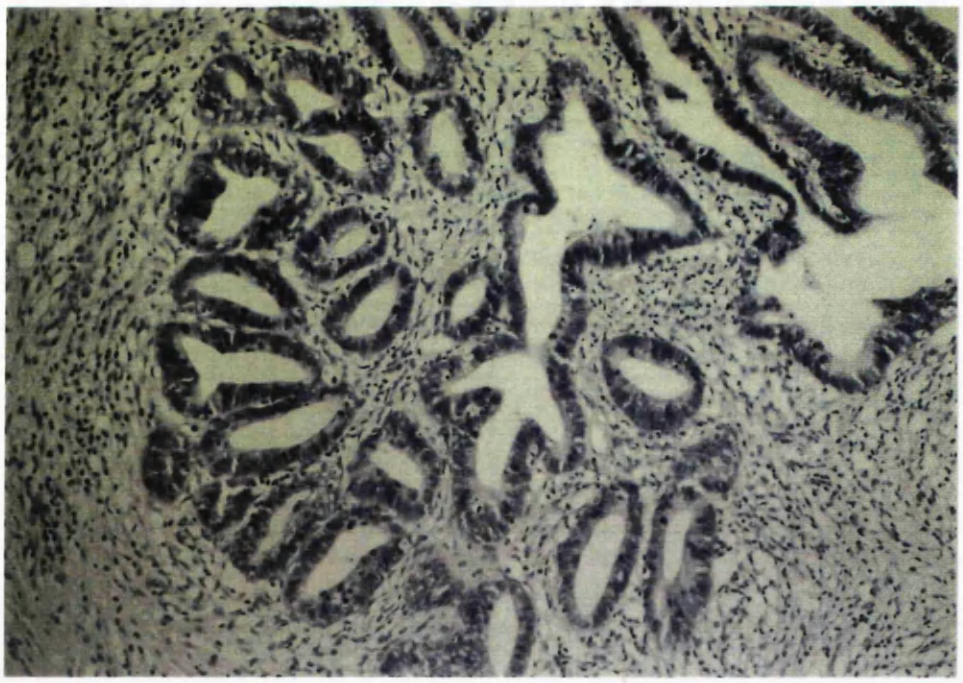


Figure 1-5 A case of florid AIS

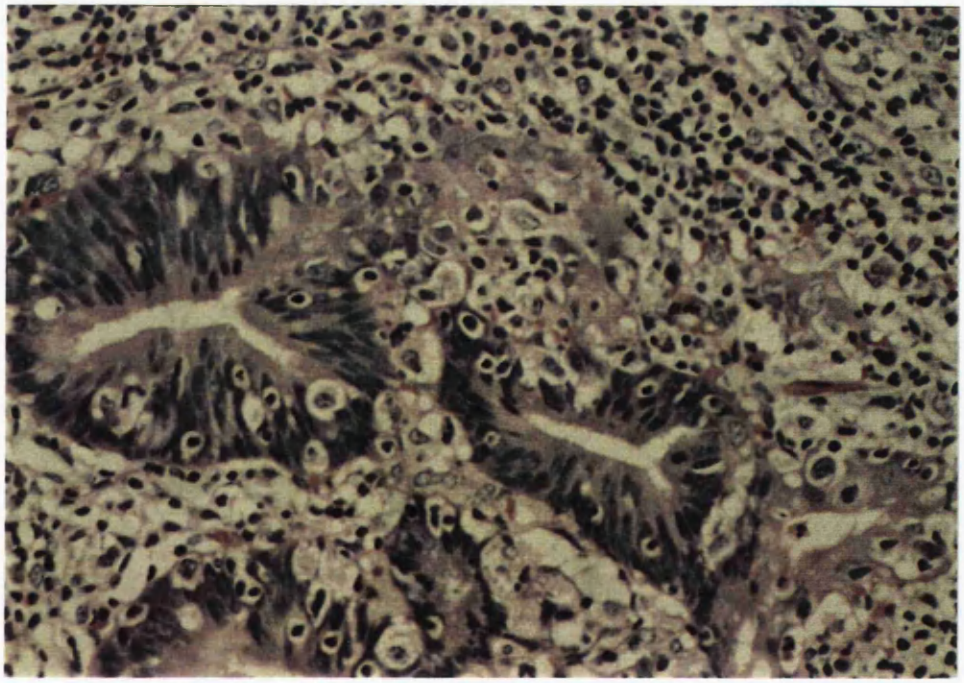


Figure 1-6 AIS/ High grade CIGN in association with foci of early stromal invasion

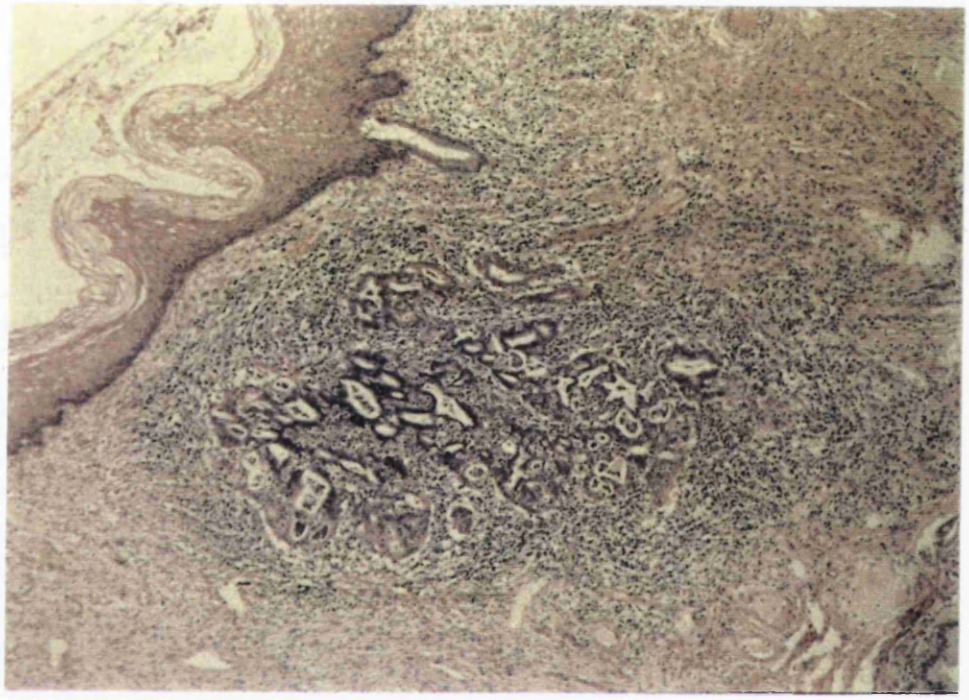


Figure 1-7 Early invasive adenocarcinoma in close proximity to ectocervical squamous epithelium

Figure 1-8 A group of atypical glandular cells in a cervical smear from a case of high grade CIGN

CHAPTER 11

A CASE - CONTROL STUDY OF CERVICAL INTRAEPITHELIAL GLANDULAR NEOPLASIA

AIM

To investigate the aetiology of cervical intraepithelial glandular neoplasia (CIGN).

OBJECTIVES

a) To compare the risk factor profiles of squamous CIN and CIGN.

b) To establish risk factors associated with CIGN.

c) To assess whether herpes virus has an aetiological role in CIGN

STUDY DESIGN

A matched case control comparison has been carried out.

Definition and selection of study population

i) Cases.

Definition of a case

A 'case' was defined as a patient with histologically confirmed CIGN .

Identification of cases

All cases recruited to the prospective study of conisation in the management of CIGN (described in chapter 111), were potentially available for inclusion in the study. Cases which did not fulfil all the entry criteria for this study, (i.e. those who had the disease diagnosed or

managed by hysterectomy) ,were available for inclusion in the study of risk factors . The patients studied originated from the centres illustrated in Figure 3-1. The cases have all been recruited since the study commenced in May 1986 , and therefore represent a proportion of the incident cases.

Case Selection

Subjects were included in the case-control study if :

1) Independent pathology review had confirmed the diagnosis of CIGN on a cone biopsy or hysterectomy specimen , the diagnosis having been made from mid-1985 onwards.

2) The centre registering the case had at least two cases that were suitable for inclusion in the study. (The reason being that given limitations on time and resources , it was decided to sample from centres which could offer multiple cases.)

3) Consent had been obtained from the patient and her gynaecologist.

Controls

Two control groups were recruited:

1) Women with CIN (Squamous cervical intraepithelial neoplasia) and:

2) Women with no history of cytological abnormality ("normal" controls).

In both instances , multiple controls were matched to cases in order to maximise the statistical efficiency of the study . (Gail et al, 1976)

CIN controls

Definition of CIN control group

These were patients who had CIN grade 11 or 111 diagnosed on a cone biopsy or hysterectomy specimen.

Identification of CIN controls

These controls were recruited from the same hospital which referred the case of CIGN.

Selection of CIN controls

Controls were selected by :

1) Identifying the case subject in the relevant operating theatre register.

2) Using this register to detect the next five subjects who:

a) had the same surgical procedure as the case, (and hence the same method of histological assessment)

b) were of similar age, i.e. within 5 years (+ or -) of the case subject.

The patient's notes were then reviewed in order to confirm the diagnosis of CIN. In some cases , patients identified by this method did not have histologically confirmed CIN 11 or 111.These considerations, coupled with the varying response rates between centres , led to there being variation in the number of CIN controls.

'Normal' Population Controls

Definition of normal controls

These were subjects who had :

i) a negative cervical smear within 5 years of the interview date,

ii) no history of an abnormal smear , or treatment for cervical intraepithelial neoplasia,

iii) a uterus (i.e. no history of hysterectomy.)

iv) an age within (+/-) 5 years of case

Identification of normal controls

These controls were recruited from the general practice which the case patient attended at the time of registration in the study.

Selection of normal controls

Co-operation was sought from the case patient's general practitioner. Following this , a preliminary visit was made to the practice in order to identify suitable controls from the practice age-sex register, or alphabetic index of notes .The case patient was identified in the register / index , and the 3 subjects immediately before, and the 3 immediately after her in rank within the register,were chosen as potential controls. If any of the subjects chosen did not fulfil the selection criteria , then adjacent records were examined until a total of 6 suitable subjects had been identified. The first 4 patients chosen , i.e. the 4 patients closest in rank to the case patient were designated as controls , with the remaining 2 considered as reserves. The 6 patients identified were invited to an interview with the principal investigator in the surgery. If all four of the controls attended, and both reserves also attended ,then only the data from the first four controls was

used in the study. If any of the first choice controls declined to attend, then the reserves were used as controls.

Methods

The study required:

1) collection of information on potential risk factors , and :

2) a serological test for neutralising antibodies to Herpes virus types 1 and 2.

1) Risk factor assessment

Information was collected by means of a structured interview questionnaire which was first piloted at the Birmingham and Midland Hospital for Women , and at BelleVue Surgery , Edgbaston , Birmingham.

Organisation of interviewing

Consent

Co-operation was sought from the gynaecologist responsible for the care of the case patients and CIN controls , and from the general practitioner with whom the patient was registered, prior to attempting to invite the cases and controls to participate . There were two instances where the gynaecologist refused to consent to participate in the study. In circumstances where general practitioners refused their consent,(6 instances) an alternative general practice in a similar area was approached, and consent obtained.

Invitation

Cases and controls were invited to interview by letter. A reply paid envelope with a response slip was provided. A standard letter of invitation was sent to cases and controls in which it was pointed out that a range of questions relating to the patient's social and behavioural background , general health ,gynaecological and contraceptive history would be asked.

Interview schedule

Interviews took place either in hospital out-patients (Cases and CIN controls) or at the practice surgery . (Normal controls) All interviews were conducted in a private consulting room on a one to one basis with the interviewer (JEC). Following experience gained at the pilot study stage, all interviews were planned for late afternoon / early evening in an attempt to optimise response rates.

Information given to subjects prior to commencing interview

At the outset, the purpose of the study was explained , and the general nature of the interview outlined . It was emphasised that all information would be treated confidentially , and that no question which caused embarrassment need be answered .The purpose of these explanations at the onset of the interview was not only to provide the subject with information , but to allow the subject to acclimatise to the conditions of the interview and to attempt to establish mutual rapport. Both control groups were informed that they were acting as controls for a patient of similar age , and in addition the date upon which the case was diagnosed was given to the patient . She was then asked to base her replies to the questionnaire on her experiences up to and including this date. (pseudo-diagnosis date)

Structure of questionnaire (See appendix 2)

1) Variables recorded

a) Social and demographic

Marital status

Number of marriages

Type of housing

Duration of formal education

Social class

b) Reproductive and sexual

Number of pregnancies

Number of pregnancies terminated

Age at first pregnancy

Age at Menarche

Age at first sexual intercourse

Lifetime number of sexual partners

History of sexually transmitted disease (STD) in current sexual partner

History of sexually transmitted disease (STD) in a previous sexual partner

History of attendance at an STD clinic

c) Contraceptive practice

Current contraceptive method

Ever use of oral contraceptive pill (OCP)

Ever use of low dose pills (<= 35 micrograms of ethinyl oestradiol or equivalent oestrogen)

Ever use of high dose pills (>= 50 micrograms of ethinyl oestradiol or equivalent oestrogen)

Ever use of progestagen only pill (POP)

Age at commencing OCP use

Duration of use of ;

- low dose OCP

-high dose OCP

- POP

Ever use of intrauterine contraceptive device , duration of use

Ever use of barrier contraception , duration of use

d) Alcohol intake and cigarette smoking

Current smoking

Ever smoked (see text for definition of smoking)

Duration of smoking

Number of cigarettes smoked per day

Alcohol consumption in 'units' (see text)

e) Negative checks

History of :

- specific childhood infectious illnesses

- rheumatic fever

- appendicectomy

-cholecystectomy

-haemmorhoids

-varicose veins in lower limbs

-diabetes mellitus

-myocardial infarction

-cerebrovascular accident

-epilepsy

-dyspepsia

- hypertension

-tuberculosis (continued)

-glandular fever
-(Non genital) Warts
- anal warts
-oral herpes 'cold sores'

Specific variables requiring amplification

a) Oral contraceptive pill usage

Data on specific types of OCP usage was collected with reference to a catalogue of OCP samples .This catalogue consisted of 45 different brands of both currently available brands and brands which have now been withdrawn from the market .These samples were obtained from the Birmingham Brook Advisory Centre, Edgbaston and the pharmacy of the Birmingham and Midland Hospital for Women. With respect to the collection of data concerning duration of oral contraceptive pill (OCP) use , these estimates were made by calculating the length of time elapsed from the beginning of the exposure until the pseudodiagnosis date , or until the time when the exposure ceased. With regard to use of the OCP, one year was deducted from the total duration for any interim pregnancy. In this way , it was hoped that any tendency for subjects to express number preferences could be discouraged.

b) Cigarette smoking

'Smoking' was defined as a habit of at least 1 cigarette or cigar a day for at least one year. In order to calculate duration of the smoking habit the length of time elapsed from the beginning of the exposure until the pseudodiagnosis date , or until the time when the exposure ceased was computed. Periods of abstinence from tobacco were subtracted from this estimate.

c) Social class

Women were allocated to a social class on the basis of their own occupation , by reference to the Classification of Occupations (OPCS ,1980) The classes used were ; 1 , 11 , 111N (non manual) , 111M (Manual) , 1V and V. In addition , a category 'V1' was created for the unemployed , and for retired individuals and pensioners . Students were classified according to the occupation of the father / head of family household. Women who described themselves as housewives were classified according to the occupation of their husband.

Alcohol consumption

Alcohol consumption was measured on an analogue scale from 1 to 5. This scale portrayed a spectrum of alcohol consumption from complete abstinence to 3 or more units per day .This scale was based on analysis of responses to the pilot questionnaire when asked "How often do you have an alcoholic drink " , a 'drink' being defined as a unit of alcohol equivalent to a half pint of beer or lager , a measure of sherry or other aperitif , a glass of wine , or a short measure of spirits . On the basis of the responses , median alcohol consumption proved to be 3 units per week. In the definitive questionnaire , a 'flash - card ' was presented to the subject who was then asked to choose which was the most appropriate category .

Negative checks

Some questions were included as negative checks .In other words, information was sought on exposures thought unlikely to be associated with the development of the diseases studied,e.g. a history of specific childhood infections , a history of haemorrhoids, etc . This would

facilitate the assessment of the degree to which cases and controls were comparable.

Pathological definitions

CIGN

Please refer to Chapter 1 (page 22).

Cervical Intraepithelial Neoplasia

CIN consists of disordered maturation and cellular atypia of the squamous epithelium of the cervical transformation zone. Diagnostic criteria for CIN are firmly established (Buckley et al,1982) ,the major cellular changes being qualitatively similar to CIGN. Recently it has been observed that there is a high degree of inconsistency between observers in the diagnosis of CIN grade 1, this diagnosis often being confused with the cytopathic effect of Human Papillomavirus . (Robertson et al 1989 , Ismail et al , 1989) For this reason , only CIN lesions of grade 11 or 111 were recruited , as there is likely to be greater consistency in diagnosis . However, histopathologic material from CIN controls was not subjected to histopathological review.

Pathology review

Histopathological material for CIGN cases was reviewed by a gynaecological pathologist of University Senior Lecturer status. (TPR) The case material was reviewed without foreknowledge of the precise histopathological diagnosis,other than that it was felt by the referring

department that there was evidence of CIGN. The purpose of the review was to establish the diagnosis of CIGN, to exclude invasive disease, and to confirm or exclude the presence of accompanying CIN.

Assessment of neutralising antibodies to HSV-1 and HSV-2

Laboratory Methods

At the termination of the interview , 10ml of venous blood was taken . This was subsequently spun down in a centrifuge in order to separate serum which was stored at -60c until analysed for neutralising antibodies to herpes simplex virus Types 1 and 2.

Laboratory technique

a)Neutralisation of live virus

Test sera and matched pre-immune (zero antibody) and hyperimmune rabbit sera for both HSV-1 and HSV-2 were diluted 1 in 10 in phosphate buffered saline , and heat inactivated at 56^0c for 30 minutes and tested by kinetic neutralisation at 25 ^{0}c . Each serum dilution was mixed with an equal volume of virus suspension containing 5 x 10^5 plaque forming units , of either HSV-1 'Troisbel 'strain , or HSV-2 , strain 3345 and the reaction was allowed to proceed at 25^0c for 1 hour. Pre - immune and hyperimmune sera were employed as controls .

b)Assay of remaining virus

Following neutralization , 0.02 ml of the virus solution was serially diluted (10^{-2} ,10^{-3} , 10^{-4} dilutions) in 2ml of Eagles minimal essential

medium supplemented with 10% tryptose phosphate broth and 10% newborn calf serum. The remaining viable virus was titrated using BHK 21 cells (Baby Hamster Kidney) , by adding 8×10^6 cells to each dilution . (Russell , 1962) Samples were then placed on a shaker at 37^0c for 1 hour. Following this , 8ml of ECCMC medium (Eagle's medium + calf serum + carboxymethyl cellulose) were added and the sample distributed between 2 petri dishes (5ml each) The dishes were incubated at 37^0c in 5% CO_2 for 48 - 72 hours and then fixed with formol saline and stained with dilute carbol fuchsin . A count of virus plaques was then made for each dish .

Calculation of k values

The viral titre in plaque forming units per ml was calculated , and then the $\log_{10}$ of this value .

Furthermore ;

$\log_{10}$ (preimmune) - $\log_{10}$ (hyperimmune) = Maximum log reduction

and

$\log_{10}$ (preimmune) - $\log_{10}$ (Sample) = log reduction for sample

The K value , or neutralising rate constant is calculated using the formula:

$$K = \frac{2.3 \ [\text{log reduction(sample) x dilution}]}{\text{time (minutes)}}$$

i.e ;

$$K = \frac{2.3 \ [\text{log reduction(sample) x 20}]}{60}$$

Data Handling

After the collection of crude data at interview, the information was

condensed onto a computer coding form , to which was added the results from the estimation of serum neutralising antibodies to HSV . A database was constructed using a modification of a dBase 111 package ('Foxplus') and the information stored on hard disk using an Apricot Xeni/286 microcomputer.

Statistical Methods

Outline

Because :

a) cases and CIN controls had been actively matched for method of histological diagnosis , age and time of diagnosis ,

b) cases and population controls had been actively matched for age and geography ,

a matched analysis was appropriate .The odds ratios and 95% confidence intervals for the risk factors under study were estimated using conditional logistic regression using the package Egret (Pecan) , commencing with univariate analyses. (See statistical appendix for details of the technique of multivariate analysis)

Manipulation of exposure variables

Where variables were divisible into more than 2 categories , e.g. alcohol consumption, the statistical significance of any trend in risk with varying levels of exposure was assessed by the likelihood ratio statistic (LRS). Multivariate analyses were subsequently performed to calculate the odds ratios adjusted for confounding factors . An example of the multivariate technique as applied to these data is given in the statistical appendix.

The statistical handling of some of the variables requires amplification, and the variables in question are again discussed in the statistical appendix .

RESULTS

Study population

Recruitment to the case - control study commenced in November 1987 and closed in April 1989, during which time 108 patients were registered in the BSCCP study of conisation in the management of CIGN. Of these 108 , 50 met the selection criteria for the case - control study.

Exclusions (See table 2-1)

a) Prior to invitation to participate

Of the 58 subjects excluded at this stage , 8 subjects came from centres which only registered 1 case, consent for participation was not obtained for 11 subjects and pathological material from 39 subjects had not yet undergone central pathology review .

b) Following invitation to participate

Five of these subjects failed to attend interview . Two subjects were excluded after further pathology review and 1 was excluded when it was not possible to find a suitable CIN control.

Hence , although 45 subjects with CIGN were interviewed (41.6% of the available study population) , there were 42 evaluable cases at the close of recruitment . Of these , 16 had pure CIGN in the absence of any abnormal squamous component , and 26 had a mixture of CIGN and CIN. These 42 cases generated 110 CIN , and 159 population controls . Thus a total of 314 subjects was interviewed.

TABLE 2-1 : SUMMARY TABLE OF RESPONSE RATES AND REASONS FOR EXCLUSIONS : CASE CONTROL STUDY OF CIGN

	CIGN	CIN	GPcontrols Total
Met selection criteria (CIGN cases only)	61	-	-
Consultant in charge refused consent to participate	6	-	-
Number eligible	55	162	258**
Subject refusal	5	34	83
Failed to attend after accepting	5	18	16
No CIN control match(CIGNonly)	1	-	-
No case match ++(CIN only)	-	30	-
'Reserves' not needed (GP controls only)	-	-	21
Excluded after pathology review of CIGN cases	2	7+	3+
Excluded due to inappropriate selection***	0	1^^	2
Final numbers included	42	72	133
Response rate	81.8%	68%	61.6% (overall)

KEY TO FIGURE

^^ Patient had invasive disease
+ controls belonging to CIGN case
++ due to failure of selected CIGN cases to attend
** GP controls selected after succesfully interviewing CIGN case + CIN controls
*** 1 with history of cone biopsy, 1 had hysterectomy

Response rates (Table 2-1)

CIGN

Of 55 cases invited to interview, 45 attended (81.8%). Of the 10 cases who failed to attend interview , the geographical distribution was Bristol (2), Birmingham (3) , Derby (1) , Newcastle (2) , Liverpool , (1) and Middlesborough (1)

CIN

110 out of 162 controls (68%) attended for interview. The response rate did not differ significantly between centres.

Population controls

159 out of 258 subjects (61.6%) attended for interview. The response rate did not differ significantly between practices.

Exclusions following pathology review

Two patients initially thought to have CIGN were excluded subsequent to pathology review, leading to loss of these 2 cases and their associated CIN controls. Seven controls were therefore discarded as a result of exclusion of the cases associated with them.

Sample population

After exclusions had taken place there were 42 matched sets of cases and controls Thus a total of 247 subjects were included in the analysis. (See table 3-1)

TABLE 2-2 . ORIGIN OF CASES OF CIGN (See also Figure 3-1)

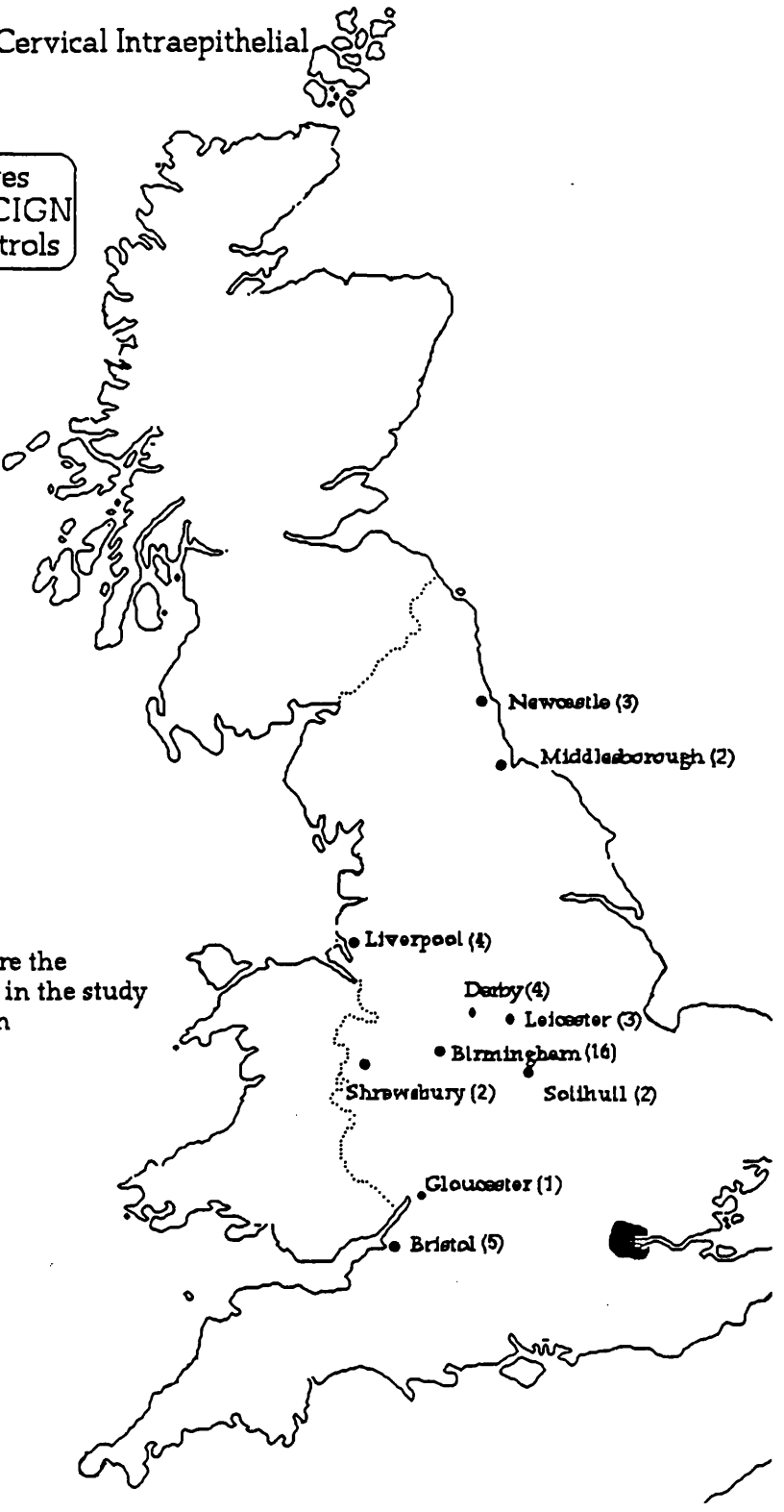
Birmingham and Midland Hospital for Women
Dudley Road Hospital,Birmingham
St. Chad's Hospital,Birmingham
Royal Infirmary Leicester
Liverpool Women's Hospital
Royal Shrewsbury Hospital
Gateshead Hospital,Tyne and Wear
Solihull Hospital
Derby City Hospital
Bristol Royal Infirmary
Leicester General Hospital
South ClevelandHospital
Gloucester Royal Hospital

Table 2-3 - Demographic characterisitics of cases and controls

	Group				
	CIN	CIGN	CIGN mixed	pure	Normal
number	72	42	26	16	133
Mean Age (SD) (years)	35.3 (8.5)	36.3 (8.3)	34.8 (8.2)	38.6 (7.9)	38.6 (7.6)
Range	21-58	25-57	20-57	25-56	20-59
Social class	numbers(percentages in parentheses)				
1 + 11	18 (25)	9 (21.4)	4 (15.4)	5 (31.3)	26 (19.5)
111n + 111m	28 (38.9)	20 (47.6)	15 (57.7)	5 (31.3)	79 (59.4)
1V + V	18 (25)	11 (26.2)	6 (23.1)	5 (31.3)	23 (17.3)
Unemployed/ Disabled	8 (11.1)	2 (4.8)	1 (3.8)	1 (6.1)	5 (3.8)
Ethnic group					
Caucasian	71 (98.6)	41 (97.6)	26 (100)	15 (93.8)	129 (97)
Other	1 (1.4)	1 (2.4)	0	1 (6.2)	4 (3.0)

Case control study of Cervical Intraepithelial Glandular Neoplasia

Major Hospital Centres contributing cases of CIGN and matched CIN controls



Numbers in parentheses are the numbers of cases included in the study from the centre in question

1 cm represents 45 km

Demographic variables

Controls were matched to within 5 years of the age of the case of CIGN , and the mean ages of each subgroup are presented in Table 2-3. When comparing CIGN with CIN , the difference in mean age was 1 year (95% C.I. on difference in mean age = +/- 3.2 years) . Similarly , the difference in mean age of CIGN and normals was 2.3 years (95% C.I. on difference in mean age = +/- 2.8 years) . However , when considering CIN and normals the difference in mean age was 3.3 years (95% C.I. on difference in mean age = +/- 2.3 years) , and for mixed CIGN and normals , the difference was 3.8 years (95% C.I. on difference in mean age = +/- 3.4 years) . Hence although some significant differences have been noted the magnitude of the age differences is , as illustrated, small , and therefore unlikely to be of any significance in relation to the risk factors for either condition.
With respect to social class, no significant differences in the distribution of social class were detected between CIGN and normals , and CIN versus CIGN patients . However 36.1% of the CIN subjects were social class 1V or lower , compared to 21.1% of normal subjects (X^2=9.78 , p=0.02) All groups were well matched for ethnic background ,with Caucasians representing over 93% of each group .

ANALYSIS

The strategy chosen for the analysis of the data and the sequence of analytical steps are first outlined;

a) Assessment of risk factors for CIN

Attention was initially focused on a case-control comparison of women with CIN and healthy controls . This was not one of the primary objectives of the study . However, the epidemiology of CIN is well documented , and therefore the results of such a comparison could be used to establish the generalisibility of the data on CIN controls.

b)Assessment of risk factors for CIGN

i) A comparison was made between cases of CIGN and normal controls.

CIGN lesions are divisible into 'pure' (CIGN only) and 'mixed' (CIGN and CIN) subtypes . In an attempt to improve the precision of the analysis, the case patients were divided into 2 groups according to histological subtype and 2 further case control comparisons made.

ii) 'Pure' CIGN versus population controls

iii) 'Mixed' CIGN versus population controls

It is acknowledged that the dilution of sample size following this subdivision would inevitably lead to some loss of statistical power to identify risk factors.

c) Epidemiological comparison between CIGN and CIN

i) A case control comparison was made between cases of CIGN and CIN.

Subsidiary analyses were made after subdividing CIGN lesions into 'pure' and 'mixed' subtypes. i.e.

ii) 'Pure' CIGN versus CIN controls

iii) 'Mixed' CIGN versus CIN controls

d) Case - Control comparisons of neutralising antibodies directed against HSV-1 and HSV-2 .

Part 1 - Interview questionnaire

CIN vs Healthy Population Controls

a) Post hoc matching procedure

As both CIN and normal controls were matched with CIGN cases for age but not with each other , it was necessary to undertake a post hoc matching procedure of CIN cases and controls before comparing these groups . This matching procedure ensured that :

1) only 1 CIN case was matched to one or more population controls,

2) normal controls were age matched to within 5 years of the CIN case,

3) the geographic relationship between CIN and population controls was maintained.

This led to there being 57 matched sets containing 118 population controls.

i) Univariate analysis

For these data , please refer to Table 2-15 in the appendix to this chapter. The results of this analysis are shown overleaf .

Summary of univariate analysis

*The odds ratio of disease was **significantly elevated** in those with the following exposures ;*

2 or more marriages
Living in rented accommodation
Current cigarette smoking
Ever smoking
Alcohol consumption in excess of 260 units per year
5 or more sexual partners
A history of having attended a clinic for sexually transmitted diseases (STD) clinic
Subjects whose current partner had a history of STD
Subjects in whom a former partner had a history of STD

*The odds ratio of disease was **significantly reduced** in those with the following exposures ;*

ever use of barrier contraception
commencing intercouse at >=19 years

*Statistically **significant dose - response relationships** were observed for :*

Alcohol consumption
Duration of the smoking habit
Numbers of cigarettes smoked
Increasing number of sexual partners
Increasing age at first intercourse (diminishing risk)

Duration of use of high dose(>=50 microgram oestrogen) contraceptive pills **

(** This was arrived at by using minimum substitutions for missing data - please refer to statistical appendix)

Multivariate analysis

Following univariate analysis , the statistically significant risk factors were assessed in a multivariate model using conditional logistic regression. The results are illustrated in table 3-5 , which illustrates the factors which confer a significant and independent effect on risk . (For a full explanation of the modelling process , turn to the statistical appendix)

Variable	Coefficient	Standard Error	P value	Odds ratio(95% CI's)
Number of sexual partners	2.006	0.521	< 0.001	7.4 (2.7 - 20.7)
Cigarette smoking (Ever / never)	2.747	0.801	<0.001	15.6 (3.2 - 74.9)
Antibodies to HSV2^ (+/-)	2.368	1.17	0.042	10.7 (1.1 - 104.9)
Partner with history of STD*	2.407	1.13	0.033	11.1 (1.2 - 101.2)

Table 2-4 CIN versus normal controls ;Conditional logistic regression ; Best fitting model

(* Sexually transmitted disease)

(^ It is of interest that HSV -2 should emerge as a risk factor having been non-significant on univariate analysis. This effect is due to confounding with number of sexual partners.When number of sexual partners is controlled for , exposure to HSV-2 becomes a significant risk factor .)

Conclusions : CIN versus normal controls

These findings are consonant with risk factors for CIN previously identified (Aurelian et al ,1973 Harris et al , 1980 Buckley et al , 1981 Lyon et al , 1983)

CIGN vs Healthy Population Controls

This analysis consisted of :

a) A case control comparison of all cases of CIGN versus population controls,

b) A comparison of the mixed variant (CIGN + CIN) with population controls,

c) A comparison of the pure variant (CIGN only) with population controls,

a) All cases of CIGN versus population controls

1) Univariate analysis ;

For these data , please refer to Table 2-16 in the appendix to this chapter.

The odds ratio of disease was significantly elevated in those with the following exposures ;

History of having ever smoked

Alcohol consumption in excess of 35 units per year

Menarche at >= 15 years of age

More than 5 sexual partners

The odds ratio of disease was significantly reduced in those with the following exposures ;

Ever use of barrier contraception

Current use of barrier contraception

Statistically significant dose - response relationships were observed for :

Number of marriages * (increasing risk)

Number of pregnancies* (decreasing risk)

Number of sexual partners (increasing risk)

Age at Menarche (increasing risk)

Duration of use of high dose(>=50 microgram oestrogen) contraceptive pills **

(* p = 0.06)

(** This was arrived at by using minimum substitutions for missing data - please refer to statistical appendix)

Multivariate analysis

Following univariate analysis , the statistically significant risk factors were assessed in a multivariate model using conditional logistic regression. The results are illustrated in table 3-6 . By reference to this table it can be seen that all the above mentioned risk factors , with the exception of number of marriages , high dose pill usage and cigarette smoking , remain as independent and significant risk factors, with use of barrier contraception being of borderline significance. When Total number of marriages was combined with number of sexual partners in a separate model, the significance of this variable disappeared (p= 0.542) .

Variable	Coefficient	S.Error	p	OR	95%C.I.
Number of sexual partners	1.006	0.336	0.003	2.74	1.4 - 5.3
Use of Barrier contraception	-0.992	0.526	0.059	0.37	.13 - 1.03
Number of Pregnancies	-0.3613	0.162	0.026	0.70	0.51 - 0.96
Age at menarche	0.3997	0.144	0.006	1.49	1.12 - 1.98
Alcohol consumption	0.5972	0.268	0.026	1.80	1.07- 3.07

Table 2-5 CIGN versus normal controls ;Conditional logistic regression ; Best fitting model

Conclusions : CIGN versus normal controls

Risk factors for CIGN were :
increasing numbers of sexual partners,
late age at menarche,
increasing alcohol consumption

Factors protecting against CIGN were:
use of barrier contraception
increasing numbers of pregnancies

b) Mixed CIGN lesions versus normal controls

1) Univariate analysis ;

Please refer to 2-17 in the appendix to this chapter.

The odds ratio of disease was ***significantly elevated*** *in those with the following exposures ;*

2 or more marriages

More than 1 sexual partner

Alcohol consumption in excess of 10 units per year

HSV-1/HSV-2 ratio >= 3 ^^

Statistically significant dose - response relationships were observed for :

Alcohol consumption (increasing risk)

Number of sexual partners (increasing risk)

Age at commencing OCP use (decreasing risk)

^^ p = 0.08

Multivariate analysis

Following univariate analysis , the statistically significant risk factors were assessed in a multivariate model using conditional logistic regression. The results are illustrated in table 3-8

Variable	Coefficient	S. Error	p	OR	95%C.I.
Number of Sexual partners	0.6599	0.285	0.021	1.94	1.11 - 3.38
Alcohol consumption	0.5696	0.304	0.061	1.77	0.97 - 3.21

Table 2-6 mixed cGIN versus normal controls ;Cond. logistic regression ; Best fitting model

Conclusions ; Mixed CIGN versus normal controls

Risk factors for *mixed* CIGN were: numbers of sexual partners, alcohol consumption

c) Pure CIGN lesions versus Normals : Univariate analysis

1) Univariate analysis ;

For these data , please refer to Table 2-17 in the appendix to this chapter.

The odds ratio of disease was ***significantly elevated*** *in those with the following exposures ;*

Menarche at 14 years or above

A history of having ever smoked (p = 0.08)

Statistically significant dose - response relationships were observed for :

Number of pregnancies (p = 0.09) [Diminishing risk]

Multivariate analysis

Following univariate analysis , these risk factors were assessed in a multivariate model using conditional logistic regression. The results are illustrated in table 3-8 . The best fitting model contained age at menarche alone .

Variable	Coefficient	S. Error	p	OR	95%C.I.
age at menarche	0.4495	0.210	0.032	1.568	1.04 - 2.36

Table 2-7 pure CIGN versus normal controls ;Cond. logistic regression; Best fitting model

The sole independent risk factor for *pure* CIGN was : increasing age at menarche

CIGN versus CIN

Orientation

This analysis consisted of :
a) A case control comparison of all cases of CIGN versus CIN controls,
b) A comparison of the mixed variant (CIGN + CIN) with CIN controls ,
c) A comparison of the pure variant (CIGN only) with CIN controls .

a) A case control comparison of all cases of CIGN versus CIN controls

Univariate analysis
Please refer to Table 2-18 in the appendix to this chapter.

The odds ratio of disease (CIGN) was ***significantly elevated*** *in those with the following exposures ;*

Menarche at >=15 years of age
HSV1K / HSV2-K ratio of 3 or more (vide infra)

The odds ratio of disease (CIGN)was ***significantly reduced*** ** in those with the following exposures ;*

10 or more sexual partners
Current sexual partner has positive history of STD
History of having ever smoked

Statistically significant dose - response relationships were observed for :

Age of menarche (increasing risk of CIGN)
Number of pregnancies** (diminishing risk of CIGN)
Increasing age at first intercourse*** (increasing risk of CIGN)
Increasing number of sexual partners (diminishing risk of CIGN)
Increasing duration of smoking (diminishing risk of CIGN)
Increasing numbers of cigarettes smoked (diminishing risk of CIGN)

(* This can be interpreted inversely , as increasing the risk of CIN)
(** $p = 0.053$ *** $p = 0.092$)

Multivariate analysis : CIGN versus CIN

Following univariate analysis , the statistically significant risk factors as well as the established risk factors for CIN ,were assessed in a multivariate model using conditional logistic regression. The results are illustrated in table 3-10.

Variable	Coefficient(R)	S. Error	p	OR	95%C.I.s
Number of sexual partners	-0.5551	0.264	0.036	0.57	0.34 - 0.96
Cigarette smoking (Ever / never)	-1.663	0.656	0.011	0.19	0.05 - 0.69
Partner with h/o STD	-1.497	0.882	0.09	0.22	0.04 - 1.26
HSV1/HSV2 >=3	+1.082	0.575	0.06	2.95	0.96 - 9.1

Table 2-8 CIGN versus CIN controls ;Conditional logistic regression ; Best fitting model

Conclusions : all cases of CIGN compared with CIN

Subjects with CIGN were :
a) *less* likely :
to have multiple sexual partners
to have smoked
to have a partner with an STD
b) *more* likely
to have neutralising antibodies with HSV-1 specificity

Mixed CIGN lesions versus CIN

Univariate analysis

Twenty six cases of CIGN were compared with 43 cases of CIN in a matched univariate analysis .

Please refer to Table 2-19 in the appendix to this chapter.

The odds ratio of disease (CIGN)was significantly reduced in those with the following exposures ;

Current cigarette smoking

Current sexual partner has positive history of STD*

History of having ever smoked

The odds ratio of disease (CIGN)was significantly increased in those with the following exposures ;

HSV-1 / HSV-2 ratio>=3

Statistically significant dose - response relationships were observed for :

Increasing duration of smoking (diminishing risk of CIGN)

Increasing numbers of cigarettes smoked (diminishing risk of CIGN)

Increasing age at menarche** (increased risk of CIGN)

* p= 0.07

** P = 0.088

Multivariate analysis : mixed CIGN lesions versus CIN

The variables for ever / never cigarette smoking , current sexual partner with a history of STD, age at menarche and HSV-1/HSV-2 (>=3) had shown significant or near significant effects on univariate analysis . However, the best fitting model is illustrated below.

Variable	Coefficient	S. Error	p	OR	95%C.I.
smoking (ever/never)	-1.821	0.795	0.022	0.16	0.03 - 0.77
Partner with h/o STD	-1.948	1.19	0.103	0.14	0.14 - 1.48
HSV-1/HSV-2 (>=3)	+1.653	0.780	0.034	5.22	1.03 - 24.06

Table 2-9 mixed CIGN versus CIN controls ;Cond. logistic regression ; Best fitting model

Conclusions : mixed CIGN versus CIN controls

Subjects with *mixed* CIGN were :
a) *less* likely :
to have smoked ,
to have a partner with an STD
b) *more* likely
to have neutralising antibodies with HSV-1 specificity

Pure CIGN versus CIN controls

Univariate analysis

Sixteen cases of CIGN were compared with 29 cases of CIN . Please refer to Table 2-19 in the appendix to this chapter.

The odds ratio of disease ('pure' CIGN) was ***significantly reduced*** *in those with the following exposures ;*

More than 5 sexual partners

Statistically significant dose - response relationships were observed for :

Increasing age at first intercourse (increasing risk)

Increasing numbers of sexual partners (increasing risk)

Multivariate analysis : Pure CIGN versus CIN

In the multivariate model, disappearance of the significant effect of age at first intercourse was observed ,the change in deviance associated with the addition of this variable being 0.028 (p = 0.867).

Variable	Coefficient	S. Error	p	OR	95%C.I.
number of sexual partners	-1.057	0.484	0.029	0.35	0.13 - 0.90

Table 2-10 pure CIGN versus CIN controls ;Cond. logistic regression ; Best fitting model

Conclusions: Pure CIGN versus CIN

Subjects with *pure* CIGN were *less* likely to have had multiple sexual partners than CIN controls

Part 11

Antibodies to Herpes simplex virus types 1 and 2

Prevalence

Neutralising antibodies directed against HSV-1 were detected in 202 (81.8%) subjects , whereas anti HSV-2 neutralising antibodies were detected in 211(85.4%) of subjects. A breakdown of the prevalence of neutralising antibody in relation to histology is presented in Table 2-11 . The relationship between age and prevalence of antibody against both viruses is illustrated in Table 2-13a , and similarly the relationship between social status and antibody prevalence is illustrated in Table 2-13b. The prevalence of both types of antibody did not appear to be related to age or social status .

Identification of type specificity

Because neutralising antibody against either HSV-1 or HSV-2 will target itself against both type-common and type-specific antigens and hence neutralise both virus types to a varying extent , the ratio of K values for HSV1/HSV-2 has been calulated , and may give a better indication of previous type exposure.(Royston and Aurelian , 1970 Prakash et al , 1985)

A ratio >=3 is considered to indicate that the serum possesses type 1 specificity , and sera with ratios <=1 were considered to be type 2 specific . (Skinner et al,1976 Royston and Aurelian,1970)

In situations where there was no detectable neutralising antibody to HSV-2 in the presence of antibody to HSV-1, an arbitrary value of 10 was chosen for this ratio.

Analysis

The following variables were examined:

Qualitative comparisons

- presence / absence of antibody to HSV-1
- presence / absence of antibody to HSV-2

Quantitative comparisons

- k values : i) HSV-1 ii) HSV-2

Specificity of sera:

a) Ratio of k values : HSV-1/HSV-2

b) Presence / absence of HSV-1/HSV-2 ratio >=3

c) Presence / absence of HSV-1/HSV-2 ratio <=1

Each variable was assessed : i) in a matched univariate analysis. If significant , the variable was included in ii) multivariate analyses as previously described .

Qualitative comparison

When cases and controls were compared for the presence or absence of antibodies to HSV-1 or 2 , no significant differences were observed in univariate analysis. However the presence of any antibody to HSV-2 emerged as a significant and independent risk factor for CIN , OR = 10.7 (1.1 - 104.9) in multivariate analysis.

Quantitative comparison

When case-control comparisons of k values for individual sera were made , no significant associations were observed between k values for either virus type and risk of disease.

*Comparison of type specificity *1*

When case control comparisons of HSV-1/HSV-2 ratios were made , there were no significant relationships between these ratios and disease.

*Comparison of type specificity *2*

By defining type 1 specificity as a ratio of HSV-1/HSV-2 >=3 , in a case control comparison of CIGN with CIN , a ratio of >=3 was associated with a 2.5 times risk of CIGN on univariate analysis , and this was significant [OR = 2.5 (1.03 - 6.0)] By subsequently analysing the histologic sub-types of CIGN separately , it was found that for mixed CIGN lesions , a ratio>=3 was associated with a significantly elevated risk of mixed CIGN [OR = 3.4 (1.02 - 11.1)] .

In multivariate analysis of CIGN versus CIN, an HSV1/HSV2 ratio of 3 or more remained as an independent risk factor for CIGN , OR = 2.95 (0.96 - 9.1) , p = 0.06. Similarly , when considering mixed CIGN lesions versus CIN , A ratio of 3 or more was associated with a significantly elevated odds ratio of mixed CIGN. [OR = 5.2(1.1 - 24.6)] , p= 0.034.

There were no significant differences in any of the other case control comparisons of type specificity.

Summary of significant Herpes related risk factors following multivariate analysis

Those with CIN were 10.7 times *more* likely to have had antibodies to HSV-2 than normal controls

Those with CIGN were 2.95 times more likely to have type 1 specific sera than CIN controls

Those with mixed CIGN were 5.2 times more likely to have type 1 specific sera than CIN controls

Table 2-11

Numbers of subjects with neutralising antibody to HSV-1 and HSV-2

(Percentages in parentheses)

	CIN	GIN (all)	Pure	Mixed	Normals
Anti HSV-1	57(79.1)	38(90.5)	15(93.8)	23(88.5)	107(80.5)
Anti HSV-2	65(90.3)	36(85.7)	15(93.8)	21(80.8)	110(82.7)

Table 2-12

Prevalence of antibody according to type specificity and group

(Percentages in parentheses)

GROUP	HSV1/HSV2>=3 (Type 1)	HSV1/HSV2<=1 (Type 2)
Normal controls n=133	57(43)	35(26.3)
CIGN n= 42	22(52)	7(16.7)
CIN n=72	20(27.8)	20(27.8)

Table 2-13 a

Age versus prevalence of antibody to HSV

	HSV-1 +ve	HSV-2+ve
<30	37(82.2)	39(87)
31-35	52(82.5)	53(84.1)
36 - 40	50(79.4)	55(83.3)
41 - 50	48(81.4)	52(88.1)
51+	12(85.8)	12(85.7)

Table 2-13 b

Social class versus prevalence of antibody against HSV

	HSV-1 +ve	HSV-2+ve
1+ 11	39(73.6)	47(88.7)
111N + 111M	107(85.4)	106(83.5)
1V + V	37(74)	41(82)
V1	16(94.1)	17(100)

Summary table of significant risk factors after multivariate analysis and size of relative risk values

STATISTICALLY SIGNIFICANT ODDS RATIOS AFTER MULTIVARIATE ANALYSIS ARE IN BOLD TYPE

STATISTICALLY SIGNIFICANT ODDS RATIOS ON UNIVARIATE ANALYSIS ONLY IN PARENTHESES

	CIN	GIN	GIN	Pure variant		Mixed variant	
	vs Norms	vs Norms	vs CIN	vs Norms	vsCIN	vsNorms	vsCIN
Risk factor							
Number of Sexual partners	**7.4**	**2.7**	**0.57**	2.6***	**0.35**	**1.94**	0.5***
Partner had STD	**11.1**	1.7	**0.22**	4.0	0.3	1.0	**0.14**
Use of barrier Contraception	0.5	**0.37**	1.1	1.0	2.0	0.4	2.0
Cigarette smoking	**15.6**	1.7	**0.19**	2.6	1.1	1.2	**0.16**
Alcohol consumed	(8.4)*	**1.80**	0.95*	3.4*	0.4*	**1.77**	0.4*
Number of pregnancies	1.4^	**0.70**	0.35**	0.22^^	0.5^	0.4^	0.5^
Age at menarche	2.0^^	**1.49**	(5.2)^^	**1.57**	2.6^^	2.9^^	3.1^^
HSV1/HSV2 >=3	0.6	1.58	**2.95**	0.7	1.5	2.4^^^	**5.22**
Antibody to HSV-2	**10.7**	1.5	0.6	2.6	1.8	1.2	0.2

KEY

* = for >=260 units of alcohol per year ^^^ p = 0.08

^ = for >=3 pregnancies

**= for >=4 pregnancies

^^= for menarche >=15 years

*** =for 5 or more sexual partners

DISCUSSION

The aetiology of CIGN has never previously been explored in an analytical epidemiological study. The major obstacle to such a study has undoubtedly been the rarity of the condition . The objective of this study was to describe the risk factor profile of CIGN , to compare this with CIN , and to determine whether there was any association between herpes simplex virus and CIGN .

Main study findings

It is well established that CIN has the epidemiological characteristics of a sexually transmitted disease. This study demonstrates that CIGN and CIN share some sexual behavioural risk factors . Both are associated with multiple sexual partners and both are less likely to occur when barrier contraception is used. There are , however , notable differences between the risk factor profiles of CIGN and CIN :

i) The association with number of sexual partners is less strong for CIGN , and those with CIN had risk factors emphasising the importance of previous sexually transmissible disease in the male .

ii) Smoking is a risk factor for CIN but not CIGN.

iii) Those with CIGN were more likely to have neutralising antibodies with type 1 herpes virus specificity than those with CIN.

In addition to the above characteristics , CIGN was distinguished from normal controls , (as well as CIN) by its association with a late menarche and low parity .

The subdivision of CIGN into pure and mixed varieties and subsequent analysis according to histological sub-type reveals some differences in the risk factor profile of pure and mixed CIGN. However , these conclusions are obviously limited by the reduction in sample size

resulting from this subdivision.

Potential biases

A number of methodological problems were encountered in the design and analysis of this study. These are now considered:

Choice of control groups

Two control groups were used. Given the rarity of the condition , the use of multiple control groups may be advantageous for 2 reasons : i) if the results obtained indicate similar associations when comparing cases with either control group , this will generate increased confidence in the results : ii) no single control group will ever be ideal.

CIN Controls

Controls with CIN were selected in order to compare the epidemiology of CIGN and CIN. The use of CIN controls diminished the potential for recall bias. Further advantages were that these controls arose from the same catchment population , were subject to the same diagnostic process, and the time of diagnosis of disease was similar . The disease under study arises from the same organ and risk factors for CIN have been well documented.

Some disadvantages are associated with the use of CIN controls. They are not truly healthy, and hence any conclusions based on this comparison alone may not have general applicability. This concern is magnified by the observation that the exposures under study might well cause both cancers , and some sharing of aetiological factors seems likely , given that these lesions have been observed to co-exist in the same cervix . (Ostor et al , 1984) The effect will be to bias the relative risk associated with any exposure, yet this bias will be in a known direction , i.e. it will tend

towards unity. This will tend to mask any true differences between the 2 lesions , and consequently real risk factors of low magnitude of relative risk could be 'hidden' as a result. However , any significant association which is demonstrated is likely to be an underestimate. The concern with respect to excessive similarity between the 2 lesions provides justification for a separate analysis of the 'pure' and 'mixed' histological subtypes of CIGN . However the disadvantage incurred by this manoeuvre is that it reduced the available sample size and hence the precision of any apparently significant estimate of association.

The risk factors observed for CIN are those which have been reported in previous case - control studies . (Harris et al ,1980 Buckley et al ,1981 Aurelian et al ,1973 Lyon et al,1983) This observation provides reassurance that this group of controls is representative of the general population of women with CIN, and in turn enhances confidence in the results of the analysis of risk factors for CIGN. A further check on 'quality control' was achieved by the use of negative checks or 'dummy' questions. The lack of any significant associations for any of these exposures provided further reassurance on the comparability of cases and controls.

Normal controls

The above reservations concerning the use of a CIN control group justified the use of a group of 'normal' (i.e. population) controls. These subjects have the advantage of being free of the diseases under study and should more accurately reflect exposure prevalences in the general population. Some potential disadvantages were associated with the use of population controls : i) recall bias may have been more likely : ii) it was more costly in terms of time and resources to identify a suitable group of population controls . However, it was felt that in order to ensure that the

controls were comparable to the source population of cases then it was essential to recruit controls from the same locality as the cases . The logistic problem was overcome by virtue of co-operation on behalf of the majority of the General Practitioners concerned : iii) response rates were lower than hospital controls , but not significantly so : iv) though it was stipulated that these individuals must be healthy by reference to an up-to-date cervical smear , the possibility remains that some healthy subjects were misclassified as a result of a false negative cervical smear . However, the effect of this would be to make the case and control groups more alike , therefore any differences detected would be underestimates.

Interviewer bias

It was not possible, employing a single interviewer , and in the context of this study , to maintain observer blindness to case-control status. Indeed this is often difficult to accomplish even when the status of subjects is deliberately withheld from interviewers. This potential problem was largely overcome by having a structured questionnaire that was administered in a consistent manner with attempts to avoid differential questioning of groups. The attempt to standardise the interview procedure pre-dated the interview itself. Each subject , whether case or control , was invited to participate in the study by means of a standardised contact letter , which differed in only minor respects between population controls and other participants . In this letter , the general nature of the questionnaire was stressed , but no specific indication of a study hypothesis was made . However , general public awareness of the aetiology of carcinoma of the cervix may have made women more conscious of the likely sexual nature of the questionnaire. However , this consideration would affect all groups equally.

Response rates

There were no significant differences in response rates between CIN controls and population controls, and CIN controls and CIGN cases. However , the response rate from CIGN cases was significantly higher than population controls. ($X^2 = 7.28$ $p<0.01$) It was not possible to determine the extent to which the non-responders in the control group differed from controls who attended interview.

Sample size

The absence of any baseline studies of risk factors for this condition prevented any estimate of sample size requirements. Indeed , given the rarity of the condition , these considerations are really academic. As many cases as possible were ascertained over a 2 year period. The risk estimates provided in this study should facilitate the planning of subsequent case-control investigations of the disorder.

RISK FACTORS FOR CIGN

The risk factors highlighted for CIGN can be subdivided into :

a) Behavioural (Number of sexual partners , use of barrier contraception and Alcohol consumption) and

b) Reproductive / Endocrine (number of pregnancies and age at menarche)

Behavioural factors

Some of these factors have previously been identified as risk factors for CIN (Harris et al ,1980) , and suggest that CIGN also has some of the features of a sexually transmitted disease.

i) Sexual variables

The strongest association was with number of sexual partners, with those with 5 or more sexual partners being at 6.3 times the risk of disease than normal subjects . This variable remained as an independent risk factor in a multivariate model. A dose-response relationship between number of sexual partners and risk of disease provides further evidence in favour of a cause and effect relationship. In multivariate analysis the odds ratio (OR) for 'number of sexual partners' was 2.7 . This seems modest in comparison to the OR for this variable when CIN cases were compared with normals (7.4). However it was observed that CIN subjects had significantly more sexual partners than CIGN cases, this OR being 1.8 . It can be concluded that the trend in risk across the categories of CIGN and CIN implies consistency in the results , and these observations concur with the findings of Horowitz et al,1988 and Parazzini et al,1988 in relation to sexual behaviour , adenocarcinoma and squamous carcinoma. These authors have also identified both sexual and reproductive factors as important in the aetiology of invasive adenocarcinoma.

The observation that 'ever' use of barrier forms of contraception has a protective effect (OR =0.37) is also consistent with the hypothesis that sexual transmission may be important in the aetiology of CIGN.

ii) Smoking

It is interesting that the chief difference between CIGN and CIN was in relation to cigarette smoking. The study : i) confirmed the previously documented association between smoking and CIN. (Clarke et al, 1985 La Vecchia et al, 1986 Buckley et al , 1981) and : ii) indicated that there is no significant association between smoking and CIGN. Parazzini et al , 1988 reported similar findings in a comparison of cases of invasive adenocarcinoma with normal controls.

In addition , subjects with CIGN were significantly less likely to have smoked than CIN controls, and in this comparison there were statistically significant dose-response relationships demonstrating a decreasing risk of CIGN in association with increased duration of smoking , and with increasing numbers of cigarettes smoked. The findings were identical for mixed CIGN. In addition , the odds ratio associated with ever smoking was protective for 'pure' CIGN (0.25 [0.02 - 2.2]) . The findings in this study are similar to those of Brinton et al, 1987b who reported that smoking was a significant predictor of squamous tumours only. Likewise , Horowitz et al ,1988 in a case control comparison of invasive squamous and glandular tumours , reported that patients with squamous cancer were significantly more likely to be current smokers .

The experimental findings are consistent with previous observations concerning the relationship between squamous carcinomas (i.e. cervix , lung , larynx ,oral cavity and oesophagus) and tobacco ingestion (Williams and Horm , 1977) Interestingly , there appears to be a parallel with the risk factor profile of neoplastic lung disease : there is a well established relationship between bronchial carcinoma and cigarette smoking , and the effects of smoking are much greater for squamous tumours than adenocarcinomas (Lubin et al , 1984) , while other workers have found no clear link between adenocarcinoma and smoking (Doll et al , 1957 Haenszel et al , 1958)

iii) Alcohol consumption

In this study , alcohol consumption was found to be a risk factor for CIGN. A dose-response relationship was observed between levels of consumption and disease risk , and the association between alcohol consumption and disease was not due to confounding by any of the other

variables studied. However , this association might still represent the effect of an unknown confounder. Although in univariate analysis , alcohol consumption also appeared to be a risk factor for CIN , the effects of this variable became non-significant when controlled for number of sexual partners. Similar effects were observed in the study of Harris et al , 1980. Thus , an association between alcohol consumption and squamous neoplasia has been noted, but not consistently demonstrated and no satisfactory biological hypothesis has been advanced to explain it. On the basis of the interview study from the Third National Cancer Survey , (Williams and Horm, 1977) , the cancer sites which are associated with alcohol consumption are usually those of the upper respiratory and digestive tracts , or colorectal tumours . These may be conveniently explained by the exposure of these sites to alcohol in high concentrations. However , the associations of breast , thyroid and skin (malignant melanoma) cancers with alcohol has led to the hypothesis that alcohol may act via a pituitary mediated mechanism. Given the current lack of understanding , it is unclear how alcohol exposure may directly influence disease of the cervix.

Reproductive/Endocrine factors

The possibility that the aetiology of CIGN is influenced by reproductive and endocrine factors is supported by the experimental observations.

i) Pregnancy

Cases of CIGN had significantly fewer pregnancies than normal controls . In a univariate comparison of CIGN with CIN , the risk of CIGN fell with an increase in number of pregnancies and this trend was of borderline significance, (p= 0.053) although this was not sustained in multivariate

analysis. Nevertheless, this implies some consistency in results for this particular exposure and risk of CIGN. The size of effect of this exposure is relatively small , (OR=0.70 in multivariate analysis) and this implies that in itself it may not be the primary aetiological factor . However , it could be confounded by another as yet unrecognised risk factor which has a reproductive or endocrine basis . Individuals with CIGN may have fewer pregnancies because of an unfavourable endocrine environment , the aetiological basis of which is uncertain . However, the findings of a significantly later menarche , and significantly fewer pregnancies are consistent in implying a somewhat reduced fertility (vide infra), and the association of these variables appears plausible by virtue of the fact that those with a later menarche have been observed to have a higher likelihood of anovulatory menstrual cycles , than those with an early menarche. (MacMahon et al, 1982 Apter and Vikho,1985) .

While there is concordance between the observations in this study and those of Parazzini et al ,1988 with respect to some sexual behavioural risk factors , the reproductive factors identified by these authors highlighted an *increase* in parity as a risk factor . This finding is not in agreement with the studies of Brinton et al ,1987 or Horowitz et al,1988 and in this study , increasing parity appears to diminish the risk of CIGN . However , this is in agreement with other studies which indicate that low parity is associated with cervical adenocarcinoma. (Saigo et al,1986 Silcocks et al,1987 Kohronen , 1980 Milsom and Friberg, 1983)

ii) Menarche

Age at menarche was a risk factor for CIGN , (vs. normal controls) and on analysing histologic subtypes , age at menarche emerged as a risk factor for the 'pure' variant. When comparing CIGN with CIN it was noted in

the univariate analysis that increasing age at menarche was a significant risk factor for CIGN. It is necessary to consider whether there is a plausible biological mechanism underpinning this apparent association. An early menarche has been shown to be a risk factor for breast carcinoma (Vikho and Apter ,1986 Statszewski,1971 Henderson et al,1981) and functional ovarian cysts (Parazzini et al,1989) , and a late menarche as a risk factor for endometrial carcinoma (Wynder et al , 1966). The common factor linking these lesions is that the target tissue is responsive to the prevailing endocrine milieu, and breast carcinoma is a hormone dependent neoplasm.

The endocrinology of the menarche has recently been studied (Vikho and Apter , 1986) and marked differences are described between girls with an early as opposed to a late menarche. The former have been shown to have an early rise in serum FSH levels at the onset of puberty and increased levels of circulating oestradiol prior to , and for some years after the menarche. An early menarche is associated with early onset of regular ovulatory cycles , and the luteal (progestagenic) phase is more likely to be adequate (Apter and Vikho , 1985) . The levels of sex hormone binding globulin (SHBG) are lower in those with an early menarche , leading to a higher free circulating level of oestradiol .(Apter et al , 1984) Hence it appears that women who have a late menarche will be exposed to a later oestrogenic stimulus which will be quantitatively less than those who have early menarche. In addition , this reduced oestrogen stimulus will not be balanced by progesterone as is the case with regular ovulatory cycles. MacMahon et al ,1982 demonstrated that the prevalence of anovular cycles was positively related to age at menarche , but not significantly so . However , by analysing number of years elapsed since menarche , the prevalence of anovular cycles was inversely and significantly related to this variable. Finally , Henderson et

al , 1981 in a case-control study of breast carcinoma reported that cases , who had a significantly earlier menarche than controls , had established regular cycles significantly earlier than controls.

The endocrinological differences between those with early and late menarche may be of relevance to the aetiology of cervical cancer. It has been suggested that the manifestations of genital tract infection and cervical cancer could be modified by the prevailing endocrine environment , and age at menarche may provide a convenient dividing line between 2 quite different endocrine states which are associated with differing susceptibility to sexually transmitted diseases including cervical cancer. (Duncan et al ,1990) These authors argue that sexual intercourse prior to menarche is associated with a significantly higher rate of infection with many genital tract pathogens including Gonococci and Chlamydia , and a (non - significantly) increased rate of cervical cancer. It is postulated that this could relate to the lack of protective effect of reproductive maturity, such protection being acquired by virtue of : a) oestrogen mediated thickening of the lower genital tract epithelium, thus improving the physical barrier to infection: b) The development and maintenance of vaginal microorganisms , i.e. lactobacilli .The latter are sustained by progesterone, which in turn is dependent on regular ovulatory cycles . Such cycles may not be established until some years after menarche, especially in the case of those with a late menarche. c) Enhanced immunological defence : it has been noted that levels of secretory IgA are reduced in cervical mucus prior to menarche . (Lantner,C, 1984).

These observations provide some biologic credibility to the argument that increasing age at menarche, and consequent prolongation of an endocrinologically immature and potentially less immunologically competent state, is a risk factor for CIGN.

iii) Oral contraceptive pill

In theory , the oral contraceptive pill , which directly affects the endocrine environment and thus prevents pregnancy , might be a risk factor for CIGN . There is some support for this hypothesis from : i) the study of Peters et al,1986 , in which it was suggested that the increase in incidence of cervical adenocarcinoma might be causally related to OCP use : ii) a case control study of oral contraceptive usage and risk of invasive cervical cancer , (Brinton et al ,1986) where it was reported that there was a significant risk of *adenocarcinoma* in association with long term 'high potency' pill use. (oestrogen > 50 micrograms per pill). This excess risk (OR=3.9) was statistically significant despite being based on only 6 exposed cases.

The findings from this study indicate that the duration of use of the high dose OCP was associated on univariate analysis with an increased risk of disease, when minimum substitutions were made for missing data .Using such substitutions , the regression coefficient of +0.007985 per month of use , was statistically significant . ($p = 0.039$, OR = 1.008 (1.000 - 1.016) per month of use . However , when the variable for duration of high dose pill use , corrected for minimum substitutions was entered into the multivariate model , it failed to alter the deviance significantly (LRS = 0.982 , $p = 0.322$) Hence while the available data are insufficient for any firm conclusion to be drawn , it seems most unlikely that high dose pill use is a risk factor and this would appear to be underlined by the observation that the lower confidence interval constructed around the elevated odds ratios for ever / never use of high dose pills were both below unity . Furthermore , the case control studies of Parazzini et al , 1988 and Silcocks et al ,1987 found no relationship between OCP use and adenocarcinoma, and although Brinton et al , 1986

reported that extended use of the OCP might be a risk factor for adenocarcinoma , this relationship was not statistically significant when re-explored in a later study (Brinton et al 1987b) . Hence the observations in this study of CIGN, coupled with the available evidence from the literature , fail to support a causal relationship between OCP use and adenocarcinoma.

Histological subdivision of CIGN

Pure CIGN

In univariate analysis of pure CIGN versus CIN , there were significant trends in risk of pure CIGN with increasing age at first intercourse , and a reducing risk with increasing numbers of sexual partners. In multivariate analysis, subjects with pure CIGN had significantly fewer sexual partners than those with CIN.

Mixed CIGN

These subjects appear to be more epidemiologically similar to CIN in terms of sexual behavioural variables. There were no significant differences in numbers of sex partners or age at first intercourse between mixed CIGN and CIN, yet these CIGN subjects had significantly more sexual partners than normals. Hence while sexual behaviour appears important in the aetiology of mixed CIGN , the results of the analysis suggest that smoking and possibly exposure to differing infectious agents can determine whether the ultimate histological picture is squamous ,or a mixture of squamous and glandular. (Table 2-9)

Brinton et al,1987b have also reported some differences in risk factor profiles of invasive adenosquamous neoplasms and adenocarcinomas. Notably , adenosquamous cancer shared some risk factors for squamous

cancer , i.e. number of sex partners and reluctance to attend for screening. Further elucidation of the differences in risk profiles was limited by the small numbers available for comparison.

Herpes virus types 1 and 2

The objective of this part of the study was to explore possible qualitative and quantitative case control differences in the prevalence of antibodies to HSV-1 and 2 and the serum type specificity. This work was stimlated by studies which had suggested that there might be an aetiological link between these viruses and cervical adenocarcinoma. (Menczer et al,1981 Wentz et al,1981)

The results for CIN revealed that these patients were significantly more likely to have antibodies to type 2 virus than normal controls, thus confirming a plethora of previous investigations on this subject. (Royston and Aurelian,1970 Nahmias et al,1970 Rawls et al,1969 Plummer and Masterson,1971)

However, no significant associations were found between CIGN and normal controls when considering the presence/absence of antibodies to HSV-1 or 2. When considering type specificity of sera, patients with the mixed variant of CIGN were more likely to have type 1 specific sera (OR=2.36 [0.9 - 6.1]) but this was not statistically significant. (p=0.08) A larger study is required to clarify the significance of this association.

When comparing CIGN with CIN , there were significant differences in the type specificity of sera, with CIGN patients significantly more likely to have a ratio HSV1/HSV2>=3. (Type 1 specific sera) Similarly, after dividing into histological subtypes , patients with mixed CIGN were significantly more likely to have type 1 specific sera. It is possible that the differences in ratios between CIN and CIGN could be

explained by the well documented association between type 2 HSV antibody and CIN , without there being any need for an association between CIGN and HSV-1 . However , if subjects with CIGN had a higher prevalence of prior exposure to HSV-1 than CIN subjects , this might imply that there was a role for HSV-1 in CIGN . Prior exposure to HSV-1 was assessed for the puposes of the study by (i) a history of oral herpes simplex and (ii) the prevalence of antibody to HSV-1 . Infact , fewer subjects with CIGN (38%) had a positive history of cold sores , than those with CIN (43.8%) and there were no significant differences in the prevalence of antibody to HSV-1 between CIGN and CIN. Hence there is little support for this hypothesis.

This study provides no clear indication of a significant association between HSV-1 or HSV-2 and CIGN. With reference to Menczer et al , who first postulated the relationship between HSV and adenocarcinoma these findings are open to criticism on the grounds that the study only involved 16 case subjects , and that these subjects were survivors , and therefore possibly an unrepresentative sample. Similarly ,Wentz et al were unable to reproduce the high incidence of adenocarcinoma achieved in 1975 in the second published series (Wentz et al,1981).

It is necessary to bear in mind the limitations of the technique employed in the current study ,which will limit any conclusions that might be drawn in relation to HSV . Firstly , both HSV-1 and HSV-2 possess type common and type specific antigens (Skinner et al , 1976) Hence any subject exposed to either of these viruses will manufacture antibodies which will neutralise both types. It has been noted that antiserum to Herpes B virus of monkeys will neutralise HSV-1 to a greater extent than the B virus itself (Watson , 1967) , and other studies have demonstrated that certain HSV-1 types are neutralised to a greater extent than HSV-2 by antiserum prepared against Type 2 virus. For these reasons the use of

ratios of values of HSV1 and HSV2 K values may provide a better way of judging previous type exposure , but it is believed that ratios in the range 1-3 will not reliably indicate the specificity of the serum. (Skinner et al,1976) . Such ratios were encountered in 96 of the subjects studied (39%). Secondly , it is recognised that herpetic infections in different body sites are associated with different patterns of antibody production , notably those with recurrent genital infection produced significantly less neutralising antibody against type 1 or type 2 virus , than patients with recurrent oral , orogenital or genital and perigenital disease. (Woodman et al , 1983) Hence the precise relationship between genital , and therefore cervical infection and the production of antibody is not straightforward , and therefore any conclusions made on the basis of antibody testing with respect to the aetiological role of cervical herpetic infection must bear this observation in mind.

Given that : i) the experimental work supports the possibility that a sexually transmitted agent is involved in the aetiology of CIGN : ii) The findings in relation to HSV-1 are inconclusive : iii) there are reports of a possible association between Epstein Barr virus and adenocarcinoma (Singh et al,1989 ; see page 49) , then it may be worth exploring the relationship between EBV and CIGN.

Summary and recommendations for future study

CIGN has a risk factor profile which suggests that it behaves as a sexually transmitted disease, the manifestation of the disorder being in some way dependent on a more immature reproductive/endocrine milieu. With respect to the identification of a possible infective agent , the study fails to provide clear evidence of an association between either HSV-1 or HSV-2 and CIGN.

This is the first study of the aetiology of CIGN , and therefore it is not possible to make a firm judgement on the cause and effect relationship between the risk factors outlined and disease. However , this work suggests the need for:

a) Further case - control studies directed at the risk factors outlined in this work . It is important that these studies be carried out in different populations in differing cultural settings.

Given the rarity of this disorder , there would appear to be little scope for fruitful cohort studies of those with /without risk factors for the disorder.

b) Review of the relationship between Epstein-Barr virus and CIGN.

c) Further review of the role of the endocrine environment in CIGN by analysis of sex hormone binding globulin levels (SHBG) in cases and controls.*

(* Given that CIGN cases have a significantly later menarche than controls, then in order for the hypothesis outlined earlier to be consistent one would expect to find higher SHBG levels in the serum of CIGN cases.)

APPENDICES

1. STATISTICAL APPENDIX

a) Oral Contraceptives

In order to calculate the risk of disease in association with types and duration of use of contraceptive pills , it was necessary to take account of the individuals who could not provide a full history of the type of pill(s) ingested or duration of use . Using the example of CIN cases versus normal controls , there were 9 subjects (4 cases , 5 controls) with uncertain histories. Therefore in accordance with the recommendations of Breslow and Day , 1980 (page 113) substitutions were made for usage in the variables LP,HP and MP . (i.e. ever / never usage of low dose , highdose and minipills respectively) Similarly , substitutions were made into the variables which coded for duration of pill use . By calculating the odds ratios and confidence intervals for those with an uncertain history of pill usage , initally using the exposure '0' for variables LP , HP , MP , and subsequently by substituting a positive history of pill use , '1' , for each of these 3 variables, the odds ratios and confidence intervals are re-calculated. This technique yields 2 sets of odds ratios and confidence intervals for the extreme and opposite situations where a) none of these individuals using the pill in question , or b) all the individuals use this pill. The 'truth' almost certainly lies between the 2 extreme values given.

The odds ratio for a negative history of low dose pill use was 1.7(0.7 - 3.9) and for a positive history, the result was very similar [1.7 (0.7 - 4.0)]. These findings indicate that both these odds ratios could have easily arisen by chance , as they both overlap unity. Similarly , with respect to duration of pill usage , the regression coefficient for 0 exposure to low dose pill use was -0.0024 , p=0.573 , whereas the coefficient calculated by substituting

the observed maximum duration of use into those records with an uncertain history was - 0.001759 , p = 0.623 . These p values indicate that both these (negative) coefficients could have arisen by chance , and on this evidence , risk of disease appears unrelated to duration of low dose pill use.

With respect to duration of use of high dose pills , maximum /minimum duration substitutions were used for missing data . With maximum substitutions, the positive regression coefficient obtained could have arisen by chance. (p = 0.09) . However, with minimum substitutions , the coefficient of 0 . 0074 per month of use was statistically significant [p = 0.037 , OR = 1.007 (1.000 - 1.014) per month of use]. Nevertheless , when the variable for duration of high dose pill use , with minimum substitutions was entered into the multivariate model , it failed to alter the deviance significantly (LRS=0.883 , P=0.347) . Hence although it is difficult to reach firm conclusions regarding the role of this variable, it appears *highly unlikely* that high dose pill use is a risk factor for CIN.

By employing the same manoeuvres for progestagen only pills , non significant results were obtained for ever / never use , and for duration of use of these preparations.

11 - Example of Conditional Logistic Regression analysis - CIN vs normal controls

In view of the known association between CIN and numbers of sexual partners , early intercourse , cigarette smoking , failure to use barrier contraception and infection with HSV-2 , it was decided to include the variables representing these factors in an initial multiple regression model. Therefore , NPAR (number of sexual partners) , CIGEVER (History of having ever smoked) , AGESEX(Age at first sexual intercourse)

BARUSE (a history of ever use of Barrier contraception) and EVERHSV2 (presence or absence of neutralising antibodies to HSV-2) formed the first model. Because some of the exposure variables within certain risk categories were related , e.g. smoking , a number of variables were consequently excluded from further analysis. It was felt that the smoking variables concerning ever/never use , current use , duration of use and numbers of cigarettes smoked each conveyed essentially similar information , and therefore only CIGEVER was included in the multivariate model. After the initial modelling , the model was successively re - fitted by entering those additional variables which had been found to be significant on univariate analysis.

For variables which were subdivided into more than 2 categories which had shown significant trends in risk on univariate analysis , these variables were included in the multiple regression model in unfactored form. The reason for this being that by representing , for example , NPAR by a single variable , the potential existed for maximising statistical significance . (by retaining degrees of freedom = 1)

The final multiple regression model contained those variables which were significantly and independently associated with disease risk.

In the initial model , NPAR and CIGEVER showed significantly elevated independent effects on risk. BARUSE showed a protective effect of borderline significance , and EVERHSV2 was a risk factor , again of boderline significance. AGESEX was non-significant in the initial model , and the model was repeated without it . The effect was a reduction in total deviance of 0.847 , d.f. = 1 , p = 0.358 , (non-significant). Hence , AGESEX was dropped from the model.

Next , PVD (History of current partner having had an STD) was added in. This led to a reduction in total deviance of 4.504 , d.f. = 1 , p =0.034 . The p value for the variable PVD in the multivariate model was 0.063 . Hence

, although this variable was of only borderline significance in the regression model , its presence led to a significant improvement in the explanatory power of the equation , and it was therefore retained . The effect of including the former was to further reduce the significance of BARUSE (p= 0.176) and therefore the model was repeated in its absence. BARUSE contributed only 1.898 to total deviance , and was therefore removed. The effect of this was to improve the significance of the variables EVERHSV2 (p = 0.042) , and PVD (p = 0.033) . The addition of VDC (A history of the subject having ever attended an STD clinic) led to no significant alteration in deviance. (Reduction in deviance 0.051, 1 d.f., p = 0.822) . Similarly the addition of ALC (Alcohol consumption) in both factored and unfactored forms failed to improve the explanatory power. (LRS = 0.966 for ALC in unfactored form , d.f.=1) The addition of NMAR (number of marriages) , HOUS (living in owner-occupied or rented accommodation) , WARTS (positive history of genital warts) and HIGHPILL(minimum substitutions) led to no significant reduction in deviance. The best fitting model is illustrated.

Variable	Coefficient	Standard Error	P value	Odds ratio(95% CI's)
NPAR	2.006	0.521	< 0.001	7.4 (2.7 - 20.7)
CIGEVER	2.747	0.801	<0.001	15.6 (3.2 - 74.9)
EVERHSV2	2.368	1.17	0.042	10.7 (1.1 - 104.9)
PVD	2.407	1.13	0.033	11.1 (1.2 - 101.2)

CIN versus normal controls : Conditional logistic regression : best fitting model

Appendix 2
Sample Questionnaire

CONFIDENTIAL

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Edgbaston,
Birmingham.

November 1987

Date of interview __/__/__

Time commenced _____

SUBJECT DETAILS STUDY NO

Name; I__I__I__I

Date of birth; --- /--- /---

dd mm yy

A d d r e s s ;

POSTCODE; _ _ _ _ _ _ _

1, STUDY GROUPING

1 cGIN

2 CIN

3 General Practice control

GP Name _____

Address_____

2, ETHNIC ORIGIN [____]

1=Caucasian 2=Afro-Caribbean 3=Indopakistani 4=0riental 5=Other

First , I would like to ask some general questions

3, What is your marital status?

1= Single
2= Married
3=Divorced / Separated /Widowed [_____]

If 3 : Was this your first marriage or have you been married before?
Note number of marriages here [___]

4 a) What is your occupation ?

[If a housewife , proceed to b) , then d)]
[If student , proceed to c) , then d)]
b) What is your husbands occupation?_____
c) What is the occupation of your father?(or if no father , the occupation of the head of your household)_____

d) Does this job have an official description or grade?_____

Is this job :
1) Managerial or Professional
2) Of foreman or supervisor status
3) Of employee status
4) Self employment (Works for himself / own business)
5) None of these

5 , What type of housing do you occupy? Is it:

1=Owner occupied

2= Rented or:

3= Do you live with parents?

[___]

6 , Up to what age did you receive full time education?

1= up to 16

2=up to 18

3=Higher education/Professional training

[___]

PERSONAL HABITS

SMOKING

7 , Have you ever smoked cigarettes or cigars ?

(By this I mean at least one cigarette / cigar a day for at least one year)

[___]

Do you smoke cigarettes at the moment? [__]

If yes to either of above

At what age did you start smoking ? _____ years

At what age did you stop smoking ? _____years

So , can I check , it would appear that you smoked for _____(insert number) years ,rounding things off to the nearest year?

[__I__]

If you do /did smoke ,how many cigarettes do/did you smoke per day?

[__I__]

8 , What is your consumption of alcohol?

(a 'drink' is the equivalent of a short' measure of spirits , a glass of wine, a measure of sherry or other aperitif , or a half pint of beer)

USE FLASH CARD 'ALCOHOL'

(Alcohol consumption , continued)

0 = Nil

1 = less than 10 drinks per year

2 = 1-3 drinks per month

3 = 1-5 drinks per week

4 = 1-2 drinks per day

5 = 3 or more drinks per day [____]

MEDICAL HISTORY

Now , I would like to ask you questions relating to any pregnancies you may have had , your general health and past medical history

9. Pregnancy

How many live children have you given birth to? [__]

Have you had a pregnancy that has resulted in a stillborn baby? [__]

if YES , How many stillbirths have you had? [__]

Have you ever miscarried a pregnancy ? [__]

if YES , How many miscarriages have you had ? [__]

Have you ever had an abortion or termination of a pregnancy?

[__]

if YES , How many abortions [___]

Now summarise the total numbers of pregnancies

"Just to check , in total you have had _____ pregnancies (Say number)

OR

"Just to check , you have never been pregnant" (Then go to 10)

How old were you when you first became pregnant?

_____ years old

10 , How old were you when your periods began? [__I__]

11 , Have you suffered with any of the following illnesses?.

1= Yes 2= No 3=Dont know

1 Measles [___]
2 Chickenpox [___]
3 German Measles [___]
4 Mumps [___]
5 Rheumatic fever [___]

Have you had any of the following operations or surgical conditions ?

1 Has your gall bladder been removed? [__]
2 Have you had your appendix out? [__]
3 Have you had Piles or haemmorhoids ? [__]
4 Have you had varicose veins ? [__]
5 Have you had anal warts ? [__]

I would now like to ask some questions about your contraceptive usage and also some questions about sexual matters . If you dont wish to answer any of these questions then please say so and we'll skip them.

12 , How old were you when you first had intercourse? [__I__]

13 , How many partners have you had intercourse with in total?
[____]

14 ,What form of contraception are you using at present?
(USE FLASH CARD , 'CONTRACEPTION')

0=none
1=Sheaths 2=Diaphragm 3=IUCD 4=Progestogen only pill
5=Combined oral contraceptive pill 6=Female sterilization 7=Male sterilization
8=Depot progestogens [__I__]

15 , Have you ever used ,or do you currently use any contraceptive pills? (' Birth pills') Yes / No

(refer to samples folder)

16 , Which brand or brands of pill did you use?

BRAND OF PILL/Computer code	DURATION(months)
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]
[I]	[I I]

17 , How old were you when you first started taking the pill ? (It doesnt matter whether this was for contraceptive purposes or for just regulating periods)

[___I___]

18 , Have you ever used any of the following methods of contraception?

1 = yes 2 = No

METHOD	DURATION (Ascertain total months of use)
The 'coil' or 'IUCD' or Intrauterine device YES / NO	[_____MONTHS]

Have you used sheaths/or condoms (sometimes called durex)

YES / NO

Have you used them as :

1] a regular method of contraception ? _____

(if YES record duration in months below)

2] an occasional method only ? _____

(If YES record duration as 001 below)

SHEATHS/CONDOMS

YES / NO [_____MONTHS]

DIAPHRAGM/ other BARRIER

YES / NO [_____MONTHS]

DEPOT PROGESTOGEN / 'Depot Provera'

YES / NO [_____MONTHS]

19 , Have you used any other form of contraception ?

YES / NO [_____MONTHS]

Description

---

20 , Have you had any of the following medical illnesses or disorders?

1 Yes 2 no 3 Dont know

1 Diabetes Mellitus (Sugar diabetes) [__]

2 Hypertension (High Blood Pressure) [__]

3 Heart attack [__]

4 Stroke [__]

5 Fits [__]

6 Indigestion [__]

7 Cold sores on the lips or mouth [__]

8 Tuberculosis ('TB') [__]

9 Glandular fever [__]

10 Warts on fingers or feet or any other part of the body excluding the genital organs? [__]

21 , Have you had any of the following sexually transmitted diseases ?
(USE FLASH CARD 'STD')

1 =YES 2 = NO 3 = DONT KNOW

1 GONORRHOEA _____[__]
2 SYPHILIS _____[__]
3 TREATMENT FOR VAGINAL THRUSH _____[__]
4 GENITAL WARTS _____[__]
5 GENITAL HERPES _____[__]
6 Any illness that could be considered 'sexually transmitted' which I have not mentioned ? e.g the 'clap' or 'pox' or 'NSU' _____[__]

22 , HAVE YOU EVER ATTENDED A VENEREAL DISEASES CLINIC ? This is sometimes called an STD clinic or "special" clinic ?

1=YES 2=NO [____]

23 , As far as you are aware has your husband or current male partner ever had any of the following diseases or disorders ?

USE FLASH CARD - MALE STD's
1 YES 2 NO 3 DONT KNOW

1 COLD SORES (LIPS/MOUTH) _____[__]
2 GENITAL WARTS _____[__]
3 GENITAL HERPES _____[__]

4 PENILE WARTS _____[__]

5 NON SPECIFIC URETHRITIS _____[__]

6 GONORRHOEA _____[__]

7 HAS HE EVER HAD TO VISIT A VENEREAL DISEASES CLINIC ?____[__]

24 , As far as you are aware , have any of your previous sexual partners ever had any of the following diseases or disorders ?

1 = YES 2= No 3 = Dont know

1 COLD SORES (LIPS/MOUTH) _____[__]

2 GENITAL WARTS _____[__]

3 GENITAL HERPES _____[__]

4 PENILE WARTS _____[__]

5 NON SPECIFIC URETHRITIS _____[__]

6 GONORRHOEA _____[__]

7 DID HE / THEY EVER HAVE TO VISIT A VENEREAL DISEASES CLINIC?

_____[__]

Time of terminating interview _____

Appendix 3

Results of matched univariate analyses

TABLE 2-15 : CIN versus Normal controls

Matched univariate analysis

FACTOR	CIN	Controls	OR	95% C.I.'s
Mean age	37.1	38.7		
Marital Status				
Single	8	9	1	1
Married	38	92	0.4	(0.1-1.3)
Divorced/Sep/Widowed	11	17	0.7	(0.2-2.4)
Number of marriages				
0 or 1	8	9	1	1
2+	49	109	6.9	(1.9-24.5)
Social Class				
I + II	13	23	1	1
IIIN + IIIM	22	72	0.5	(0.2-1.2)
IV + V	15	18	1.4	(0.5-3.9)
Unemployed/Retired	7	5	2.2	(0.5-9.6)
			trend test p = 0.213	
Housing				
Owner	38	98	1	1
Tenant/Parental home	19	20	2.8	(1.2-6.6)
Education				
until 16	43	90	1	1
until 18	7	13	0.9	(0.3 -2.7)
Higher education	7	15	0.9	(0.3-3.3)
Total pregnancies				
0	6	11	1	1
1	14	15	2.4	(0.6-9.2)
2	12	40	0.9	(0.2-3.1)
>=3	25	52	1.4	(0.4-4.9)
pregnancy termination				
0	48	100	1	1
1 or more	9	18	1.3	(0.8-2.1)
Age at menarche				
<12	26	52	1	1
13-14	24	56	0.9	(0.4-1.9)
15+	7	10	2	(0.6-6.6)
Age at 1st pregnancy				
<=18	11	24	1	1
19 - 25	25	50	1.2	(0.5 - 2.9)
26+	15	33	1	(0.4 - 2.7)
Never Pregnant	6	11	0.8	(0.2 - 2.7)
			trend test p = 0.691	

FACTOR	CIN	Controls	OR	(95% C.I.'s)
Age 1st Intercourse				
<16	13	7	1	1
16 - 18	31	50	0.3	(0.1 - 1.3)
19 +	13	61	0.1	(0.04 - 0.5)
			Trend test p< 0.001	
No. of sexual partners				
0,1	5	65	1	1
2-4	24	39	7.6	(2.2-26.2)
5+	28	14	41.6	(9.5-181)
			Trend test p<0.001	
Partner had STD				
Yes	10	3	8.2	(1.8-38.4)
No	47	115		
Former partner had STD				
yes	9	4	12.5	(1.5 - 105.8)
no	48	114		
Attended VD clinic				
YES	10	8	4	(1.2-13.7)
NO	47	110		
Current smoker				
yes	34	34	3.5	(1.8-7.0)
no	23	74		
Ever smoked				
yes	49	52	8.2	(3.2-21.1)
no	8	66		
Duration co-efficient	R=0.08318	P<.001		
[OR=1.087 (1.045-1.131) per year smoked]				
Number smoked	R=0.08831	P<.001		
[OR=1.092(1.046-1.141) per cig per day]				
Alcohol consumption - units per year				
0-9	10	48	1	1
10-35	10	24	1.7	(0.6-5.1)
36-259	18	37	2.4	(0.9-6.4)
>= 260	19	9	8.4	(2.7 - 26.4)
			Trend test p=0.004	
HYPERTENSION				
Yes	6	11	1.2	(0.4-3.7)
No	51	107		
EVER USE OF OCP				
YES	51	97	2	(0.6-6.4)
EVER LOW PILL				
YES	39	63	1.7 *	*(0.7-3.9)
EVER HIGH PILL				
YES	32	56	1.8 *	*(0.8-3.9)
EVER MINIPILL				
YES	12	22	1.0 *	*(0.4-2.4)

* no change in OR having taken account of missing data

FACTOR	CIN	Controls	OR	(95% C.I.'s)
AGE COMMENCING PILL				
<=17	12	15	1	1
18-19	14	21	0.9	(0.3 - 2.6)
20+	25	62	0.6	(0.2 - 1.7)
Never	6	20	0.3	(0.1 - 1.5)
			Trend test p=0.122	

DURATION OF PILL

Taking account of missing data using maximum / minimum substitutions
(See statistical appendix)

LOWPILL	coefficient per month of use	
a)Minimum	-0.0024	p=0.573
b)Maximum	-0.001759	p=0.623
HIGHPILL		
a)Minimum	0.0074	p=0.037
b)Maximum	0.00473	p=0.091
MINIPILL		
a)Minimum	0.0147	p=0.529
b)Maximum	0.00542	p=0.714

IUCD USE				
YES	17	26	1.7	(0.8-3.7)
NO	40	92		
BARRIER CONTRACEPTION USED				
YES	40	96	0.5	(0.3-1.0)
NO	17	22		
CURRENT CONTRACEPTION				
Barrier	5	20	0.4	(0.1 - 1.4)
IUD	5	9	1	(0.3 - 3.2)
PILL	8	15	0.9	(0.4 - 2.2)
STERILISATION	18	42	0.9	(0.5 - 2.0)
SPECIFIC SEXUALLY TRANSMITTED DISEASE				
Genital warts	5	2	4.9	(0.7 -26.0)
Gonorrhoea	2	0		
Herpes genitalis	3	2		
TREATMENT FOR THRUSH				
YES	33	68	1	(0.5 - 1.9)
NO	24	50		
ANTIBODIES TO HSV-1				
present	49	96	1.1	(0.5 - 2.7)
absent	8	22		
ANTIBODIES TO HSV-2				
present	51	98	2.8	(0.8 -10.0)
absent	6	20		

FACTOR

HSV-1K VALUE
R = 0.02867 OR = 1.029
P = 0.913

HSV-2K VALUE
R = 0.05363 , OR = 1.055
P = 0.935

HSV-1K/HSV-2K RATIO
R=- 0.01773 p= 0.779

FACTOR	CIN	Controls	OR	(95% C.I.'s)
HSV-1K/HSV-2K >=3	16	51	0.6	(0.3 - 1.3)
HSV-1K/HSV-2K <=1	16	31	1.14	(0.54-2.4)
NEGATIVE CHECKS				
Positve history of :				
Chickenpox	40	92	0.6	(0.3 -1.4)
Measles	36	90	0.5	(0.3 - 1.1)
German measles	29	55	1.2	(0.7 - 2.4)
Mumps	30	63	1	(0.5 - 1.8)
Rheumatic fever	0	2	Not computable	Not computable
Cholecystectomy	1	0	Not computable	Not computable
Appendicectomy	5	15	0.7	(0.2 - 2.1)
Haemmorhoids	28	52	1.4	(0.7 - 2.7)
Varicose veins	11	27	0.9	(0.4 - 2.0)
Anal warts	2	0	Not computable	Not computable
Diabetes	0	1	Not computable	Not computable
Heart attack	0	1	Not computable	Not computable
Cerebrovascular accident	0	0	Not computable	Not computable
Epilepsy	1	4	0.4	(0.03 - 3.5)
Dyspepsia	44	91	1	(0.4 - 2.3)
Oral herpes simplex	25	61	0.7	(0.4 - 1.4)
Tuberculosis	0	2	Not computable	Not computable
Glandular fever	5	6	1.5	(0.4 - 5.2)
Non genital warts	31	48	1.7	(0.8 - 3.1)

TABLE 2-16 : CIGN versus Normal controls : Matched univariate analysis

FACTOR	CIGN	NORMAL	OR	(95% C.I.'s)
Mean age	GIN=36.7	Normal=38.6		
Marital Status				
Single	2	9	1.5	(0.2-3.9)
Married	32	106	0.9	(0.3-2.3)
Divorced/Sep/Widowed	8	18	1.8	(0.7-4.8)
No of marriages				
0	2	9	1	
1	34	113	2.4	(0.3-23.5)
2+	6	11	4.7	(0.4-55.3)
				trend test p=0.06
Social Class				
I + II	11	26	1	
IIIN + IIIM	20	79	0.5	(0.2-1.3)
IV + V	9	23	0.8	(0.3-2.3)
Unemployed	2	5	1	(0.2-5.6)
Housing				
Owner	32	112	1	
Tenant/Parental home	10	21	1.6	(0.6-4.3)
Education				
until 16	29	103	1	
until 18	7	14	1.6	(0.6-4.8)
> 18	6	16	1.3	(0.5-3.8)
Total pregnancies				
0	7	11	1	
1	9	15	1	(0.2-2.7)
2	14	46	0.6	(0.2-1.6)
>=3	12	61	0.4	(0.1-1.1)
				trend test p= 0.06
[Nulliparous subjects versus rest , RR= 1.7 (0.6 - 1.7)]				
Termination of pregnancy				
YES	10	21	1.4	(0.6-3.3)
NO	32	112		
Age at menarche				
<12	12	58	1	
13-14	18	64	1.1	(0.5-2.4)
15+	12	11	5.1	(1.7-15.2)
				trend test p = 0.013

FACTOR	CIGN	NORMAL	OR	(95% C.I.'s)
Age at 1st pregnancy				
<=18	6	26	1	
19-25	20	62	1.1	(0.4 - 3.0)
26+	9	34	1	(0.3 - 3.1)
Never pregnant	7	11	1.5	(0.4 - 5.5)
				trend test p=0.685
Age 1st intercourse				
<16	5	8	1	
16-18	22	57	0.56	(0.1-1.7)
19+	15	68	0.35	(0.1-1.1)
				trend test p= 0.086
Number of sexual partners				
0,1	11	75	1	
2-4	17	42	2.6	(1.0-5.3)
5-9	14	16	6.3	(2.5-24.9)
				trend p < 0.001
Partner had STD				
Yes	2	3	1.7	(0.3-11.1)
No	40	130		
Previous partner had STD				
Yes	1	5	0.8	(0.1 - 6.9)
No	41	128		
Attended VD clinic				
YES	6	9	2.7	(0.8-8.6)
NO	36	124		
Current smoker				
yes	18	41	1.7	(0.8-3.5)
no	24	92		
Ever smoked				
yes	26	59	2.1	(1.0-4.2)
no	16	74		
Duration co-efficient	0.02879	p=.112		[OR = 1.029 per year smoked]
No. of cigs smoked	0.03321	p=.097		[OR = 1.034 /cig. smoked / day]
Alcohol consumption - units per year				
<10	5	52	1	
10-35	11	28	3.2	(1.0-10.3)
36-259	20	44	5.8	(1.9-17.7)
>= 260	6	9	7.6	(1.8-32.4)
				trend test p<0.001
HYPERTENSION				
Y	7	12	1.6	(0.6-4.7)
N	35	121		

FACTOR	CIGN	NORMAL	OR	(95% C.I.'s)
EVER USE OF OCP				
YES	36	110	0.9	(0.3-2.6)
NO	6	23		
EVER LOW PILL*				
YES	25	73	1	(0.4-2.5)
EVER HIGH PILL*				
YES	24	64	2	(0.9-4.5)
EVER MINIPILL*				
YES	4	25	0.6	(0.2-1.6)

* no change in RR/OR having taken account of missing data

AGE COMMENCING PILL				
<=17	11	16	1	
18-19	7	28	0.4	(0.1 - 1.3)
20+	18	67	0.4	(0.1 - 1.4)
Never	6	22	0.4	(0.01 - 1.8)
				trend test p=0.252

DURATION OF PILL
Taking account of missing data

LOWPILL	R = regression coefficient per month of use			
a) Maximum	-0.004033	p = 0.675		
b) Minimum	-0.00337	p = 0.768		
HIGHPILL				
a)Maximum	0.004342	P=0.171		
b) Minimum	0.007985	p=0.033	OR = 1.008	(1.000-1.016)
MINIPILL				
a)Maximum	-0.00142	p= 0.582		
b) Minimum	0.02045	p = 0.757		

IUCD USE				
YES	8	30	0.8	(0.3-2.1)
NO	34	103		

SPECIFIC SEXUALLY TRANSMITTED DISEASE

Genital warts	3	3	4.7	(0.7 - 28.4)
Gonorrhoea	0	0		
Herpes genitalis	0	2		

TREATMENT FOR VAGINAL THRUSH				
YES	11	56	0.5	(0.2 - 1.1)
	31	77		
BARRIER METHODS USED				
YES	30	110	0.4	(0.2-0.9)
NO	12	23		

R = -0.005346 per month of use p = 0.143

FACTOR	CIGN	NORMAL	OR	(95% C.I.'s)
CURRENT CONTRACEPTION				
Barrier	1	25	0.1	(0.01 - 0.8)
OCP	10	18	2.1	(0.7 - 5.4)
IUCD	1	8	0.3	(0.04 - 3.0)
Sterilised	11	52	0.6	(0.2 - 1.3)
ANTIBODIES TO HSV-1				
present	38	107	2.1	(0.7 - 6.5)
absent	4	26		
ANTIBODIES TO HSV-2				
present	36	110	1.5	(0.5 - 4.2)
absent	6	23		
HSV-1 K value				
R= -0.3043	p=0.313			
HSV-2 K Value				
R= -0.1256	p=0.845			
HSV1/HSV2 RATIO				
R= +0.6343 p = 0.284				
HSV1/HSV2 >=3	22	57	1.6	(0.8-3.3)
HSV1/HSV2 <=1	7	35	0.6	(0.3-1.5)

FACTOR	CIGN	NORMAL	OR	(95% C.I.'s)
NEGATIVE CHECKS				
Positve history of :				
Chickenpox	31	100	1	(0.4 - 2.2)
Measles	36	99	2.2	(0.8 - 5.8)
German measles	21	65	1.1	(0.5 - 2.4)
Mumps	21	72	1	(0.5 - 2.0)
Rheumatic fever	0	2		Not computable
Cholecystectomy	0	0		Not computable
Appendicectomy	3	16	0.6	(0.2 - 2.1)
Haemmorhoids	18	61	1	(0.5 - 2.0)
Varicose veins	7	28	0.8	(0.3 - 2.0)
Anal warts	1	0		Not computable
Diabetes	0	2		Not computable
Heart attack	0	1		Not computable
Cereb/vasc. accident	0	0		Not computable
Epilepsy	0	4		Not computable
Dyspepsia	29	101	0.8	(0.4 - 1.8)
Oral herpes simplex	16	66	0.6	(0.3 - 1.3)
Tuberculosis	0	2		Not computable
Glandular fever	2	6	1.1	(0.2 - 6.0)
Non genital warts	21	58	1.4	(0.7 - 2.9)

TABLE 2-17
Subdivision of CIGN into pure and mixed variants , comparison with normal controls

Univariate analyses for 1) Mixed CIGN vs normals 2) Pure CIGN vs normals

FACTOR	**Mixed**	**Controls**	OR(95%CIs)	**Pure**	**Controls**	OR(95%CIs)
Mean age	**34.8**	**38.6**		**38.6**	**38.6**	
Marital Status						
Single	1	4	0.8(0.1-11.6)	1	5	-
Married	21	67	0.7(0.2-2.6)	11	39	0.8*(0.2-3.3)
Divorced/Sep/Widow	4	9	1.6(0.4-5.7)	4	9	2.2*(0.5-10.3)
No of marriages						*vs 2 others combined
0 or 1	20	72	1	16	50	
2+	6	8	2.8(0.9-8.9)	0	3	-
Social Class						
I + II	5	13	1	5	13	1
IIIN + IIIM	14	47	0.9(0.2-3.2)	5	32	0.4(0.1-1.4)
IV + V	6	17	1.0(0.2-3.9)	3	6	1.3(0.2-7.2)
Unemployed/Disabled	1	3	0.9 (0.1-11.4)	3	2	1.6(0.1-19.9)
Housing						
Owner	21	66	1	11	46	1
Tenant/Parents home	5	14	0.7(0.2-3.3)	5	7	3.0(0.7-12.5)
Education						
until 16	19	64	1	10	39	1
until 18	4	11	1.1(0.3-4.6)	3	3	2.6(0.5 -14.7)
> 18	3	5	2.0(0.5-8.4)	3	11	1.1(0.2 - 4.9)
			trend test p=0.389			
Total pregnancies						
0	5	8	1	3	3	1
1	6	10	1.7 (0.4-7.2)	2	5	0.2(.01-3.1)
2	9	26	0.9(0.2-3.7)	5	20	0.2(0.03-1.5)
>=3	6	36	0.4(0.1-1.9)	6	25	0.2(0.03-1.4)
			trend test p= 0.154			
Termination of pregnancy						
YES	3	14	0.8(0.3-2.7)	6	7	1.4 (0.6-3.4)
NO	23	66		10	46	
Age at menarche-1						
<12	8	29	1			-
13-14	13	42	1.0(0.4-2.9)			-
15+	5	9	2.9(0.7-11.7)			-
			trend test p=0.178			
Age at menarche-2			<=13	8	42	1
			14+	8	11	5.1(1.2-20.9)

FACTOR	Mixed Controls		OR(95%CIs)	Pure Controls		OR(95%CIs)
Age at 1st pregnancy						
<=18	4	16	1	2	10	1
19-25	12	39	0.9(0.3 - 3.1)	8	23	1.4 (0.3 - 8.2)
26+	5	17	1.0(0.2 - 4.2)	4	17	1.1 (0.2 - 7.7)
Never	5	8	1.0(0.2 - 4.7)	2	3	3.6 (0.4 - 35.9)
						trend test p= 0.475
Age 1st intercourse						
<=18	20	43	1	7	22	1
19+	6	37	0.4(0.1-1.3)	9	31	1.1 (0.3 - 3.7)
Number of sexual partners						
0,1	6	47	1	6	28	1
2-4	10	24	3.9(1.2-12.6)	6	18	1.6(0.4-6.1)
5+	10	9	12.2(2.9-52.2)	4	7	2.6 (0.6-11.9)
			trend test p<0.01			trend test p = 0.416
Partner had STD						
YES	1	2	1.0(0.1-11.3)	1	1	4.0 (0.3-64.0)
Previous partner had STD						
YES	0	5	-	1	2	2.0(0.2 - 22.0)
Attended VD clinic						
YES	2	4	2.0(0.3-12.7)	4	5	3.3(0.7-15.3)
NO	24	76		12	48	
SPECIFIC STD's						
Warts	3	1	6.4(0.6 - 73.2)	1	1	-
Gonorrhoea	0	0		0	0	-
Herpes	0	0		0	2	-
TREATMENT FOR VAGINAL THRUSH						
YES	11	34	1.2 (0.5 - 3.0)	0	23	-
Current smoker						
yes	9	24	1.2(0.5-3.2)	9	17	2.6(0.9-7.8)
no	17	56		7	36	
Ever smoked						
yes	15	36	1.7(0.7-4.3)	11	23	2.7(0.9-8.4)
no	11	44		5	30	

Duration co-efficient R = 0.0205 per year smoked P = 0.455 R=0.0389 , p = 0.155

Number cigs smoked R = 0.0254 per cigarette smoked per day, P = 0.452 R=0.0402 p=0.148

FACTOR	Mixed Controls		OR(95%CIs)	Pure Controls		OR(95%CIs)
Alcohol consumption						
<10 units(per year)	2	31	1	4	21	1
10-35 units	7	12	12.8(1.4-122.1)	3	15	1.0 (0.2-4.9)
36-259 units	13	32	16.14(1.9-343.3)	7	13	3.0(0.6-14.1)
>= 260 units	4	5	22.2 (2.0-244)	2	4	3.4 (0.4-31.8)
			trend test p = 0.048			trend test p=0.345
HYPERTENSION						
Y	3	10	1.3(0.4-4.9)	3	2	0.2 (0.04-1.4)
N	23	70		13	51	
EVER USE OF OCP						
YES	22	65	1.1(0.3-4.3)	13	45	0.7(0.1-3.3)
NO	4	15		3	8	
EVER LOW PILL*						
YES	19	45	3.3(0.8 -13.9)	7	28	0.5(0.2-1.9)
EVER HIGH PILL*						
YES	15	35	2.3(0.8-6.1)	9	29	1.4(0.4-5.9)
EVER MINIPILL*						
YES	2	18	0.4(0.08-1.6)	3	8	1.3(0.3-6.11)
* no significant change in OR/CIs after allowing for missing data						
AGE COMMENCING PILL						
<=17	8	10	1	3	6	1
18-19	5	17	0.4(0.1 - 1.7)	1	11	0.2 (0.01 - 4.0)
20+	9	39	0.2 (0.04 -1.0)	9	28	1.0 (0.1 - 10.5)
Never	4	14	0.2 (0.02-1.3)	3	8	1.3 (0.1 - 19.3)
			trend test p=0.045			p = 0.432

DURATION OF PILL

Taking into account missing data

LOWPILL	coefficients (R) per month of use	
a)maximum	-0.00485 p=0.359	-0.001118 p=0.885
b)minimum	-0.001102 p=0.844	-0.009414 p=0.332
HIGHPILL		
a)maximum	0.002152 p=0.594	0.008346 p=0.124
b)minimum	0.01072 p=0.066	0.005272 p=0.340
MINIPILL		
a)maximum	-0.007641 p=0. 600	0.03211 p=0.154
b)minimum	0.02318 p=0.309	0.01718 p=0.546

IUCD USE						
YES	5	18	1.0(0.3-3.0)	3	12	0.9 (0.7-1.1)
NO	21	62		13	41	

Univariate analyses for 1) Mixed CIGN vs normals 2) Pure CIGN vs normals

BARRIER CONTRACEPTION USED						
YES	19	70	0.4(0.1-1.3)	10	40	1.0(0.99-1.01)
NO	7	10		6	13	
CURRENT CONTRACEPTION						
Barrier	0	14	-	1	15	0.3 (0.03 - 2.2)
IUCD	0	3	-	1	5	0.5(0.04 - 5.6)
Pill	16	35	2.0 (0.7 - 5.9)	1	6	0.5 (0.05 - 4.4)
Sterilised	6	33	0.4(0.1 - 1.3)	5	19	0.9 (0.2 - 2.9)
ANTIBODIES TO HSV-1						
+	23	62	2.1(0.6 - 7.8)	15	45	2.2(0.3 - 18.3)
_	3	18		1	8	
ANTIBODIES TO HSV-2						
+	21	63	1.2 (0.4 - 4.0)	15	47	2.6(0.3 - 24.4)
-	5	17		1	6	

HSV-1K value
R= -0.1003 p=0.783

HSV-2K value
R=+0.2467 p=0.785

HSV-1K/HSV-2K Ratio
R= +0.000924 p=0.725

HSV-1K/HSV-2K>= 3
17 34
OR= 2.4[0.9 - 6.1]
R= +0.8576 p=0.079

HSV-1K/HSV-2K<=1
4 21
OR = 0.5(0.2 - 1.7)

HSV-1K value
R=-0.7043 p=0.211

HSV-2K value
R=-0.4899 p=0.600

HSV-1K/HSV-2K Ratio
R=-0.004067 p=0.403

HSV-1K/HSV-2K>= 3
5 23
OR=0.7(0.2 - 2.74)

HSV-1K/HSV-2K<=1
3 14
OR=0.7(0.2-2.8)

TABLE 2-18 CIGN VERSUS CIN - Matched univariate analysis

FACTOR	CIGN	CIN	OR	95% C.I.'s
Mean age	36.7	35.3		
Marital Status				
Single	2	11	1	
Married	32	48	2.6	(0.5-14.0)
Divorced/Sep/Widowed	8	13	2.5	(0.4-17.0)
No of marriages				
0	2	11	1	
1	34	45	2.7	(0.5-14.5)
2+	6	16	1.3	(0.2-9.0)
Social Class				
I + II	11	19	1	
IIIN + IIIM	20	27	1	(0.4-2.5)
IV + V	9	18	0.7	(0.2-2.1)
Unemployed	2	8	0.2	(0.02-2.3)
			trend test p = 0.235	
Housing				
Owner	32	48	1	
Tenant/Parental home	10	24	0.7	(0.2-1.8)
Education				
until 16	29	53	1	
until 18	7	9	1.5	(0.4-5.0)
> 18	6	10	1.1	(0.4-3.1)
Total pregnancies				
0	7	7	1	
1-2	23	32	0.7	(0.2-2.5)
3-4	10	24	0.4	(0.1- 1.5)
>4	2	9	0.4	(0.02-1.4)
			trend test p = 0.053	
Termination of pregnancy				
NO	32	58	1.7	(0.6-4.5)
YES	10	14		
Age at menarche				
<12	12	35	1	
13-14	18	29	2	(0.8-5.1)
15+	12	8	5.2	(1.5-18.1)
			trend test p = 0.024	
Age at 1st pregnancy				
<=18	7	18	1	
19-25	20	31	1.4	(0.5-4.1)
26+	9	16	1.2	(0.4-3.6)
Never	6	7	2	(0.5 - 8.0)
			trend test p=0.511	
Age 1st intercourse				
<16	5	18	1	
16-18	22	40	1.5	(0.5-5.0)
19+	15	14	2.6	(0.8-8.4)
			trend test p= 0.092	

FACTOR	CIGN	CIN	OR	95% C.I.'s
Number of sexual partners				
1	11	8	1	
2-4	17	27	0.6	(0.2-1.7)
5-9	12	21	0.4	(0.1-1.5)
10+	2	16	0.1	(0.02-0.7)
			trend test p=0.005	
Partner had STD				
Yes	2	16	0.2	(0.05-1.0)
Previous partner STD				
Yes	1	10	0.2	(0.02 - 1.6)
Attended VD clinic				
YES	6	15	0.8	(0.3-2.1)
SPECIFIC STD's				
Genital warts	3	7	0.7	(0.1 - 3.5)
Gonorrhoea	0	3	-	
Herpes genitalis	0	4	-	
Treatment for vaginal thrush				
Yes	11	28	0.7	(0.3 -1.4)
Current smoker				
yes	18	44	0.5	(0.2-1.2)
no	24	28		
Ever smoked				
yes	26	62	0.3	(0.1-0.8)
no	16	10		
Duration co-efficient	R = -0.05426 per year smoked, p=0.022 [OR=0.9472 (0.9043-0.9921)]			
Number of cigs smoked	R = -0.06468 per cig smoked per day,p=0.014, [OR= 0.9374 (0.89-0.98)]			
Alcohol consumption - Units per year				
<10	5	13	1	
10-35	11	16	2.7	(0.5-13.8)
36-259 units	20	23	2.9	(0.7-11.9)
>= 260	6	20	1	(0.2-5.2)
			trend test p = 0.893	
HYPERTENSION				
Y	7	6	3	(0.7-12.2)
EVER USE OF OCP				
YES	36	64	0.7	(0.2-2.3)

FACTOR	CIGN	CIN	OR	95% C.I.'s
EVER LOW PILL*				
YES	26	48	0.9	(0.4-2.2)
EVER HIGH PILL*				
YES	24	34	1.1	(0.5-2.6)
EVER MINIPILL*				
YES	5	12	0.7	(0.2-2.1)

* No change in OR/CI's after taking account of missing data

AGE COMMENCING PILL				
<=17	11	19	1	
18-19	7	17	0.6	(0.2 - 2.1)
20+	18	28	0.9	(0.3 - 2.6)
Never	6	8	1.1	(0.3 - 4.8)
			trend test p = 0.897	

DURATION OF PILL
Taking account of missing data

LOWPILL		
a) Minimum	R = 0.00266	p = 0.639
b) Maximum	R = 0.000912	p = 0.841
HIGHPILL		
a) Minimum	R= 0.000487	p=0.896
b) Maximum	R = 0.00091	p=0.774
MINIPILL		
a) Minimum	R = 0.00675	p =0.706
b) Maximum	R = 0.000233	p =0.983

IUCD USE				
YES	8	24	0.6	(0.2-1.4)
BARRIER CONTRACEPTION USED				
YES	30	51	1.1	(0.5-2.5)

R = 0.0004618 per month of use , p = 0.913

CURRENT CONTRACEPTION				
Barrier	1	7	0.2	(0.03 - 2.1)
IUD	1	8	0.2	(0.03 - 1.9)
Pill	10	10	2.4	(0.8 -7.5)
Steri	11	18	0.8	(0.3 - 2.2)

FACTOR	CIGN	CIN	OR	95% C.I.'s
ANTIBODIES TO HSV-1				
present	38	57	2.4	(0.6 - 9.0)
ANTIBODIES TO HSV-2				
present	36	65	0.6	(0.2 - 2.2)
'K' values				
HSV-1	R =-0.2679	p = 0.435		
HSV-2	R = 0.09416	p = 0.902		
HSV1/HSV2 RATIO				
R= +0.08541 p = 0.263				
HSV1/HSV2 >=3	22	20	2.5	(1.03 - 6.0)
HSV1/HSV2<=1	7	20	0.5	(0.2 - 1.5)

TABLE 2-19 Subdivision of CIGN into pure and mixed variants

Univariate analyses	**1) Mixed CIGN vs CIN**			**2) Pure CIGN vs CIN**		
FACTOR	**Mixed**	**Controls**	**OR(95%CIs)**	**Pure**	**Controls**	**OR(95%CIs)**
Mean age	34.8			38.6		
Marital Status						
Single	1	7	1	1	4	-
Married	21	30	3.6(0.3 -41.0)	11	18	1.8(0.2-17.9)
Divorced/Sep/Widowed	4	6	2.9(0.2 - 52.0)	4	7	2.0(0.2-25.6)
No of marriages						
0 or 1	20	34	1	16	22	
2+	6	9	0.8(0.3 - 2.5)	0	7	-
Social Class						
I + II	5	8	1	5	10	1
IIIN + IIIM	14	19	1.5(0.4 - 5.4)	5	9	0.9(0.2 - 4.4)
IV + V	6	12	0.9* (0.2 - 3.9)	3	6	0.8(0.1 - 6.0)
V1	1	4		3	4	1.7(0.2-14.6)
			(* IV+V+VI)	trend test p=0.731		
Housing						
Owner	21	30	1	11	18	1
Tenant/Parents home	5	13	0.6(0.1 - 2.4)	5	11	0.8 (0.2 - 3.0)
Education						
until 16	19	31	1	10	22	1
until 18	4	6	1.3(0.3-5.3)	3	3	2.5(0.2-33.2)
> 18	3	6	0.5(0.1 - 3.3)	3	4	2.0(0.4-10.5)
			trend test p=0.775			
Total pregnancies						
0	5	7	1	3	0	1
1	6	12	1.3(0.2 - 7.4)	2	6	1
2	9	8	2.2(0.4 - 13.2)	5	6	0.7(0.1 - 8.5)
>=3	6	16	0.5 (0.1-3.3)	6	17	0.5(0.1 - 2.4)
			trend test p=0.596		trend test p= 0.403	
Termination of pregnancy						
YES	3	9	0.5(0.1 -2.2)	6	5	2.6(0.7-11.6)
NO	23	34		10	24	
Age at menarche						
<15	20	39	<=13	8	21	1
15+	6	4	3.1(0.7-13.4) >13	8	8	2.6(0.6-10.9)

FACTOR	Mixed	Controls	OR(95%CIs)		Pure	Controls	OR(95%CIs)
Age at 1st pregnancy							
<=18	4	11	1				
19-25	12	16	1.4(0.4-5.1)		<18 2	7	1
26+	5	9	1.1(0.2-5.4)		19-25 7	15	2.3(0.4-15.2)
Never	5	7	1.1(0.2-5.4)		26+ 7	7	1.4 (0.3 -8.3)
			trend test p=0.987				
Age 1st Intercourse							
<=18	20	36	1	<16	1	7	1
19+	6	7	1.2(0.4-3.8)	16-18	6	15	2.6(0.2-28.4)
				19+	9	7	6.8(0.7-70.4)
						trend test p= 0.038	
Number of sexual partners							
0,1	6	5	1		6	3	1
2-4	10	21	0.5(0.1-2.2)		6	6	0.5 (0.1 -3.2)
5+	10	17	0.5(0.1-2.1)		4	20	0.1(0.01-0.8)
			trend test p=0.310			trend test p = 0.003	
Partner had STD							
Yes	1	12	0.2(0.02-1.2)		1	4	0.5 (0.1-4.7)
No	25	31			15	25	
PREVIOUS PARTNER HAD STD							
+	0	5	-		1	2	0.3(0.03-2.3)
Attended VD clinic							
YES	2	9	0.4(0.1 - 1.9)		4	6	1.5(0.4-6.4)
NO	24	34			12	23	
SPECIFIC STD's							
Warts	2	5	0.3(0.04 - 3.1)		1	2	2.8(0.2-47.2)
Gonorrhoea	0	2	-		0	1	-
Herpes	0	1	-		0	3	-
TREATMENT FOR VAGINAL THRUSH							
YES	11	12	2.3(0.8-6.8)		0	16	-
Current smoker							
yes	9	28	0.3(0.1-0.96)		9	16	1.1(0.3-3.5)
no	17	15			7	13	
Ever smoked							
yes	15	37	0.3(0.1-0.96)		11	25	0.3(0.02-2.2)
no	11	6			5	4	

Duration co-efficient R = -0.07393 per year smoked P = 0.033
Number of cigs smoked R = -0.08666 per cigarette smoked per day, P = 0.012

FACTOR	Mixed	Controls	OR(95%CIs)	Pure	Controls	OR(95%CIs)
Alcohol consumption- units per year						
<10	2	9		4	4	1
10-35	7	9	1	3	7	0.5(0.06 -4.1)
36-259	13	13	2.0(0.7 - 6.0)	7	10	0.7(0.1 - 3.8)
>= 260	4	12	0.4(0.1-2.0)	2	8	0.4(0.04-3.3)
			trend test p=0.755		trend test p=0.287	
HYPERTENSION						
Y	3	4	0.6(0.04 - 3.3)	3	2	0.3(0.05-2.0)
N	23	39		13	27	
EVER USE OF OCP						
YES	22	40	0.5(0.1 - 3.1)	13	24	0.9(0.2 - 4.2)
NO	4	3		3	5	
EVER LOW PILL*						
YES	19	30	2.4(0.6 - 9.5)	7	18	0.4(0.1 - 1.6)
EVER HIGH PILL*						
YES	15	19	1.8(0.6-5.7)	9	15	0.7(0.2 - 2.4)
EVER MINIPILL*						
YES	2	7	0.9(0.2-5.0)	3	5	0.6(0.1 - 3.3)

* no significant change in OR/CIs after allowing for missing data

AGE COMMENCING PILL

<=17	8	13	1	3	6	1
18-19	5	11	0.8(0.2-2.8)	1	6	0.2(0.01-4.0)
20+	9	16	0.7(0.2-2.5)	9	12	1 (0.1-10.5)
Never	4	3	1.3(0.2-9.1)	3	5	1.3(0.1-19.3)
			trend test p=0.855		trend test p = 0.432	

DURATION OF PILL
Taking into account missing data

LOWPILL	coefficients per month of use	
a)maximum	R =0.00267 p=0.687	R= -0.000035 p=0.960
b)minimum	R= 0.01032 p=0.208	R= -0.004721 0.581
HIGHPILL		
a)maximum	R= 0.3304 p=0.539	R=0.00025 p=0.952
b)minimum	R= 0.001549 p=0.790	R=-0.001879 p =0.700
MINIPILL		
a)maximum	R=0.01169 p=0.627	R=0.00838 p=0.665
b)minimum	R= 0.01208 p=0.617	R= -0.001226 p=0.966

IUCD USE						
YES	5	15	0.5(0.2-1.6)	3	9	0.6 (0.1-2.7)
NO	21	28		13	20	

FACTOR	Mixed	Controls	OR(95%CIs)	Pure	Controls	OR(95%CIs)
BARRIER CONTRACEPTION USED						
YES	19	27	2.0(0.6 - 5.8)	10	24	0.5(0.1 -1.8)
NO	7	16		6	5	
CURRENT CONTRACEPTION						
Barrier	0	4	-	1	2	0.8(0.1-8.9)
IUCD	0	4	-	1	2	1.2(0.1-19.9)
Pill	9	6	3.3 (0.9 - 10.2)	1	4	0.4(0.04-4.1)
Sterilised	6	11	0.7(0.2 - 2.4)	5	7	1.1(0.3 - 4.8)
ANTIBODIES TO HSV-1						
+	23	33	2.6(0.5 -13.9)	15	24	2.0(0.2-17.2)
–	3	10		1	5	
ANTIBODIES TO HSV-2						
+	21	40	0.2(0.08-1.8)	15	25	1.8(0.2-17.1)
-	5	3		1	4	

K values — Regression coefficients

HSV1-K — R=0.00357 p=0.997 — R = -0.6558 , OR = 0.52(0.1 - 2.2)

HSV-2K — R=-0.1425 p=0.718 — R=+0.2631 ,OR=1.3(0.1-3.6)

HSV-1/HSV-2 RATIO
R=-0.000201 p=0.964

HSV-1/HSV-2 RATIO
R=-0.000103 p=0.999

HSV-1/HSV-2 >=3

17 14

OR= 3.4[1.02 - 11.1]

R=+1.212 p=0.046

HSV-1/HSV-2 >=3

5 6

OR= 1.5(0.4 - 6.4)

HSV-1/HSV-2 <=1

4 13

OR=0.3 (0.1 - 1.7)

HSV-1/HSV-2<=1

3 7

OR= 0.8(0.2 - 3.4)

CHAPTER 111

A PROSPECTIVE STUDY OF CONISATION OF THE CERVIX IN THE MANAGEMENT OF CERVICAL INTRAEPITHELIAL GLANDULAR NEOPLASIA

Introduction

CIGN is an uncommon lesion whose incidence, aetiology and natural history are unclear . Not surprisingly , the management of this lesion is problematic and clinical opinion has been influenced by the results of small retrospective clinico-pathological studies. These studies have usually advocated hysterectomy as definitive management. (Weisbrot et al ,1972 Qizilbash,1975 Christopherson et al,1979 Hopkins et al, 1988). While the primary aim of treatment is to prevent the development of invasive carcinoma , it is important not to recommend unnecessarily radical treatments, especially as these treatments compromise reproductive function. A growing body of opinion suggests that cone biopsy may be sufficient management provided the margins of the specimen pass through normal tissue. (Ostor et al,1984 Luesley et al , 1987 Bertrand et al ,1987 Andersen and Arffmann,1989) However , there has been no prospective evaluation of this policy of management , and all the series quoted involve small numbers. A prospective cohort study has therefore been carried out to assess the role of conisation in the management of CIGN.

Aim of study

To define the optimum management of CIGN.This optimum form of management should be associated with minimum surgical intervention.

Objectives of study

To prospectively assess the effectiveness of cone biopsy in the management of histologically proven CIGN.

Subjects and Methods

Recruitment to the study took place along the lines indicated in Chapter 1. (See 1-4) Subjects were included in the study if they had histologically proven CIGN with or without an associated squamous component on a cone biopsy specimen. Subjects were considered ineligible for the study if they had concurrent atypical endometrial pathology , if there was concurrent benign gynaecological pathology that would normally necessitate hysterectomy , if there was evidence of invasive adenocarcinoma , or if it was unlikely that regular cytological follow-up could be guaranteed .

For those registered in the study , a request was made for representative histopathological material to be submitted for central independent pathology review. A questionnaire was sent to consultants registering patients in the study in order to ascertain the following information:

1) Cytological findings at referral to hospital

2) Colposcopic findings , including results of colposcopically directed biopsy,

3) Length of cone biopsy in millimetres from apex (endocervical margin) to base (ectocervical margin)

4) Status of excision margins of cone biopsy (Free of disease or involved with disease),

5) Details of cytological and colposcopic follow-up examinations,

6) Details of any surgery subsequent to initial cone biopsy,

After enrolment in the study a questionnaire was sent at 6 monthly intervals requesting information on the patient's progress , notably the results of the most recent cytological examination , and details of any further surgery .

Pathology review

All pathological material was submitted for central pathology review . Such review was felt to be essential to verify this diagnosis which : a) is made infrequently in routine hospital practice , and b) can be easily confused with benign conditions of the endocervix, e.g. tubal metaplasia. (Wells and Brown, 1986) . The review panel consisted of 3 pathologists with a special interest in gynaecological pathology.(See 1 - 7) Suitability for trial inclusion and confirmation of abnormality in subsequent specimens was dependent on central review. A standard proforma was completed by the histopathologist for each specimen examined. All subjects described in this report had undergone review by at least one member of the panel (TPR , See 1-4) .

Study protocol

i) Management

Following registration in the study , further management was determined by the results of examination of the margins of excision of the cone specimen.(See Figure 3-1) This assessment was made by the pathologist at the referring institution who had access to all the diagnostic material. If cone margins were free of CIGN, no further operative treatment was recommended. However , regular cytological follow-up was advised . (see below) If the margins of excision were involved by CIGN it was recommended that hysterectomy or a second cone biopsy should be performed 4 months later .The choice of surgical procedure was left to the discretion of the referring gynaecologist and the 4 month interval was advised to allow time for the post-surgical inflammatory reaction to subside , which might theoretically enable more accurate histopathological and cytological analysis. It was advised

that further surgery should be preceeded by one further cytological assessment thus allowing a comparison of the predictive accuracy of excision status and cytological assessment in determining the presence/absence of residual disease. The length of the cone specimen as measured from the apex of the cone (i.e. the upper endocervical margin) to its base (the ectocervical margin) was routinely recorded .

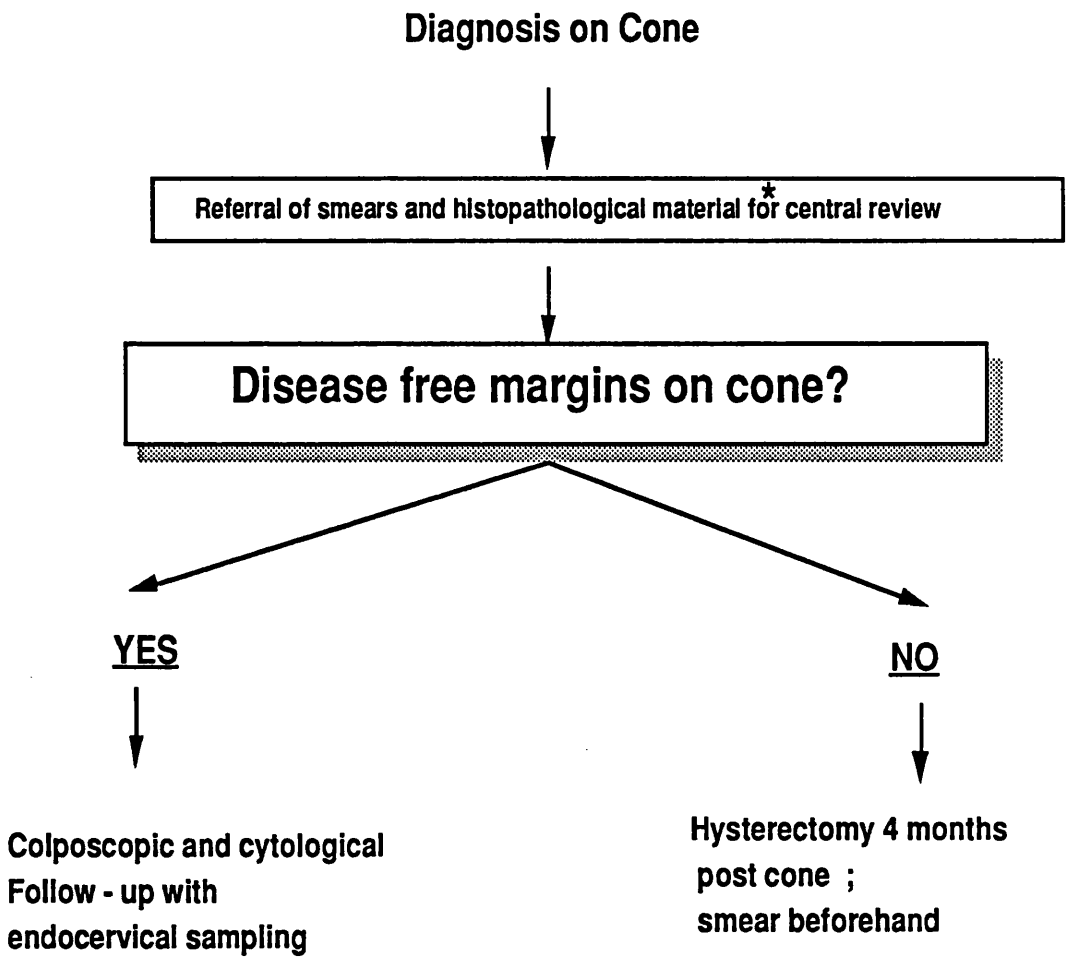
ii) Follow-up

It was advised that all patients be followed up at 4 monthly intervals for 2 years and annually thereafter. Cytological samples were collected at each follow up visit using the endocervical cytobrush (Medscand) in addition to the standard Ayre's spatula. In all cases where persistently abnormal cytology was found , a request was made for further histological material to be obtained. However , the precise management in individual cases was left to the discretion of the referring clinician.

iii) Consent

Consultants registering cases in the study were asked to inform subjects that they were being managed in the context of a clinical trial. The study protocol stressed that informed consent should be given by patients and the onus was with the consultant registering the case to explore whether local ethical committees required that written consent be obtained .

TREATMENT SCHEDULE



* 'representative' material

FIGURE 3-1

Results

The data which follows represents the results of 51 cases of CIGN , where:

a) the diagnosis of CIGN has been confirmed by central pathology review , and invasive disease has been excluded .

b) Follow up cervical cytology has been carried out following conisation.

c) Full information on the patient's management has been received from referring clinicians.

Exclusions

At registration , 4 subjects were excluded as they had not been managed in accordance with the study protocol. Insufficient clinical information is available on 15 subjects despite repeated requests , and they have therefore not been considered further. At the time of writing , central histopathological review has led to exclusion of 5 cases on the grounds that there was a suspicion of invasive disease . These cases are illustrated in Table 3-2 . In addition, 9 cases have been rejected on the grounds that no CIGN has been demonstrated in the specimen. This leaves 51 cases of confirmed CIGN available for study.

Patient characteristics

The mean age of the 51 patients studied was 35.7 years , range 19 - 56 years . The median parity was 1.5 , range 0 - 6 . Fourteen patients (27.5%) were 30 years or less when diagnosed , and fifteen subjects (29.4%) were nulliparous . A breakdown of grade of CIGN, age and parity is given in table 2-1. The 51 patients were recruited from 24 centres across the country .

TABLE 3-1 ; CHARACTERISTICS OF 51 PATIENTS WITH CIGN

a) In relation to degree of CIGN

Degree of CIGN	Number	Mean age	Mean Parity
mild / moderate	6	34.3	1.7
severe / AIS	45	36.4+	1.7

b) In relation to presence or absence of CIN

	Number	Age	Parity
Pure CIGN	25	37.7**	1.7^
CIGN + CIN	26	32.9**	1.2^

** Significant difference in age distribution , p=0.029 , unpaired t-test,

^ Non-significant difference in parity

+ Non-significant difference in age

Method of diagnosis

In 27 cases (52.9%) , the cervical smear taken prior to diagnosis was reported by the local laboratory as showing atypical glandular cells . Of these , 13 (25.5%) were also in association with squamous cell dyskaryosis. Four patients had evidence of a glandular abnormality on a colposcopically directed biopsy , at the same time as the cervical smear showed atypical glandular changes. Ten patients (19.6%) had evidence of glandular abnormality on a colposcopically directed biopsy , in the absence of suggestive cytological changes. The remaining 14 patients (27.4%) had the diagnosis of CIGN made on cone biopsy , in the absence of any forewarning of glandular abnormality .

Subjects where there was suspicion of invasive disease

Five patients registered in the study were excluded for these reasons. In 2 instances , all three pathologists diagnosed the presence of invasive disease. In one other instance , two of the three pathologists reported the presence of a fragment of possible invasive disease , which was separate from the remainder of the diagnostic material. It was suggested that this fragment represented 'carry over' from a different case. In the 2 remaining subjects, 2 pathologists reported a 'strong suspicion' of invasive disease , however there were no unequivocal features of invasive adenocarcinoma and in these cases the 3rd pathologist reported the presence of adenocarcinoma - in - situ only.

Two of the above patients have had a hysterectomy and both are well at intervals of 6 and 10 months post hysterectomy (Subjects 1,3 Table 3-2). One patient has repeatedly defaulted from follow up (Subject 4) and her current status is unknown . (Invasive adenocarcinoma diagnosed by all 3 pathologists) Two patients (Subjects 2 and 5) have had no other surgical procedure except for cone biopsy, and they are both well with negative cytological follow-up at 22 and 24 months following cone biopsy.

Table 3-2- Subjects excluded after pathology review had indicated the likelihood of invasion

Subject	Original diagnosis	Reviewer 1	Reviewer 2	Reviewer 3
1	AIS	AIS Invasion in fragment ? carry over	AIS + Invasion Invasion in fragment ? carry over	AIS only
2	AIS (+ CIN3)	AIS + ?Invasion	AIS + ?Invasion	AIS only
3	AIS	AIS + Invasion	AIS + Invasion	AIS + Invasion
4	AIS	Invasive adenoca	Invasive adenoca	Invasive adenoca
5	AIS	AIS,?invasion	AIS,?invasion	AIS only

Follow up and further management

Cytological follow-up

There have been 10 instances of abnormal cytology at follow-up (4 squamous dyskaryosis , 1 glandular atypia and 5 Borderline abnormalities) leading to 8 further surgical diagnostic procedures (vide infra) . The median period of cytological follow up was 14 months (range 4 - 160 months) .The median numbers of follow up smears was 2 (Range 1 - 10)

Management

Subgroup 1

Diagnosis on Cone ---> CIGN confirmed ---> Disease free margins

There are 43 subjects in this category. Of these , 7 have manifested cytological abnormality following cone biopsy. Thirty six subjects have had no further treatment since the diagnostic cone biopsy.

Further surgery

Additional surgical procedures have been carried out in 5 cases. (Table 3-3) Of these 5 , 1 had a hysterectomy at 33 months post cone biopsy because of persisting borderline cytological abnormality. No residual CIGN or CIN was found. The remaining 4 patients had a second cone biopsy . Two of the 4 had cone margins which were positive for CIN only . Of these 4 , there was one case of CIN , and 1 case of low grade CIGN with disease free margins. In this latter case , the initial cone biopsy (which revealed Adenocarcinoma in situ and CIN111 with disease free margins) was performed some twelve years beforehand ,

and follow-up cytology demonstrated persistent borderline abnormalities.

Cytological abnormality managed conservatively

Two patients with cytological abnormality have had no further surgery. The first , whose original cone specimen showed AIS/high grade CIGN only , has had a smear showing mildly dyskaryotic squamous cells, at 33 months following the original cone biopsy. Colposcopy at this time showed a small wart like area on the posterior lip of the cervix. This area was biopsied , and shows CIN 111. The patient was offered hysterectomy but declined any treatment. One subject has a smear reported as showing borderline changes at follow-up 18 months post-cone, colposcopic examination at this time was normal. It is felt that unless this minor degree of cytological abnormality persists, cytologic and colposcopic follow up will be continued.

Patients who have had a hysterctomy for incidental reasons

Two subjects have had a hysterectomy because of benign gynaecological disorders unrelated to CIGN (1 vaginal prolapse , 1 menorrhagia/dysmenorrhoea) . Neither of these subjects had abnormal cytology post conisation and no residual disease was found in the cervix post hysterectomy.

Patients managed by cone biopsy with no evidence of cytological abnormality

There are currently 34 subjects in this category.

Subgroup 2

Diagnosis on Cone —> CIGN confirmed —> Margins involved with CIGN

Subjects managed by Hysterectomy

This category comprises 8 subjects . Only 1 case was found to have residual CIGN. This subject had a smear prior to hysterectomy which suggested the presence of glandular cell abnormality.

A summary table of case histories of subjects requiring further procedures in accordance with the study protocol is presented. (Table2-3)

Relationship between cytological follow-up and status of excision margins

In 1 subject it was not possible to assess the status of the cone excision margins due to heat artefact following a laser cone . Three subjects who had involved margins on cone biopsy did not have a further cytological assessment prior to hysterectomy. This leaves 47 subjects on whom the above relationship is analysed.

a) Margins involved with either CIN or CIGN

The results are presented in Figure 3-2 and Table 3 - 4. Ten subjects had involved margins on cone biopsy (5 positive for CIGN, 5 for CIN), 5(50%) were subsequently shown to have abnormal cytology , and one of these subjects had residual CIGN discovered at hysterectomy. None of the 5 subjects with involved margins who had a negative smear post conisation (2 involved with CIGN ,3 with CIN) , were shown to have any residual disease at hysterectomy.

Amongst those with disease free margins on cone , 32/37 (86.5%) had

negative cytology after a median follow-up period of 14 months. (X^2 with Yate's correction= 4.04 , $p < 0.05$)

b) Margins involved with CIGN only

Of these 5 subjects , 3 (60%) developed abnormal cytology , and of those who had negative margins for CIGN (42subjects) , 35 (83.3%) have negative cytology after median follow-up of 14 months.(X^2 with Yate's correction= 2.76 , $p>0.05$) (Figure 3-5)

Relationship between cytological follow-up and length of diagnostic cone biopsy

In 4 subjects ,the length of the cone biopsy was not recorded. In 3 subjects a follow up smear was not taken after conisation and prior to hysterectomy. This leaves 44 subjects upon whom the above analysis is based. Figure 3-3 and Table 3-5 illustrate the findings in these patients where a cone length of 25mm has been chosen as the dividing line between cones designated as long (>= 25mm) and short (< 25mm) . Of 16 subjects who had a cone biopsy measuring 25mm or more, there was only 1 instance of further abnormal cytology.

Relationship between cone biopsy length and status of excision margins

Figure 3-4 illustrates that incomplete excision of both CIGN and CIN occurs less frequently with increasing cone length. Using our optimum defined length of cone specimen (Bertrand et al,1987) , there was only 1 instance where a cone >= 25mm was associated with margins positive for CIGN.

DISCUSSION

This study aims to examine the efficacy and safety of cone biopsy in the management of cervical intraepithelial glandular neoplasia and it is the first study which attempts to do this in a prospective manner. The data on cone length , status of excision margins and cytologic follow-up provide some support for the view that conisation may be sufficient *primary* management. However, the outcome does appear to be related to the status of the excision margins on the original cone specimen.

Status of excision margins

In the present study, 83.3% of those subjects with margins free of CIGN on the initial cone biopsy have negative cytological follow-up after a median follow up period of 14 months. Of the five subjects with disease free margins on the initial cone who were subjected to a further surgical procedure , only one out of the 5 had low grade CIGN on the second specimen. However this procedure was carried out some 12 years after the original cone biopsy after 11 years of negative cytological examinations , and may be considered as a recurrent rather than a residual lesion.

By considering the effect of marginal involvement with either CIGN or CIN , those patients with negative cone margins were significantly less likely to develop abnormal cytology following conisation (Table 3-4)

Length of cone biopsy specimen

With respect to this variable , there was only one instance where a cone biopsy of >=25mm length was associated with involved excision margins . Although the relationship between the length of the cone and subsequent cytological follow-up did not reach statistical significance , these data concur with the views of Bertrand et al ,1987 and Colgan and Lickrish,1990 that a cone biopsy of length 25mm should be successful

from the point of view of clearing the lesion. With respect to cytological follow up , of those 16 subjects who had a cone biopsy measuring 25mm or more , there has only been one instance of cytological abnormality at follow up. Interestingly , the mean cone length in those who developed abnormal cytology was 18.5mm compared to a mean length of 22mm in those with persisting negative cytology , although this difference was not statistically significant .(p = 0.16 Student's t test)

Abnormal cytology following conisation

Given that 10/51 (19.6%) of subjects have manifested abnormal cytology following initial treatment, this suggests that these subjects are at high risk of either residual CIN or CIGN and hence subjects managed by conisation as primary therapy require continuing cytological and , I believe , colposcopic follow-up. (see 'follow-up')

Experience so far suggests that the presence of abnormal cytology post conisation is a more sensitive indicator of the presence of residual disease (4/10 abnormal smears had some residual pathology) than is the presence of positive cone margins (1 out of 10 had pathology) . However,the numbers are too small for firm conclusions to be drawn.

Histological subtypes of CIGN

A significant difference in age distribution was noted between those subjects with 'pure' and 'mixed' lesions, with the latter being on average, 4.8 years younger . The explanation for this finding may lie in the fact that the presence of CIN , which is efficiently detected by exfoliative cytology, accelerates the diagnosis of CIGN which is less well recognised. This study gives support to this latter hypothesis , as CIGN was a chance finding on histological examination of a cone specimen in 27.4% of our study population where there was no forewarning of glandular abnormality on either cytology , colposcopy or directed biopsy of the cervix.

Previous studies have indicated that the frequency of glandular epithelial atypias of lesser degree than adenocarcinoma in situ , occur in up to 15% of subjects who have CIN111.(Brown and Wells , 1986) , and of these the majority (68%) were believed to be 'low grade'. There have been no other reports of such a high prevalence of low grade glandular atypias, and our findings of only 6/51 (11.8%) subjects with low grade atypia ,(as defined by Brown and Wells,1986) , suggests either that such atypias are indeed infrequent or conversely that there is considerable subjectivity in grading between pathologists. This question is being addressed by the pathology review panel who aim to assess the degree of agreement between observers in the grading of this lesion.

Problems with diagnosis of early invasive adenocarcinoma

While there appears to be a high level of agreement concerning the major diagnostic features of this lesion, (Rollason et al, 1988b) it can be difficult to confidently exclude early invasive disease in a small proportion of these lesions ,where diagnosis may be difficult and highly subjective. A minority (6%) of patients registered in the study had some evidence of early invasive disease, and were therefore excluded. Of the 5 excluded , in 3 cases the diagnosis of invasion was *not* certain and by no means unanimous. Other workers have recognised that the distinction between early invasive adenocarcinoma and AIS can be difficult (Brand et al , 1988) . Subjects where at least one pathologist felt that there was invasion present have been excluded , no matter how 'early' this appeared to be, although it is reassuring that in these cases the disease appears to follow a benign course and this agrees with the findings of Teshima et al , 1985. This study was established to assess the management of pre-invasive disease , and a separate study is required to establish the optimum management for these difficult cases with early invasion.

Follow-up

Although the early results of this management protocol are encouraging , the results imply that cytological detection of CIGN is less efficient than CIN . Approximately half of those with CIGN had some forewarning of glandular abnormality compared to 88.5% of CIN which was accurately predicted by cytology .(X^2=7.9 , p <0.01). How can this apparent lack of sensitivity for detection of glandular abnormalities in smears be reconciled with our objective to carry out cytologic examination as the mainstay of follow up? ; Firstly , this represents the situation prior to diagnosis , where routine cytologic sampling of the endocervical canal was *not* employed. Secondly , follow - up cytological assessment of the patients in this study consisted of the routine Ayre spatula combined with a brush smear from the endocervical canal . Thirdly , given that a) the cytological identification of specific glandular lesions is less generally accepted than CIN , and b) these subjects are at high risk of residual CIN , then cytological follow-up should be supplemented with colposcopic and clinical pelvic examination of the patient.

Conclusions

Where the diagnosis of CIGN has been established and where cone margins appear to be free of disease , no instances of residual CIGN or invasive disease have so far been encountered after using conisation as definitive management in 43 cases studied prospectively, although there has been 1 arguably 'recurrent' case. Where conisation has failed to yield a specimen with disease free margins , further surgery has been carried out . Although the conclusions from these data are limited by small numbers , the majority of those with positive margins for CIGN had no residual disease at hysterectomy , and more reassuringly ,there was no instance of unsuspected invasive disease in the remaining cervix; indeed there has been only one case of residual moderate grade

CIGN in a subject where the cone margins were involved with disease, and cytology prior to hysterectomy showed atypical glandular cells. It can therefore be concluded that the application of this management protocol is *to date* , safe and effective.

The findings clearly require confirmation by long term follow up .

Figure 3-2-Relationship between cytological follow-up and status of excision margins

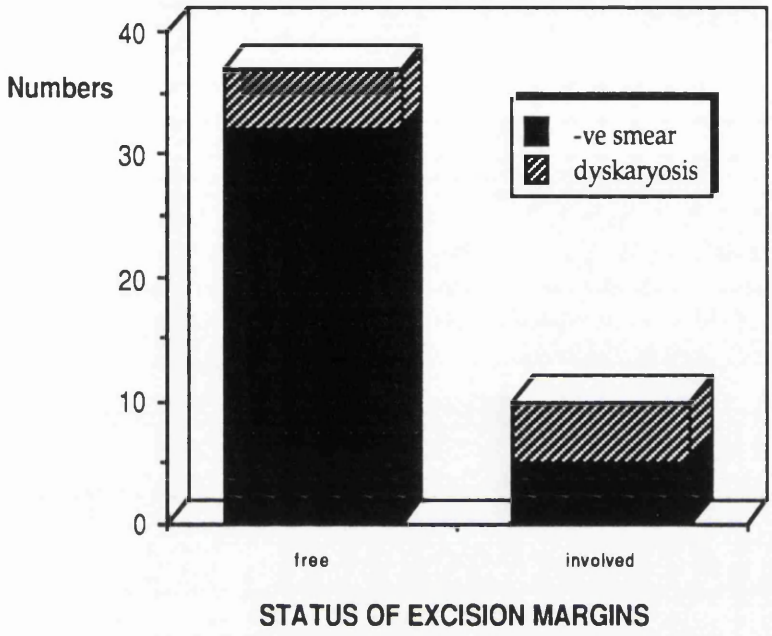


Table 3 -4 - Status of excision margins on cone biopsy and results of subsequent follow up cytological assessments, n=47

	Abnormal cytology	Negative cytology
Involved margins (n=10)	5* (50%)	5 (50%)
Free Margins (n=37)	5^ (13.5%)	32(86.5%)

X^2 with Yate's correction= 4.04 $p<0.05$

Figure 3-3 - Relationship between cone length and cytological follow - up

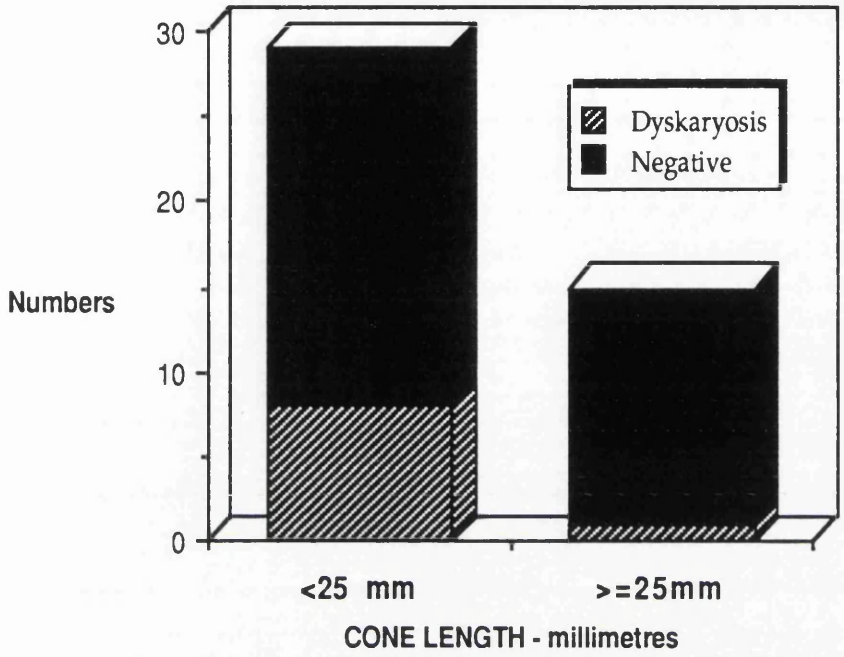
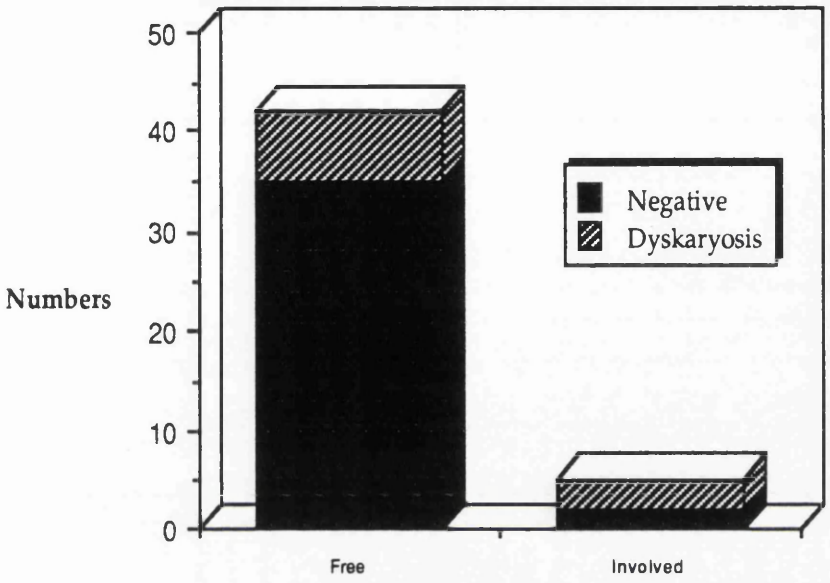


Table 3- 5 - Relationship between length of cone specimen and cytological follow up (n=44)

	Abnormal cytology	Negative cytology
Cone length < 25 millimetres (n=28)	8(28.6)	20(71.4%)
Cone length >= 25 millimetres (n=16)	1(6.3%)	15(93.7%)

X^2 with Yate's correction = 1.90 p >0.05

Figure 3-5: Status of excision margins for CIGN and subsequent cytological follow up



STATUS OF EXCISION MARGINS FOR CIGN

Cone length versus excision margins

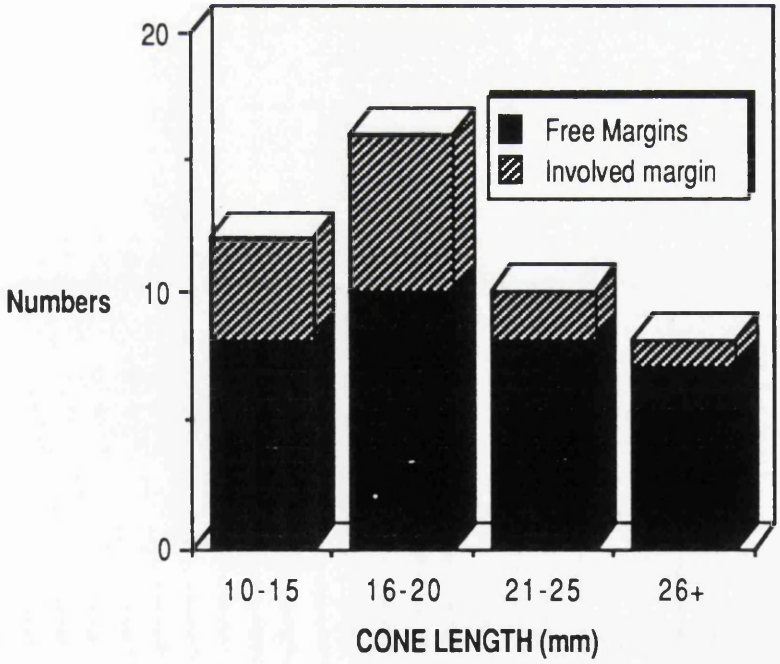


FIGURE 3-4

Table 3 - 3 - SUMMARY OF CASE HISTORIES OF PATIENTS REQUIRING FURTHER PROCEDURES (in accordance with study protocol)

Subject	Age	Review diagnosis (Cone biopsy)	Margins	Post cone smear	Histology (hysterectomy or 2nd cone)
1	43	Severe atypia, No CIN	Involved	Negative	No residual disease
2	39	AIS + CIN3	Involved	-	No residual disease
3	35	AIS , No CIN	Involved	-	No residual disease
4	37	AIS + CIN3	Free	Borderline changes	No residual disease
5	30	AIS	Involved	-	No residual disease
6	31	AIS , CIN3 with ESI	Free AIS Inv.CIN3	Borderline changes ?Gland atypia	No residual disease (Second cone)
7	24	AIS + CIN3	Free	mild squamous dyskaryosis, ?glandular atypia	CIN1 , No Gl. atypia (Second cone)
8	25	AIS only	Involved	?glandular atypia Severe squamous dyskaryosis	No residual disease
9	47	AIS only	Involved	Borderline changes	No residual disease
10	46	Moderate atypia only	Involved	Glandular atypia	Mild Glandular atypia
11	56	AIS only	Involved	Negative	No residual disease
12	26	AIS + CIN3	Free	Borderline	Mild/Moderate Glandular atypia (Second cone)
13	27	AIS +CIN3	Free AIS Involved CIN	Mild dyskaryosis	No residual disease (Second cone)

CHAPTER IV

A study of Nucleolar Organiser Regions in adenocarcinoma-in-situ of the endocervix

Introduction

Cervical Intraepithelial Glandular Neoplasia (CIGN) has been found by a variety of authors to be strongly associated with cervical squamous intraepithelial neoplasia (CIN) , (Ostor et al,1984 Van Roon et al , 1983) and one group has observed CIGN lesions of lesser grade than adenocarcinoma in situ (AIS) in crypts adjacent to areas of CIN111 in 15% of cases . (Brown and Wells,1986) These lesions were called 'cervical glandular atypia' by these authors . Whilst such a high incidence of glandular atypia in association with CIN111 has not been observed in our laboratory , it is possible that we might be overlooking most low grade CIGN . Given the observation by the same workers that AIS has been found to be separated from normal crypts by intervening areas of lesser grades of atypia, or 'transition zones' , an investigation was undertaken to determine whether there are areas adjacent to AIS which demonstrate differences in 'nuclear activity' .The method used was an assessment of nucleoloar organiser regions (NORs) using the silver impregnation or AgNOR technique. For , if it could be shown that there was a measurable alteration in nuclear 'activity ' , this might support the concept of the existence of a transitional zone of pre-neoplastic mucosa which is not easily recognisable on routine histological staining . If such a finding was confirmed , then this might have implications for the treatment of the lesion.

Review of relevant literature

Nucleolar organiser regions (NORs) are loops of ribosomal DNA which transcribe for ribosomal nucleic acid , rRNA (Gall and Pardue, 1969) and determine the production of 18S and 28S ribosomes . NORs are therefore of crucial importance in the regulation of cell protein synthesis. In humans , NORs are located on the 5 acrocentric chromosomes (13/14/15/21/22), appearing as achromatic gaps on the short arms in metaphase spread

preparations. Since 1975 , cytogeneticists have investigated these regions using a silver staining technique, the AgNOR method, which has proven useful in the investigation of chromosomal disorders , notably trisomy 21 , where there are additional AgNORs on inappropriate chromosomes.

The silver stain identifies acidic non - histone proteins associated with the NOR. The silver staining reaction gives rise to "black dots" which are primarily within nucleoli , but also scattered throughout the nucleus. Experiments using gel electrophoresis have shown that the proteins which bind silver do so by virtue of the possession of sulphydryl groups . RNA polymerase 1 (which catalyses the transcription of rDNA to rRNA), and C23 and B23 proteins have been positively identified as AgNOR proteins.(Ochs and Busch,1984) Ultrastructural studies have shown that AgNORs cluster in compact regions within nucleoli and these correspond to the fibrillar centres . The most intensely staining areas are directly related to the dense fibrillar component , the place where rDNA transcription occurs (Ruschkoff et al , 1989) . Recent developments of the technique of detection of AgNORs enable it to be applied to paraffin embedded sections, (Ploton et al , 1986) and therefore it is possible to assess the role of AgNORs in a variety of conditions . Study of the relationship between AgNORs and malignancy was stimulated by observations from cytogenetic studies which showed unusual AgNOR patterns in certain malignancies (Lancet editorial ,1987) . Using the one step silver colloid method (Ploton et al , 1986) , the number of AgNOR dots are an indication of both the number and dispersion of NOR's, and any apparent increase may reflect either active cellular proliferation , cell transformation , or even overt malignancy. (Ruschkoff et al , 1989)

AgNOR counting has recently been shown to be of use in the distinction between high and low grade lymphomas (Crocker and Nar, 1987) , benign melanocytic lesions and malignant melanoma (Crocker and Skilbeck , 1987) , and small cell malignancies of childhood (Egan et al , 1987) . I decided to apply this technique to cases of adenocarcinoma in situ (AIS) of the cervix

in order to assess whether its use could give more information on the existence of possible morphologically inapparent areas of epithelial atypia associated with AIS.

Aim of study

To investigate cases of adenocarcinoma-in-situ for the existence of an adjacent zone of atypical epithelium , which may not be easily recognisable on routine histological staining.

Objectives of study

a) To assess whether there are zones of increased nuclear activity adjacent to areas of adenocarcinoma in situ of the cervix, using a histochemical marker of nuclear proliferation, the AgNOR method.
b) To determine whether the sites of predilection for neoplastic transformation, i.e. the crypts adjacent to the squamocolumnar junction (SCJ) , differed from the rest of the endocervix using the same technique in healthy cervices.

Materials and Methods

i) Staining Procedure and AgNOR counting

Cases of classical AIS were identified from the pathology files of the Birmingham and Midland Hospital for Women. 3um sections were cut from routinely processed paraffin wax blocks.These were dewaxed in xylene and hydrated through ethanols to double distilled deionised water. A staining solution was prepared consisting of gelatin dissolved in 1 g/dl aqueous formic acid at a concentration of 2 g/dl. , mixed with 50 g/dl aqueous silver nitrate (1 volume gelatin/formic acid to 2 volumes silver nitrate). This mixture was poured over tissue sections ,which were left in a

humidity chamber in the dark for 1 hour at room temperature.The silver colloid was then washed off with deionised water , and sections were counterstained with haematoxylin. Sections were dehydrated to xylene and mounted in synthetic medium. The sections were viewed under a x100 oil immersion lens using a green filter , and counts were made of discrete AgNOR dots within nuclei. In counting each cell nucleus, the focus control was carefully adjusted to enable dots to be enumerated. One hundred cells were counted in each area chosen. Large mulberry shaped aggregates were always counted as one dot.

ii) Areas counted (See Figure 4 - 3)

a) Normal tissue

Sections of histologically normal cervices (obtained from subjects who had undergone hysterectomy for dysfuctional uterine bleeding) were stained and AgNOR counts were made in endocervical epithelium :
a) immediately adjacent to and beneath the Squamocolumnar junction (SCJ) and : b) At least 5mm distant from the SCJ , along the axis of the endocervical canal. Cells were chosen 'randomly' within the defined areas but the selection method excluded a standard random sampling technique.

Adenocarcinoma - in situ

Counts were made in the following areas :
1) In endocervical epithelium showing obvious AIS. In every case these areas were closely related to the endocervical border of the SCJ.
2) In endocervical epithelium not more than 1mm away from the neoplastic cells, subsequently referred to as 'transitional' areas .
3) in histologically normal endocervical crypts at a distance of at least 5mm further along the axis of the endocervical canal, subsequently referred to as

'distant' areas.

4) In all cases where crypts were partially involved by AIS ,these were included in the counting to include transitional areas as close as possible to the neoplastic cells.

iii) Statistical analysis

The differences between AgNOR counts in different areas were assessed by analysis of variance, using a square root transformation of the counts. (Armitage,1971)

iv) Check for intraobserver variation

In order to check for the reproducibility of the counting procedure , it was decided that in the case of the third example of AIS which was counted , and the third specimen of healthy cervix , that repeat counts be made of all the areas examined . and that these counts be compared to see if there were any significant differences (Table 4-3).

Results

Eleven cases of cervical AIS and five examples of normal cervices from hysterectomy specimens removed for non cervical pathology were assessed in the way described above. After counting 100 cells in each category the mean number of AgNOR dots per cell nucleus was calculated .The results are illustrated in Tables 4-1 and 4-2 and summarised in Figure 4-3. In every case of AIS except one, the numbers of AgNOR dots within cell nuclei of the neoplastic epithelium were found to be increased compared to normal. (See Table 4-2) The differences in AgNOR counts between AIS and all other areas were significant ($p<0.01$) . In addition, the AgNOR

staining dots in the histologically normal areas usually appeared as well defined rounded areas, whereas in the areas demonstrating AIS the AgNORs were smaller, sometimes irregular, and dispersed throughout the whole nucleus.

There were no significant differences between transitional and distant areas, including those transitional areas immediately adjacent to AIS tissue which occupied only part of a crypt. There were no significant differences between distant areas in diseased cervices and in normal cervices. There were no significant differences in AgNOR counts at different sites in the group of normal cervices examined (Table 4-1).
There were no significant differences between AgNOR counts in those cases where the counts were repeated as a check on observer variation in counting.

DISCUSSION

AgNOR counting has recently been extensively employed in the assessment of a variety of tumours . In some malignancies AgNOR counts are significantly greater than in normal or benign conditions at the same site (Crocker and Skilbeck,1987) , and this may be in part because in malignancy , NORs become dispersed throughout the nucleus. The AgNOR count is therefore more likely to be a measure of nucleolar dispersal than of an absolute increase in nucleolar material. In support of this hypothesis, some authors have observed an inverse relationship between increasing AgNOR counts and the size of individual AgNORs . (Egan et al , 1990) . Similar observations were made in this study (See figures 4-1 and 4-2) although no formal measurements of AgNOR size were made. AgNOR counting has been suggested to be of use in the distinction between high and low grade lymphomas (Crocker and Nar,1987) and benign melanocytic lesions and malignant melanoma

(Crocker and Skilbeck,1987) amongst other conditions. (for summary see Walker,1988 Underwood and Giri,1988) All these studies have concentrated on conditions distinguished for the purpose of investigation by standard histological techniques . It might therefore be argued that the AgNOR technique has simply been used to confirm an already established diagnostic method. In the present study I have utilized the technique simply to attempt to detect epithelial alteration adjacent to AIS with no pre-determined histological differences evident. To my knowledge, this is the first time the technique has been used in this manner.

It has been postulated that in areas adjacent to adenocarcinoma in situ of the cervix, there is a transitional zone of epithelial atypia of lesser grade than AIS , i.e. low grade CIGN , which separates AIS from histologically normal epithelium (Wells and Brown,1986). The extent of these areas was not stipulated by these authors . Whilst not denying the existence of glandular atypias of lesser grade than AIS, it has not been possible to appreciate such atypia commonly occurring adjacent to our cases of AIS, which usually distinctly abut upon adjacent histologically normal glands. (T.P. Rollason , personal communication). However, the presence of a broad zone of neoplastic potential might have implications for the treatment of these lesions, especially if such a zone extends more than a few millimetres from the microscopically diseased areas. Such an observation might prejudice the clinician in favour of more radical treatments than cone biopsy for cervical AIS/ high grade CIGN . A cone specimen ,which on conventional histopathological assessment contains AIS/high grade CIGN with apparently disease free margins , might still lead to recurrence of glandular atypia if there is histologically normal but nevertheless potentially pre-neoplastic epithelium in the putatively AgNOR rich areas. However , the negative findings obtained in this study provide no evidence to contradict the opinion which favours conisation as rational management for the condition.

In the experience of this institution, glandular epithelial atypia which is clearly identifiable as of lesser severity or grade than AIS is no more common than AIS itself, and the differentiation of the conditions is difficult. As the results of AgNOR counting show no appreciable difference in counts between areas immediately adjacent to AIS, and distant from it, this offers no support for the possibility that we may be failing to appreciate subtle histological changes in glands adjacent to AIS. This series examined classical cases of AIS, unlike Brown and Wells, who looked for glandular epithelial atypias in subjects with CIN111. To date,we have not applied the AgNOR technique to examine the cervical crypts in cases of CIN111 , but if there was a broad glandular change indicative of pre-malignancy we might expect to see lesser degrees of glandular atypia in AIS more readily than in cases of CIN111.

I failed to appreciate any difference in AgNOR staining at differing sites within the cervices of normal subjects, and therefore, there appears to be no intrinsic difference in the degree of nuclear activity as measured by the AgNOR method at different sites in the endocervix in the normal subject.

I would not extend the findings on this small group of cases using this additional technique to deny the presence of a transitional zone adjacent to AIS or CIN, but the present study adds no weight to the debate in favour of its existence.

Table 4-1

Mean numbers of AgNOR dots in normal cervices.Median numbers in parentheses

Subject	at SCJ	>5mm from SCJ
1	1.3(1)	1.2(1)
2	0.9(1)	1.0(1)
3	1.4(1)	1.6(1)
4	0.9(1)	0.7(1)
5	1.0(1)	0.9(1)

Table 4-2

AIS CASES

Mean numbers of AgNOR dots within nuclei(Bold type) with median numbers (in parentheses) in different areas of diseased cervices.

				Partially involved glands	
Subject	AIS tissue	Transitional	Distant	AIS	normal
1	4.9(5)	1.4(1)	1.5(1)	3.9 (3)	1.0(1)
2	3.6(4)	1.9(2)	1.0(1)	2.7(3)	1.2(1)
3	4.0(4)	1.3(1)	1.3(1)	3.5(3)	1.5(1)
4	7.5(8)	1.2(1)	1.1(1)	9.0(8)	1.3(1)
5	1.3(1)	1.3(1)	1.2(1)	-	-
6	4.9(5)	1.1(1)	1.1(1)	-	-
7	5.3(5)	1.4(1)	1.2(1)	-	-
8	4.5(4)	0.6(1)	0.9(1)	-	-
9	6.1(6)	1.1(1)	1.2(1)	5.4(5)	1.0(1)
10	5.2(5)	0.9(1)	1.1(1)	4.9(5)	1.1(1)
11	3.9(4)	1.2(1)	1.3(1)	-	-

Table 4-3 - Check for Intraobserver variation in counting

Example	Mean count	SD	SE	MW*(z)	p
Healthy cervix *1					
a) SCJ					
First count	1.28	.766	.077	-0.878	.15<p<.20
Second count	1.19	.748	.075		
b) 5mm distant					
First count	1.21	.701	.070	-0.736	.20<p<.25
Second count	1.13	.661	.066		
AIS * 11					
a) AIS tissue					
First count	3.9	1.367	.137	-0.771	.20<p<.25
Second count	3.7	1.33	.133		
b) Transitional area					
First count	1.22	0.524	0.052	-0.698	.20<p<.25
Second count	1.17	0.637	0.064		
c) Distant area					
First count	1.28	0.514	0.051	-1.084	.10<p<.15
Second count	1.38	0.648	0.065		

* Mann Whitney test

FIGURE 4-1

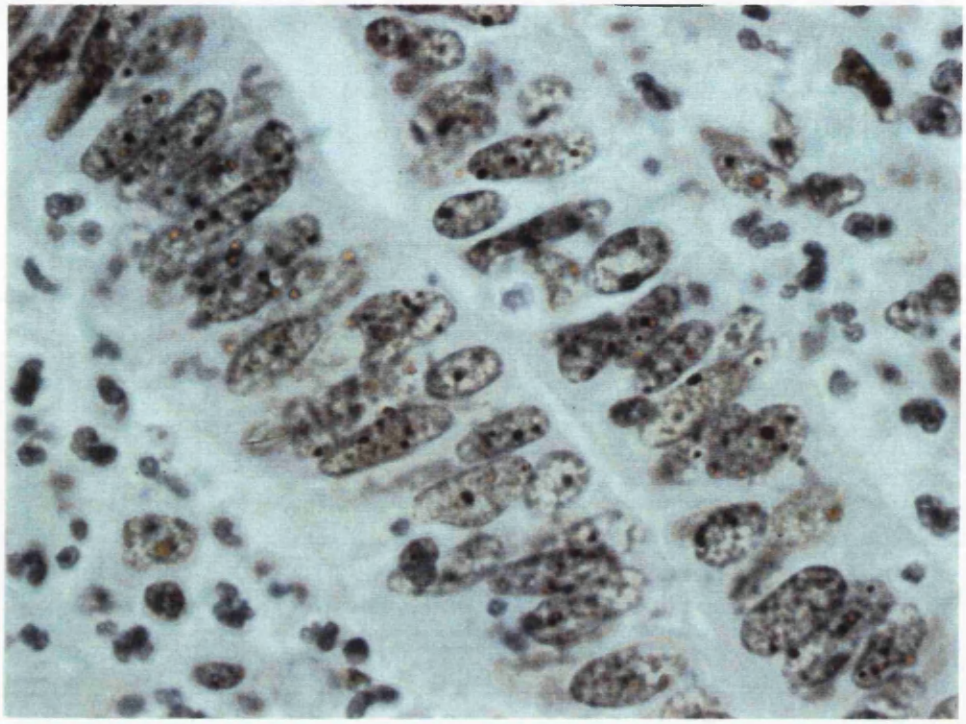
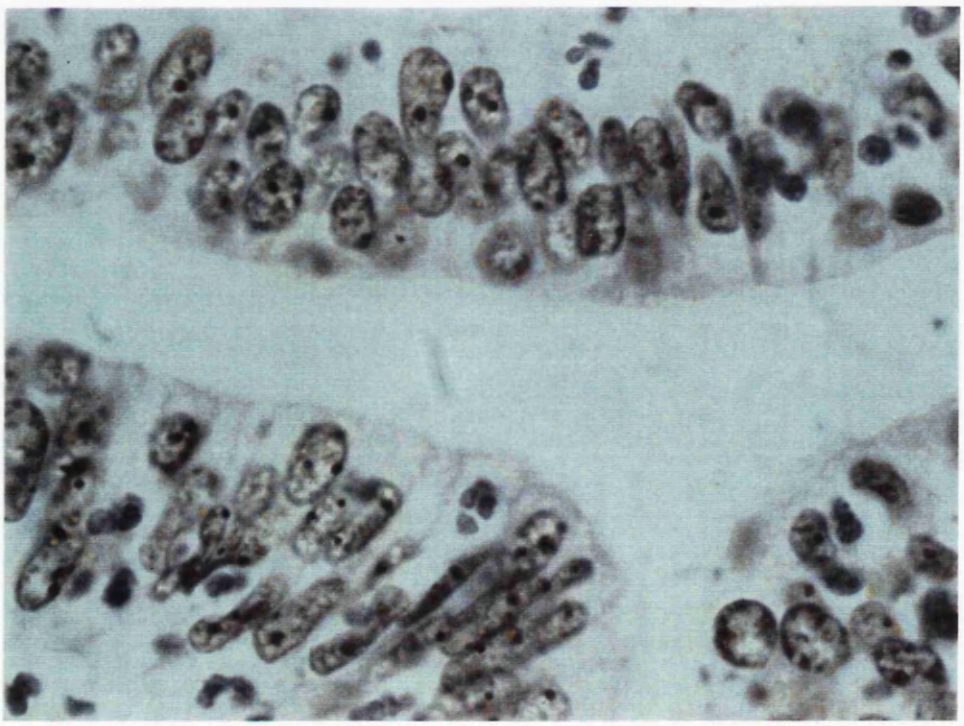


FIGURE 4-2

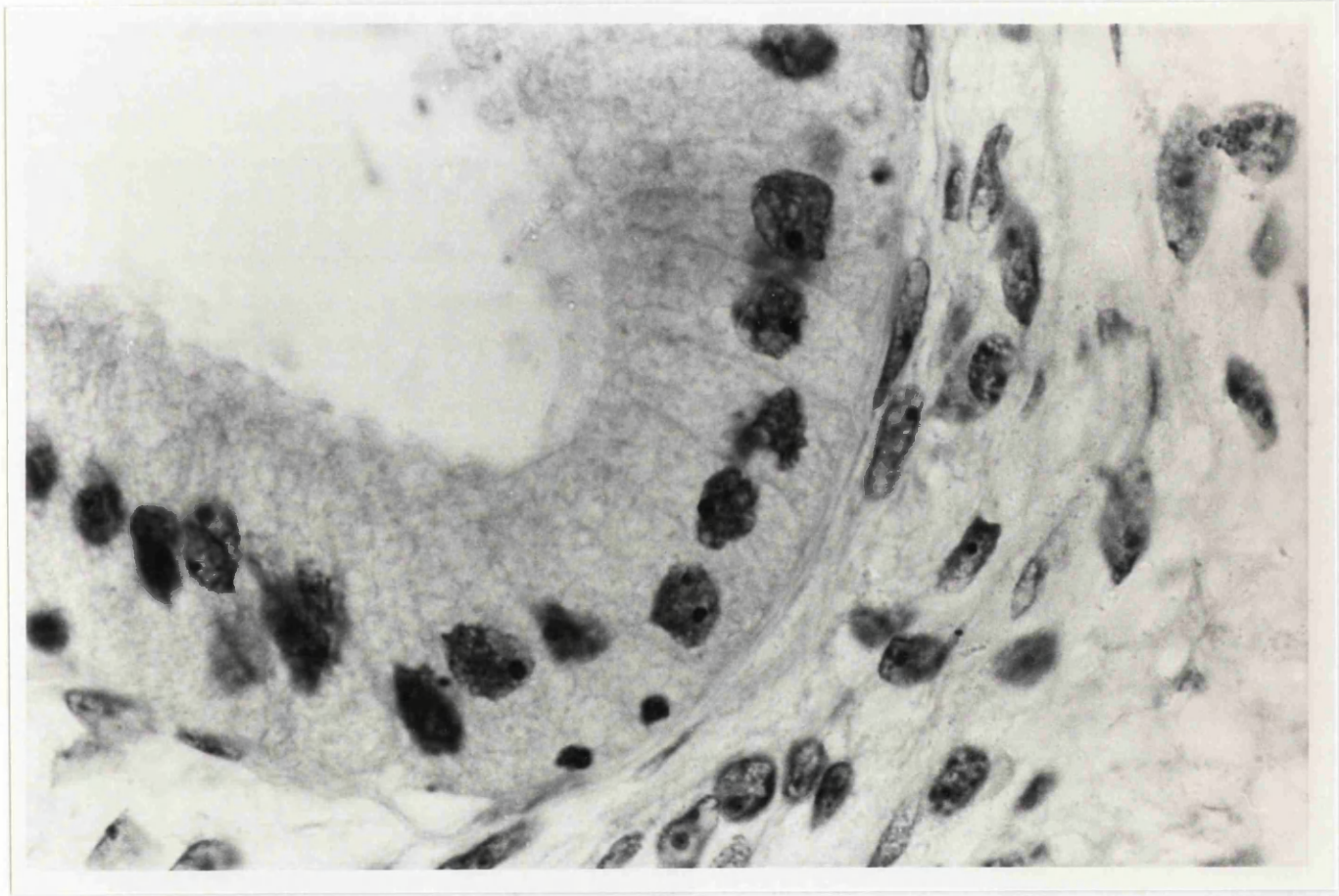


FIGURES 4- 1 , 4-2

Adenocarcinoma in situ of cervical gland crypts,stained by AgNOR technique. Multiple small NORs are seen in nucleii .

FIGURE 4-3

Histologically normal gland crypt,stained by AgNOR technique . A solitary large NOR is seen in nucleii .



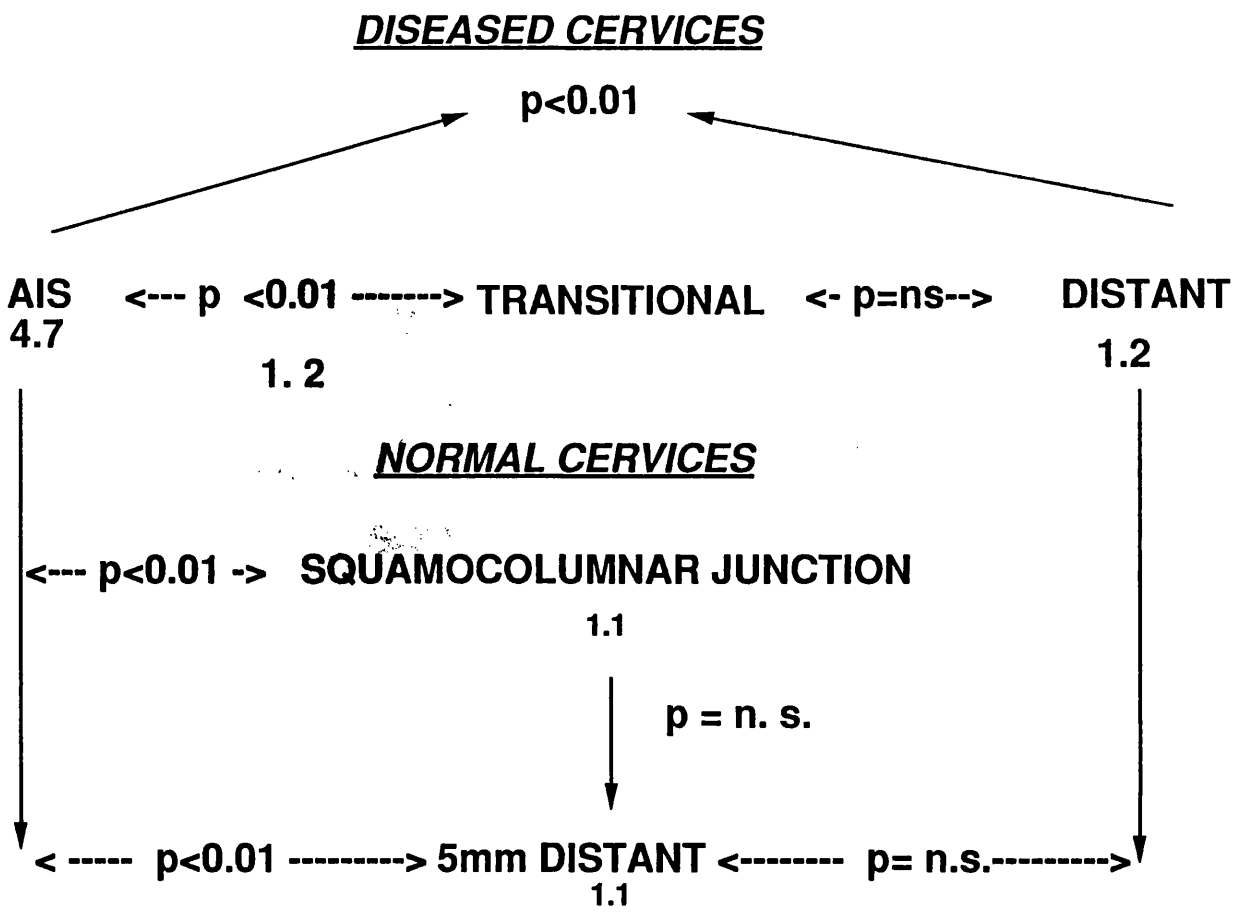


FIGURE 4-4 Mean nubers of AgNOR dots in each cell in different areas , and significance of differences

OVERALL CONCLUSIONS

Primary prevention of cervical adenocarcinoma

Prevention of this disease depends on the ability to identify those at risk of disease , to identify factors which increase disease susceptibility , and finally to modify exposures which confer risk so that the likelihood of disease is reduced. Therefore , an age matched case-control study has been carried out to elucidate risk factors for the development of Cervical Intraepithelial Glandular Neoplasia. (CIGN) . Cases of CIGN were compared with controls consisting of : a) subjects with Cervical (squamous) intraepithelial neoplasia (CIN) , recruited from hospitals and , b) 'normal' controls recruited from general practice registers. A standard questionnaire concerning lifestyle , medical, reproductive, sexual and contraceptive factors was administered and serum was assayed for the presence of neutralising antibodies to HSV-1 and HSV-2 .

Forty two cases of CIGN , 72 cases of CIN and 133 general population controls have been recruited. The diagnosis of CIGN was confirmed by independent pathology review. Analysis of data by conditional logisitic regression revealed that;

1) CIGN showed some of the characteristics of a sexually transmitted disesase , in that these subjects were more likely to have had multiple sexual partners , and less likely to have used barrier contraception than normal controls,

2) Subjects with CIGN had features indicating that their hormonal/reproductive milieu differed from normals in that they experienced a later menarche and fewer pregnancies than normal controls,

3) CIGN had a risk factor profile that displayed both similarities and differences with CIN. Notably , Both disorders were associated with multiple sexual partners and both were less likely to occur when barrier contraception was used. However, when compared with CIN , cases of CIGN had fewer sexual partners , were less likely to smoke

and were more likely to have neutralising antibodies with HSV-1 specificity than CIN controls.
It is recommended that further case control studies be carried out in order to attempt to validate the findings in this study, and that the relationship between disease and other sexually transmitted agents , notably Epstein Barr virus, be explored.

Secondary prevention of adenocarcinoma

i) Prospective study of conisation in the management of CIGN.

A multicentre prospective study of conisation in the management of cervical intraepithelial glandular neoplasia has been carried out . The preliminary results of the first 51 patients with confirmed CIGN managed by conisation are presented.
Fourteen patients (27.5%) were aged 30 or less and 15 (29.4%) were nulliparous. Thirty six patients who had a cone biopsy showing margins free of CIGN have been managed by conisation alone. After a median follow-up period of 14 months , there are no cases of residual CIGN or invasive disease in this group. Thirteen patients have had further surgical procedures in accordance with the study protocol, and 2 have had a hysterectomy for benign gynaecological disorders . Eight further procedures were carried out primarily because the original cone biopsy showed margins involved with CIGN. Of this subgroup , there was only 1 instance of residual CIGN. Five procedures were carried out solely because of abnormal cytology , leading to diagnosis of 1 case of CIGN and 1 of CIN 1. A total of 10 patients manifested cytological abnormality following cone biopsy. Amongst these , there were two cases of CIGN, 1 case of CIN 1 ,and 1 case of CIN111.
When a diagnosis of CIGN is made upon a cone biopsy , analysis of the data so far suggest that further surgery is unnecessary in those

subjects where the margins of the conisation specimen are free of disease. Cytological and colposcopic follow up is recommended for these patients . These results require confirmation by longer term follow-up.

ii) Study of nucleolar organiser regions in adenocarcinoma in situ of the endocervix.

It has been reported that cervical 'glandular atypia' has been observed to occur adjacent to 15% of cases of CIN3, and also in areas bordering adenocarcinoma-in-situ (AIS), forming transition zones between neoplastic and normal epithelium. The AgNOR technique was employed to analyse 11 cases of adenocarcinoma-in-situ of the endocervix and 5 examples of non diseased cervices to assess whether areas of 'increased nuclear activity' can be located adjacent to AIS.

Areas of AIS had significantly more AgNOR staining dots than apparently normal areas bordering AIS ('transitional areas'), and areas of endocervical epithelium remote from AIS. There were no significant differences between AgNOR counts in transitional areas and areas remote from AIS , and between these areas and histologically normal cervices. These observations provide no support for the hypothesis that areas of increased nuclear activity (indicative of glandular atypia /CIGN of lesser severity than AIS) exist adjacent to AIS. These findings provide further experimental support for the hypothesis that conisation with disease free margins is adequate primary treatment of CIGN.

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The treatment of cervical intraepithelial neoplasia

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Keywords: Cervical intraepithelial neoplasia (CIN), colposcopy, traditional excisional therapies, local destructive therapies, transformation zone excision, treatment failure.

Summary

Treatment of cervical intraepithelial neoplasia, both squamous and glandular, is based on the premise that this condition may ultimately progress to invasive cancer. Treatments have evolved in a conservative direction resulting in

reduced morbidity yet also achieving satisfactory control of disease. As yet there is no way in which those cases that have malignant potential can be distinguished from those that do not. Satisfactory management is dependent upon the exclusion of an invasive process. Cytologic, colposcopic and histologic confirmation of preinvasion are therefore natural precedents to management.

I Introduction

The histological identification of cervical intraepithelial neoplasia (CIN) was initially fortuitous and until this asymptomatic condition could be detected by a relatively simple screening technique there was no motivation to define appropriate therapies. It might be regarded as unfortunate that detection techniques paved the way for treatment modalities before the development of an understanding of the aetiology and natural history, for it is still the case that the major premise for treatment is the belief that invasive carcinoma of the cervix will develop unless intraepithelial disease is eradicated.

Many would now consider a conservative non-treatment or observational approach unethical, for although spontaneous regression has been frequently observed (Peterson, 1955; Stern and Neely, 1964; Brown and Phillips, 1985; Nasiell *et al*, 1986) there is also a weight of circumstantial evidence suggesting that intraepithelial disease, particularly high grade lesions, do not regress and have the potential to become invasive (McIndoe *et al*, 1984).

The lack of understanding of the natural history has prevented accurate identification of those lesions that will progress to invasive disease and, similarly, those that will not. While low grade lesions (CIN I) and high grade lesions (CIN III) may behave differently, treatment approaches encompass all grades and will continue to do so until more reliable prognostic information is available. This situation should be set against a background of public anxiety and the personal emotional trauma of the woman with an abnormal cervical smear. The pressure to offer treatment is not inconsiderable.

1 Evolution of therapies

Radical treatment (radiotherapy and radical hysterectomy), although once considered the norm, would no longer be seen as appropriate for intraepithelial disease, reflecting a trend toward more conservative management. That patients are often young and wish to preserve fertility and that disease is confined to the cervical epithelium has formed the basis for conservative therapy. Clinicians treating this disease must now bear in mind outcome not only in terms of disease eradication but also in terms of cervical structure and function. To achieve this end and at the same time to provide accurate and representative histological material, colposcopic evaluation combined with directed biopsy has become the foremost diagnostic procedure. Intraepithelial neoplasia diagnosed in this way may then be managed by a number of therapeutic modalities (Table 1).

Table 1. Therapeutic modalities for cervical intraepithelial neoplasia

Traditional excisional techniques:
1. Hysterectomy
2. Cold knife conization
Local ablative techniques:
1. Electrodiathermy coagulation
2. Cryocautery
3. Cold coagulation
4. CO_2 laser vapourization
Transformation zone excision:
1. CO_2 laser excision
2. Loop diathermy excision
Topical chemotherapy:
1. 5-Fluorouracil
2. Dinitrochlorobenzene
Experimental techniques:
1. Photodynamic therapy

II Pretreatment assessment

Several histological abnormalities of both the squamous and columnar components of the cervix may result in recognizable cytological abnormalities, hence referral for a colposcopic assessment and diagnosis. The primary objective of assessment is to define the population with squamous intraepithelial disease. This can only be achieved if several criteria can be met. The whole cervical transformation zone, or the area of metaplastic epithelium lying between columnar epithelium cranially and native squamous epithelium caudally, must be fully visualized. This area of the cervix is currently believed to be the site of neoplastic transformation. Ideally, assessment should be performed under optimal conditions, thus affording better diagnostic accuracy, a reduction in the need for conization and, even in those who still require conization, optimal visualization of the position of the squamocolumnar junction improves the ability to plan the cone size.

The cervix is an organ where appearances are dependent upon the prevailing endocrine milieu and also on local inflammation. All colposcopists are aware of the pronounced effects of pregnancy and the menopause on cervical morphology and the presence of cervicovaginitis can adversely affect assessment by masking CIN. Even in premenopausal non-pregnant women Cartier believes that colposcopy is easier and more informative when the cervix is under oestrogenic influence (Cartier, 1984). Prendiville *et al* (1986) stated that the squamocolumnar junction was more likely to be fully visualized and to be situated nearer the external os when patients had been given a short course of oral ethinyloestradiol before colposcopy. These patients who had initially had unsatisfactory examinations were subsequently made amenable to local destructive methods of treatment. A further therapeutic approach using oestrogen was reported by Toplis *et al* (1986), who observed a higher rate of technically satisfactory colposcopy in

postmenopausal women taking hormone replacement therapy compared to those receiving no such treatment.

There are situations where even adequate visualization of the transformation zone cannot safely direct local therapy. Such situations include a suspicion of invasive disease (based on colposcopic or cytologic recognition criteria (Benedet *et al*, 1985; Luesley *et al*, 1987a)) or a suspicion of glandular intraepithelial neoplasia (GIN) (Luesley *et al*, 1987b).

1 Suspicion of invasive disease

Several colposcopic characteristics have become associated with early stromal invasion, microinvasion or frank invasion. These features have included atypical vasculature, contact bleeding and irregular surface contour. There are grounds to doubt these facts. Based on a case controlled retrospective analysis of 73 cases of invasive disease (Table 2) the sensitivity of colposcopy and directed biopsy in the recognition of invasive disease, even when the whole transformation zone is visualized, is still far from adequate and while most clinicians would obviously err on the side of caution there are reasonable grounds for suggesting that some early lesions are underdiagnosed and treated by local ablation (Anderson, 1985b) and current consensus would deem this as inappropriate.

Pretreatment assessment must also include a balanced evaluation of the presenting cytology. While not a diagnostic technique, there are cytological characteristics suggestive of invasion, and when analysed, again in retrospect (Table 3), sensitivity is relatively high and one might suggest that given a smear indicating an invasive process, local destructive therapies should not be considered.

2 Suspicion of glandular intraepithelial neoplasia

Intraepithelial neoplasia in endocervical epithelium has been recognized more recently than its squamous counterpart. Our data have led us to believe that the condition is underdiagnosed (Luesley *et al*, 1987b), a feeling echoed by others (Christopherson *et al*, 1979; Boon *et al*, 1981). Cytological techniques, although developed to identify squamous lesions, can also detect glandular abnormalities with a sensitivity of up to 70%; specificity is poor, however (Table 4). This situation may be compounded by the not infrequent coexistence of a squamous lesion. Colposcopy and directed biopsy are of little value in diagnosis or rationalizing management, as theoretically the whole endocervical canal may be at risk and therefore beyond the visual capabilities of the colposcope. It is also of relevance that no specific characteristics can be identified to suggest a glandular lesion, hence even if these areas were readily accessable they could not be reliably recognized. Given these drawbacks, assessment of a smear containing malign glandular cells requires a large excision biopsy removing at least the lower part of the endocervical canal as morphometric studies of early adenocarcinoma (Teshima *et al*, 1985) and adeno-in-situ (Jaworski *et al*, 1988) indicate that the majority of lesions lie close to the squamocolumnar junction.

Table 2. Role of colposcopy in the diagnosis of invasive disease (73 cases)

	Colposcopic opinion				
	No abnormality seen	*No comment*	*CIN only +/− warts*	*CIN ↑ canal*	*ESI or invasion*
Group A	2	5	4	10	13
Group B	—	1	3	6	8
Group C	—	1	4	4	12
Total	2 (3%)	7 (10%)	11 (15%)	20 (27%)	33 (45%)

Group A Early stromal invasion (ESI)
Group B Microinvasion (measurable lesions)
Group C Occult stage Ib invasive cancer

	Directed biopsy (36 cases)		
	Normal	*CIN +/− warts*	*ESI/invasion*
Group A	1	10	5
Group B	2	4	3
Group C	1	5	5
Total	4 (11%)	19 (53%)	13 (36%)

All cases eventually diagnosed by large excision biopsy, ie at least cone

Table 3. Cytological prediction of invasive disease

	Histology			
Cytology	*No invasion*	*ESI/microinvasion*	*Invasion*	*Total*
Grade IV	546 (99%)	2 (0.5%)	2 (0.5%)	550
Grade V	11 (31%)	9 (25%)	16 (44%)	36

ESI = early stromal invasion

3 Unsatisfactory colposcopy

Colposcopy is unsatisfactory if the whole transformation zone has not been visualized. In this situation a representative biopsy of the most atypical part of the transformation zone cannot be taken. The inability to fully visualize the squamocolumnar junction is the most frequent reason for performing cone biopsy. The most recent prospective audit of our clinic practice in Birmingham records 49 of 400 (12.5%) consecutive new patients in whom the cervical transformation zone could not be seen. This figure will be dependent upon the age structure of the referral population as unsatisfactory colposcopy occurs more frequently in elderly patients and particularly in

Table 4. Cytology in the detection of glandular lesions

55 cases: subsequent pathology (all cone biopsies):
Positive for malign glandular elements: 24
Negative for malign glandular elements: 31
Specificity of 'glandular smear' = 44%

Sensitivity of glandular cytology
19 cases of adeno-in-situ and nine cases of glandular atypia 1978–86

	Cytological opinion	
	Glandular pathology	*No glandular pathology*
Pure glandular lesion	10	1
Mixed lesion	10	7
Total	20	8

Sensitivity of 'glandular smear' = 71%

Cytology: lesion severity

	Cytological opinion	
	Glandular pathology	*No glandular pathology*
Adeno-in-situ	14	5
Atypia	6	3

postmenopausal patients (Toplis *et al*, 1986; Constantine *et al*, 1987). Occasionally the cervical epithelium may improve and the squamocolumnar junction become more easily visualized after the administration of oestrogen, but this has not been subjected to prospective analysis. It could be argued that as there is less need to be conservative in elderly patients, and as such patients have a potentially greater risk of harbouring neoplastic disease, then cone biopsy should always be performed. Cone biopsy still requires a general anaesthetic and has well documented morbidity (Luesley *et al*, 1985a).

4 Making the diagnosis

Assuming that none of the aformentioned conditions arise, directed biopsy can be used to achieve a histological diagnosis. Some techniques such as laser excision and ring diathermy excision (see below) provide more histological material by excising the whole transformation zone. Occasionally, colposcopic assessment fails to reveal any epithelial abnormality and further management then rests on other factors such as the presenting smear and cytological history (Luesley *et al*, 1987a).

Biopsy is often deferred in pregnant patients for two reasons. Firstly, the procedure may result in excessive haemorrhage, and secondly, treatment would almost certainly be deferred until after the pregnancy is completed. Should intervention be contemplated, this would only be justifiable in the presence of an invasive lesion, the latter being far more accurately diagnosed

on a larger biopsy (cone or wedge). It would therefore seem logical only to perform large biopsies in pregnancy and only when invasive disease is suspected on colposcopic or cytological grounds.

III Treatment options

Treatment selection (Table 1) follows an appraisal of both assessment outcome and patient characteristics; these include the patients' age, menopausal status, fertility status, likelihood of consistent follow-up, anxiety and pain threshold and the presence or absence of coexistent benign gynaecological disease.

1 Traditional excisional treatment

Hysterectomy, cone biopsy and cervical wedge biopsy all retain a place in the diagnosis and management of intraepithelial disease. There is no longer any justification for radical surgical procedures and certainly no place for pelvic lymphadenectomy.

i *Hysterectomy*

Hysterectomy might be regarded as the treatment of choice when confirmed intraepithelial disease coexists with benign pelvic pathology that would best be managed by total abdominal hysterectomy. Colposcopy is still mandatory before treatment, not only to direct confirmatory biopsies but also to ensure that the caudal extension of the abnormal transformation zone is correctly identified to avoid incomplete excision of the lesion which in turn might result in posthysterectomy vaginal intraepithelial neoplasia (Woodman *et al*, 1984).

Ideally, hysterectomy should not be performed until a diagnosis has been made, the underlying logic being that invasive disease might otherwise be managed by a non-radical approach. The incidence of occult invasion is low and our own data suggest that these occult lesions are generally small with no parametrial or nodal involvement, hence satisfactorily extirpated by total abdominal hysterectomy. There are also many retrospective data demonstrating that such patients fare no worse than those treated with a primary radical procedure if radiotherapy or further surgery is performed immediately after a primary hysterectomy (Witherspoon *et al*, 1979; Papavasiliou *et al*, 1980; Heller *et al*, 1986; Orr *et al*, 1986).

Persistently abnormal cytology after previous conization might be treated by hysterectomy even though invasive disease cannot be confidently excluded. Repeat cone biopsies have been performed in this situation but they may be difficult to interpret and difficult to perform, particularly in the postmenopausal patient.

ii *Cone biopsy*

Cone biopsy remains the most frequently used treatment and diagnostic modality on a global scale. Even in centres where more conservative therapy

is available, conization is still required in certain situations (Table 5). Between 15% and 20% of patients are treated by conization in our institution where alternative conservative methods of management are available.

Pooled data (Jordan, 1980) would suggest that cone biopsy and hysterectomy are both effective forms of therapy with a 0.3–0.4% incidence of invasive disease being observed after these procedures. These data are somewhat difficult to interpret as the indications for conization are not itemized. Obviously, if cones are performed because of a suspicion of invasion then this will not be the case. In our own series, we have not seen any patients develop invasive disease if none was present in the initial cone specimen. However, the occurrence of CIN and the development of invasive carcinoma in adequately treated patients have been reported by others (Kolstad and Klemm, 1976; Creasman and Rutledge, 1972).

The pressure to develop more conservative treatment modalities stems from the morbidity associated with conization. This has been extensively documented (Davis *et al*, 1972; Claman and Lee, 1974; Rubio *et al*, 1975; Ohel, 1981; Luesley *et al*, 1985a). The major problems are postoperative haemorrhage and stenosis, the latter occurring in up to 40% of patients (Luesley *et al*, 1985b) and being dependent upon the length of cervical canal removed (Luesley *et al*, 1985a). Stenosis may result in symptoms such as dysmenorrhoea or amenorrhoea and/or disturbance of function, particularly with regard to subsequent fertility or performance in pregnancy. The latter two concepts have been difficult to prove in practice and while such sequelae appear logical, controlled studies would be necessary to substantiate such an outcome. A further problem associated with stenosis is inadequate follow-up, both colposcopy and adequate cytology being difficult to achieve in the scarred cervix.

Post-cone morbidity has been approached in terms of avoidance and definitive management. Several novel techniques have been employed in an attempt to prevent morbidity. These have included varying haemostatic techniques (Codling, 1967; Villasanta, 1973) and the use of the carbon dioxide laser as a cutting tool (Dorsey and Diggs, 1979; Larsson *et al*, 1983; Wright *et al*, 1983). Some success with the latter has been claimed (Fenton *et al*, 1985); however, lasers suitable to perform excisional cones are expensive and not universally available, hence strategies to deal with the complications such as stenosis, when and if it arises, are still required. Laser canalization of stenotic cervices is feasible and relatively effective (Luesley

Table 5. Indications for cone biopsy

1. Suspicion of invasion (includes early stromal invasion and microinvasion) based upon colposcopic, cytological or directed histological findings
2. Colposcopically abnormal transformation zone that is not fully visualized, cytology confirms dyskaryosis or worse
3. Colposcopically normal transformation zone, cytology confirms dyskaryosis or worse
4. Suspicion of glandular intraepithelial neoplasia based on cytological or directed histological findings
5. Recurrent suspicious cytology with negative directed biopsies
6. Recurrent dyskaryotic or worse cytology after previous local ablative therapy for CIN

et al, 1986); whether simple mechanical dilatation is as effective remains to be proved.

A further problem associated with conization is the management of incomplete excision. Hysterectomy has been recommended in such situations (Garcia *et al*, 1975) but seems unduly radical. Further management depends primarily on the histology. Should the cone contain microinvasive or early stromal invasive elements a further large biopsy will be required, as according to recent FIGO guidelines such staging cannot be made unless the whole lesion is contained within the biopsy material, hysterectomy is usually contemplated in this situation. Our own data on cases with early stromal and microinvasion have demonstrated that a high proportion of incompletely excised cones containing these variants also harbour disease in the subsequent hysterectomy specimen (Buxton *et al*, 1987).

Incompletely excised CIN need not be managed by hysterectomy. In a retrospective analysis of cases having hysterectomy after cone biopsy the sensitivity of abnormal cytology was found to be greater than involved excision margins in predicting residual disease. No patients were found to have disease if the post-cone smears were negative. In severely stenosed cervices, where cytology may not be reliable, hysterectomy might be contemplated. (Buxton *et al*, 1987).

iii *Wedge biopsy*

Wedge biopsy does not remove the whole transformation zone and therefore cannot be considered as an appropriate therapeutic procedure. The major indication for wedge biopsy is during pregnancy where invasive disease is suspected. In this situation a punch biopsy may be unreliable and a cone biopsy excessively morbid. Prior colposcopic assessment allows a wedge of the most atypical part of the transformation zone to be removed; if CIN only is confirmed then definitive treatment can be delayed until after the pregnancy.

iv *Conclusions*

Apart from the more obvious morbidities associated with excision all these procedures require inpatient management and general anaesthesia. There has been some interest in outpatient cone biopsy using the carbon dioxide laser. This technique should not be confused with laser excision of the transformation zone (see below). A cone biopsy, no matter what method is used to cut it, usually removes up to or more than 20 mm of the endocervical canal and is performed for specific indications. 'Mini-cones' or laser excision are an alternative to laser ablation, albeit providing more histological material, and consequently similar criteria should be met before performing laser excision.

2 Local destructive therapies

The rationale for local destruction depends on accurate colposcopic assessment and correctly targeted biopsy. As local destruction is only

considered appropriate for intraepithelial disease it follows that the above assessments should exclude invasive disease. All grades of intraepithelial neoplasia may be treated by local ablation and several different techniques have been developed and are in frequent use. A further point that should be considered is the depth of tissue destruction. Anderson (Anderson and Hartley, 1980) has demonstrated by morphometric studies that the maximum depth of gland crypts within the transformation zone is 5 mm. As intraepithelial disease may involve gland crypts, destruction should ideally be beyond this depth and 7 mm has been recommended although deeper destruction also has its advocates.

i *Electrocoagulation diathermy*

Electrocoagulation diathermy (ECD) destroys tissue by heat generated by an electric current passing through tissue. Depth of destruction is related to the current and tissue resistance. Deep destruction can be achieved, as the diathermy probe (needle or knife) can be inserted deeply into the tissues. The apparatus required to provide such a current is available in most operating theatres and is relatively cheap and easy to maintain. The technique also allows for concurrent haemostasis. Because of the inability to control the heat transfer processes within the cervix thermal necrosis may extend considerably beyond the intended field of destruction which may result in excessive discharge and sloughing of tissue after treatment. Radical diathermy destruction also requires general anaesthesia. The technique has been well documented by Chanen (1982) and excellent rates of disease control achieved (in excess of 95%).

Utilizing a wire loop or ring allows ECD to be used as an excisional procedure thus providing more material for histological interpretation. Coagulation artefact may render such biopsies as unsuitable for reporting on excision status, but the larger surface area of sections might allow more accurate histological interpretation.

ii *Cryocautery*

Destruction by freezing can be achieved using many different types of cryoprobe and in essence disease control is dependent on the size of the 'ice-ball' generated in the tissue. Cryoprobes are cheap and widely available and can be used in an outpatient setting without analgesia. Various combinations of freeze and thaw have been employed in an attempt to enhance the destructive potential, yet reliable destruction beyond a depth of 4 mm is difficult to achieve because of the inherent heat exchanging properties of any vascularized organ. These theoretical considerations would appear to be justified in terms of disease control, as Savage *et al* (1982) noted a failure rate of 27% (160 patients) in cases where gland clefts were involved. Success in controlling CIN has been demonstrated (Richart, 1980), although less than acceptable control has been noted with large areas of CIN 3 (Monaghan and Townsend, 1981). Sevin *et al* (1979) have reported on cases of invasion following cryocautery. Though this must be considered as a cause

for concern, it might not necessarily reflect on the treatment modality but rather on pretreatment assessment and the correct use of the method.

Cryocautery is frequently used to treat benign ectopy. In this situation the cervix should be free of intraepithelial disease, as if this is present it will inevitably be at the periphery of the ectopy and may not be fully destroyed.

iii *Cold coagulation*

The use of the adjective 'cold' is misleading as cold coagulation technique destroys tissue by the local application of heat (up to 140°C). The device is cheap and easy to maintain and can be used in unanaesthetized patients. A proportion of patients experience discomfort and local analgesic infiltration is being increasingly employed. It might be argued that attempting to destroy tissue by the surface application of heat may be subject to the same problems as the application of a cold probe in that depth of destruction could be limited by the vasculature of the cervix conducting heat away from the tissues. Some recent observations (Haddad *et al*, 1988) have suggested a maximum depth of destruction of 4 mm; however, the method involved in such assessments must take into account immediate surface stripping after application of the probe, and this may appreciably increase the amount of tissue destroyed. Using this technique, Duncan (1981) has demonstrated good control of disease.

iv *Laser vapourization*

The carbon dioxide laser has become a popular tool in many areas of medicine to effect precise tissue destruction. CO_2 laser light has a wavelength of 10.6 microns. This is less than visible light but greater than microwaves. The radiation is non-ionizing and its biological effect is predominantly thermal. Infra-red radiation is strongly absorbed by living tissues because of their high water content and the initial effect after impacting tissue is to vapourize. The greater the absorptive capacity of the tissue the greater the precision with which the beam can be used. This precision allows good control of destruction depth, good haemostasis and, as correct use minimizes the adjacent zone of thermal necrosis, excellent healing usually follows.

Most lesions can be dealt with without recourse to general anaesthesia, although local infiltration of the cervix is again being increasingly used. Excellent rates for disease control have now been reported (Jordan *et al*, 1985). The major disadvantage is the initial cost and maintenance of such machines and the necessity of providing adequate safety precautions not only for laser beam use but also for tissue vapour and particle extraction—the potential hazards of the latter remain unknown.

3 Ablation or excision?

The ability to confidently exclude invasive disease on colposcopic examination depends on the skill of the observer, the quality of the biopsy specimen taken and the ability of an early malignancy to produce alterations in the surface epithelium that will be reliably recognized. It is clear that errors can occur and one might predict that an increase in demand for colposcopic

evaluation without a concurrent increase in available expertise will exacerbate this situation. There have been several reports documenting invasive disease after local destruction performed in referral centres (Jordan *et al*, 1985). There are no consistently reproducible data documenting the prevalence/incidence of early stromal invasion. Anderson (1985b), however, has noted an increase in the observed incidence of microinvasion and early stromal invasion when excisional techniques are employed. It is likely that the true incidence of glandular intraepithelial neoplasia has also been underestimated (Luesley *et al*, 1987b).

4 Transformation zone excision

In view of the above considerations conservative excisional techniques have been described, notably laser excision of the transformation zone (Dorsey and Diggs, 1979; Baggish, 1986; Partington *et al*, 1987) and diathermy excision of the transformation zone (Cartier, 1981).

Laser excision compares favourably with vapourization but can be technically more demanding and requires a laser that can perform in a cutting mode (ie high power density). Laser excision also takes longer to perform—an average of 12 minutes compared to four minutes for vapourization (Baggish, 1986). Excision using a diathermy loop connected to a low voltage output has been practised by Cartier *et al* (1981). This technique can be used to excise the whole transformation zone including 10 mm or more of the endocervical canal. Both techniques produce some thermocoagulation artefact which is of haemostatic advantage but theoretically might prejudice histopathological interpretation. Well preserved epithelial surfaces can still be obtained, however, as demonstrated in a series of 102 women treated by Prendiville *et al* (1986).

A potential advantage of excisional techniques is the ability to treat the patient at the time of first assessment. Should the colposcopic criteria for local destruction be met (ie whole transformation zone visualized) the transformation zone can be completely removed. Any invasive disease found on subsequent histological assessment of the specimen would then direct further therapy. Some patients would undoubtedly be overtreated by such an approach and there would be an increased pathological workload. The major logistical advantage lies in the decrease in clinic time (and resulting waiting time for assessment) and from the patients' point of view a more rapid resolution of their abnormal cytology.

IV Treatment failure: identification and management

Treatment of CIN is advocated to prevent progression to invasive cancer. Treatment might be said to have failed when either invasive cancer occurs at a later date or there is residual CIN after treatment.

1 Invasive cancer after treatment of CIN

There is agreement that, while treatment of CIN prevents progression to cancer in some patients, even those who have been successfully treated carry

an elevated risk of invasive disease. Long term follow-up studies of patients treated for CIN 3 (Kolstad and Klemm, 1976; Burghardt and Holzer, 1980; McIndoe *et al*, 1984) indicate that invasive disease of the cervix and vaginal vault occurred in 1.1% (Kolstad and Klemm, 1976), 2.1% (Burghardt and Holzer, 1980) and 4.3% (McIndoe *et al*, 1984) respectively of patients treated for CIN 3 with an interval to invasion varying from two to 28 years. Therefore patients treated for invasion require some form of prolonged follow-up. The risk of invasion increases if exfoliative cytology is persistently abnormal, but normal cytology one or two years after treatment is no guarantee against future invasion. In the above studies, treatment was predominantly surgical, ie cone biopsy or hysterectomy with no clear advantage for hysterectomy in the prevention of subsequent invasion. However, treatment methods have become more conservative in recent years and although comparative long term follow-up is not yet available there have been isolated reports of invasive disease after local destructive methods. Some of these failures have been ascribed to inappropriate selection of cases (Townsend *et al*, 1981). Our experience after 10 years of treatment using laser vapourization is that 0.22% of patients have developed invasive disease. We have recently reported seven cases of invasive cancer after laser treatment; in six of these selection criteria might have been considered inappropriate (when assessed retrospectively).

2 Identification of invasive disease

Little information is available with respect to diagnosis of invasion after local destructive therapy. However, cases have been described where infiltrating tumour is present beneath intact hyperplastic squamous epithelium after cryotherapy (Sevin *et al*, 1979) and we have recently seen the same phenomenon after laser treatment.

3 Residual CIN after treatment

Currently available treatment modalities for CIN have high success rates for short term eradication of disease, consequently little space is devoted to treatment failure in the literature. There is no consistent definition of treatment failure. Several features have been cited as evidence of failure including abnormal cytology, atypical colposcopic findings, biopsy confirmed CIN or human papillomavirus alone or in combination. Follow-up intervals have also varied widely.

4 Colposcopic follow-up: is it necessary?

With respect to diagnosis, we believe that cytology used alone misses 20% of residual CIN (unpublished data). MacLean *et al* (1987) quote a false-negative rate for cytology of 33% at follow-up. These data would support the routine use of colposcopic follow-up, yet there is no consensus as to the ideal programme for each patient and this is usually determined by local resources rather than data generated from studies.

Is there any factor which increases the likelihood of residual disease? With respect to laser vapourization and cryotherapy several groups have noted that failure was more frequent with increasing grade of CIN, although no explanation for these findings is discussed (Wright and Davies, 1981; Anderson, 1982; Baggish, 1986; Wright *et al*, 1983). However, Ferenczy (1985) has noted that CIN 3 lesions are larger than CIN lesions of lesser grades and believes that the difference in failure rates may be mediated by lesion size. Jordan and Myelotte (1981) and others emphasize the importance of adequate depth of destruction in eradicating diseased tissue, while Ali *et al* (1986) believe that the inexperience of the operator, the presence of human papillomavirus infection and increasing age of the patient contribute to a diminished success rate. A case–control analysis of treatment failures at the Birmingham and Midland Hospital for Women has shown that all the factors mentioned above fail to exert any appreciable effect on risk of failure.

5 Management of residual/recurrent CIN

In the absence of any guidance from prospective studies, patients with residual disease after treatment should be assessed by cytology, colposcopy and directed biopsy. Colposcopic assessment of the cervix is more difficult after treatment, and it should be borne in mind that islands of CIN and invasive tumour can be buried beneath an apparently normal surface epithelium. Until more data are available pertaining to colposcopic follow-up after local treatment it may be advisable to treat 'failures' by excisional methods.

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Large loop excision of the transformation zone (LLETZ). A new method of management for women with cervical intraepithelial neoplasia

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Summary. The paper describes the technique of 'LLETZ' (large loop excision of the transformation zone), a new method of management for women with an abnormal cervical smear which offers the advantages of conization with those of local destruction. A large loop of thin wire forms a diathermy electrode that allows deep excision of the transformation zone with minimal tissue damage. The tissue removed can be examined histologically. The technique was used to investigate and treat 111 women with abnormal smears referred to the Bristol Royal Infirmary during 1986. Microinvasive disease was revealed in one woman where it was not suspected by cytology or colposcopic examination. Of 102 women followed up for at least 1 year by cytology, colposcopy and, where appropriate, histology, two women were found to have residual/recurrent cervical intraepithelial neoplasia.

Women with a dyskaryotic smear should have a colposcopic examination (Jordan *et al.* 1982). Subsequently the transformation zone in women with cervical intraepithelial neoplasia (CIN) may be managed either by excision or by destruction. Traditionally excision has been equated with cone biopsy (or rarely hysterectomy). Cone biopsy achieves complete removal of the transformation zone and the dysplastic tissue within it. This has the distinct advantage over destructive techniques in that the specimen may be examined histologically; microinvasive/invasive disease can be excluded with confidence and the limits of the lesion clearly defined. But, excision has well-recognized short- and long-term morbidity (Claman & Lee 1974; Jones *et al.* 1979; Editorial 1980; Moinian & Andersch 1982; Larsson *et al.* 1982). In contrast, local destructive techniques (laser, cold coagulation, cryocautery and radical diathermy) do not require general anaesthesia, are associated with minimal morbidity and are highly successful (Townsend 1978; Anderson 1982; Duncan 1983; Chanen & Rome 1983). However, they are reserved for women in whom the transformation zone is fully visible and in whom invasive disease has been ruled out, therefore a colposcopic assessment must be performed before the treatment visit. At this visit the extent of the transformation zone is delineated and the degree of abnormality assessed. A colposcopically directed biopsy is then taken and sent for histological examination so that invasive disease may be excluded.

The confidence with which invasive disease may be excluded depends on the colposcopist being sufficiently experienced so that the biopsy

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is taken from the most abnormal area, and on the biopsy being of sufficiently good quality so that the pathologist can give a confident opinion. These conditions are not always satisfied (Stafl 1983; Choo *et al.* 1984; Prendiville 1986). Also early invasive disease may occur without detectable surface epithelial changes (Cartier 1984).

We describe here a new method of managing CIN which combines the advantages of local destructive techniques with those of cone biopsy. The technique involves large loop excision of the transformation zone (LLETZ) and was used in 111 women with an abnormal smear referred to the Bristol Royal Infirmary in 1986.

Subjects and methods

Equipment

Power is supplied by a Valleylab Force 2 electrosurgical unit. This machine allows an exact and variable blend of cutting and coagulation at low output levels. The unit is connected to the loop by a holder containing a hand switch. Cutting, coagulation or a blend of both may be activated by two buttons on the holder or by a foot pedal (Fig. 1a). The loop consists of an insulated shaft connected to an insulated transverse arm to which the loop wire is attached. The wire is a very thin (0·20 mm) hard stainless steel wire (Ormiston, London). These loops may be fashioned to any size or shape and are reusable.

Selection of patients

Whilst it is possible to use a modified LLETZ technique to perform a cone biopsy, this paper is confined to the description of LLETZ for women with CIN in whom the transformation zone is fully visible and confined to the cervix. Microinvasive disease (suspected cytologically or colposcopically) can also be treated with LLETZ as tissue is obtained for histological examination, in contrast to locally destructive techniques.

Anaesthesia

After demarcation of the upper and lower margins of the transformation zone, a generous volume (5–10 ml) of 3% prilocaine hydrochloride with 0·03% octapressin is infiltrated into the cervical stroma surrounding and underneath the transformation zone.

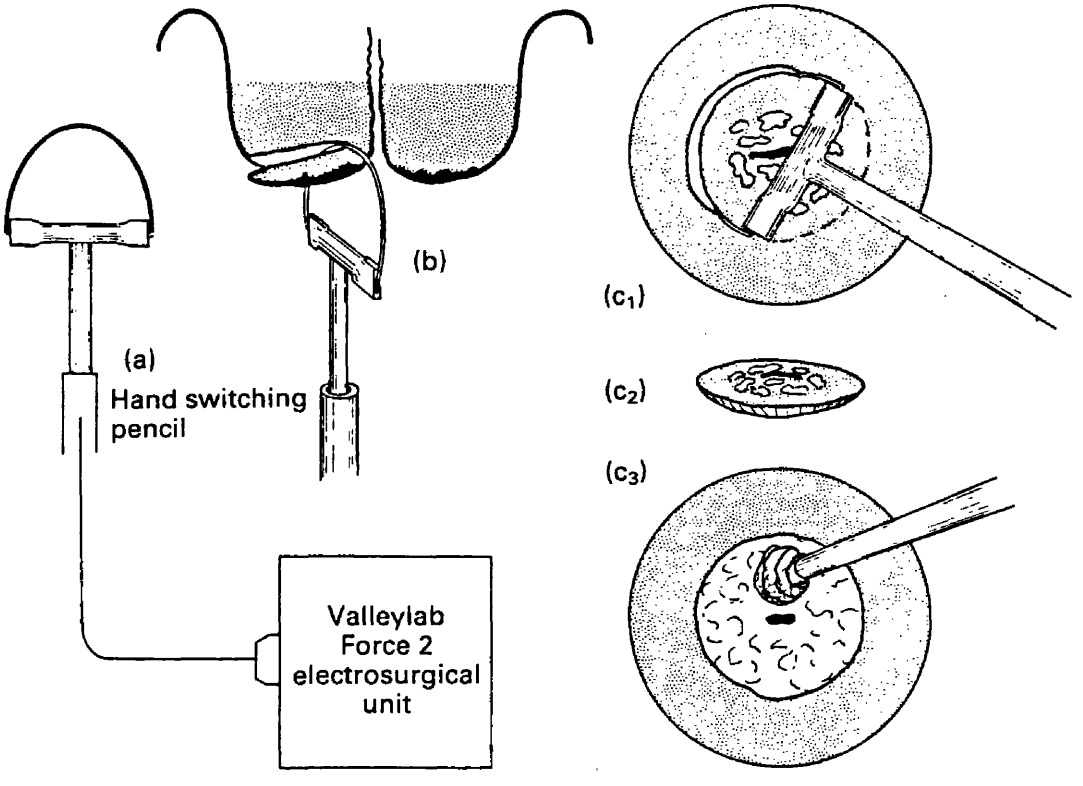

Fig. 1. Large loop excision of the transformation zone (LLETZ). (a) The excision loop; (b) and (c_1) excising the transformation zone; (c_2) the specimen; (c_3) ball diathermy coagulation of cervical wound.

Principle

The diathermy power required depends upon the amount of wire in contact with tissue. The maximum drain upon the power will be when the wire is in contact with the maximum amount of tissue (Fig. 1b and c_1). This is when the loop is exactly half-way through the procedure. In selecting power levels, the aim should be to use sufficient power to cut through the tissue whilst using as little as possible in order to limit tissue damage in both the specimen removed and the wound in the cervix. Figure 2 lists the optimal power settings relative to the size of loop being used. Blend 1 on the 'Force 2' unit combines approximately 20% of whatever coagulation power output has been rung up, with the desired cutting component.

Practice

Like any operative procedure the technique is best taught in practice on a one-to-one basis. A video of the technique is available. LLETZ is performed with colposcopic guidance by simultaneously cutting around and underneath the transformation zone with the loop (Fig. 3). For right-handed operators, starting approximately 0·5 mm outside the periphery of the transformation zone at its right lateral margin the loop is advanced vertically until the required depth of 7–8 mm has been achieved. The loop is then slowly taken from right to left across the cervix underneath the transformation zone. It is then withdrawn at a similar (0·5 mm) distance beyond the left lateral margin of the transformation zone. In this way the transformation zone will be removed as one piece.

Perhaps the most important technical point is that the loop should be advanced very slowly and gently across the cervix underneath the transformation zone. In this way the current will jump ahead of the wire producing a clean cut with a superficial coagulative or fulgurating effect. This produces the cleanest and driest type of cut and is associated with the minimum of diathermy artefactual damage both to the biopsy and the cervical wound bed. If the wire is pushed across the cervix too quickly or too forcibly the wire will remain in contact with the tissue throughout. This results in greater tissue heating, deeper coagulation but less cutting effect, and the artefactual damage produced is more widespread.

A further important practical point is the choice of loop size. The transformation zone varies in shape and size, perhaps partially dependent upon the degree of dysplasia. In most patients the transformation zone can be removed in its entirety by choosing a loop 2 cm in diameter and 1·5 cm in depth. Where the transformation zone is larger or wider in any diameter so that it cannot be excised with this loop, one can either choose a deeper or wider loop or else remove the transformation zone in two pieces. A larger loop will increase the power needed and usually also the amount of tissue damage. Although removing the transformation zone in more than one piece produces a slightly less

	Loop dimensions (cm) Diameter A	Depth B	Cutting	Coagulation	Blend
(a)	1	1	25	20	1
(b)	2	1·5	30	25	1
(c)	2	2	35	30	1
(d)	2·5	2	35	30	1
(e)	3	2	40	30	1

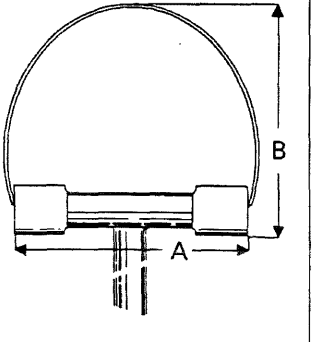

Fig. 2. Power settings using the Valleylab Force 2 electrosurgical unit for large loop excision of the transformation zone (LLETZ).

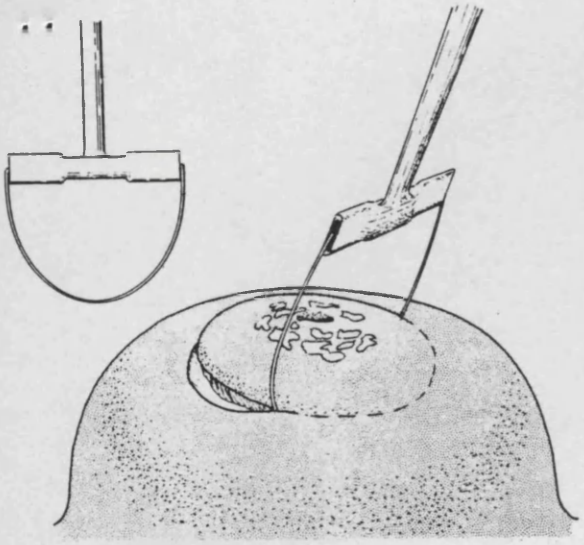

Fig. 3. The excision loop in use.

satisfactory specimen it is a straightforward procedure.

When removing the transformation zone in more than one piece it is preferable to remove the central portion—that which contains the squamocolumnar junction—first, as one piece, and to remove the periphery subsequently usually with the small loop (Fig. 2(a)). In this way the architecture of the squamocolumnar junction remains undisturbed, and comprehensive histological examination is possible.

After excision, the specimen may be cut open and pinned flat before fixation to facilitate orientation and sectioning in the laboratory. The cervical wound is then inspected colposcopically. Although there are sometimes individual bleeding points that may be diathermy coagulated, the bed is often dry. Despite this, it is important to coagulate the cervical wound with the diathermy ball, utilizing the coagulation mode with the same power setting. A superficial yet effective haemostasis may be achieved if one attempts superficial fulguration rather than deep coagulation. To do this the ball is kept moving quickly across the surface of the wound.

Study population

Every new patient referred to the Bristol Royal Infirmary colposcopy clinic, and seen by one of the authors (W.P.), was considered for loop excision. Following colposcopic examination, patients were treated by LLETZ if they satisfied the usual criteria for outpatient local ablative techniques. These are that: (i) the transformation zone is fully visible; (ii) the transformation zone is confined to the cervix; (iii) neither invasive nor glandular disease is suspected cytologically or colposcopically; (iv) follow-up compliance is assured.

One patient in whom microinvasive disease was suspected at cytology but not at colposcopy was also included. During the first 6 months of the study a colposcopically directed biopsy was taken using a 'small' loop (Prendiville 1986) and invasive disease was ruled out in this biopsy by histological examination before treatment at a second visit (45 patients). Subsequently this practice was altered so that patients were treated by LLETZ at their first visit (66 patients). Following LLETZ the cervical wound was left open; no packs or other haemostatic agents were used. Patients were advised to avoid intercourse and vaginal tampons for 4 weeks. Prophylactic antibiotics were not used in this study.

Patients were subsequently seen in the colposcopy clinic at intervals of 6 months. At these visits a cytological smear was taken with an Ayre's spatule before colposcopy. When the new squamocolumnar junction was not fully visible an endocervical brush smear was also obtained. At colposcopy the new squamocolumnar junction was scrutinized. Signs of residual transformation zone were sought (gland openings, nabothian follicles and iodine negative epithelium) outside the new squamocolumnar junction. Finally, when there was a colposcopic suspicion of residual or recurrent CIN, loop excision biopsy was taken and sent for histological examination.

Results

Overall, 111 women were treated by LLETZ during 1986, nine of them have been lost to follow-up. All of the remaining 102 women have been followed up for at least 12 months (median length of follow-up 18 months, range 12–36, mean 19·2, SD 6·6). Three patients were teenagers, 21 were aged between 20 and 29, 66 between 30 and 39, and 12 between 40 and 49 years. The degree of abnormality suspected before excision and the histological diagnosis made from examination of the excised transformation zone are shown in Table 1. In 52 women the transformation zone was removed in its entirety as one piece, in 47 it was removed in two pieces, and in three women in three pieces. Two patients were pregnant at the time of treatment (both less than 6 weeks gestation). These preg-

Table 1. Degree of abnormality suspected cytologically and subsequent colposcopic and histological diagnosis in 102 women treated by large loop excision of the transformation zone (LLETZ)*

	Severe inflammation or HPV changes; CIN not suspected	CIN I	CIN II	CIN III	Micro-invasion	Total
Cytology	5	13	32	51	1*	102
Colposcopy	3	14	36	48	1	102
Histology	9	7	21	64	1	102

*For the patient in whom a cytological suspicion of microinvasive disease was reported, the colposcopic and histological diagnosis was CIN III. In another patient in whom cytology and colposcopy suggested CIN III, histological examination revealed microinvasive disease.

nancies continued uneventfully and have resulted in normal spontaneous vaginal deliveries at term.

Table 2 details the abnormal cytological and histological findings in the 102 women who attended for adequate follow-up (minimum of 12 months). At colposcopy the squamocolumnar junction was visible in 94, seven had evidence of residual transformation zone epithelium, and in five there was a suspicion of CIN.

The complications of LLETZ may be divided into perioperative, postoperative and long-term. The last of these cannot be properly assessed as yet. However, follow-up colposcopic examination of women who have been treated by LLETZ has given us the subjective impression that these cervices suffer a similar degree of fibrosis as those treated by laser or cold coagulation which we have used in our clinic for several years. Two patients were admitted to hospital because of perioperative bleeding. Both responded to dry gauze packing of the cervical wound bed and the vagina in combination with broad spectrum antibiotics. Two further patients had secondary haemorrhages and were treated in the same way. A further nine patients were diagnosed as having a secondary infection which occurred between 8 and 21 days after operation, and all responded to broad spectrum antibiotics. None required admission to hospital. In all of these, bleeding was a component of their complaint but it was always less than the patient's normal menstrual loss.

Discussion

Cartier (1984) has long advocated the low voltage diathermy loop as a means of both investigating and treating cervical intraepithelial neoplasia. He uses a small (0·5×0·5 cm) loop both to take biopsies and to remove the transformation zone in strips. It was Cartier's pioneering work which stimulated us to investigate and develop this system.

Cartier's small loop produces cervical biopsies that are superior to those from punch biopsy forceps (Prendiville 1986). Cartier's system does not incorporate a cutting/coagulation blend facility and it is necessary to change to the coagulation mode after the biopsy has been taken in

Table 2. Abnormal cytological and histological findings at follow-up in 102 women treated by large loop excision of the transformation zone (LLETZ)

Histological diagnosis	Cytology			
	Severe inflammation or HPV changes only	Dysplasia		
		Mild	Moderate	Severe
No biopsy taken, colposcopy normal	3	—	—	—
Normal or HPV	2	—	1	—
CIN I	—	—	—	—
CIN II	—	—	1	—
CIN III	—	—	—	1

order to effect haemostasis. LLETZ attempts to overcome these problems by changing the system as follows:

(1) The Valleylab Force 2 unit allows for variable blends of small amounts of cutting and coagulation diathermy power.
(2) The actual loops have been considerably enlarged so that the entire transformation zone may be removed in one or two pieces.
(3) The base of the loop is insulated to minimize the risk of diathermy damage to the (surface) epithelium removed.

Where colposcopic examination of the stroma underlying the transformation zone after treatment reveals apparent incomplete excision of some gland crypt bases, the ball may be used to destroy this residual tissue. An alternative of course is that advocated by Cartier—further and deeper loop excision. As he describes, one of the advantages of excision by low voltage diathermy is that it allows one to examine colposcopically the connective tissue after extirpation of the transformation zone epithelium (Cartier 1984).

This first series of patients suggests that LLETZ is an effective method of treatment. Comparison of methods of treatment is confounded by differing criteria used for patient selection, the length of follow-up and whether this follow-up included colposcopy (and, where appropriate, histology). However a 'success' rate of 98% after a single treatment in this study compares favourably with similar follow-up studies for laser, 91% (Reid 1985); cold coagulation, 95% (Duncan 1983); radical diathermy, 97% (Chanen & Rome 1983); or cryosurgery, 90% (Creasman 1985). In our study four women (4%) suffered peri- or postoperative bleeding sufficient to warrant admission to hospital; this compares with values of 1·5% (Jordan 1986) to 2% (Reid 1985) for laser. In retrospect we believe that this complication can be avoided by excluding patients with a cytological or colposcopic suspicion of cervicitis. The problem of minor bleeding which occurred in a significant number of patients (9/102) is, however, less avoidable and this rate has persisted. The main complication associated with cryotherapy appears to be a watery discharge which occurred in all the women reported by Townsend & Richart (1983) and was profuse in 80%. Duncan (1983) did not report complications associated with cold coagulation. Recently we have investigated a rollerball electrode for post-LLETZ coagulative haemostasis. This may produce less tissue damage and therefore be associated with a less complicated healing process but this is as yet speculative.

The principal advantage of LLETZ is that it removes rather than destroys the tissue under suspicion. In our series, one patient with a cytological and colposcopic suspicion of CIN III actually had microinvasive disease on histological examination of the LLETZ specimen. It is well established that destructive methods may fail (Ali *et al.* 1986; Cullimore *et al.* 1988). Invasive disease may ensue for more than one reason: inadequate depth of destruction may leave a focus of CIN which may subsequently progress to invasive disease; also, microinvasive disease may be missed (Choo *et al.* 1984; Stafl 1983; Cartier 1984) when the lesion is destroyed. Finally, even where a lesion is correctly suspected of being worse than CIN III, a punch biopsy specimen may not be deep enough to allow confident histological appraisal (Prendiville 1986). The present series is small, and one case out of 102 may not reflect the true number of cases of unsuspected microinvasion. However, performing LLETZ and sending the entire transformation zone for histological examination minimizes the possibility of missing such disease. Furthermore histology can confirm that the limits of the lesion are clear of the lines of resection. A further advantage of LLETZ is that it may be performed at a patient's first visit to the colposcopy clinic. This has obvious advantages both to the patient and to a hard-pressed colposcopy service.

Boulanger *et al.* (1984) have reported a comparative study of treatment with CO_2 laser and diathermy loop excision using the loops described by Cartier (1984). They concluded laser was a superior treatment because it was less painful, easier to handle and more precise. It has long been our practice to use local anaesthesia for laser or LLETZ so that pain is no longer an issue. Atkinson (1984) has also described the use of Cartier's loop both as a means of taking large biopsies and to extirpate the whole transformation zone, again in strips. There is a need for a large randomized controlled trial of LLETZ versus destructive techniques for the treatment of women with CIN in order to establish its relative efficacy, complication rate and acceptability to patients and colposcopists.

The principal advantages of LLETZ are that it is an outpatient local anaesthetic procedure and that the tissue is removed rather than destroyed.

It is therefore possible to rule out invasive disease and confirm that the lesion has been removed in its entirety. LLETZ uses cheap simple equipment. It is a simple matter to choose a loop of appropriate dimensions and remove the transformation zone in its entirety, doing so in a smooth and symmetrical way. This contrasts with the greater difficulty experienced in cutting cones with the laser beam and the far greater cost of laser equipment. A further advantage over destructive methods is that it may be performed at the first/assessment colposcopic visit, thereby cutting in half the number of visits that women with CIN make for investigation and treatment.

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The management of diabetic pregnancy in a regional centre. A five year review

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Summary

A five year review (1981–1985) of the management and outcome of 85 diabetic pregnancies is presented. Management was based on self blood glucose monitoring and frequent outpatient attendances. With increasing experience of this system the median number of days spent as an inpatient during pregnancy progressively decreased. A good standard of diabetic control was maintained, and we did not observe any increase in perinatal morbidity. In the last three years of the study there was an increase in mean birth weight and in the number of babies whose birth weight exceeded the 90th centile for gestational age.

There was no relationship between blood glucose control in the last 10 weeks of pregnancy and the incidence of neonatal problems, or the incidence of birth weight >90th centile. There was a relationship between elevated mean glycosylated haemoglobin levels in the last 10 weeks of pregnancy and an adverse neonatal outcome.

INTRODUCTION

THE risks to the baby of a diabetic mother have decreased considerably in the last 50 years. While the perinatal mortality rate for this group now approaches that for non-diabetics, there remains an appreciable incidence of well recognised fetal and neonatal complications (Gamsu, 1978). The improvement in mortality rates has been sustained, despite changing from inpatient to outpatient based management. The latter has been reported to be associated with good blood glucose control, is more economical for the Health Service, and is highly acceptable to patients (Burke *et al.*, 1985). Our management policy of the pregnant diabetic is outlined and the results of this policy are presented for a five year period, 1981–1985.

PATIENTS AND METHODS

Eighty-five pregnancies occurred in 70 insulin dependent diabetics in the interval from 1.1.81 to 31.12.85. These data do not include patients who aborted, who were managed at another hospital. White's classification (White, 1965) has been applied to this population, yielding the following distribution: B-26 C-23, D-17, R(proliferative retinopathy)-3, F-1. There were 37 primigravid pregnancies. Patients were seen in a joint obstetric and medical clinic by an obstetrician and a physician, where they were instructed in the technique of using 'BM' sticks (Boehringer Corporation (London) Ltd) for self blood glucose monitoring. The patient estimated capillary glucose levels before meals, 1–2 h after meals, and just before retiring, on 3 days per week. As a check on patient reliability, the laboratory arranged a weekly quality control estimate of glucose levels using capillary blood spots on filter paper strips (Wakelin *et al.*, 1978). We aimed for pre-prandial glucose levels $<5{\cdot}0$ mmol/l, and post-prandial glucose $<7{\cdot}5$ mmol/l. Glycosylated haemoglobin (HbA_{1c}) was measured once monthly (Ambler *et al.*, 1983). The aim of management was to allow the patient to monitor blood glucose levels and to correct her own insulin requirements. In well controlled diabetics, insulin adjustments were often made after telephone consultation with the physician. There were no routine admissions for pregnant diabetics, unless the patient required education in insulin

administration, or unless there was inadequate understanding of self blood glucose monitoring. All patients were managed on at least two injections of mixtures of short and intermediate acting insulin. Ultrasound was used to assess gestational age accurately and to detect significant congenital anomaly. Fetal well-being was assessed by monthly ultrasound scans. Patients were seen fortnightly until 30 weeks, and weekly thereafter.

Fetal well-being was assessed by daily fetal movement charts (from 30 weeks) and weekly unstressed cardiotocography. Patients in whom blood sugar levels were well controlled who did not develop obstetric problems were allowed to continue to 40 weeks as outpatients. After this, induction of labour was arranged. Where indicated, elective caesarean section was carried out at 39 weeks. In labour, intravenous infusion of insulin was administered to maintain glucose levels between 4 and 7 mmol/l. Continuous fetal monitoring was employed. A paediatrician attended all deliveries. After delivery, the patient's pre-pregnancy insulin regimen was reinstated. In the absence of problems, the neonate accompanied the mother to the postnatal ward, where hourly 'Dextrostix' (Miles Ltd, Ames Division, Stoke Poges, England) estimations were made, and early feeding of the baby was standard practice.

RESULTS

Diabetic control

To facilitate the assessment of blood glucose control, an overall mean glucose level was determined for each patient in each consecutive 10 weeks of pregnancy, from the filter paper spot results. These mean glucose levels were normally distributed. As pregnancy progressed, there was a reduction in mean glucose levels. A similar progressive reduction in mean HbA_{1c} levels was noted (Table I). By analysing these data on a yearly basis, there were no differences in quality of control as assessed by mean glucose and HbA_{1c} levels. Daily insulin requirements increased as pregnancy progressed. In 1984–1985, mean insulin dose at booking was 50 u, whereas at delivery mean insulin dose was 91 u. There was no relationship between the occurrence of neonatal problems as defined in Table II, and mean glucose levels in the final 10 weeks of pregnancy. There was a relationship between elevated mean HbA_{1c} levels in the final 10 weeks and an adverse neonatal outcome (Table II). There was no relationship between our chosen parameters of diabetic control and the incidence of birth weight >90th centile, adjusted for gestational age and sex.

Table II. Diabetic control in the final 10 weeks of pregnancy and neonatal outcome

	Number of babies with	
	*Neonatal complications**	*No complications*
Mean maternal glucose (χ^2 not significant)		
< = 5 mmol/l	18	24
>5 mmol/l	11	20
Mean maternal HbA_{1c} ($P < 0{\cdot}05$)		
< = 7·9 per cent	18	33
>7·9 per cent	16	9

*Any of the following: hypoglycaemia, major congenital anomaly, significant neonatal jaundice, intraventricular haemorrhage or polycythaemia requiring exchange transfusion.

Hospital admission

Over the course of the study there was a progressive reduction in the number of antenatal days spent in hospital, the median being 14 in 1981 and 0 in 1985.

Onset of labour and mode of delivery

Spontaneous labour occurred in 28 per cent of patients; 33 per cent had labour induced and 39 per cent had an elective caesarean section. The delivery rates were: normal vaginal 30 per cent; low forceps 9 per cent; rotational forceps 7 per cent; caesarean section 54 per cent. There was one case of shoulder dystocia in the series. Primary indications for caesarean section were: (a) planned elective procedures, 74 per cent. Just over one-

Table I. Diabetic control in each 10 week interval of pregnancy

	Weeks of pregnancy			
	0–10	*11–20*	*21–30*	*31–40*
Mean glucose (mmol/l)	7·1	5·8	5·6	5·0
Mean HbA_{1c} (per cent)	10	8·6	7·8	7·7

third of these patients had had a previous caesarean section. (b) emergency section, 26 per cent. Two-thirds of these were for fetal distress, the remainder for poor progress in labour.

Neonates

There was one stillbirth, and one infant death in the series. The stillbirth occurred in relation to an episode of maternal ketoacidosis at 33 weeks. The patient failed to follow her treatment and took her own discharge from hospital when admitted for stabilisation.

The infant death occurred at 6 months in a baby shown to have suffered from congenital hypertrophic cardiomyopathy (diabetic type).

The mean gestational age for the whole series was 37 weeks and 5 days (range 26–42 weeks). The mean overall birth weight was 3220 g (700–5140 g). Twenty-four babies (28 per cent) weighed greater than the 90th centile for gestational age and three babies (4 per cent) weighed less than the 10th centile for gestational age.

The incidence of preterm delivery was 23 per cent of all births in the series, those born at less than 34 weeks accounting for 10 per cent of all births.

There were four examples of major congenital anomaly (hypertrophic cardiomyopathy, diabetic type, 1; transposition of great vessels, 2; caudal regression syndrome, 1). There were seven minor anomalies (inguinal hernia or hydrocoele, 4; syndactyly, 1; hypospadias, 1; talipes, 1). Thus 11 babies (13 per cent) had congenital anomalies.

Five babies (6 per cent) developed hypoglycaemia in the neonatal period and required intravenous glucose. Twenty-two babies (26 per cent) developed jaundice (serum unconjugated bilirubin $>205\ \mu mol/l$. One baby required an exchange transfusion for polycythaemia (packed cell volume, 75 per cent). Fifteen babies suffered respiratory distress syndrome; of these four required continuous positive pressure ventilation, and 11 required oxygen alone.

Yearly analysis of data (Table III)

Over the course of the study period, despite increased reliance on outpatient management, there was no change in the rate of vaginal delivery, the caesarean section rate or mean gestational age at delivery.

There was a progressive increase in mean birth weight and percentage of infants whose birth weight was >90th centile. The incidence of neonatal problems, excluding neonatal hypoglycaemia, did not vary appreciably between years of the study.

DISCUSSION

The improvement in perinatal mortality and morbidity rates in diabetic pregnancy has been associated with the adoption of well defined principles of management. The effect of improving blood glucose control on perinatal outcome has been well illustrated (Fuhrmann *et al.*, 1983; Karlsson and Kjellmer, 1972).

Table III. Analysis of data by year

	Year				
	1981	*1982*	*1983*	*1984*	*1985*
Live births	18	11	13	18	23
Stillbirths	1	0	0	0	0
Neonatal deaths	0	0	0	0	1
Mode of delivery					
Vaginal	31%	54%	46%	50%	48%
Caesarean section	69%	46%	54%	50%	52%
Patients who booked in first 10 weeks	39%	55%	29%	39%	70%
Mean gestational age at delivery	37+4	36+5	37+6	38+2	37+3
Mean birth weight (kg)	2·95	2·85	3·36	3·42	3·50
>90th centile for gestational age	21%	11%	25%	31·6%	47·6%
Congenital anomalies					
Major	0	0	1	1	2
Minor	3	1	0	1	1
Neonatal hypoglycaemia (no. of episodes)	0	1	0	1	3
Neonatal jaundice (>205 μmol/l)	26%	18%	15%	22%	32%
Respiratory distress syndrome	28%	18%	15%	17%	14%

Having accepted the importance of good control, there has been much debate in recent years as to how best to achieve this. There is much evidence to support a policy of home self blood glucose monitoring, backed up by easy telephone access to hospital physicians, and frequent hospital outpatient attendance (Varner, 1983; Murphy *et al.*, 1984). This approach has been shown to be as safe as hospital based management and achieves equally good diabetic control (Hanson *et al.*, 1984; Peacock *et al.*, 1979). It is also popular with patients. It involves and educates patients in the management of their disorder. This method of management is also more economical, with considerable savings on the cost of inpatient beds (Varner, 1983; Murphy *et al.*, 1984).

This study confirms the finding that the home based management of diabetic pregnancy is safe for diabetic mothers and is associated with an acceptable standard of diabetic control, and a low perinatal mortality rate, which for this series was 2·3 per cent. This compares well with other series (Gyves *et al.*, 1977; Coustan *et al.*, 1980; Lavin *et al.*, 1983; Murphy *et al.*, 1984).

We could not demonstrate any association between blood glucose control in the final 10 weeks of pregnancy, and neonatal outcome or fetal macrosomia. This has also been the experience of other investigators (Lavin *et al.*, 1983). On the other hand, it may merely reflect the shortcomings of the parameter used to assess diabetic control, mean blood glucose. Artal *et al.* (1983) have demonstrated that by taking account of the daily variability in blood glucose levels there is a significant correlation between the coefficient of variation for 'within day' plasma glucose variability and neonatal outcome. Gillmer *et al.* (1975a,b) have also postulated the importance of wide excursions in blood glucose levels in the genesis of complications of diabetic pregnancy.

Mean glycosylated haemoglobin levels in the last 10 weeks of pregnancy appear to be related to neonatal outcome. This parameter of diabetic control may be more informative in this context than mean blood glucose, as it is not subject to such wide variability. Its measurement correlates well with plasma glucose levels in the 4 weeks before measurement (Leslie *et al.*, 1978).

HbA_{1c} measurements are also of value when performed in the early part of pregnancy and indicate those who are at significantly higher risk of fetal anomaly (Ylinen *et al.*, 1984). In our study group there were 13 patients with mean HbA_{1c} in the first 10 weeks of pregnancy in excess of 10 per cent, implying poor periconception control. Of these, three gave birth to babies with major congenital anomalies. Good control of diabetes before conception and in the early first trimester be the way to reduce the incidence of congenital abnormalities, and our numbers, though small, support this view. Preconception counselling is therefore vital for the diabetic woman.

This series demonstrates a low antenatal admission rate for pregnant diabetics. There has been a trend towards fewer antenatal admissions as the study period progressed, and by 1985 the average patient could expect to spend just 2 days in hospital during the whole of her pregnancy (excluding delivery) for problems related to diabetic control. There also appears to have been a trend towards increased birth weight, and the number of babies whose birth weight exceeded the 90th centile for gestational age and sex. The maximum prevalence of macrosomia occurred in 1985 (48 per cent of births), and this differed appreciably from previous years. Those delivered in 1985 may have been atypical for a variety of reasons. Notably, just over half of them had had a previous successful pregnancy earlier in the study period. Hence increasing parity and maternal age could explain some difference in birth weight. Likewise, in 1985 compliance with antenatal management seemed to be greater, based on the large number of patients who booked within the first 10 weeks of pregnancy. It is conceivable that this group of patients may have smoked less than before, although we did not routinely collect information on patterns of cigarette consumption. We believe that it is difficult to reach any firm conclusions on the relationship between outpatient based management and birth weight, based on such short term data, and that other factors may be involved in the observed pattern of birth weight change.

Good control of blood sugar is not the only determinant of fetal size; neonatal outcome does not seem to be adversely prejudiced by outpatient management.

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Routine repeat uterine curettage after primary evacuation of hydatidiform mole. Does it affect the prognosis?

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Summary

The outcome in 80 patients who had routine repeat uterine curettage following primary evacuation of hydatidiform mole was compared with that of 76 patients in whom a routine repeat curettage was not performed. The incidence of need for chemotherapy at the end of a 1 year follow up was 16 per cent in the 'curettage' group and 18 per cent in the 'no curettage' group. This difference was not significant. In only 7·5 per cent of patients was trophoblastic tissue obtained during the routine repeat curettage. The histological yield did not predict the course of the disease. Repeat curettage after primary evacuation of molar pregnancy should not be performed as a routine procedure.

INTRODUCTION

SUCTION evacuation followed by immediate sharp uterine curettage is currently a standard method of primary treatment of hydatidiform mole. It has been said that this primary sharp curettage renders routine repeat curettage to remove residual trophoblastic tissue unnecessary (Tow, 1966; Morrow, 1984), but some centres adopt a policy of routine repeat curettage a week or so after the first suction curettage. The argument is that the uterus is then hard and can be curetted boldly (Chun *et al.*, 1964; Lao *et al.*, 1987). However, the prognostic value of a routine repeat curettage has not yet been fully evaluated.

At the Maternity Hospital of Kuwait, a routine repeat curettage was performed in all patients a week after suction evacuation of molar pregnancy. After January, 1986, the routine repeat curettage was abandoned. This retrospective controlled study evaluates the role of routine repeat curettage on the prognosis of the disease.

PATIENTS AND METHODS

Two groups of patients were selected for the study. The first group included 80 patients in whom a hydatidiform mole was evacuated by suction curettage which was followed by a routine repeat curettage within 7–10 days after the primary evacuation. These patients were treated between January 1983 and December 1985, at the Maternity Hospital of Kuwait. The second group included 76 patients who had evacuation of moles by suction curettage between January 1986 and June 1988 at the same hospital. In these patients, a routine repeat curettage was not performed. In all patients studied, hydatidiform mole was confirmed histologically following the primary evacuation.

All patients were followed up regularly at the Trophoblastic Registry Clinic of the Maternity Hospital of Kuwait. During follow up, they were evaluated clinically and by serial estimation of serum β-subunit of human chorionic gonadotrophin (β-hCG). The results were plotted against a standard -hCG regression curve. Other investigations included chest X-ray, complete blood counts, liver function tests and thyroid function tests. Lung and brain CAT scan were done when indicated. Contraception was advised for 6–12 months. The low dose combined contraceptive pill was used by the majority of patients while others relied on barrier contraceptives. Chemotherapy was indicated when there was clinical evidence of choriocarcinoma or when there was abnormal β-hCG regression. The histological findings after the repeat curettage were analysed. The two groups in the study were compared regarding

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Entrapment of the fetal head in a unilateral imperforate vagina in association with complete duplication of uterus and cervix. Case report

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Case report

A 20-year-old primigravida attended the antenatal clinic for booking in March 1984. Her dates were uncertain, but her menstrual cycle was regular and she did not complain of any dysmenorrhoea. Bimanual assessment of the uterus suggested an 8-week gestation. No abnormal vaginal swelling was noted, although the cervix was described as being flush with the vault of the vagina. An early ultrasound assessment confirmed a viable 8-week pregnancy.

The subsequent progress was uneventful until 26 weeks gestation when she was admitted with lower abdominal pain. The clinical picture was of a urinary tract infection and she was given ampicillin; culture of her mid-stream specimen of urine revealed an ampicillin-sensitive coliform infection. After 24 h the patient experienced regular painful uterine contractions after apparent spontaneous rupture of membranes. A speculum examination of the vagina revealed large quantities of a dark red/brown offensive fluid draining, but it was impossible to visualize the cervix, because of a cystic bulge (6 × 5 cm) on the right anterolateral aspect of the vagina. The fluid was believed to be amniotic fluid as a nitrazine swab was strongly positive. Her initial management was conservative, but 12 h later the patient complained of constant severe lower abdominal pain with the uterus diffusely tender on palpation. Cardiotocography revealed a baseline fetal tachycardia (160–180 beats/min). The vaginal loss continued with large quantities of brown malodorous fluid which remained strongly positive to amnicator. Vaginal examination confirmed the presence of a cystic mass 6 × 5 cm indenting the right anterior vaginal wall displacing the cervix upwards and to the left. The cervix was noted to be uneffaced and closed; the right lip was flush with the vaginal vault. Intra-amniotic infection in association with a right para-vaginal abscess was diagnosed and intravenous oxytocin was given to expedite delivery; intravenous antibiotics were continued. As signs of fetal distress developed the patient had another vaginal assessment and this confirmed progressive enlargement of the vaginal wall bulge which was now extremely tender, the cervix on the left remaining closed and uneffaced. Oxytocin was discontinued and the patient was examined under anaesthesia. A right anterolateral paravaginal mass was confirmed now measuring 8 × 8cm, pressure upon which produced large quantities of malodorous brown fluid followed by clear amniotic fluid. Transvaginal incision and drainage released approximately 300 ml of malodorous old blood having the appearances of a haematocolpos. Culture of this fluid revealed bacteriodes, anaerobic streptococci and *E. coli*. The fetal heart was audible after the procedure. Uterine contractions ceased post-operatively and then 20 h later clear amniotic fluid was noted to be draining vaginally. Strong labour became established; then and at subsequent vaginal examination the cervix admitted one finger. The fetal head was palpable to the right below the cervix in the space previously occupied by the encysted old blood. It was possible also to palpate the fetal head through the cervix, and in view of this, sacculation of the lower segment was suspected. An immediate laparotomy was performed to elucidate the diagnosis and to prevent further trauma to the maternal genital tract. At abdominal exploration the uterus appeared heart shaped with the lower segment relatively well formed. A transverse lower segment incision was made and the fetal head was found to be

deeply impacted in an imperforate right sided vagina (Fig. 1). A live female infant weighing 770 g was born. The uterus was noted to be bicornuate, and the placenta was removed manually from the fundal aspect of the left horn. The edges of the intrauterine septum were ragged, friable and bruised at the level of the lower segment, and it was possible to insert one finger only through the cervix on the left. The right horn of the uterus was found to be in communication with a unilaterally dilated imperforate vagina on the right, although no obvious cervix was seen on the right side at the time of delivery. It was presumed that a communication had existed between the two genital tracts just above the level of the endocervical canal prior to labour and this had subsequently allowed transgression of the fetal head during the later stages of labour from the left horn to the right blind vagina. A postoperative intravenous pyelogram and renal scan both confirmed right-sided renal agenesis.

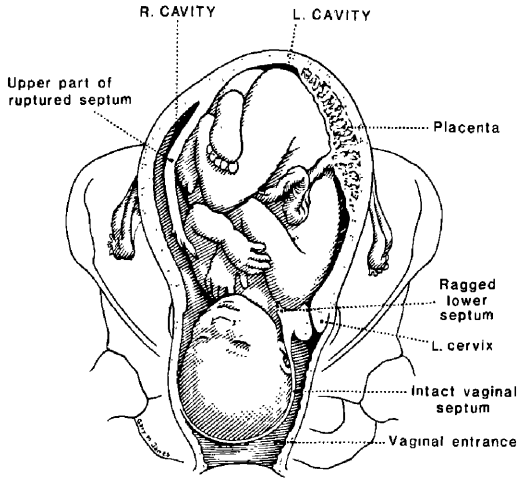

Fig. 1. Schematic drawing showing the fetal head in a blind right vagina having ruptured through the intrauterine septum from the left horn of a bicornuate uterus.

At the postnatal examination the patient had resumed normal menses with no intermenstrual bleeding or vaginal discharge, but bimanual examination confirmed a 10-cm right-sided paravaginal mass, pressure on which released old blood into the vagina through the left cervix. A hysterosalpingogram combined with real-time ultrasonography of the uterus outlined only the left uterine cavity and left fallopian tube with a filling defect seen above endocervical level with failure of the contrast medium to enter the right uterine cavity. These appearances associated with a figure-of-eight collection seen in the longitudinal axis of the uterus on ultrasound suggested the presence of a right haematometra and haematocolpos.

The infant required assisted ventilation for 2 months because of severe respiratory distress syndrome but at 4 months she is currently breathing spontaneously. She is being bottle fed with full strength 'Premium' and her present weight is 2·44 kg.

Discussion

Complete duplication of the uterus and cervix with a unilaterally imperforate vagina and ipsilateral renal agenesis has been reported previously (Rock & Jones 1980). The diversity of clinical presentation with consequent delay in diagnosis was found to depend on whether the vaginal obstruction was partial or complete with two main categories of patients being recognized—complete vaginal obstruction with a haematocolpos, and incomplete vaginal obstruction without a haematocolpos.

Rarely a third category of patient was also found, in which complete vaginal obstruction occurred with a laterally communicating double uterus. An obstetric complication in association with this third category of patient is reported.

All of these patients had similar symptoms of progressive dysmenorrhoea and intermittent lower abdominal pain in association with a paravaginal mass, yet in most instances their menstrual history was normal. Our patient similarly had normal menses from the menarche but did not have dysmenorrhoea and although a paravaginal mass was not noted at her booking examination the cervix was described as being flush with the vaginal vault.

A review of the world literature with special reference to pregnancy in association with complete duplication of the uterus and unilaterally imperforate vagina reveals only scanty details of successful pregnancy in 23 patients (Semmens 1956; Allan & Cowan 1963; Johansen 1972; Gilliland & Dych 1976; Eisenberg *et al.* 1982). In most of these pregnancies labour was uncomplicated, however, Johansen (1972) reported a series of nine successful pregnancies of which three had complications namely retained placenta in one, unstable lie in the second and arrest of the breech by the vaginal septum in the third.

The unusual feature of our patient was the transgression of the fetal head from the preg-

nancy in the left horn of the uterus into the blind right vagina which must have occurred via communication between the two genital tracts. This pre-existing communication could explain why the patient did not experience dysmenorrhoea. Such a fistulous connection between the two cavities could allow egress of menstrual fluid from the right side and explains why pressure on the right sided vaginal swelling was followed by drainage of amniotic fluid stained by old blood through the left cervix.

The planned surgical management in this case is incision and drainage of the vaginal pouch and its contents, to be followed by complete excision of the vaginal septum at a later date.

Acknowledgments

We wish to thank Mr Gary James for the medical illustration, Mrs F. Potter for typing the script, and Professor G. M. Stirrat for permission to present the case and for his advice and that of Mr M. G. R. Hull in preparing the manuscript.

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PUBLICATIONS SUBMITTED IN SUPPORT OF CANDIDATURE FOR THE DEGREE OF M.D.
AND,
STATEMENT OF PERSONAL SHARE OF WORK IN CONJOINT PUBLICATIONS

J.E. CULLIMORE M.B. B.S.

[A]
Publications arising from thesis work

1) Cullimore , J.E. Rollason, T.P. and Marshall , T (1989) Nucleolar Organiser regions in adenocarcinoma in situ of the endocervix . Journal of Clinical Pathology , 42 : 1276 - 1280
'Please refer to statement of work carried out' (Chapter 1- Page 7 of thesis)

[B]
Publications arising from study of CIGN but not directly related to thesis work

1) Rollason, T.P. Cullimore , J.E. and Bradgate, M (1989) A suggested columnar cell morphological equivalent of squamous carcinoma in situ with early stromal invasion . International Journal of Gynaecological Pathology , 8 , 3 : 230-236

2) Cullimore , J.E. Luesley, D.M. Rollason , T.P. Waddell, C and Williams , D.R. (1989) A case of glandular intraepithelial neoplasia involving the cervix and vagina . Gynaecologic Oncology , 34 : 249 - 252

These 2 publications arose as a result of research into CIGN and endocervical carcinoma.
My role in the first of these publications was of authorship of the clinical case reports on the 3 patients , and criticism of the draft stages of the paper prior to the writing of the final report.
This paper describes a small series of subjects with adenocarcinoma in situ (AIS)

who also demonstrated foci which were indistinguishable from squamous early stromal invasion , which arose directly from AIS. (See figure 1-6 , page 53). These observations support the theory that squamous and glandular neoplasms may have similar aetiological factors.

For the second publication , I collected all the data on this subject and was author of the preliminary draft and the final report , following criticism by my co-authors. This paper constitutes only the second case of vaginal intraepithelial glandular neoplasia ever described , and it was associated with CIGN. This case report suggests the need for cytologic and colposcopic assessment of the vagina in subjects with CIGN.

OTHER CONTRIBUTIONS TO MEDICAL LITERATURE

(i) Relating to the secondary prevention of cervical carcinoma.
(ii) Relating to other areas of Obstetrics and Gynaecology

Contributions relating to the secondary prevention of cervical carcinoma.

1) Cullimore , J.E. Marshall,T and Woodman C.B.J. (1988) Can risk factors be identified for failure of laser treatment to the cervix? British Journal of Obstetrics and Gynaecology , 95 : 1206 - 1208

This was the first of a group of publications concerning research into failure of local destructive treatment of cervical intraepithelial neoplasia. My role in this study was of collection and analysis of data . I performed the statistical calculations in conjunction with Dr. Marshall. I prepared the first and subsequent drafts of this paper as well as the final report.
This paper was the first to systematically address the question why some laser treatments failed. It confirmed a widely held belief that high grade lesions are more difficult to eradicate successfully , and provided confirmation that inadequate depth of destruction is a risk factor for failure. In addition , discomfort experienced by the patient during the operative procedure was , for the first time , highlighted as a risk factor for treatment failure.

2) Cullimore , J.E. Woodman C.B.J. Luesley , D.M. Jordan , J.A. and Byrne,P (1989) When laser vaporisation for CIN fails , what next? Lancet , i : 561-562

This study was the first ever prospective assessment of re-treatment of individuals who had documented failure of laser vaporisation. My role was to recruit suitable patients , to perform cytological and colposcopic assessment prior to re-treatment , which took the form of cone biopsy or loop diathermy excision. I shared the treatment of these patients with my co-authors. I analysed the data and prepared the first and subsequent drafts of this paper prior to producing the final report.
The conclusions were :
a) Invasive disease was encountered in 5% of laser failures,
b) The frequency of unsuspected invasive disease treated by laser vaporisation was 0.19%,
c) 'Laser failures' are best manged by cone biopsy rather than repeat laser treatment.

3) Cullimore , J.E. Rollason , T.P. Luesley, D.M. Ward,K Waddell, C and Jordan,J.A. (1990) Invasive cervical cancer after laser vaporisation for cervical intraepithelial neoplasia : A 10 year experience . Journal of Gynecologic Surgery , 6: 2, 103 - 110

I instigated this investigation to assess the numbers of patients and the likely reasons for the occurrence of invasive cancer following laser treatment. I collected and analysed all the data and wrote the initial draft of the paper. Following criticisms by co - authors and advice on histopathological terminology , I produced the final report.
This case study provided a reasonably accurate estimate of the frequency of invasive cancer after laser vaporisation (0.22%) and discussed potential reasons for failure.

4) Ferryman , S.R. Rollason , T.P. and Cullimore ,J.E. (1990) A simple morphometric study of cone biopsies following failure of laser ablation therapy for cervical intraepithelial neoplasia . Journal of Obstetrics and Gynaecology , 10 : 5 , 440 - 443

My role in this study was:
i) identification , assessment and re-treatment of patients as outlined above
ii) Authorship of the clinical aspects of the 'patients and methods' section
iii) Criticism of the preliminary and final drafts of the paper (which were prepared by the first author)
This study indicates that the effect of laser vaporisation is to cleanly excise a ring of cervical tissue with the formation of a new squamocolumnar junction deeper into the endocervical canal. The study provided no evidence for 'concealment' of residual disease in 'buried' cervical crypts.

5) Luesley , D.M. and Cullimore , J.E. (1988) The treatment of cervical intraepithelial neoplasia . Cancer Surveys , 7 , 3 : 529 - 545 (Invited Chapter)

This chapter formed a contribution to a work on the preventitive aspects of cervical carcinoma. My authorship consisted of the sections devoted to : 'pre-treatment assessment' (II) and treatment failure (IV)

6) Prendiville , W Cullimore , J.E. and Norman , S (1989) Large Loop excision of the transformation zone (LLETZ) . A new method of management for women with cervical intraepithelial neoplasia . British Journal of Obstetrics and Gynaecology , 96 : 1054 - 1060

My role in this work was of participation in the assessment of this technique of treatment which was first developed in the clinic of which I was a member. This work comprises the results of the first 111 patients managed by the technique. I personally treated approximately 20% of these subjects , and followed up a similar proportion after treatment. I was involved in the re-drafting of this paper prior to its submission.

<u>Contributions relating to other areas of Obstetrics and Gynaecology</u>

1) Cullimore , J.E. Roland ,J and Turner ,G.M. (1990) The management of diabetic pregnancy in a regional centre. A five year review. Journal of Obstetrics and Gynaecology , 10 : 171 - 175

I was responsible for the collection of all the data for the study . I analysed these data , and drafted the first report. Following criticism by co-authors , I prepared the final report for submission.
This article was essentially an audit of clinical practice . However , it provided evidence in support of the use of home based management of pregnant diabetics.

2) Davies , W.A.R. and Cullimore , J.E. (1985) Entrapment of the fetal head in a unilateral imperforate vagina in association with complete duplication of uterus and cervix. Case report

This report concerns the unique case of a patient managed by the authors. I prepared the initial draft of this paper , which included a review of the world literature on the subject.

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Nucleolar organiser regions in adenocarcinoma in situ of the endocervix

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J Clin Pathol 1989;**42**:1276–1280

Nucleolar organiser regions in adenocarcinoma in situ of the endocervix

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SUMMARY The AgNOR technique was used to analyse 11 cases of adenocarcinoma in situ of the endocervix and five examples of healthy cervices to assess whether areas of "increased nuclear activity" could be located adjacent to the malignant tissue. Areas of adenocarcinoma in situ had significantly more AgNOR staining dots than apparently normal bordering areas ("transitional areas") and areas of endocervical epithelium remote from adenocarcinoma in situ. There were no significant differences between AgNOR counts in transitional areas and areas remote from adenocarcinoma in situ, and between these areas and histologically normal cervices.

These observations provide no support for the hypothesis that areas of glandular atypia of lesser severity or zones of "increased nuclear activity" exist adjacent to adenocarcinoma in situ.

Adenocarcinoma in situ is believed to be a precursor of invasive cervical adenocarcinoma. It is an uncommon lesion, although its true incidence has probably been underestimated.[1] Various authors have found that glandular atypia of the cervix was strongly associated with cervical squamous intraepithelial neoplasia (CIN),[2] and one group observed glandular atypia of lesser severity than adenocarcinoma in situ in crypts adjacent to areas of CIN3 in 15% of cases.[3] Although we have not observed such a high incidence of cervical glandular atypia in association with CIN3, we might have overlooked most glandular atypias. Given the observation by the same workers that adenocarcinoma in situ was found separated from normal crypts by intervening areas of lesser grades of atypia, we investigated whether there are areas adjacent to adenocarcinoma in situ which show differences in the numbers of nucleolar organiser regions (NORs), as measured by the silver impregnation (AgNOR) method. For if it could be shown that there was a measurable change in nuclear "activity", this might support the concept of the existence of a transitional zone of pre-neoplastic mucosa which is not easily recognisable on routine histological staining. We also felt that it would be useful to determine whether the preferred sites for neoplastic transformation—that is, the crypts adjacent to the squamocolumnar junction—differed from the rest of the endocervix by using the same technique in healthy cervices.

Accepted for publication 27 July 1989

Material and methods

Cases of classic adenocarcinoma in situ were identified by one of us (TPR) from the pathology files of the Birmingham and Midland Hospital for Women. Sections 3 μm thick were cut from routinely processed paraffin wax blocks. These were dewaxed in xylene and hydrated through ethanols to double distilled deionised water. A staining solution was prepared which consisted of gelatin dissolved in 10 g/l aqueous formic acid at a concentration of 20 g/l, mixed with 500 g/l aqueous silver nitrate (1 volume gelatin/formic acid to 2 volumes silver nitrate). This mixture was poured over tissue sections which were left in a humidity chamber in the dark for one hour at room temperature. The silver colloid was then washed off with deionised water and sections were counterstained with haematoxylin. Sections were dehydrated to xylene and mounted in synthetic medium. The sections were viewed under a × 100 oil immersion lens using a green filter and counts were made of discrete AgNOR dots within nuclei. In counting each cell nucleus, the focus control was carefully adjusted to allow the dots to be counted. One hundred cells were counted in each area chosen. Large mulberry shaped aggregates were always counted as one dot.

Sections of histologically normal cervices were stained and AgNOR counts were made in endocervical epithelium (i) immediately adjacent to and beneath the squamocolumnar junction and (ii) at least 5 mm distant from the squamocolumnar junction along the axis of the endocervical canal. Cells were

Table 1 *Mean numbers of AgNOR dots within nuclei with median numbers in different areas of diseased cervices*

Case No	Adenocarcinoma in situ	Transitional	Distant	Partially affected glands	
				Adenocarcinoma in situ	Normal
1	4·9 (5)	1·4 (1)	1·5 (1)	3·9 (3)	1·0 (1)
2	3·6 (4)	1·9 (2)	1·0 (1)	2·7 (3)	1·2 (1)
3	4·0 (4)	1·3 (1)	1·3 (1)	3·5 (3)	1·5 (1)
4	7·5 (8)	1·2 (1)	1·1 (1)	9·0 (8)	1·3 (1)
5	1·3 (1)	1·3 (1)	1·2 (1)		
6	4·9 (5)	1·1 (1)	1·1 (1)		
7	5·3 (5)	1·4 (1)	1·2 (1)		
8	4·5 (4)	0·6 (1)	0·9 (1)		
9	6·1 (6)	1·1 (1)	1·2 (1)	5·4 (5)	1·0 (1)
10	5·2 (5)	0·9 (1)	1·1 (1)	4·9 (5)	1·1 (1)
11	3·9 (4)	1·2 (1)	1·3 (1)		

chosen "randomly" within the defined areas but the selection method excluded a standard random sampling technique.

ADENOCARCINOMA IN SITU

Counts were made in the following areas:

(i) In endocervical epithelium showing obvious adenocarcinoma in situ. In every case these areas were closely related to the endocervical border of the squamocolumnar junction.

(ii) In endocervical epithelium not more than 1 mm away from the neoplastic cells, subsequently referred to as "transitional" areas.

(iii) In histologically normal endocervical crypts at a distance of at least 5 mm further along the axis of the endocervical canal, subsequently referred to as "distant" areas.

(iv) In all cases where crypts were partially affected by adenocarcinoma in situ these were included in the counting to include transitional areas as close as possible to the neoplastic cells.

The differences between AgNOR counts in different areas were assessed by analysis of variance, using a square root transformation of the counts.[4]

Results

Eleven cases of cervical adenocarcinoma in situ and five examples of normal cervices from hysterectomy specimens removed for non-cervical pathology were assessed. After counting 100 cells in each category the mean number of AgNOR dots in each cell nucleus was calculated. The results are recorded in tables 1 and 2 and illustrated in figs 1–3. In every case of adenocarcinoma in situ except one the numbers of AgNOR dots within cell nuclei of the neoplastic epithelium were increased compared with those of normal controls (table 1). The differences in AgNOR counts between adenocarcinoma in situ and all other areas were significant ($p < 0·01$). The AgNOR staining dots in the histologically normal areas usually appeared as well defined rounded areas; in the areas with adenocarcinoma in situ the AgNORs were smaller, sometimes irregular, and dispersed throughout the whole nucleus.

There were no significant differences between transitional and distant areas, including those transitional areas immediately adjacent to adenocarcinoma in situ tissue which occupied only part of a crypt. There were no significant differences between distant areas in diseased cervices and normal cervices. There were also no significant differences in AgNOR counts at different sites in the group of normal cervices examined (table 2).

Table 2 *Mean and median numbers of AgNOR dots in normal cervices*

Case No	At squamocolumnar junction	> 5 mm from squamocolumnar junction
1	1·3 (1)	1·2 (1)
2	0·9 (1)	1·0 (1)
3	1·4 (1)	1·6 (1)
4	0·9 (1)	0·7 (1)
5	1·0 (1)	0·9 (1)

Discussion

Nucleolar organiser regions (NORs) are chromosomal segments in which ribosomal RNA is encoded and are the interphase equivalent of the pars amorpha within the nucleolus. NORs can be located by silver staining, hence the term AgNORs. The stain identifies acidic proteins associated with the NOR. In some malignant diseases AgNOR counts are significantly greater than in normal or benign conditions at the same site, and this may be in part because in malignancy NORs become dispersed throughout the nucleus. The AgNOR count is therefore more likely to be a measure of nucleolar dispersal than of an absolute increase in nucleolar material. It has been suggested that AgNOR counting can be used to distinguish between high and low grade lymphomas[5] and benign melanocytic lesions and malignant melanoma[6] among

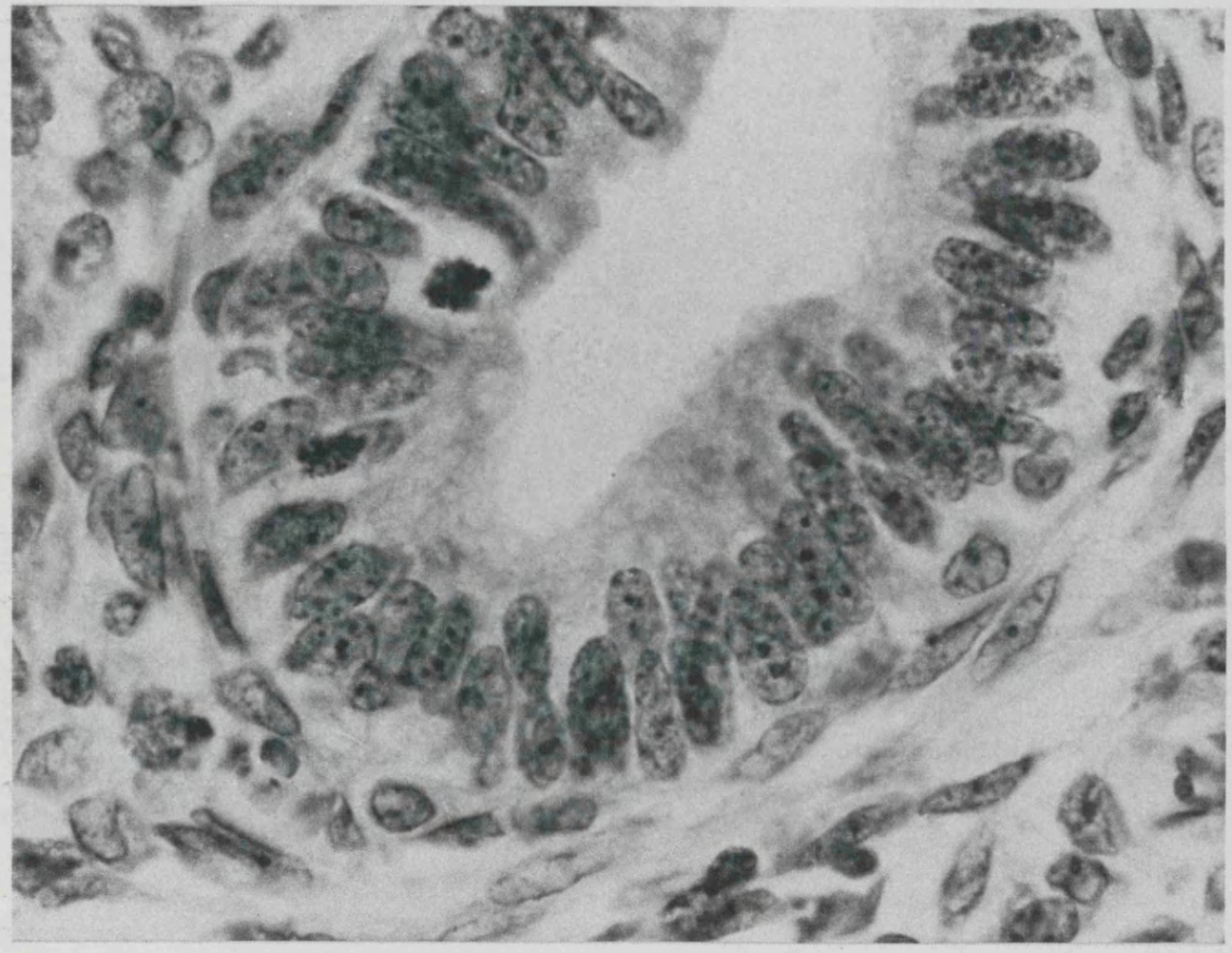

Fig 1 *Adenocarcinoma in situ of cervical gland crypts stained by AgNOR technique. Multiple small NORs are seen in nucleii.*

other conditions.[7,8] All these studies have concentrated on conditions distinguished for the purpose of investigation by standard histological techniques. It might therefore be argued that the AgNOR technique has simply been used to confirm an already established diagnostic method. In this study we used the technique simply to attempt to detect epithelial change adjacent to adenocarcinoma in situ with no predetermined histological differences evident. To our knowledge, this is the first time the technique has been used in this way.

It has been suggested that in areas adjacent to adenocarcinoma in situ of the cervix there is a transitional zone of epithelial atypia of lesser grade than adenocarcinoma in situ, which separates adenocarcinoma in situ from histologically normal epithelium.[9] The extent of these areas was not stipulated by the authors. While we certainly do not deny the existence of glandular atypias of lesser grade than adenocarcinoma in situ, we have not been able to confirm such atypia commonly occurring adjacent to areas of adenocarcinoma in situ, which usually distinctly abut on adjacent histologically normal glands. The presence of a broad zone of neoplastic potential, however, might have implications for the treatment of these lesions, especially if such a zone extends more than a few millimetres from the microscopically diseased areas. In our experience clearly identifiable glandular atypia of lesser severity or grade than adenocarcinoma in situ is no more common than adenocarcinoma in situ itself, and the differentiation of the conditions is difficult. As the results of AgNOR counting show no appreciable difference in counts between areas immediately adjacent to adenocarcinoma in situ and distant from it this offers no support for the possibility that we may be failing to appreciate subtle histological changes in glands adjacent to adenocarcinoma in situ. This series examined typical cases of adenocarcinoma in situ, unlike that of Brown and Wells, who looked for glandular atypias in patients with CIN3. To date, we have not applied the AgNOR technique to examine

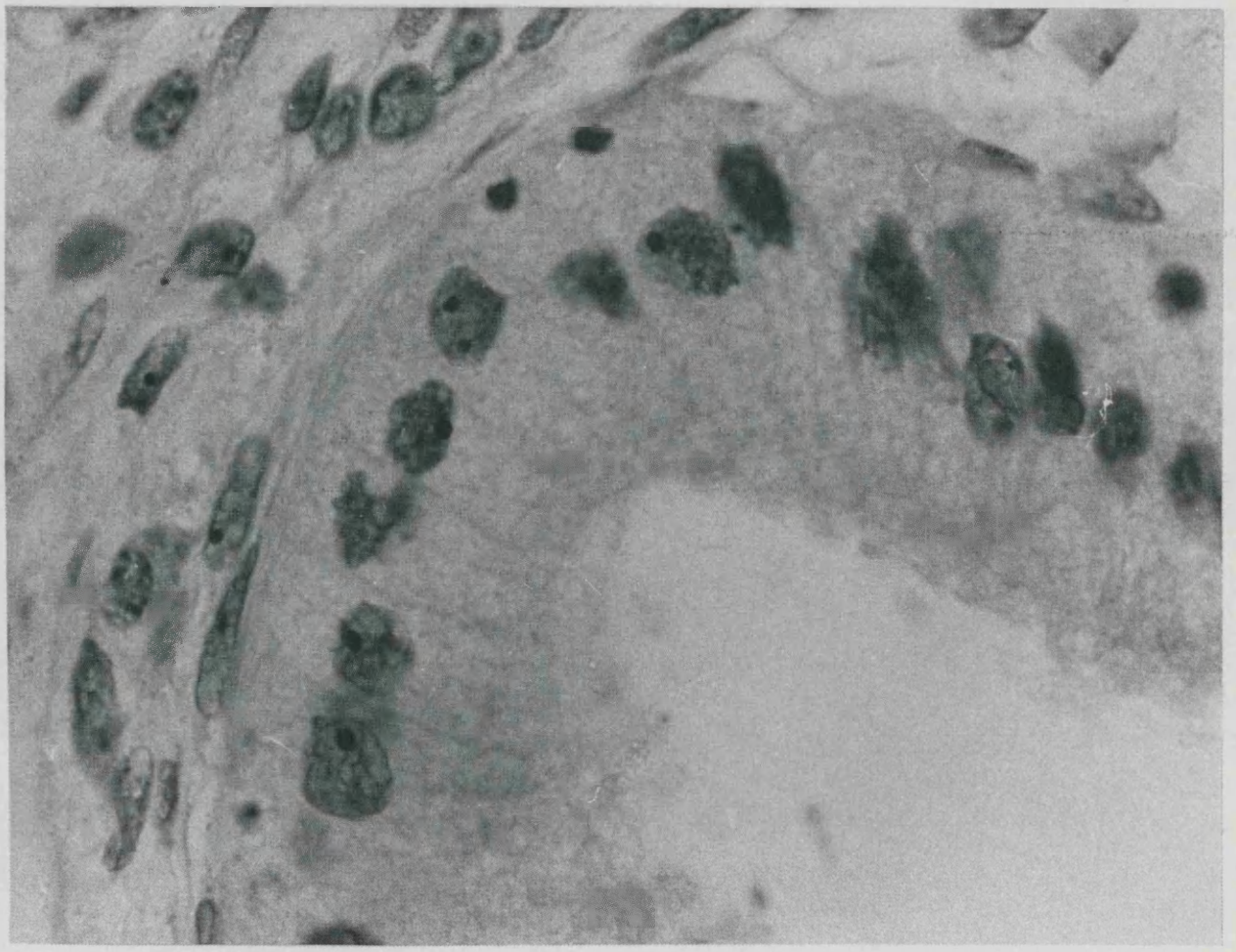

Fig 2 *Histologically normal gland crypt stained by AgNOR technique. A solitary large NOR is seen in nucleii.*

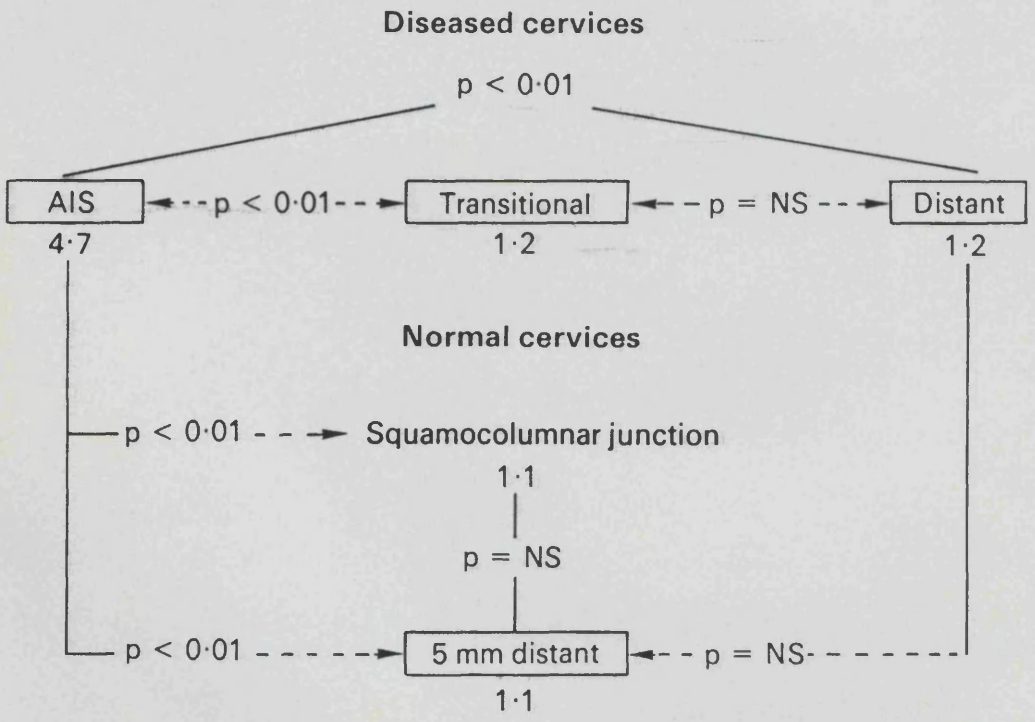

Fig 3 *Mean numbers of Agnor dots in each cell in different areas and significance of differences.*

the cervical crypts in cases of CIN3, but if there was a broad glandular change indicative of pre-malignancy we might expect to see lesser degrees of glandular atypia in adenocarcinoma in situ more readily than in cases of CIN3.

We failed to appreciate any difference in AgNOR staining at differing sites within the cervices of normal subjects and, therefore, there seems to be no intrinsic difference in the degree of nuclear activity as measured by the AgNOR method at different sites in the endocervix in the normal subject.

We would not extend our findings on this small group of cases using this additional technique to deny the presence of a transitional zone adjacent to adenocarcinoma in situ or CIN, but the present study in our opinion adds no weight to the debate in favour of its existence.

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A Suggested Columnar Cell Morphological Equivalent of Squamous Carcinoma In Situ with Early Stromal Invasion

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Summary: Cervical squamous carcinoma in situ with early stromal invasion has recently been defined as a strict histologic entity. Two cases of extensive classical adenocarcinoma in situ of the uterine cervix and one of very early invasive adenocarcinoma are reported that showed foci indistinguishable from squamous early stromal invasion arising directly from unequivocally glandular in situ malignant foci. This finding is further evidence in support of the theory that glandular and squamous intraepithelial neoplasia in the cervix are closely related conditions, possibly with a common origin in the subcolumnar reserve cell. Key Words: Cervix—Adenocarcinoma in situ—Intraepithelial neoplasia—Microinvasive carcinoma.

The determination as to whether early invasion is present in cases of cervical adenocarcinoma in situ (high-grade glandular intraepithelial neoplasia) is difficult and highly subjective. Suggested criteria for the diagnosis of "microinvasion" have been presented by several authors (1–3), but the problem remains acute, particularly with the recent increased awareness of adenocarcinoma in situ by both histopathologists and cytopathologists.

In the case of cervical squamous neoplasia, the adoption by many pathologists of the recently published Royal College of Obstetricians and Gynaecologists study group classification (4), which separates microinvasive carcinoma into two categories (early stromal invasion and "measurable lesions") on the basis of depth of invasion and tumour confluence, has greatly clarified the previously confused situation. The following three cases, all seen in patients appearing for primary treatment at one hospital in the past 2 years, illustrate what we believe may be a columnar cell morphologic equivalent of early stromal invasive disease. We have also seen a fourth virtually identical case in consultation.

CASE REPORTS

Case 1

A 25-year-old woman, parity 1 + 1, was referred for colposcopic assessment after a smear that showed primitive undifferentiated cells with both squamous and glandular features.

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Colposcopy revealed a large cervical transformation zone. A raised area with frond-like projections and atypical vascular appearances was seen. This showed intense whitening after application of acetic acid and was iodine negative. She underwent wedge biopsy under general anaesthesia, and a Wertheim's hysterectomy was later performed.

At 3-month follow-up examination, there are no signs of recurrence.

Case 2

A 31-year-old woman, parity 3 + 2, was referred after a smear had shown "deep dyskaryosis . . . with some dyskaryotic endocervical cells." There was a history of several months of intermenstrual and postcoital bleeding. Colposcopy revealed a raised, friable lesion with atypical vasculature on the lower lip of the cervix. The lesion exhibited marked acetowhitening and was iodine negative. Conisation was performed.

At 4-month follow-up examination, colposcopy showed an acetowhite patchy lesion, and an abdominal hysterectomy was later performed. She is well at follow-up examination 18 months after conisation.

Case 3

A 38-year-old woman, parity 3 + 0, was referred for colposcopy after a routine cervical smear had shown changes consistent with CIN 3. In the visible transformation zone, there was an acetowhite area that failed to stain with Schiller's iodine. The vascular pattern was normal. A smear taken at the time of the colposcopy showed "undifferentiated dyskaryotic cells and severe keratinizing squamous cell dyskaryosis," and the possibility of a mixed glandular and squamous lesion was raised. Conisation was subsequently performed.

She later underwent an abdominal hysterectomy and bilateral salpingo-oophorectomy. The patient is free of disease at 2-year follow-up examination.

MATERIALS AND METHODS

All specimens were fixed in Bouin's fluid and embedded in paraffin wax. Sections were cut at 4 μm and stained with haematoxylin and eosin. Serial sections were cut from the foci of particular interest and representative sections stained by the diastase–periodate-Schiff's method.

PATHOLOGIC FINDINGS

Case 1

Wedge biopsy. Extensive, incompletely excised, in situ adenocarcinoma was present involving both the surface epithelium and gland clefts. Both endometrioid and goblet cell differentiation was seen. Small foci were seen in one area that showed tongue-like extensions into the inflamed and oedematous adjacent stroma (Fig. 1). These tongues were strikingly eosinophilic in contrast to the clearly and unequivocally glandular epithelium from which they arose (Fig. 2). The pattern was strikingly similar to the usual appearance seen in squamous carcinoma in situ with early stromal invasion. There were foci of koilocytosis in the squamous epithelium, favouring human papilloma virus infection, but no CIN was evident.

Wertheim's hysterectomy specimen. Extensive but completely excised adeno-

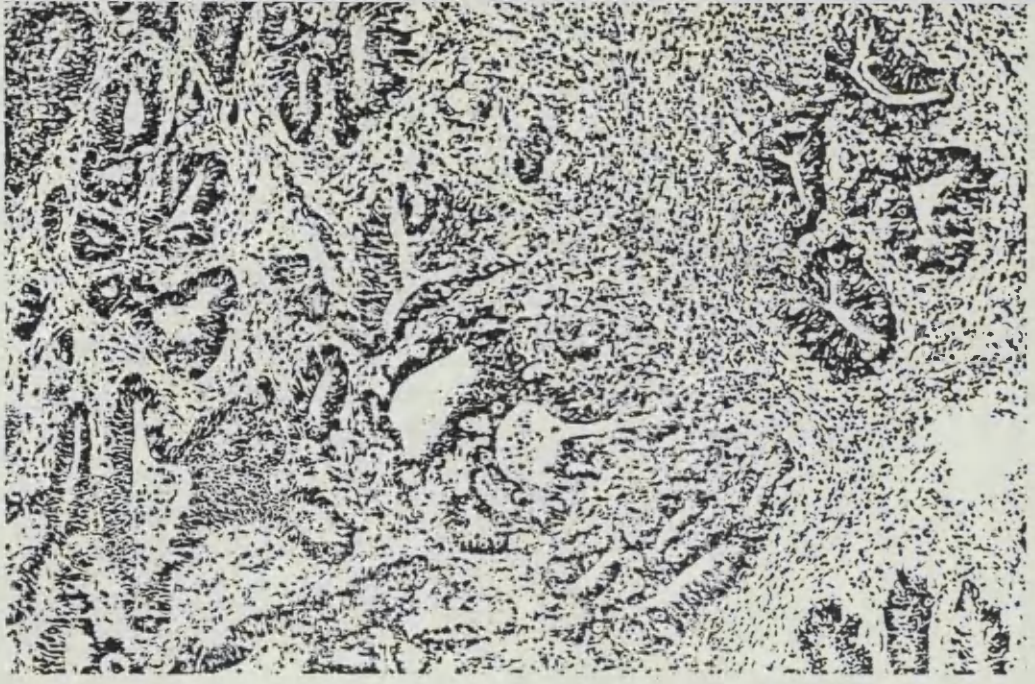

FIG. 1. Case 1. There is intense inflammation seen related to small, pale tongues of malignant glandular epithelium. The glandular architecture is clearly abnormal (haematoxylin–eosin, ×180 approximately).

carcinoma in situ was again present. The whole circumference of the cervix was involved, and in one area several very early invasive foci, similar to those seen in the wedge biopsy sample, were present. A small focus of CIN 3 was present in a separate quadrant, but no residual human papilloma virus-associated changes

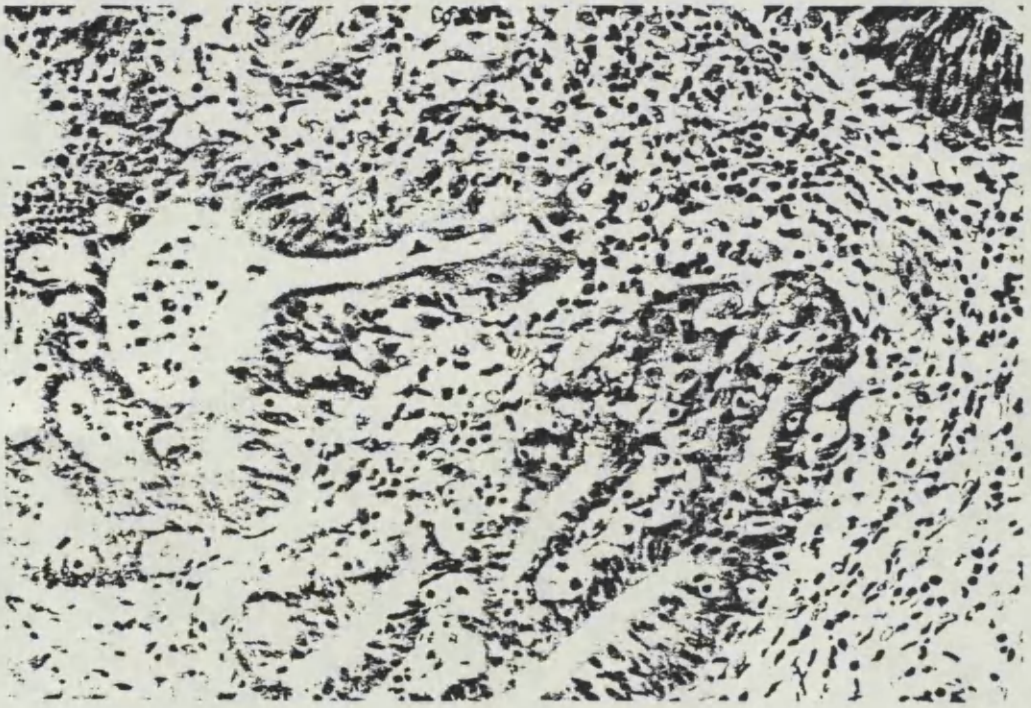

FIG. 2. Case 1. Higher magnification shows the small tongues to be composed of pale "squamoid" cells with large pale nuclei (haematoxylin–eosin, ×450 approximately).

were seen. No vessel involvement was present. Lymph nodes, parametrial, and other extrauterine tissues were all free of disease.

Case 2

Cone biopsy. The cone measured 4.5 by 2.5 cm at the base and was a maximum of 2.5 cm deep. The endocervical canal exited a minimum of 1.3 cm from the nearest ectocervical resection margin (i.e., the cone was eccentric). Extensive adenocarcinoma in situ was seen encircling the majority of the cone circumference and involving both the surface mucosa and the gland clefts. In one area, arising from the glandular cells were foci of very early invasion of the stroma (well within 1 mm of the parent epithelial base), and exactly the same "squamoid" eosinophilic pattern was seen as described in the previous case (Fig. 3). Despite the very shallow invasion, a small vessel space was involved at one point. Excision of the glandular neoplasia appeared complete.

Scattered foci of CIN, including a zone of CIN 3, were also seen but were clearly separated from the adenocarcinoma in situ. The CIN extended to the endocervical resection margin.

Hysterectomy specimen. No residual CIN or glandular neoplasia was found.

Case 3

Cone biopsy. The specimen measured 2 by 1.5 cm at the base and was 2 cm deep. The endocervical canal exited a minimum of 1.4 cm from the nearest ectocervical margin. Areas of distinct CIN 3, adenocarcinoma in situ, and intimately admixed CIN/glandular dysplasia (5,6) were evident around the whole circumference of the cone, but gland cleft involvement by classical adenocarcinoma in situ was most widespread. At one point, the foci of glandular neoplasia extended to 6 mm from the surface mucosal base, and this finding, together with the altered

FIG. 3. Case 2. Large, pale multinucleate "squamoid" cells arise from architecturally atypical, malignant glandular elements (haematoxylin–eosin, ×450 approximately).

architectural pattern, favoured early invasion of the usually described type. Nearer to the surface there were, however, separate areas showing the tongue-like processes and eosinophilia seen in the previous cases (Figs. 4 and 5), and an inflammatory reaction was also seen here. No vessel–space involvement was evident, and excision of all abnormal epithelium appeared complete.

Hysterectomy specimen. No relevant pathologic findings were seen.

DISCUSSION

The Royal College of Obstetricians and Gynaecologists study group proposal defines squamous early stromal invasive carcinoma as tumor ". . . in which invasive buds are present either in continuity with an in-situ lesion, or apparently separated cells not more than 1 mm from the nearest surface or crypt basement membrane." To our knowledge, no one has applied the term "early stromal invasion" to adenocarcinoma in this strict sense, and where the term "microinvasion" has been used it has often not been clear what criteria were used in coming to this diagnosis (7). Burghardt, in his exhaustive account of the pathologic appearance of early cervical cancer (1), indicates that in only one isolated case did he see solid epithelial buds arising from adenocarcinoma in situ. His impression was that these represented the earliest stage of glandular epithelial invasion. He illustrates in one photograph (page 357) a process that appears very similar, if not identical, to that in the cases we have presented. We have been able to find only one other similar illustration (8). While it is undoubtedly the case that the "usual" form of invasive adenocarcinoma starts to invade as solid cell groups without a central lumen, it should be stressed that the major difference between that appearance and the one seen in the cases reported here is the eosinophilic

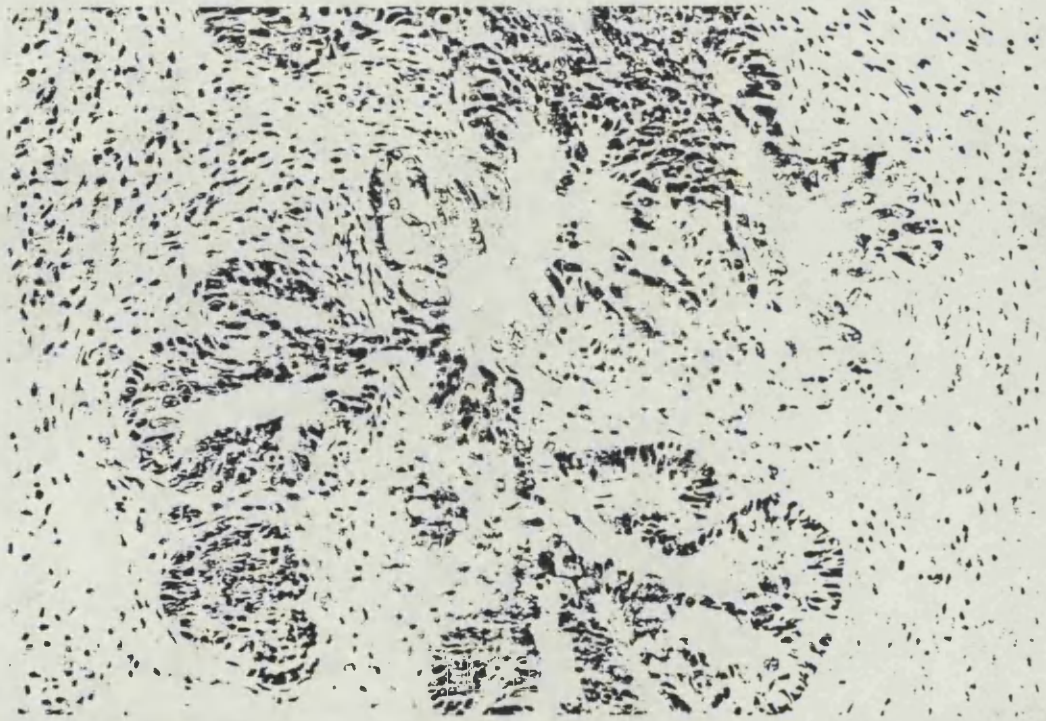

FIG. 4. Case 3. Multiple foci of differentiation to a pale cell form are seen in this group of glands showing adenocarcinoma in situ with architectural atypia (haematoxylin–eosin, ×240 approximately).

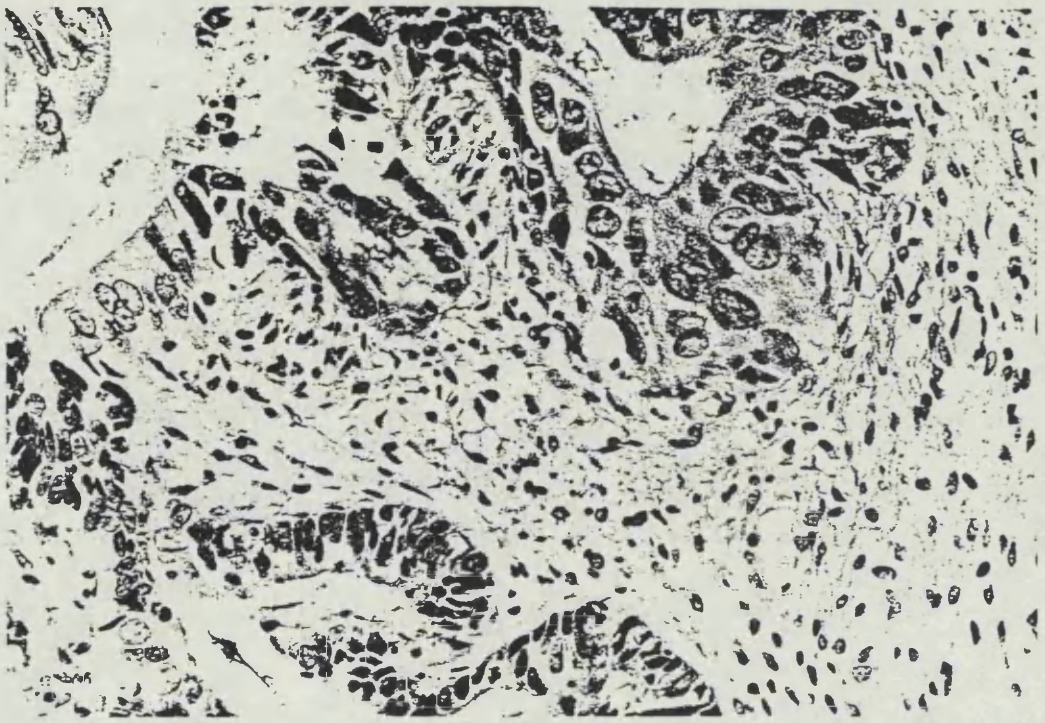

FIG. 5. Case 3. The pale cells again show enlarged, vesicular nuclei, fine cell membranes, and multinucleation (haematoxylin–eosin, ×570 approximately).

"squamoid" cytoplasmic staining and nuclear enlargement that are so characteristically seen in squamous carcinoma in situ with early stromal invasion. We would suggest that this morphologic similarity adds further weight to the theory that both squamous and glandular cervical intraepithelial neoplasia have a common origin in the subcolumnar reserve cell, as opposed to the view that other conditions, such as microglandular hyperplasia, antedate adenocarcinoma (9).

The common coexistence of cervical squamous and glandular carcinoma in situ, seen in two of our cases, also favours a common reserve cell origin, as does the existence of a mixed adenosquamous in-situ variant (2,5,6), which was evident in case 3 (which also showed "pure" adenocarcinoma and squamous carcinoma in situ).

The generally accepted histologic changes accompanying early invasion in adenocarcinoma have been stated to be exuberant glandular budding, confluent glandular foci, an inflammatory cell and stromal cellular response, extension of the glands beyond the normal maximal gland cleft depth, and papilla formation (2). These changes in our experience also have been of most benefit in determining whether invasion was present, and foci meeting some of these criteria were evident in one of the presented cases remote from the "squamoid" foci. What we suggest is that the foci we illustrate simply represent an altered pattern of morphologic expression, from glandular to squamous, and not the "usual" pattern of early invasion, as an in-situ tumour able to express both differentiation patterns begins to invade the stroma.

Whether the findings described have any prognostic significance remains to be seen. This would, in any event, be dependent on the gynaecologist's willingness to adopt a more conservative approach to the treatment of adenocarcinoma than is presently usually the case.

Acknowledgment: We wish to thank Mr. Alan Cooper for his invaluable photographic assistance. Our thanks also go to the clinicians of The Birmingham and Midland Hospital for Women for allowing us to publish details of their cases and to the laboratory staff of the same hospital for technical assistance.

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GYNECOLOGIC ONCOLOGY 34, 249–252 (1989)

CASE REPORT

A Case of Glandular Intraepithelial Neoplasia Involving the Cervix and Vagina

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A case of high grade glandular intraepithelial neoplasia (GIN) of the vagina is described. This lesion developed 5 years after hysterectomy, which had been carried out because of histologically incomplete excision of cervical adenocarcinoma *in situ*, despite two conizations. The vaginal lesion was treated by local excision and subsequent radiotherapy to the vagina. The literature contains reference to only one case of vaginal adenocarcinoma *in situ*, which was successfully treated by local excision. The possible histogenesis of this lesion is discussed and recommendations made for follow-up of patients who have received treatment for high grade cervical or vaginal GIN. © 1989 Academic Press, Inc.

CASE REPORT

The patient was initially referred in 1964 having had a positive cervical smear. She was 37 years old, gravida 12, parity 9, and she had been taking the oral contraceptive pill for 3 months prior to the consultation. There was no history of intrauterine exposure to DES (Diethylstilboestrol). Cervical cytology at consultation showed dyskaryotic squamous cells and grossly enlarged endocervical cell nuclei. A cone biopsy was reported as showing "marked epithelial dysplasia." Cervical mucus glands were noted to be plentiful in number, but not abnormal. Subsequent review of this specimen has shown that extensive areas of classical adenocarcinoma *in situ* (AIS) were present together with zones of glandular dysplasia of lesser severity (Fig. 1). Areas showing the goblet cell or Type 2 variant of AIS [4] were prominent. A small focus of CIN II was evident in an area of incomplete, i.e., partial thickness, squamous mucosa in one area of the cone. No invasion was evident but excision of the glandular dysplasia appeared incomplete on the endocervical margin.

Annual follow-up cervical cytology remained normal for the next 10 years. The patient was referred back to gynecology OPD in 1978, at age 51, complaining of postmenopausal bleeding. She was noted to have atrophic cervico-vaginitis. A diagnostic curettage failed to disclose an endometrial source for this bleeding. Two years later cervical cytology was reported as showing atypical columnar cells. Colposcopic examination in 1981 revealed some atypical vasculature on the ectocervix. The squamo-columnar junction was not visualized. Cervical cytology performed just prior to this examination revealed many groups of malignant appearing columnar cells suggestive of adenocarcinoma. Repeat cone biopsy (dimensions 2 × 1.2 × 2.5 cm deep) and diagnostic curettage were carried out. In two transverse sections from the endocervical resection margin, adenocarcinoma *in situ* was again seen extending to the endocervical line of resection. The lesion involved most of the relatively small number of glands present and lined the whole circumference of the canal. There was no evidence of invasion.

Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed because of histologically incomplete excision of disease in the second cone. The patient recovered uneventfully from the operative procedure. Histopathological examination of the operative specimen revealed normal fallopian tubes and ovaries, a normal myometrium, and a small cervix, the squamous epithelium covering which was normal. Subsequent review and resectioning of this material has revealed that extensive AIS was present in the isthmic region extend-

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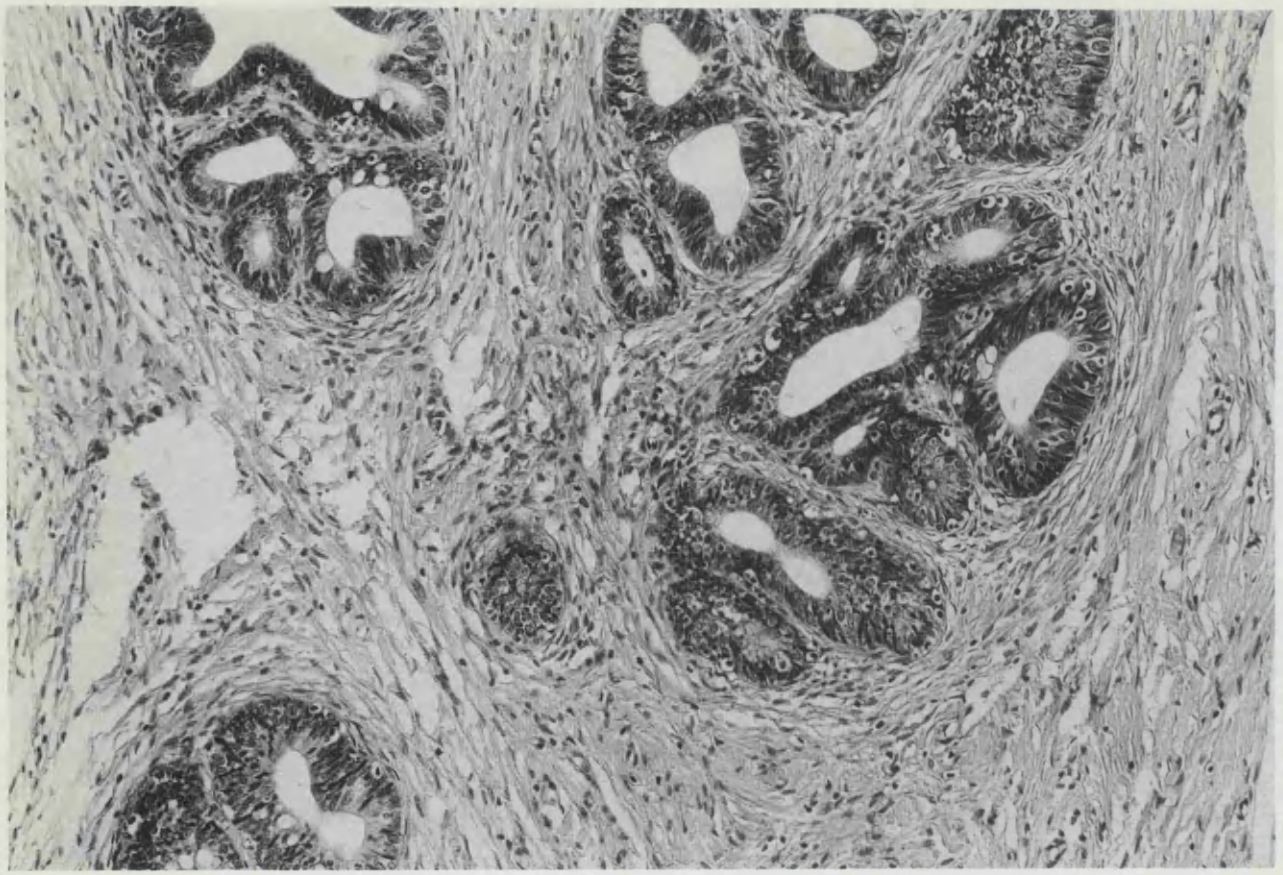

FIG. 1. Section from first conization specimen. Crowded glands lined by hyperchromatic pleomorphic cells showing focal goblet cell differentiation. No stromal response is seen (×200).

ing to replace the surface epithelium and some glands in the lower uterine segment. No invasion was evident and as previously indicated the ectocervical resection margins appeared free from glandular or squamous intraepithelial neoplasia. In 1986 cytology was abnormal, with many malignant cells of glandular origin (Fig. 2). Colposcopic examination revealed an area 10 × 5 mm situated in the left angle of the vaginal vault suture line, which bore a close resemblance to normal columnar epithelium. The area was biopsied, and microscopy showed normal squamous epithelium, with highly atypical glandular elements, representing adenocarcinoma *in situ* (Fig. 3). In view of this, and because the whole of the abnormal area was not removed by the biopsy, the patient was admitted for excision biopsy under general anesthesia.

The specimen excised consisted of both squamous and columnar epithelium. No dysplasia was seen in the squamous elements. The columnar mucosa showed marked atypia with nuclear crowding, representing high grade glandular intraepithelial neoplasia. No invasion was seen. In view of the possibility of residual disease in the vaginal vault, the patient was referred subsequently for radiotherapy and received two cylinders of intravaginal radium for 96 hr.

Cytology 1 year later revealed dyskaryotic metaplastic and columnar cells. Colposcopy of the vaginal vault revealed the presence of smooth vaginal vault mucosa containing superficial irregular corkscrew vessels, thought to be consistent with a postradiation effect [1]. Most recent vault cytology (March 1988) shows many abnormal glandular cells with active metaplastic cells. Colposcopic findings are unchanged. In view of this patient's current poor general condition (severe osteoarthritis, hypertension) no active intervention is planned at present.

DISCUSSION

Adenocarcinoma *in situ* of the cervix (AIS) is a relatively uncommon lesion. Christopherson *et al.* quote

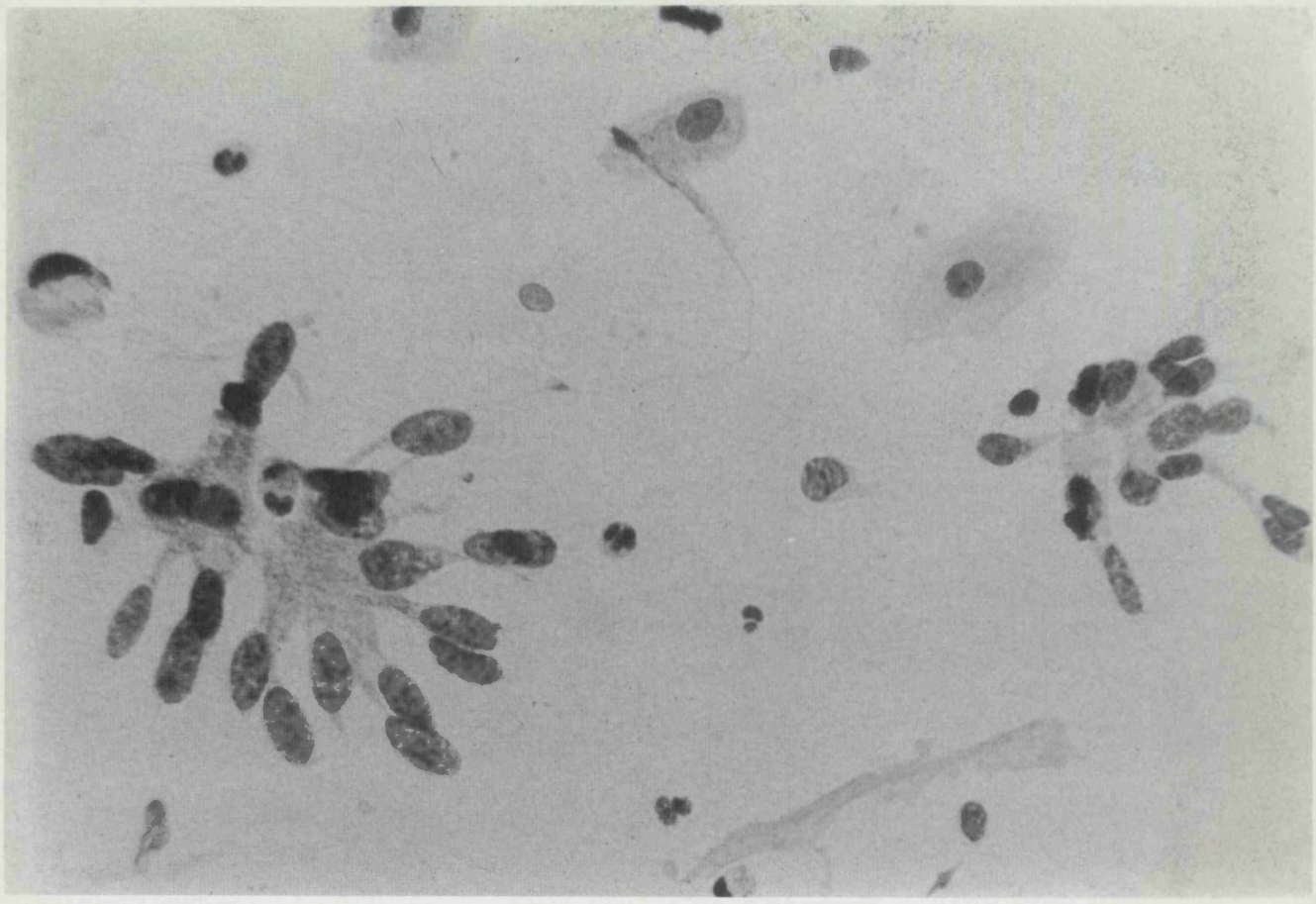

FIG. 2. Two clusters of abnormal endocervical cells demonstrating characteristic stellate formation, associated with adenocarcinoma *in situ* of the vagina (×1000).

an incidence ratio of 239 cases of squamous carcinoma *in situ* for every single case of AIS diagnosed [2].

However, adenocarcinoma *in situ* of the vagina has only been reported once previously in the literature by Clement and Benedet [3]. Their case was similar to ours in that it demonstrated *in situ* epithelial change in the cervix and vagina, and their patient's lesion was also brought to light after the finding of abnormal vaginal vault cytology several months after a total hysterectomy. The lesion was successfully treated by local excision. Our case is unusual in view of the rarity of this lesion, and also in that such an extensive distribution of glandular intraepithelial neoplasia has not previously been described.

There would seem to be three possible explanations for the histogenesis of this lesion. First, an area of extension of AIS to the ectocervical margin may have been missed in both the cone biopsies and the hysterectomy specimen. This seems highly unlikely although it is theoretically possible that when surgery was performed, some epithelium containing glandular dysplastic elements was implanted into the vagina at the time of suturing the bed of the cone biopsy. Second, it is possible that glandular dysplasia could have arisen in an area of vaginal adenosis, although the predominantly surface involvement in this case and the lack of any adjacent adenosis are against this, as, possibly, is the "endometrioid" differentiation of the glands. However, sporadic cases of vaginal adenosis can occur in women with no history of DES exposure [5]. Clement and Benedet [3] believed that the most likely explanation of the histogenesis of their case was that preinvasive epithelial transformation occurred within such areas.

Third, it is believed that in the transformation zone, both squamous and columnar epithelia are derived from a common precursor, the subcolumnar reserve cell. This raises the possibility as to whether squamous basal cell differentiation to a glandular epithelial variety may have occurred within the vaginal epithelium.

In summary, this case provides further evidence that women found to have high grade cervical glandular intraepithelial neoplasia (cGIN) are at risk of further preinvasive glandular changes in the lower genital tract. We therefore believe:

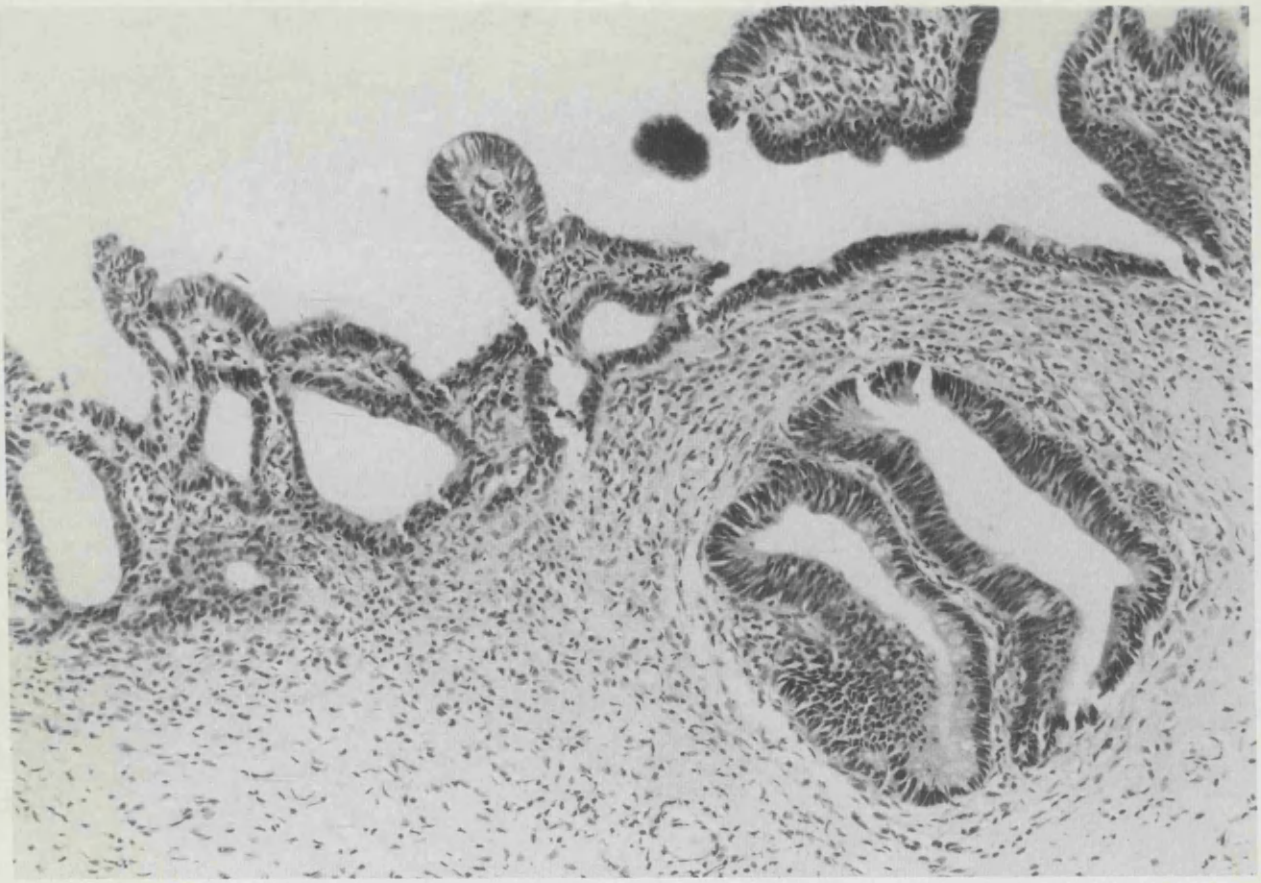

FIG. 3. Section from vaginal biopsy. The surface mucosa is thrown into folds and gland-like clefts are seen. The epithelium is pleomorphic (×200).

(a) that where dyskaryotic glandular elements are identified by cervical cytology, that careful colposcopic assessment is required of the vagina as well as the cervix;

(b) that when the diagnosis of adenocarcinoma *in situ*/high grade cGIN is made following hysterectomy or cone biopsy, that the patient requires careful follow-up with vault cytology (as well as cervical cytology post-conization), and if abnormal, careful colposcopic assessment of the upper vagina is indicated.

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Can risk factors be identified for failure of laser treatment to the cervix?

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Carbon dioxide laser vaporization of the cervical transformation zone is currently a popular method of treating cervical intraepithelial neoplasia (CIN). It is commonly performed as an outpatient procedure, and published success rates vary between 87% and 98% (Jordan *et al.* 1985). However, invasive disease following laser vaporization has been reported (Evans & Monaghan 1983). Various hypotheses have been advanced to explain treatment failure, but none has yet been tested. The aim of this study was to investigate factors possibly responsible for the failure of treatment.

Patients, methods and results

All patients in this study were treated in the outpatient department of the Women's Hospital, Birmingham, between 1984 and 1985. The selection of patients, technique of vaporization and the schedule of outpatient follow-up after treatment have been described previously (Jordan *et al.* 1985). Information relating to patient and disease characteristics, operative procedure and patient follow-up, were recorded on a form in routine use in the colposcopy clinic.

Laser treatment was considered to have failed when histological examination of a colposcopically directed biopsy or cone biopsy revealed CIN within 1 year of treatment (21 patients) or when repeated cytological and colposcopic examination showed abnormality to persist (3 patients).

Laser treatment was considered successful when repeated cytological and colposcopic examination detected no evidence of abnormality within 1 year of treatment.

Failures were identified by a search of registration forms for colposcopy clinic attenders between 1984 and 1985 inclusive. For each laser failure identified, the next four patients fulfilling the criteria for laser success were taken as controls. The following risk factors were studied: age, parity, grade of CIN, presence of human papillomavirus (HPV) infection (either cytological, colposcopic or histological evidence), histological evidence of HPV infection alone, seniority and experience of laser surgeon, use of local anaesthesia, depth of destruction achieved, discomfort experienced, and bleeding during treatment. Patients were not included in the study if the operator had recorded that the degree of bleeding was such as to prevent adequate completion of treatment or if the degree of discomfort experienced by the patient resulted in the procedure being abandoned.

As cases and controls were not actively matched with respect to any relevant characteristic, an unmatched analysis was appropriate. Relative risks (RR) for treatment failure were estimated by the odds ratio, and 95% confidence intervals for RR were calculated.

There were 24 cases and 96 control subjects in the study. The distribution of subjects according to the various factors, relative risks and confidence intervals are shown in Table 1. No factor was found to have a significantly elevated or reduced RR for laser failure, and exploration of subclasses of parity, grade of CIN and seniority of surgeon did not change this finding.

Discussion

The inexperience of the operator, age of the patient and the presence of HPV infection have

Table 1. Cases and controls by risk factors, relative risks (RR) and 95% confidence intervals (CI)

Factor	Failure (Cases) (n = 24)	Success (Controls) (n = 96)	RR	95% CI
Age (years)				
≥35	5	19	1·07	0·35 to 3·22
<35	19	77		
Parity				
≥3	4	13	1·28	0·38 to 4·34
<3	20	83		
Histology				
CIN III	15	46	1·81	0·72 to 4·54
Other	9	50		
Evidence of HPV infection				
Present	6	42	0·43	0·16 to 1·17
Absent	18	54		
HPV histology				
Positive	3	19	0·58	0·16 to 2·15
Negative	21	77		
Operator				
Consultant	8	28	1·21	0·47 to 3·16
Other	16	68		
Depth of destruction (mm)				
<7	2	4	2·09	0·36 to 12·15
≥7	22	92		
Local anaesthesia used				
Yes	2	5	1·66	0·30 to 9·10
No	22	91		
Discomfort noted				
Yes	17	53	1·97	0·75 to 5·19
No	7	43		
Bleeding noted				
Yes	7	48	0·41	0·16 to 1·08
No	17	48		

been reported to increase the risk of failure of laser treatment (Ali *et al.* 1986). Our point estimates do not support these opinions. They support but cannot confirm the importance of achieving an adequate depth of destruction (Andèrson 1982) and the increased risk of failure associated with CIN III lesions (Wright & Davies 1981).

In this study patient discomfort during treatment was associated with a higher risk of failure. We did not observe any beneficial effect from the local anaesthetic agent which was given to 10% of patients in this series, although it was only given when the patient was already experiencing discomfort and we have no information as to the effectiveness of the analgesia obtained. It may be, therefore, that the outcome in this small group reflects the elevated risk associated with mild degrees of discomfort. We now routinely administer a local anaesthetic and vasoconstrictor agent before laser treatment. The minor degrees of bleeding reported by operators in this series did not increase the risk of laser failure.

There are two problems encountered when investigating the subject of failure of laser treatment. The first relates to the definition of failure. Previous studies have employed differing criteria: either cytological or colposcopic abnormality at follow-up (Popkin 1983; Baggish 1980), evidence of residual HPV infection alone or in combination with CIN (Goldberg *et al.* 1985), or the need to undertake further treatment other than repeat vaporization (Ali *et al.* 1986). Definition of failure based on cytology alone may be inadequate, as the false-negative rate of cytology in this situation has been reported as 33% (Maclean *et al.* 1987). Definitions of failure which rely on the presence of colposcopic abnormality alone are unsatisfactory because immature metaplastic epithelium

and CIN can have similar colposcopic appearances (Cartier 1984).

We have therefore chosen a definition of treatment failure based where possible on the histological demonstration of residual CIN in 21 patients or on the presence of both cytological and colposcopic abnormality in three patients.

The second problem we have encountered relates to the sample size necessary to show clinically important and statistically significant association. This study leads us to believe that high-grade CIN lesions, inadequate depth of destruction and discomfort experienced during treatment are factors which may lead to increased risk of treatment failure. The point estimates of relative risk associated with these factors are all approximately 2·0. In order to demonstrate such a risk to be statistically significant, with exposure rates in the control population being (approximately) 50, 5 and 50% respectively, we should require a study size of 118 cases (and 4 controls per case) for the first and third factors, and 328 cases and 1312 controls for the second, at the conventional 5% level of significance with 90% power. These calculations could not be made in advance of the study for there were no published estimates of relative risk for the factors studied. If our point estimates of relative risk are indeed of the correct order of magnitude, it would require a study almost five times the size of this one to demonstrate the effect statistically.

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A simple histo-morphometric study of cone biopsies following failure of laser ablation therapy for cervical intra-epithelial neoplasia

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Summary

Cone biopsies from 22 patients previously treated by laser ablation therapy for cervical intra-epithelial neoplasia (21 cases) or human papillomavirus effect alone (1 case) were examined histologically and by simple morphometric means.

Of the 19 patients with 'recurrent' cervical intra-epithelial neoplasia, all but two showed involvement of the 'new' squamo-columnar junction. No consistent stromal abnormalities were seen and healing appeared complete. The average depth of crypts at the squamo-columnar junction was greater in laser treated patients than in controls but less than the average depth of the crypts in the canal of laser treated and control groups.

A correlation was seen between mean maximum crypt depths at the squamo-columnar junction and in the canal in the laser treated patients. This was not seen in the controls. Taken in conjunction with the increase in depth at the squamo-columnar junction in the laser treated compared to the control patients this suggests that the effect of laser ablation therapy, as expected on clinical evidence, is to cleanly 'excise' a ring of cervical tissue with the formation of a new squamo-columnar junction deeper into the original endocervical canal.

INTRODUCTION

FAILURE of laser treatment appears to occur in less than 10 per cent of cases treated for cervical intra-epithelial neoplasia (Jordan and Mylotte, 1982). Histological studies and morphometric analyses have not been previously reported on 'laser failure' cases but clearly this information may be important in the future management of the disease, particularly if further laser treatment rather than cone biopsy is considered. We have looked at cone biopsy specimens from 22 patients known to have developed recurrent cervical disease after laser destructive therapy, and in particular to attempt to further define the effects of laser ablation on cervical structure.

PATIENTS AND METHODS

The patients in this study had all been treated with a Coherent or Sharplan laser at a power density of 500–1200 W/cm^2 and with a spot size of 1·6–1·8 mm. The age range of the patients was 18–42 years with a mean age of 30·3 years. Two patients had had laser vaporisation to a depth of 5 mm, one patient to 5–7 mm, 17 patients to 7 mm and two patients to a depth of 8 mm or more. In all cases the operator believed that an adequate depth of destruction had been achieved and no intra-operative complications were recorded. The pre-operative diagnoses in the study group were cervical intra-epithelial neoplasia (CIN) grade III in 14 patients, CIN II in five patients, CIN I in two and warty changes alone in one.

Abnormalities in the group under study were detected after the original treatment by a combination of cytology and colposcopy with biopsy. In our centre failure of laser treatment was considered to have occurred if within one year of apparently adequate destructive treatment: (a) Invasive cancer was diagnosed (no cases in this study; in one patient there was a colposcopic suspicion of invasive disease). (b) There was histological evidence of CIN on a colposcopically directed biopsy (five patients). (c) Cytological examination following treatment revealed severe dyskaryosis or raised the possibility of an invasive lesion (nine patients). (d) Cytological examination predicted lesser degrees of squamous dyskaryosis

on at least two consecutive occasions (eight patients). (e) Cytological examination predicted the presence of a glandular cell lesion (no patients).

Cone biopsies were performed on average 12 months after laser treatment with a range of 5–21 months. The cone biopsies were performed purely on the basis of 'failed laser treatment' and not on the basis of any other criteria such as failure to visualise the squamo-columnar junction.

The cone biopsies were fixed in Bouin's solution and trimmed in a standard manner taking parallel blocks, lengthwise, perpendicular to the endocervical canal, after first removing the endocervical resection margin in one transverse slice. Sections were examined from the 22 cone biopsies to determine the nature of the recurrent disease and the site of recurrence and to detect any changes in the underlying stroma evident on routine histological staining.

With the use of a camera lucida microscope attachment and a projected 5 mm graticule for standardisation, measurements were made of the deepest endocervical crypt (measured from the base of the overlying surface epithelium and perpendicular to it), the deepest crypt involved by CIN and the deepest crypt within 1 mm on either side of the squamo-columnar junction. Comparative measurements were taken of the deepest crypt and the deepest crypt at the squamo-columnar junction on a control series of ten cervices, similarly fixed and trimmed but obtained from hysterectomy specimens for other than cervical disease.

One representative section of every block was used for measurement as there is good evidence that the findings would not be significantly altered by the examination of multiple levels (Anderson and Hartley, 1980). Measurements on histological slides differ from those on fresh tissue due to shrinkage from fixation and processing but the difference is likely to be less than 5 per cent (Anderson and Hartley, 1980) and the results on fixed tissue are therefore sufficiently reliable.

RESULTS

Twelve of the 'laser failure' cases (55 per cent) had CIN III, three (13 per cent) had CIN II, four (18 per cent) had CIN I, two (9 per cent) had evidence of human papillomavirus infection only and one (4.5 per cent) had follicular cervicitis only. Sixteen women (73 per cent) with CIN also had evidence of papillomavirus infection. Excision appeared incomplete in two cases on the endocervical plane of excision. All were completely excised on the ectocervical margin.

Of those women with CIN, nine (41 per cent) had involvement immediately adjacent to and confined to the surface epithelium at the squamo-columnar junction only (range of surface extension of CIN 1·2–3.0 mm, mean 2·18 mm). Five (23 per cent) had involvement of the squamous epithelium adjacent to the junction and underlying crypts. Two (9 per cent) had more extensive surface epithelial involvement within the canal. One woman (4·5 per cent) had involvement of the surface epithelium within the canal continuous with the ectocervical epithelium in combination with endocervical crypt involvement. One patient (4·5 per cent) had apparent surface and crypt involvement within the canal only, unconnected with the squamo-columnar junction, and in one patient involvement of the epithelium adjacent to the junction with apparently unconnected ectocervical involvement was seen. In total 18 cases (82 per cent) had recurrent CIN involving the new squamo-columnar junction.

Minor changes were seen in the superficial cervical stroma including oedema, a scanty lymphocytic infiltrate and capillary dilatation. There was no clear evidence of scarring or granulation tissue formation and the changes seen were inconsistent and non-specific.

Results of the overall mean maximum crypt depths and mean maximum crypt depths at the squamo-columnar junction for test and control groups are shown in the Table together with mean maximum depths of crypts involved by CIN in the test group. In three cases the surface epithelium immediately at the junction was stripped and crypt depths at the junction were unmeasurable. These cases were not included in the statistical analysis for this reason.

The mean maximum crypt depths for test and control groups (Figures 1 and 2) were very similar, 3·36 and 3·29 mm, respectively (95 per cent C.I. −0·86–1·0 mm; t using unpaired two-tail test 0·15, 30 d.f., $P=0·88$). There was a difference between mean maximum crypt depths at the squamo-columnar junction for laser treated and control groups, which were 2·62 and 1·52 respectively, ($P<0·05$; 95 per cent C.I. 0.26–1·94 mm). Clearly the depths at the junction in the laser treated group lie intermediate between the control group and the overall depths throughout the canal in both control and laser treated groups. No difference was found between the laser treated depths at the squamo-columnar junction and the control group overall depths (difference between means 0·68 mm, 95 per cent C.I. −0·2–1·55 mm), but a difference still remained between the depths at the junction in the laser treated cases and the overall canal depths for the laser treated group (difference between

Table. Maximum crypt depths for treated and control groups

	Mean (s.d.	95% Confidence limits	
		Lower	Upper
Laser treated group (n=22)			
Maximum uninvolved crypt depth	3·36±1·19	2·86	3·86
Maximum depth CIN within crypts	0·60±0·91	0·22	0·98
Maximum crypt depth at squamo-columnar junction*	2·62±1·06	2·14	3·09
Control group (n=10)			
Maximum crypt depth	3·29±1·24	2·53	4·06
Maximum crypt depth at squamo-columnar junction	1·52±0·99	0·90	2·13

* Minus three cases where the junction was not visible.

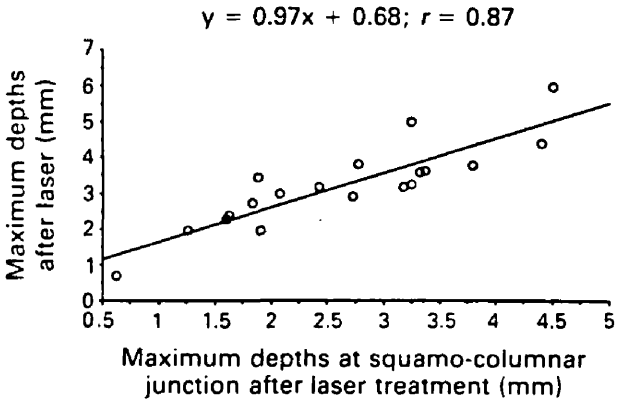

Figure 1. Mean maximum crypt depths at the squamo-columnar junction *versus* mean maximum gland depths throughout the canal for laser treated patients.

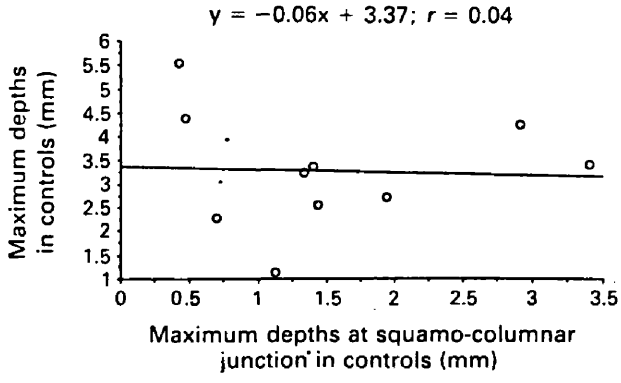

Figure 2. Mean maximum crypt depths at the squamo-columnar junction *versus* mean maximum gland depths throughout the canal for control group of untreated patients.

means 0·75 mm, $P<0·05$, 95 per cent C.I. 0·01–1·48 mm). On the basis of sampling area alone one would expect a slight difference between squamo-columnar junction measurements and those from the whole canal as mean maximum depths were used rather than maximum depths.

In the control group no correlation was seen between mean maximum depths at the squamo-columnar junction and in the whole canal ($r=0·04$) but in the laser treated group a correlation between these two groups was seen ($r=0·87$). This may be taken to support the suggestion that before treatment the area beneath the junction is biologically different to the rest of the canal whereas the area beneath the 'new' junction after treatment is more closely similar to the endocervical canal generally; this cannot be proved on the available data.

DISCUSSION

The mean maximum uninvolved crypt depths in this study for control and test groups compare closely with previously reported mean maximum depth measurements (Anderson and Hartley, 1980) where the depth was measured from the top of the overlying surface epithelium and not from the basement membrane as in this study.

Recurrent CIN involving crypts was always associated with surface involvement either adjacent to the squamo-columnar junction or in the endocervix. The crypt involvement was predominantly within the neck of the crypts but occasionally the whole crypt was involved. It seems likely therefore that crypts are recolonised by residual superficial disease. In these cases there was no evidence of 'concealment' of disease within crypts.

The finding of a shift in crypt depths at the squamo-columnar junction in the treated group to become closer to the maximal depths throughout

the endocervical canal suggests that laser treatment alters the relative position of the junction, moving it upwards into the endocervical canal. Clearly the difference in crypt depths is likely to be simply due to the smaller area available for measurement at the junction but this does not detract from findings after laser treatment. Support for an alteration of the area around the junction to resemble more closely the rest of the canal after laser treatment is also evident in the correlation seen between depths at the two sites, which did not exist in the control group.

Although the new squamo-columnar junction after treatment may be higher in the endocervix it is normally visible at colposcopy. 'Prolapse' of the cervical canal as a consequence of an alteration in connective tissue in the endocervix (Ober, 1959) seems a highly unlikely explanation, as this is thought to occur as a result of major change in ovarian hormone influences (Hutchins, 1987) for which there is no evidence in this laser treated group. The findings of absence of scarring and granulation tissue after laser treatment combined with the altered crypt depths suggests that a ring of tissue is completely destroyed by the treatment and the remaining cervix is directly re-epithelialised without extensive granulation tissue formation or fibrous tissue proliferation, a finding supported by the colposcopic appearances after treatment (Jordan and Mylotte, 1982).

In this small series of patients comment on the aetiology of recurrence is of doubtful value but the presence of only two cases of 'recurrent' CIN unassociated with contiguous disease adjacent to the squamo-columnar junction is of interest. As in a proportion of cases no crypt disease was seen and the time interval to 'recurrence' was never more than 21 months, the findings suggest that the new junctional region, in some cases at least, is recolonised by marginal epithelium that either already is, or has the potential to rapidly become, neoplastic. Why this did not happen in those cases where isolated foci of CIN were seen unconnected with the junction is unclear. It has often been suggested that these foci seen after destructive therapy represent residual foci previously connected to the main 'sheet' of CIN; perhaps in fact they are debarred from recolonisation by intervening normal epithelium.

In this study only one patient (5 per cent) had CIN within the canal (with crypt involvement) separated from the squamo-columnar junction by non-neoplastic epithelium. This type of case is often quoted as one of the pitfalls of colposcopic assessment of the cervix following destructive or excisional therapy. From the present study it would seem to be an uncommon occurrence but the small numbers in the present study must be borne in mind and the fact that it arises at all is of importance. In this patient the abnormality was detected both cytologically and colposcopically.

In conclusion we would suggest that more extensive use of simple morphometric techniques on larger series of cases may well provide sounder principles than those at present in use upon which to base future treatment.

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LEFT-VENTRICULAR SUPPORT BY INTRAVENTRICULAR BLOOD PUMP DURING HIGH-RISK CORONARY ANGIOPLASTY

SIR,—Technical improvements have led to a new design of arterial pump, permitting non-invasive, closed-chest temporary mechanical cardiac assistance. The new design ('Hemopump') is based on the Archimedes screw principle and consists of an inlet cannula, an axial-flow blood pump, a drive cable contained in a polymer sheath, and a rotor. The pump head is in the 21F cannula and is actuated by a rotating electromagnetic field. The spiral vanes of the pump head, rotating at 25 000 rev/min displace blood from the tip of the cannula in the left ventricle to the outflow port in the ascending aorta, at flow rates of 2·5–3·5 l/min. The cannula is inserted into the ventricle under fluoroscopic control via the femoral artery. The system has been evaluated in animals[1] and the major potential risk, blood cell trauma, has been shown almost non-existent. The first clinical studies were in patients in cardiogenic shock after myocardial infarction or graft rejection.[2] Simplicity, safety, and rapidity of insertion make the new pump potentially ideal for prophylactic temporary left-ventricular cardiac assistance during high-risk coronary angioplasty.

A 61-year-old man was selected for circumflex artery balloon angioplasty. He had had heart surgery in 1978, being given a single saphenous vein graft on the left anterior descending (LAD) artery for unstable angina. After 10 years free of any major myocardial event he presented on July, 1988, with impending myocardial infarction and was operated on. A double bypass was done on the right and distal LAD arteries. A 95% circumflex artery stenosis could not be bypassed. 2 months postoperatively he deteriorated rapidly and angina recurred, with left-ventricular dysfunction. Permanent ischaemic damage to the left-ventricular posterior wall was demonstrated by thallium scintigraphy stress test. The ejection fraction was 16%. Surgery seemed to contraindicated, because of the previous unsuccessful attempt. Both grafts were patent and the circumflex artery stenosis was unchanged. Angioplasty was proposed. Because of the risk of such a procedure, due to poor left-ventricular function and progressive ischaemia in a ventricular area unprotected by collaterals, we decided to try prophylactic implantation of the hemopump. The patient gave his informed consent.

After right catheterisation and contrast injection into the left main artery the guidewire was left in the coronary vessel. Under local anaesthesia the pump was introduced retrogradely into the ventricle via the superficial femoral artery. The pump speed was set to achieve maximal left-ventricular unloading (table) and bypassed flow. Prolonged atrioventricular block occurred but this had no effect on consciousness and mental alertness and the patient did not report any discomfort. The block resolved spontaneously after 1½ min. During the two inflations of the balloon (60 s, 1 MPa) the patient did not feel any pain. His ECG remained unchanged. 30 min after this successful dilatation, the pump flow was progressively reduced and the left-ventricular cannula withdrawn. The plasma free haemoglobin concentration was 0·2 mg/dl pre-implant and 0·4 mg/dl afterwards. The patient remained symptom-free in the post-intervention period.

In this case the hemopump provided substantial left-ventricular support during a high-risk coronary angioplasty even during a period of cardiac arrest (table). The mechanism is probably complex and related to left-ventricular unloading and the maintenance of adequate coronary perfusion in non-occluded vessels. It reduced the danger of high-risk angioplasty by permitting adequate central blood flow.

HAEMODYNAMIC FINDINGS

Indicator	Pre-implant	Pump on		Post-implant
		During PTCA	Post PTCA	
PAP (mm Hg)	35/13 (20)	35/15 (19)	35/15 (18)	30/15 (18)
PCWP (mm Hg)	18	14	15	17
AoP (mm Hg)	125/60 (75)	75	84	110/65 (82)
CO (l/min)	2·89	4·36	3·66	3·22
CI (l/min/m²)	1·61	2·41	2·04	1·79
Bypass Q (l/min)	..	3·4	1·7	..

PAP = pulmonary arterial pressure (and mean); PCWP = pulmonary capillary wedge pressure; AoP = aortic pressure; CO = cardiac output; CI = cardiac index; bypass Q = bypass flow; PTCA = percutaneous coronary angioplasty.

D. LOISANCE

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WHEN LASER VAPORISATION FOR CIN FAILS, WHAT NEXT?

SIR,—In 1985 we argued that failure of one laser vaporisation to eradicate cervical intraepithelial neoplasia (CIN) did not contraindicate further local destructive treatment.[1] However, we have reconsidered this policy. A second laser treatment can be justified when the initial procedure is incomplete due to intraoperative complications such as bleeding, but when no complications have been recorded and an apparently adequate depth of destruction has been achieved, the argument is more difficult to sustain; the limited histological characterisation provided by colposcopically directed biopsy may result in women with early invasive disease being inappropriately selected for local destructive methods;[2] and we have observed five cases of invasive carcinoma diagnosed within 12 months of laser vaporisation. We have been evaluating the treatment of selected laser failures by cone biopsy.

Patients were selected for inclusion in the trial if they had been treated by laser vaporisation at this hospital between March, 1986, and March, 1988; if the operator believed that an adequate depth of destruction had been achieved and no intraoperative complications had been recorded; and if there was evidence of persisting abnormality within the first year of follow-up.

During the study period 1081 patients were treated with laser vaporisation. 40 patients (3·7%) met the inclusion criteria: 8 had histological evidence of CIN on a colposcopic biopsy in the presence of a negative smear, 3 had colposcopic suspicion of invasive disease, 15 had cytological evidence of severe squamous dyskaryosis on at least one occasion, and 14 had lesser degrees of squamous dyskaryosis on at least two consecutive occasions.

The mean age of patients was 30·5 years, range 18–42. The mean interval from initial laser treatment to definitive treatment was 13·5 (5–24) months. 29 of the 40 patients (73%) had a fully visible squamocolumnar junction (SCJ) at follow-up. 5 of these patients had colposcopic evidence of involvement of the endocervical canal with premalignant disease. 3 patients had colposcopic findings suspicious of an invasive lesion. Informed consent having been obtained, 40 patients were treated by cone biopsy.

Histological examination of cone biopsy material revealed invasive carcinoma in 2 cases (stages 1a and 1b), CIN in 29, and no evidence of invasive or intraepithelial disease in 9. On our operational definition of laser failure, the frequency of unsuspected invasive disease treated by laser vaporisation was 0·19%.

The utility of the suggested management policy must be considered in terms of costs and benefits compared with our previous protocol. Costs are defined in terms of unnecessary cone biopsies; benefits follow the diagnosis of unsuspected carcinoma. 19 of the 40 patients would not have been suitable for a second destructive treatment due to unsatisfactory colposcopic findings (16) or the suspicion of invasive disease (3). Thus the additional costs attributable to this management policy arise from the 21 "unnecessary" cone biopsies, because these cases would have been suitable for further local destructive treatment. No benefits could be attributed to this management policy because the 2 cases of invasive carcinoma were predicted before cone biopsy. However, colposcopic recognition of early invasive disease is not always so certain. Sensitivities of 73% and 86% have been reported for the

detection of microinvasive and stage 1b occult carcinoma, respectively,[3] yet these findings relate to adequate colposcopic assessment of the cervix before local ablation and there are no data on the sensitivity of colposcopy for detection of invasion after treatment. Given uncertainty over the ability of colposcopists consistently to exclude invasive disease, the extra cost of 21 cone biopsies per 1081 patients treated by laser vaporisation may be an acceptable price to pay for the security of a safety net. Recent developments in the application of outpatient excisional methods of treatment of CIN may reduce the need for this form of insurance.

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CORRECTING TOTAL SERUM CALCIUM

SIR,—In reply to Dr Larsson and Dr Ohman (Feb 11, p 326) we did not claim that use of albumin-adjusted total serum calcium is new. They have perhaps misunderstood the problem we addressed.

Despite the recognised limitations of total serum calcium measurements and the desirability of measuring ionised serum calcium more widely in hospital, recent UK external quality assessment schemes such as the Birmingham NEQAS or Burroughs Wellcome, show that total serum calcium continues to be the commonest method of measurement. We assume that during routine laboratory hours, laboratories often make available albumin levels so that an adjustment can be made. Serum calcium measurements, however, are also required on an urgent basis throughout the day. In UK hospital laboratories these urgent analyses, including serum calcium, are often done on a discretionary basis. We believe that, on these urgent requests, total serum calcium levels without albumin adjustment are commonly reported. We cannot comment on the way serum calcium measurements are made in Sweden. We addressed the problems that can arise from the inconsistency of providing an albumin adjustment for routine but not for urgent results.

Methods for measuring serum calcium which can be applied to a wider hospital population without requiring correction for prevailing protein levels are needed. However, until these methods become widespread and while total serum calcium measurements continue to remain in use, albumin adjustment (including urgent results) should be made.

In reply to Dr Urban and colleagues (Feb 11, p 326) we did point out that we do not consider albumin-adjusted total serum calciums to be ideal. Neither are we arguing against the introduction and use of ionised calcium measurements. Hitherto, however, the modular design of ionised calcium analysers has restricted these measurements to a relatively small and selected group of inpatients. Until these methods are applicable to a wider population our study has merely emphasised the importance of using consistent serum calcium indices throughout the day. With the current state of the art, albumin-adjusted total serum calcium seems the optimum option.

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SIR,—I would temper the enthusiasm for measuring ionised calcium on a much wider scale, as proposed by Dr Larsson and Dr Ohmann and by Dr Urban and colleagues. Adjustment of total calcium for albumin in patients who are not acutely ill can avoid misinterpretation, misinvestigation, misdiagnosis, and even mistreatment.[1,2] There is also little doubt that measurement of ionised calcium activity in an anaerobic blood sample is invaluable in acutely ill patients who have acid-base disturbances that alter calcium binding to plasma proteins, or who have increased plasma concentrations of anions such as lactate or citrate that chelate free calcium. For example, during liver transplantation or cardiopulmonary bypass surgery the large volumes of citrated blood given may result in a greatly increased total and albumin-adjusted calcium because of chelation of administered calcium, while the ionised calcium remains dangerously low.[3,4]

My caution concerns the use of ionised calcium measurements to attempt to detect disturbances of calcium homoeostasis in patients who are not acutely ill. Although true ionised calcium is independent of chronic changes in plasma protein concentration, measurements made with commonly used commercial instruments have shown significant positive regressions of ionised calcium on albumin concentration, both in hospital patients with no obvious disturbance of calcium homoeostasis and in normal laboratory staff.[5] Thus, the problem of interpretation in patients with abnormal plasma proteins remains: in those with hypoalbuminaemia, measurement of ionised calcium may result in the overdiagnosis of hypocalcaemia and the underdiagnosis of hypercalcaemia. A probable explanation is that protein is dehydrated and precipitated by the hypertonic reference electrode liquid junction of ionised calcium instruments.[6,7] Recent experiments with Dr B. M. Buckley have shown that protein interference, which is also evident in ionised sodium and potassium measurements, depends on the composition and design of manufacturers' reference electrodes.

The albumin-adjusted plasma calcium concentrations of siblings cluster within the reference range.[8] Thus, if a patient with a value near the upper limit of normal has symptoms that could be attributed to hypercalcaemia, it may well be that he is truly hypercalcaemic in relation to his normal set point within the reference range. In such circumstances it would seem more appropriate to pursue a diagnosis by the measurement of urine calcium excretion per unit volume of glomerular filtration rate in a fasting morning urine sample than to go on to measure ionised calcium. The upper limit of normal is not the lower limit of values in disease in the individual.

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SIR,—Cheesbrough[1] recommends that serum albumin be measured in conjunction with serum total calcium, which is adjusted by the addition of 40 minus the albumin (g/l), all divided by 40. We found this correction to be of special importance in 11 inpatients with eclampsia (7 ante-partum, 4 post-partum), of average age 19 years. Blood samples, drawn within 2 days of the first seizure, were analysed for albumin and total calcium by the *o*-cresolphthalein complex and bromocresol-green manual methods.[1] The total serum calcium averaged 2·08 (SD 0·35) mmol/l. The reference range is 2·25–2·60; 7 patients had values below this range and 1 was above it. However, after albumin adjustment only 3 values remained below the reference range whilst

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Invasive Cervical Cancer After Laser Vaporization for Cervical Intraepithelial Neoplasia: A 10-Year Experience

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ABSTRACT

The treatment of cervical intraepithelial neoplasia by laser vaporization has been in progress at the Birmingham and Midland Hospital for Women since September 1977. In this interval, 3182 patients have been treated. Seven women have developed invasive cancer at intervals 4–34 months postlaser. The lesions diagnosed were stage Ia (3), Ib (1), IIb (1), IIIa (1), and IV (1). These case histories are presented. On retrospective assessment, there were contraindications to local destructive treatment in six of the seven cases. This series emphasizes the need for thorough evaluation of patients before embarking on local destructive treatments and emphasizes the need for careful follow-up of treated patients. (J GYNECOL SURG 6:103, 1990)

INTRODUCTION

THE TREATMENT OF PREMALIGNANT DISEASE of the cervix has changed dramatically in recent years. Surgical excision in the form of cone biopsy or hysterectomy was believed to be overtreatment for the majority of cases, and the increasing numbers of young women referred with abnormal smears made it imperative that treatment for cervical intraepithelial neoplasia (CIN) should be associated with minimal surgical morbidity and minimal interference with reproductive potential. Local destructive methods, therefore, became popular, in particular, laser vaporization.

Laser treatment is a highly successful method of treating CIN, with success rates in excess of 90% for eradication of CIN.[1,2] However, these success rates are based on short-term follow-up. It is debatable whether failure to eradicate CIN is a true failure of treatment, since the aim of the screening program is to prevent invasive disease. There is, however, no doubt that the appearance of invasion after laser represents treatment failure. This article analyzes the case histories and the process of assessment of those individuals who, having had laser vaporization for CIN, were diagnosed subsequently as having invasive cervical cancer. The likely reasons for treatment failures are discussed.

MATERIALS AND METHODS

All patients had been referred for colposcopic assessment of abnormal cervical cytology at the Birmingham and Midland Hospital for Women. This institution acts as a tertiary referral center for gynecologic oncology and has a long-standing interest in the research and treatment of premalignant disease of the lower genital tract. Patients were selected for laser vaporization in accordance with well-established criteria,[3] and routine policy was to treat all grades of CIN/cervical dysplasia. Colposcopy clinics were staffed by gynecologists who

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had undergone training in colposcopy and laser ablative surgery. These individuals were of both consultant and registrar (resident) grade. We believe that the standard of colposcopic expertise was high and unrelated to the clinical status of the colposcopist, and previous research carried out at this institution supports this contention.[4]

Assessment of colposcopic biopsies was performed by a consultant pathologist with a special interest in gynecologic pathology (TPR).

For laser treatment, a CO_2 laser was employed at a power density between 500 and 1200 W/cm^2, using a spot size of 1.6–1.8 mm. Direct intracervical injections of local anesthetic with vasoconstrictor were made outside the abnormal transformation zone, and destruction was carried out to a measured depth of 5–7 mm. Standard follow-up of treated patients consisted of cytologic and colposcopic examinations at 4–6 months and 12 months postlaser. If these examinations proved normal, annual Pap smears were taken at hospital-based cytology clinics staffed by cytopathologists. Cytologic assessment was carried out in the laboratories of the Birmingham and Midland Hospital for Women (CAW) and the Birmingham Maternity Hospital (KW). We believe that these factors led to a consistently high standard of cytological and histopathological assessment. The number of laser vaporizations was calculated from colposcopy clinic records for out-patient treatment, and operating room records for in-patient procedures. Cases of invasive cancer were ascertained from computerized hospital statistics.

In the interval from September 1, 1977, until December 31, 1987, inclusive, 3182 patients were treated for CIN using laser vaporization. In the same interval, 368 cases of invasive cervical cancer were diagnosed, of which 7 cases followed laser treatment. This represents 0.22% of all those lasered and 1.9% of all invasive cervical cancer during this period (Fig. 1). The median interval from laser treatment was 4 years (4 months–10 years).

CASE REPORTS

Patient 1

The patient was 27 years old and nulliparous. A cervical smear showed severe dyskaryosis. Colposcopy showed grade 2 acetowhite epithelium with a mosaic pattern extending 2–3 mm into the canal where the squamocolumnar junction (SCJ) was seen. A colposcopically directed biopsy was reported as showing moderate dysplasia. Two months later, laser vaporization was carried out to a depth of 7 mm without complication. The patient was reviewed 6 months after treatment by the same colposcopist, when examination revealed acetowhite areas with faint mosaic pattern thought to represent metaplasia. The SCJ was seen. A

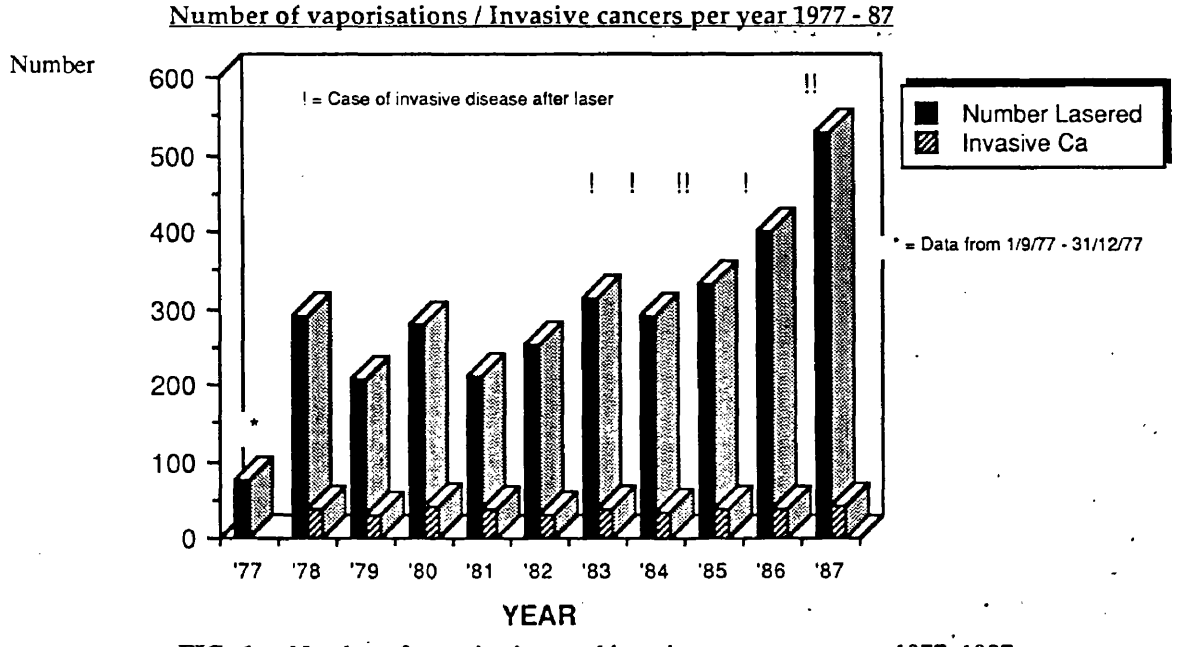

FIG. 1. Number of vaporizations and invasive cancers per year, 1977–1987.

colposcopic biopsy was reported as being unsatisfactory for assessment, and the smear was technically unsatisfactory. At review 6 months later, the colposcopic findings were unchanged, but the cytology was now positive with atypical glandular cells. A further colposcopic examination 1 month later, again by the same examiner, revealed dense acetowhite areas. No CIN or glandular atypia was seen on punch biopsy. Conization was carried out 5 months later. Histologic study showed a microinvasive adenosquamous lesion and areas of adenocarcinoma in situ. Wertheim's hysterectomy showed no residual disease in the cervix, and the nodes were clear. The patient is alive and well 2 years posttreatment.

Patient 2

The patient was aged 28 years, G3P3, and came to the gynecology clinic with vaginal discharge, urinary symptoms, and intermenstrual bleeding. The cervix appeared eroded. A smear was taken, and the cervix was treated by cryocautery before obtaining the cytologist's report. The smear was reported as showing moderate to severe dyskaryosis and was repeated 2 months later, immediately before colposcopic examination. This showed severe squamous dyskaryosis and abnormal endocervical cells. Colposcopy showed grade 3 acetowhite epithelium with coarse punctuation extending onto the anterior fornix. The SCJ was seen at the external os. A punch biopsy from the anterior lip of the cervix was reported as CIN III with gland cleft involvement, and in some areas the basal layer of epithelium was irregular and poorly demarcated from the stroma. However, there was no unequivocal evidence of invasion. Laser vaporization was carried out 1 month later under general anesthesia by a different colposcopist. Destruction to a depth of 7 mm was achieved, although the procedure was complicated by hemorrhage.

At colposcopic follow-up 4 months later, cytology was positive, and colposcopy showed an area of grade 1 acetowhite epithelium in a fully visible transformation zone (TZ). A punch biopsy was reported as showing CIN III with gland cleft involvement.

The patient continued to experience stress incontinence and urgency, for which a vaginal hysterectomy and pelvic floor repair were recommended. Histologic assessment of the cervix showed an area of invasive carcinoma in the anterior lip of the cervix, which was adenosquamous in type. The tumor measured 9 mm maximum diameter and 3 mm deep, and vascular permeation was present. The patient was treated with radical radiotherapy. She was alive and well when seen 33 months postdiagnosis.

Patients

The patient was aged 34 years, G0P0. Cytologic study showed moderate dyskaryosis with viral effects. Colposcopy revealed a grade 2 acetowhite lesion, and the SCJ was seen in the lower endocervical canal. The patient was reassessed under general anesthesia and thought suitable for vaporization. A biopsy immediately before vaporization showed CIN III. Vaporization was carried out to a depth of 10 mm without complication. Colposcopic follow-up at 4 months postlaser showed acetowhite epithelium with mosaic pattern, suggestive of residual disease, in a fully visible transformation zone. Cytology was positive. A punch biopsy showed CIN III, and the patient had repeat laser vaporization.

Follow-up 3 months later showed a normal cervix with a clearly visible SCJ. Cytology was positive. The patient was reviewed 6 months later, when both cytology and colposcopy were negative. Review 1 year later showed a fully visible transformation zone with the SCJ in the lower part of the endocervical canal. There was no colposcopic abnormality. However, cytology was positive, and the presence of an invasive lesion was questioned. All colposcopic examinations and both laser vaporizations were carried out by the same observer. An abdominal hysterectomy was carried out that showed a poorly differentiated squamous carcinoma 1.4 cm deep with extensive vascular invasion. The tumor (Fig. 2) had a covering of hyperplastic normal squamous epithelium that showed CIN III in only one small focus. This may explain the normal colposcopic appearances at follow-up.

The patient was treated with pelvic radiotherapy and is alive and well at 6 months review.

Patient 4

The patient was aged 36 years, G2P2. Cytologic study showed occasional mildly dyskaryotic cells. Colposcopy revealed a grade 2 acetowhite lesion extending into the canal. The SCJ was seen 5 mm inside the canal.

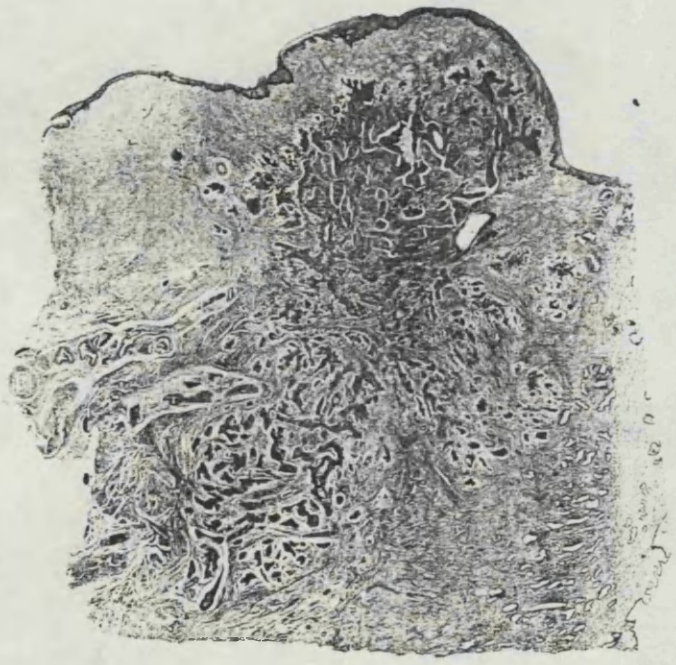

FIG. 2. Mounted section. There is extensive infiltrating squamous carcinoma with vessel space involvement beneath an intact normal ectocervical surface mucosa. ×4. Colposcopic findings were normal.

A punch biopsy showed CIN III with gland cleft involvement. After reassessment under general anesthesia 2 months later by a different colposcopist, the lesion was lasered to a depth of 10 mm without complication. Colposcopic follow-up at 3 months and 10 months was negative, cytology on both occasions showing marked inflammatory changes but no dyskaryotic cells. Sixteen months postlaser, colposcopy was performed after a smear taken at another hospital showed dyskaryotic metaplastic cells. No abnormality was found (SCJ seen), cytology was negative, and the patient was discharged again to Pap smear follow-up. She was referred back 18 months postlaser after an endocervical brush smear had shown degenerate dyskaryotic cells and debris, raising the possibility of an invasive lesion. Colposcopy showed acetowhite epithelium in the canal, but the SCJ was not seen. An endocervical curettage showed CIN III.

Cone biopsy showed microinvasive squamous carcinoma (maximum depth 1.6 mm, maximum diameter 8.7 mm, tumor volume 45 mm_3) with vascular invasion and incompletely excised CIN III. A total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed (22 months postlaser). The cervix showed an area of residual CIN only. The patient is alive and well at 9 months follow-up.

Patient 5

The patient was aged 28 years, G2P2. Cytologic study raised the possibility of an invasive lesion (grade 5). Colposcopy showed an extensive ectopy, the SCJ was clearly visible, and two areas of grade 2 acetowhite epithelium were noted on the anterior and posterior lips of the cervix. Punch biopsies were reported as CIN III with "agitation" of the basal layer of epithelium. No definite invasion was seen, although the pathologist could not unequivocally exclude this diagnosis. Laser vaporization to 7 mm was carried out, with slight hemorrhage.

The patient was referred back 4 months later complaining of postcoital bleeding. Colposcopy revealed grade 2 acetowhite areas on the posterior lip of the cervix. Punch biopsies were reported as showing the SCJ with CIN III and foci of invasion and small vessel involvement. Cytologic study suggested an invasive lesion. At conization 1 week later, examination of the pelvis before surgery revealed a necrotic posterior cervical lip and parametrial extension of tumor, clinical stage IIb cervical carcinoma. An intravenous pyelogram showed chronic pyelonephritis of the left kidney but no outflow obstruction. Treatment with radical radiotherapy was carried out. Eight months later, the patient developed hematuria and was found to have a necrotic vaginal vault. She has so far undergone two courses of cisplatin for recurrent disease and still has evidence of active disease.

Patient 6

The patient was aged 27 years G1P0. Cytologic study was reported as showing malignant cells. Colposcopy showed a visible SCJ. There was an extensive grade 3 acetowhite lesion with mosaic and punctation. Punch biopsy was reported as CIN III, with gland cleft involvement. Laser vaporization was carried out under general anesthesia to 7 mm without complication.

At review 5 months later, colposcopy was normal (SCJ visible), but cytology suggested severe dyskaryosis. At second review 11 months postlaser, which was delayed because of problems in achieving patient follow-up, colposcopy showed an invasive cervical carcinoma with vaginal extension. Cytology was positive. An EUA and biopsy confirmed the presence of a stage IIIa squamous carcinoma of the cervix, and the patient was treated by radical radiotherapy. She subsequently developed pelvic recurrence and ureteric obstruction. The obstruction was relieved with pigtail ureteric catheters, and she was commenced on chemotherapy. The patient died of massive vaginal hemorrhage 22 months postlaser treatment.

Patient 7

The patient was aged 39 years, G0P0. Cytologic study revealed moderate dyskaryosis with HPV changes. Colposcopy showed areas of leukoplakia and features of CIN and HPV. Two punch biopsies were reported as showing CIN I and CIN III. Laser vaporization was carried out to 7 mm. Cytology at 1 and 5 months was reported as showing no dyskaryosis. At 5 months, colposcopy showed atypical epithelium consistent with HPV but not thought likely to represent CIN. The patient was discharged to cytologic follow-up. Smears at 1 and 2 years postlaser were reported as normal, but review of the slides revealed dyskaryotic cells. She went to her family practitioner 34 months after laser treatment with constipation and dyspareunia and a clinical stage IV lesion. She was treated with diaminodichloroplatinum, followed by radiotherapy. She developed renal failure and died 3 1/2 years postlaser.

DISCUSSION

When local destructive methods of treatment of CIN were introduced in the late 1970s, it was recognised that there was a risk of overlooking early invasive disease at colposcopic assessment. This is the principal reason for having strict criteria for selection of patients for these treatments. These criteria are well documented.[3,5] As experience with local destructive methods increased, the selection criteria were modified, and these modifications were based on experience of cases such as those reported here.

TABLE 1. PROPOSED REASONS FOR TREATMENT FAILURE

Patient	*Factor(s)*
1	Unrecognized glandular atypia progressing to invasion.[a]
	Management guided by negative colposcopic biopsy when cytology and colposcopy were abnormal[b]
2	Local treatment to cervix prior to colposcopic assessment.[a]
	Unrecognized glandular atypia (cytology)[b]
	Inconclusive punch biopsy diagnosis[b]
	Multiple colposcopic assessors[b]
3	Endocervical disease present[a]
	Repeat destructive treatment in presence of persisting cytologic and colposcopic abnormality[b]
4	Endocervical disease present[a]
5	Occult invasion present, suggested by cytology[a]
	Inconclusive punch biopsy diagnosis[b]
6	Occult invasion present, suggested by cytology[a]
	Suboptimal follow-up
7	Residual CIN progressed to invasion[a]

Is it possible to speculate why treatment failure has occurred in these cases? Perhaps the most interesting feature of this retrospective review is that in 5 of these 7 cases, it is possible to identify more than 1 error in management of each case.

Two patients were reported as having significant involvement of the endocervix with colposcopically abnormal tissue. Such involvement makes accurate colposcopic assessment unreliable and contraindicates local destructive treatment. The presence of an unrecognized glandular cell component may have been the explanation for the two patients in the series (1 and 2) who had an adenosquamous carcinoma following laser treatment. In one of these cases, cytology suggested the presence of endocervical dyskaryosis. The significance of such a finding has only recently been appreciated. However, in case 1, the smear showed no evidence of such cellular changes prior to treatment. The sensitivity of cytology for preinvasive glandular lesions has been quoted as 70%,[6,7] and there appear to be no distinctive colposcopic recognition criteria for these lesions.[6] Hence, the diagnosis of preinvasive glandular lesions is difficult. Histopathologic review of case 1 revealed the presence of focal glandular atypia (cervical glandular intraepithelial neoplasia, or cGIN[8]) on the preoperative punch biopsy. This case illustrates that it is not uncommon for such lesions to be overlooked.[9] There may be a case for routine endocervical cytologic sampling to pick up glandular cell abnormality, and controlled studies are required to elucidate this matter.

We believe that this series illustrates two examples of cases where CIN remained after destructive treatment, which subsequently became invasive. Two patients, cases 3 and 7, were found to have abnormal cytology soon after treatment of CIN, and invasion was recognized 30 and 34 months, respectively, postlaser treatment. These cases emphasize the need for continued follow-up of treated patients. It is likely that in two of our cases, an early invasive lesion was present but was not recognized by colposcopy (patients 5 and 6). These two patients had cytologic suspicion of invasion prior to treatment. In one of them, cytology was reported as showing "malignant cells." In this case, the cytologic findings suggested the presence of an invasive lesion. However, it would appear that the cytology report was interpreted as indicating severe dyskaryosis. In a 1987 publication,[10] the use of the term "malignant cells" for severe dyskaryosis is discouraged. If an invasive lesion is suspected, the cytopathologist is asked to convey this information clearly in the report. Our example would appear to provide support for this approach.

In case 5, the histology report of the punch biopsy specimen indicated the presence of agitation of the basal epithelial layers and distinctive surrounding stromal changes, as previously described by Burghardt.[11] As a result of retrospective histopathologic review, such changes were discovered also in the colposcopically directed biopsy of case 2 before laser treatment. Multiple sections showed no definitive early stromal invasive buds in either case. In view of these findings, we believe that a prospective study is warranted to assess the significance of the finding of these changes when there is no other evidence of invasion in a punch biopsy specimen. Only then will it be possible to conclude whether such reports point to the need for conization to exclude invasion at other sites within the transformation zone.

There was one example in this series where local destructive treatments were applied to the cervix prior to colposcopic assessment (case 2). This may have prejudiced the colposcopist's ability to diagnose an early invasive lesion and may have made biopsies and subsequent smears unrepresentative. Such a finding has been reported in a previous survey of invasive cancer following local treatments to the cervix.[12] Reports of invasive cancer after local destructive treatments for CIN are uncommon.[1,12] The series of Townsend et al.[12] highlighted the fact that some patients were not comprehensively assessed before administration of local destructive treatments, and they highlighted the omission of endocervical curettage (ECC) as a major factor. In Great Britain, such a procedure is rarely used in the assessment of patients with abnormal cytology. If the whole of the transformation zone is not visible, a cone biopsy is considered to be mandatory for thorough assessment. In the presence of a fully visible transformation zone, it is debatable whether ECC is of any value in the pretreatment assessment,[13] and in those cases where invasion was presumed to have been missed by the colposcopist, we believe that the correct diagnosis would have been arrived at by obtaining a larger biopsy of the transformation zone rather than a superficial curettage specimen.

The recognition of early invasive cancer by colposcopy is not easy. The signs of invasion can be subtle and easily missed, and, therefore, it has been argued that colposcopic assessment should always be performed by experienced operators. Although it is likely that colposcopist error played some part, there is some evidence to suggest that colposcopic examination fails to pick up early invasion in some cases. Benedet et al.[14] reported on the accuracy of colposcopy in a small series of patients with early invasive lesions. Of those with a fully visible transformation zone, colposcopy failed to recognize 27% of microinvasive and 14.5% of occult invasive cancer. Although two thirds of the missed lesions in this series were thought to be due to colposcopist

error, it was acknowledged by these authors that there are "lesions whose colposcopic appearance is not sufficiently distinct or characteristic to permit a diagnosis of early invasion."[14]

The occurrence of invasive disease soon after colposcopic assessment and treatment of apparently preinvasive disease is disturbing. Invasive disease does occur even after more radical excisional treatments for CIN, i.e., cone biopsy and hysterectomy,[15,16] although the maximal incidence of invasive carcinoma following treatment of carcinoma in situ does not peak until several years after treatment, suggesting that these represent progression of residual or recurrent CIN to invasion. The incidence rate quoted by us is provisional, given that only short-term follow-up is available on many of the patients treated and that some patients have moved away from the area served by our institution and have had their follow-up continued elsewhere. It is highly likely, therefore, that our figure is an underestimate of the true prevalence of invasive disease after laser vaporization, and this figure could be calculated accurately only by a systematic audit of all the follow-up cytologic and colposcopic examinations of all treated patients. An accurate estimate of the extent to which unsuspected invasive disease is treated by destructive methods has been assessed by a prospective study of treatment failures.[17] The result we obtained was 0.19% of all laser treatments. We concluded on the basis of this study that failures of local destructive treatment would be ideally managed by conization rather than repeat laser vaporization, and our experience of cases 3 and 6 in this series adds further support to this viewpoint.

Local destructive methods offer an efficient method for treatment of CIN in the vast majority of cases. However, follow-up of treated cases needs to be prolonged before final conclusions can be drawn. Invasive disease after laser vaporization is a rare phenomenon thus far.

Increasing numbers of patients are being referred for colposcopic assessment.[18] There is also a need to follow up patients who have been treated for CIN. Given the increasing demand on colposcopy services, it is likely that there will be further cases of invasive disease after laser treatment. Outpatient excisional techniques are now available and are gaining in popularity,[19] largely because they provide insurance against the risk of overlooking early invasion at colposcopy.

In the light of these observations, there is a need to define the optimal treatment of CIN, and a large-scale comparison of colposcopically guided destructive and excisional techniques would be appropriate.

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