“EXPRESSION OF HLA 1 AND CD 8 IN CANCER CERVIX PATIENTS"

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

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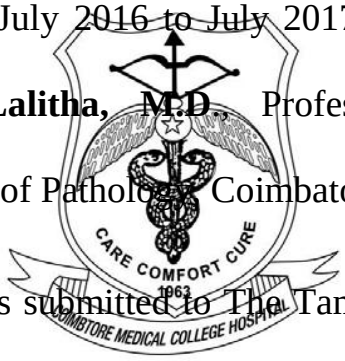
CHENNAI

APRIL 2018

DECLARATION

DECLARATION

I hereby declare that the dissertation entitled **"Expression of HLA 1 and CD 8 in cancer cervix patients"** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from July 2016 to July 2017 under the guidance and supervision of **Dr.C.Lalitha, M.D.**, Professor and Head of the department, Department of Pathology, Coimbatore Medical College.



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CERTIFICATE

CERTIFICATE

This is to certify that the dissertation entitled "Expression of HLA 1 and CD 8 in cancer cervix patients" is a record of bonafide work done by **Dr.R.PADMAVATHI** in the Department of Pathology, Coimbatore Medical College, Coimbatore under the guidance and supervision of **Dr.C.LALITHA, M.D**., Professor and Head of the department, Department of Pathology, Coimbatore Medical College and submitted in partial fulfilment of the requirements for the award of M.D. Degree (Branch III) in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai.

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The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

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ACKNOWLEDGEMENT

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And above all am dedicating this book to my Grandpa, Grandma and my father...

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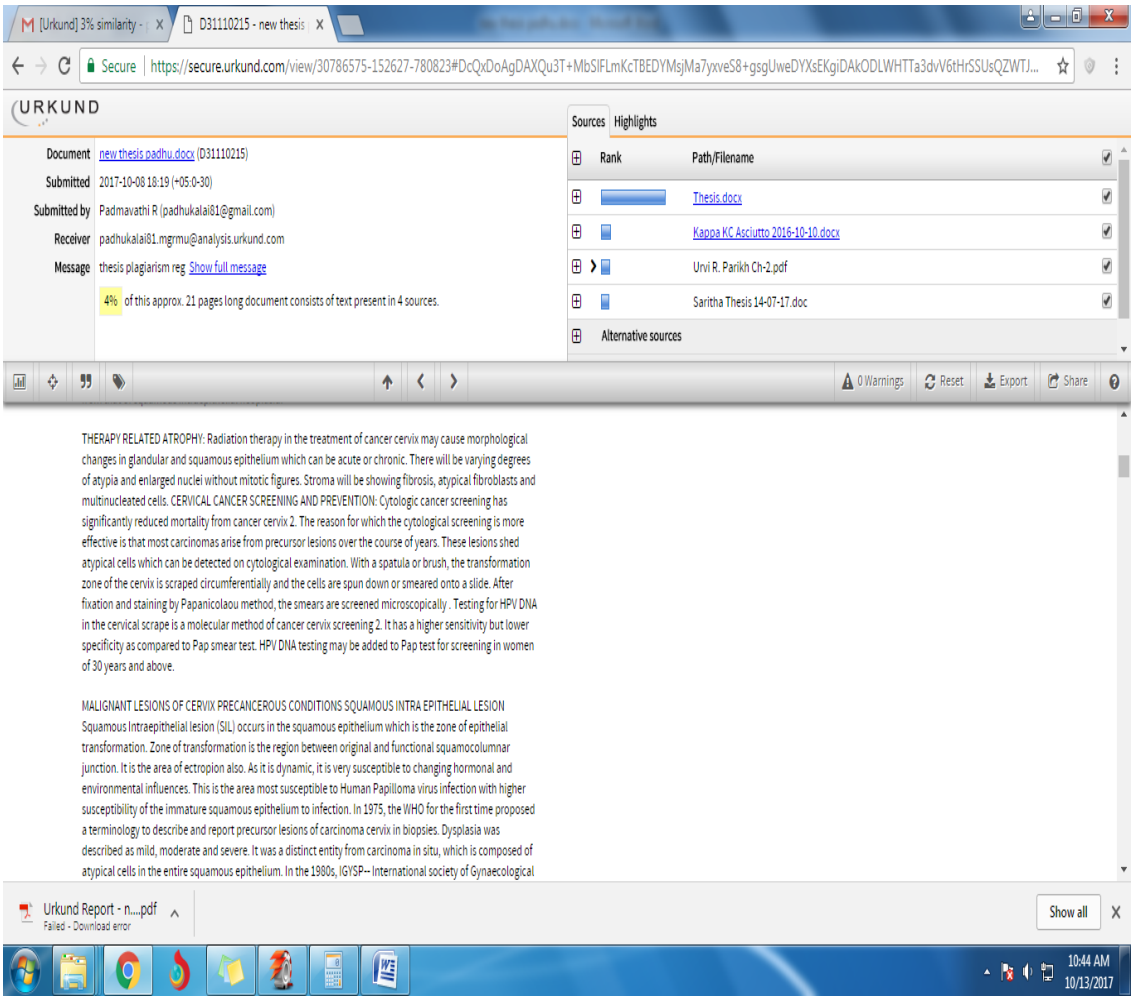
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THERAPY RELATED ATROPHY: Radiation therapy in the treatment of cancer cervix may cause morphological changes in glandular and squamous epithelium which can be acute or chronic. There will be varying degrees of atypia and enlarged nuclei without mitotic figures. Stroma will be showing fibrosis, atypical fibroblasts and multinucleated cells. CERVICAL CANCER SCREENING AND PREVENTION: Cytologic cancer screening has significantly reduced mortality from cancer cervix 2. The reason for which the cytological screening is more effective is that most carcinomas arise from precursor lesions over the course of years. These lesions shed atypical cells which can be detected on cytological examination. With a spatula or brush, the transformation zone of the cervix is scraped circumferentially and the cells are spun down or smeared onto a slide. After fixation and staining by Papanicolaou method, the smears are screened microscopically . Testing for HPV DNA in the cervical scrape is a molecular method of cancer cervix screening 2. It has a higher sensitivity but lower specificity as compared to Pap smear test. HPV DNA testing may be added to Pap test for screening in women of 30 years and above.
MALIGNANT LESIONS OF CERVIX PRECANCEROUS CONDITIONS SQUAMOUS INTRA EPITHELIAL LESION Squamous Intraepithelial lesion (SIL) occurs in the squamous epithelium which is the zone of epithelial transformation. Zone of transformation is the region between original and functional squamocolumnar junction. It is the area of ectropion also. As it is dynamic, it is very susceptible to changing hormonal and environmental influences. This is the area most susceptible to Human Papilloma virus infection with higher susceptibility of the immature squamous epithelium to infection. In 1975, the WHO for the first time proposed a terminology to describe and report precursor lesions of carcinoma cervix in biopsies. Dysplasia was described as mild, moderate and severe. It was a distinct entity from carcinoma in situ, which is composed of atypical cells in the entire squamous epithelium. In the 1980s, IGYSP-- International society of Gynaecological
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CERTIFICATE – II

This is to certify that this dissertation work titled “Expression of HLA 1 and CD 8 in cancer cervix patients” of the candidate Dr. R. PADMAVATHI with Registration Number 201513253 for the award of M.D in the branch of Pathology. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 4% (Four percentage) percentage of plagiarism in the dissertation.

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INTRODUCTION

INTRODUCTION

Cervix is the most common site in the female genital tract which is exposed to viral and bacterial infections. Also it is the target for carcinogenic agents leading to invasive cancer.

Cervical carcinoma is the second most common malignancy in women according to the worldwide cancer statistics.[1] Patients with invasive cancer cervix has an average age of 45 years.[2] Every year, almost 50% of the reported new cases have been proved to be fatal. The tumor occurs most often in the older age groups but also occurs, with relatively increased frequency in young females.[3] The decline in the rate of cancer cervix are due to the effective screening programmes via papanicolaou smear.[2,68]

Routine cytological PAP smear screening, early diagnosis and curative treatment had reduced the mortality of cancer cervix.

All the features which are of diagnostic and prognostic significance cannot be revealed by routine histological techniques. 80% of the cases are of squamous cell carcinoma subtype. The next most common is Adenocarcinoma constituting 15%. Remaining 5% of the cases are contributed by Adenosquamous and

Neuroendocrine carcinomas. Most of the histological subtypes are associated with high risk Human Papilloma viruses.[4]

Studies have shown that there is a crucial role for high risk Human papilloma viruses in the etiology of carcinoma cervix. [5] Host immune response factors contribute to the persistent HPV infection and progression to cervical neoplasia (Cervical Intraepithelial Neoplasia and Invasive cancer). Genes in the Human Leukocyte region of chromosome 6 are associated increased susceptibility to transforming properties of high risk HPV.

Cancer cells have many mechanisms to escape from immune mediated recognition and destruction. Loss of cell surface expression of Human Leukocyte Antigen (HLA) class I molecules is very important particularly, as this enables cancer cells to evade recognition and lysis by cytotoxic (CD 8 +) T lymphocytes. [12,61]

Present study intends to analyse the clinical features and histomorphology of cancer cervix and to assess the expression of HLA I and CD 8 in the differentiated forms of malignant lesions of the cervix. Also to correlate the grade of malignancy and HLA 1 and CD8 expression in carcinoma cervix.

AIM AND OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY

1. To analyse the clinical features and histomorphology of carcinoma cervix.

2. To assess the expression of Human Leukocyte Antigen 1 and CD 8 in the differentiated forms of malignant lesions of the cervix.

3. To correlate the grade of malignancy and HLA 1 and CD 8 expression in carcinoma cervix.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY

Cervix is a portion which is located at the lower end of uterus. It connects the cavity of uterus to the vagina. A narrow cervical canal passes through it.

Ectocervix is the external surface of the vaginal portion and endocervix is related to the endocervical canal of cervix. The internal os is the upper limit of the endocervical canal. [3]The external os is the opening of endocervical canal into vagina. [4]

HISTOLOGY

The endocervical glands are lined by the mucinous epithelium. The columnar epithelium changes abruptly into a non keratinizing stratified squamous epithelium at the portio vaginalis. Lamina propria connective tissue is more fibrous than in the uterus. Blood vessels, nerves, smooth muscles and elastic tissue are also seen. Smooth muscle fibres are mainly located in the endocervix. The sphincteric action of isthmus is due to concentric arrangement of smooth muscles constituting about 50 to 60% of the supporting tissue.

Majority of the ectocervix is covered by non keratinizing stratified squamous epithelium which in child bearing age is composed of basal cell, mid zone and superficial layers. The stem cells of cervical squamous mucosa are located in the suprabasal layer as proposed by some authors[3]. The mucus secreting columnar cells rest on an inconspicuous subcolumnar reserve cell which is located at or near the squamo-columnar junction. This is involved primarily in squamous metaplasia, cervical intraepithelial neoplasia and malignancy[4].

INFLAMMATORY CONDITIONS

INFECTIONS

The common organisms include bacteria, virus, fungi, parasites and protozoa. Infections are frequently due to Candida albicans, Trichomonas vaginalis, Gardnerella vaginalis, HPV and HSV.

The infectious viral agents which cause morphological alterations of cells have a site of predeliction for squamous epithelium of cervix. Anogenital HPV infections are transmitted by direct skin -to - skin or mucosa - to - mucosa contact. Both squamous and glandular lesions are associated with high risk HPV infections[4]. Inflammatory infiltrates are lymphocytes, plasma cells and histiocytes in chronic cervicitis.

Atypical morphological changes due to inflammation is difficult to differentiate from genuine atypia. But the changes are mild without increase in mitosis or reduction in cytoplasm.

REGENERATING EPITHELIUM

Chronic persistent infection can lead to reactive changes in the epithelium due to cervical erosion. This is also found in epithelial injury due to biopsy or conization. The changes include disorganization in epithelium and nuclear atypia in ectocervix and endocervix. Cytologically and histologically, these morphological changes mimics intra epithelial neoplasia. The nuclei are in uniform size and shape, clumped chromatin with normal mitotic figures and cytoplasmic membrane is well defined.

ATROPHY[6]

Hormone deficiency causes changes in maturation of squamous epithelium in pre pubertal, peri menopausal and post menopausal states. The changes are uniform basal and para basal cells with increased nuclear cytoplasmic ratio, coarse chromatin and rare mitosis. These epithelial changes are sometimes difficult from that of squamous intraepithelial neoplasia.

THERAPY RELATED ATROPHY:

Radiation therapy in the treatment of cancer cervix may cause morphological changes in glandular and squamous epithelium which can be acute or chronic inflammation and there will be varying degrees of atypia and enlarged nuclei without mitotic figures. Stroma will be showing fibrosis, atypical fibroblasts and multinucleated cells.

CERVICAL CANCER SCREENING AND PREVENTION:

Cytologic cancer screening has significantly reduced mortality from cancer cervix [2]. The reason for which the cytological screening is more effective is that most carcinomas arise from precursor lesions over the course of years. These lesions shed atypical cells which can be detected on cytological examination. With a spatula or brush, the transformation zone of the cervix is scraped circumferentially and the cells are spun down or smeared onto a slide. After fixation and staining by Papanicolaou method, the smears are screened microscopically.

Testing for HPV DNA in the cervical scrape is a molecular method of cancer cervix screening [2]. It has a higher sensitivity but lower specificity as compared to Pap smear test. HPV DNA testing

may be added to Pap test for screening in women of 30 years of age and above.

MALIGNANT LESIONS OF CERVIX

PRECANCEROUS CONDITIONS

SQUAMOUS INTRA EPITHELIAL LESION

Squamous Intraepithelial lesion (SIL) occurs in the squamous epithelium which is the zone of epithelial transformation. Zone of transformation is the region between original and functional squamocolumnar junction. It is the area of ectropion also. As it is dynamic, it is very susceptible to changing hormonal and environmental influences.

This is the area most susceptible to Human Papilloma virus infection with higher susceptibility of the immature squamous epithelium to infection.

In 1975, the WHO for the first time proposed a terminology to describe and report precursor lesions of carcinoma cervix in biopsies.

Dysplasia was described as mild, moderate and severe and carcinoma in situ is a distinct entity composed of atypical cells in the entire squamous epithelium.

In the 1980s, ISGYP--International Society of Gynaecological Pathologist introduced a terminology which have replaced dysplasia with Cervical Intraepithelial Neoplasia (CIN) and have eliminated carcinoma in situ. The CIN was divided into 1, 2 and 3 with carcinoma in situ being included into the Cervical Intraepithelial Neoplasia III.

The Bethesda system of reporting cervical cytology is recently applied for reporting of cervical lesions. [4]

It is a two-tiered system which divides dysplasia into Low grade Squamous Intraepithelial Lesions (LSIL) and High grade Intraepithelial Lesions (HSIL).

The single category of HSIL includes both CIN 2 and CIN 3. The Bethesda includes Condyloma acuminatum into LSIL, whereas WHO kept it as a seperate entity.

The Bethesda system of classification unifies the terminologies used for both cervical cytology and biopsy reporting [7].

In 2014 Bethesda system, atypical squamous cells are continued to be included in the squamous cell abnormality with subcategorization as Atypical Squamous cells of Undetermined Significance (ASC- US) and Atypical Squamous cells- cannot exclude a High grade squamous intraepithelial lesion (ASC- H).

2014 Bethesda update maintains the two-tiered reporting terminology of LSIL/ HSIL.

INVASIVE CANCER CERVIX:

Cervix cancer is the second most common form of neoplasm in women worldwide. [1,8] Statistics of cancer mortality states that after lung and breast cancer, it is the third leading cause of death. In Asian women, the incidence and mortality rate is 4 to 5 times greater than in developed nations. [50]

Squamous cell carcinoma is having two peaks of age distribution, one at 35 years and another one around 50 to 55 years.

AETIOLOGY:

Age:

The single most important factor is age at first intercourse.[3] Young and sexually active women are at increased risk for HPV infection and cervical intraepithelial neoplasia. With increasing age, there will be increased risk for carcinoma. Also the risk decreases with the menopausal approach.

Religion and race:

Jews and Muslim women have reduced risk of carcinoma cervix because of the ritual circumcision done for male children.

Social and Economic factors:

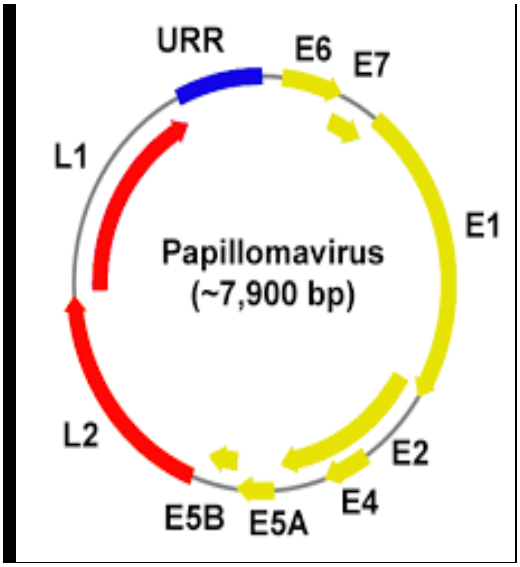
Carcinoma cervix is more common in people of low socio economic status as they have poor sanitation, early and frequent coitus.

Coitus:

It is 4 to 5 times more common in sexually active women.

Cervical Infection

HPV GENOME



URR - UPSTREAM REGULATORY REGION
ER - EARLY REGION
LR - LATE REGION

Infection due to Human Papilloma Virus plays an important role. High risk HPV types 16, 18, 31 and 45 accounts for more than 80% of cancer cervix[2,9] . HPV 16 is the most common type and 18 is the second common type[20]. It infects epithelial cells[54].

There are around 15 to 20 types acting as a cofactor in the development of carcinoma.

Hormonal Factors:

Oral contraceptives with oestrogen and progesterone favours intraepithelial lesions and also invasive cervical cancers [6].

The other risk factors include smoking, pregnancy at an early age, HIV infection, vitamin deficiency.

Predisposing Histological changes:

These include basal cell hyperplasia, squamous metaplasia and High grade Squamous Intraepithelial Lesion are significant precancerous conditions.

Histological classification of cervical carcinoma:

Carcinoma cervix starts at the squamocolumnar junction and 80 to 90 % are of squamous cell in origin.

Remaining 5 to 10% are of columnar cells in origin (Adenocarcinoma). This is more in young women who are smokers and pill users.

The remainder constitutes the mixed types.

WHO CLASSIFICATION OF UTERINE CERVIX TUMORS (2014)

Epithelial tumors

Squamous cell tumors and its precursors

Squamous intraepithelial lesions

- Low - grade squamous intraepithelial lesion
- High - grade squamous intraepithelial lesion

Squamous cell carcinoma, NOS

- Keratinizing
- Non - keratinizing
- Papillary
- Basaloid
- Warty
- Verrucous
- Squamotransitional
- Lymphoepithelioma - like carcinoma

Benign squamous cell lesions

- Squamous metaplasia
- Condyloma acuminatum
- Squamous papilloma
- Transitional metaplasia

Glandular tumors and its precursors

Adenocarcinoma in situ

Adenocarcinoma

Endocervical adenocarcinoma , usual type

Mucinous carcinoma ,NOS

Gastric type

Intestinal type

Signet - ring cell type

Villoglandular carcinoma

Endometrioid adenocarcinoma

Clear cell carcinoma

Serous carcinoma

Mesonephric carcinoma

Adenocarcinoma admixed with neuroendocrine carcinoma

Benign glandular tumours and tumour - like lesions

Endocervical polyp

Mullerian papilloma

Nabothian cyst

Tunnel clusters

Microglandular hyperplasia

Lobular endocervical glandular hyperplasia

Diffuse laminar endocervical hyperplasia

Mesonephric remnants and hyperplasia

Arias Stella reaction

Endocervicosis

Endometriosis

Tuboendometrioid metaplasia

Ectopic prostate tissue

Other epithelial tumours

Adenosquamous carcinoma

Glassy cell carcinoma

Adenoid basal carcinoma

Adenoid cystic carcinoma

Undifferentiated carcinoma

Neuroendocrine tumors

Low grade neuroendocrine tumour

Carcinoid tumour

Atypical carcinoid tumour

High - grade neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

Mesenchymal tumors and tumor- like lesions

Benign

Leiomyoma

Rhabdomyoma

Others

Malignant

Leiomyosarcoma

Rhabdomyosarcoma

Alveolar soft part sarcoma

Angiosarcoma

Malignant peripheral nerve sheath tumour

Other sarcomas

Liposarcoma

Undifferentiated endocervical carcinoma

Ewing sarcoma

Tumour - like lesions

Postoperative spindle - cell nodule

Lymphoma - like lesion

Mixed epithelial and mesenchymal tumor

Adenomyoma

Adenosarcoma

Carcinosarcoma

Melanocytic tumors

Blue naevus

Malignant melanoma

Germ cell tumours

Yolk sac tumour

Lymphoid and myeloid tumors

Lymphomas

Myeloid neoplasms

Secondary tumors

Reagan et al found three groups in squamous cell carcinoma[10]

Non - Keratinizing - large cell type (moderately differentiated grade)

Keratinizing - large cell type (well differentiated grade)

Small cell Non - Keratinizing type (poorly differentiated grade)

Other variants are

Papillary squamo transitional carcinoma

Verrucous carcinoma

Lympho epithelioma like carcinoma

Basaloid carcinoma

Condylomatous (warty) carcinoma

MICROINVASIVE SQUAMOUS CELL CARCINOMA:

In 1847, Mestwerdt was the one to introduce about microinvasive carcinoma. It is a type of carcinoma which can be diagnosed only by microscopical examination.

Invasion by tumor cells extend to a maximum depth of 5 mm and to a maximum width of 7 mm.

INVASIVE SQUAMOUS CELL CARCINOMA:

After breast carcinoma, squamous cell carcinoma of cervix is the second most common carcinoma worldwide. And it is the most common carcinoma in the women of developing countries.[1]

The overall frequency has been decreased because of the national cervical screening programmes. This has also been used in the detection of asymptomatic small and early invasive lesions.

Cervical squamous cell carcinoma has three variants[10, 21]

Non - Keratinizing carcinoma accounts for two third of cases. It shows individual cell cytoplasmic keratinization and intercellular bridges, but nesting of tumor cells, keratin pearls are not present.

Keratinizing carcinoma accounting for one sixth of cases. It contain keratin pearls and nests of tumor cells with central keratin. Keratohyaline granules, intercellular bridges and cytoplasmic keratinization are also seen.

Small cell non -keratinizing carcinoma are characterised by sheets of non - keratinizing small cells with high nuclear cytoplasmic ratio.

Squamous cell (epidermoid) carcinoma constitutes about 70% . The common pattern is non - keratinizing large cell type with better prognosis.

Well differentiated keratinizing carcinoma accounts for 25% of the cases.

Undifferentiated carcinoma is less common with poor prognosis.

Based on the differentiation, modified Broder 's method[11,23] includes

Well differentiated grade- Grade 1

Moderately differentiated grade- Grade 2

Poorly differentiated grade- Grade 3

Grade 2 is common, then grade 3 and then grade 1 are common in decreasing order.

Basaloid carcinoma has basaloid appearance. It is composed of nests of tumor cells with less amount of cytoplasm and peripheral palisading of cells.

Verrucous carcinoma is a very rare subtype characterised by exophytic growth with minimal or no atypia.

Warty carcinoma is also exophytic with koilocytic changes in the surface epithelium.

Papillary carcinoma is having papillary growth pattern. It is divided into

1) Papillary undifferentiated carcinoma
2) Papillary transitional cell carcinoma
3) Papillary squamo - transitional carcinoma

Lymphoepithelial like[1] carcinoma is characterised by undifferentiated cells with lymphocytic infiltration in the stroma and the neoplastic cells have syncitial growth pattern, poorly defined cell borders and pale eosinophilic cytoplasm.

Adenocarinoma:

Primary adenocarcinoma comprises 15% of all carcinoma cervix. Its incidence is on the rise in young women. Long term use

of oral contraceptives and its association with endocervical neoplasia has been reported.[3]

Most common pattern is well differentiated glandular pattern with mucin.

Morphological variants of adenocarinoma

Endometrioid adenocarcinoma

Papillary serous carcinoma

Adenosquamous (mixed)

Adenoid cystic carcinoma

Adenoid basal carcinoma

Clear cell carcinoma

Endometrioid adenocarcinoma is similar to its ovarian and endometrial counterparts. Its incidence is increasing. Minimal deviation adenocarcinoma is otherwise known as adenoma malignum.

Glucksmann and Cherry emphasised the importance of adenosquamous carcinoma and used the terminology of mixed carcinoma.[4] Mixed carcinoma includes adenocarcinoma with well defined squamous component.

A type of poorly differentiated adenosquamous carcinoma is Glassy cell carcinoma occuring in a younger age group and has been associated with pregnancy.

Clear cell carcinoma or mesonephric carcinoma is composed of cells arranged in tubulo cystic, papillary pattern. The cells will have abundant clear cytoplasm, enlarged and pleomorphic nuclei which protrudes into the lumen having a ' Hob nail ' appearance.

It is common in young women and have a relatively good prognosis.

Mixed Epithelial / Mesenchymal neoplasm includes

1. Adenomyoma
2. Carcinosarcoma
3. Cervical Adenosarcoma

Adenosarcoma is a biphasic tumor. Primary adenosarcoma is a very rare (2%) neoplasm in the genital tract.

Melanocytic tumors

1. Blue Nevus
2. Malignant melanoma

Malignant melanoma is a rare neoplasm of which only 30 cases have been reported. The mean age is 60 years and it is having poor prognosis.

Neuroendocrine tumors include

Carcinoid

Atypical carcinoid

Large cell neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Carcinoid

Benign tumors have a characteristic organoid pattern. The difference between typical and atypical carcinoid includes atypia of the nucleus and mitosis.

Atypical Carcinoid

Cytological atypia, areas of necrosis and increased mitotic count (around 5 to 10 per high power field) are the features of atypical carcinoid.

Small cell carcinoma

This accounts for about 1 to 6% of cervix carcinoma. Glandular or squamous component may be seen in association.

Large cell neuroendocrine carcinoma

This is composed of high grade neoplastic cells with abundant cytoplasm, vesicular chromatin, prominent nucleoli and high mitosis (> 10 per 10 high power field).

Undifferentiated sarcoma, metastasis from other sites and adenocarcinoma with neuroendocrine differentiation are the differential diagnosis and immunohistochemistry markers include neuron specific enolase, synaptophysin and chromogranin are useful to arrive at a diagnosis.

2009 MODIFICATION OF FIGO STAGING OF CARCINOMA OF THE CERVIX UTERI:

STAGE I - Cervical carcinoma confined to the uterus (extension to the corpus should be disregarded)

STAGE I A - Invasive carcinoma diagnosed only by microscopy; all macroscopically visible lesions , even with superficial invasion, are stage IB

STAGE IA1 - Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread

STAGE IA2 - Stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread of 7.0 mm or less

STAGE IB - Clinically visible lesion confined to the cervix or microscopic lesion greater than IA2

STAGE IB1- Clinically visible lesion 4.0 cm or less in greatest dimension

STAGE IB2 - Clinically visible lesion more than 4.0 cm in greatest dimension

STAGE II - Tumor invades beyond the uterus but not to pelvic wall or to lower third of the vagina

STAGE IIA - Without parametrial invasion

STAGE IIA 1 - Clinically visible lesion < or equals 4.0 cm in greatest dimension

STAGE IIA 2 - Clinically visible lesion > 4.0 cm in greatest dimension

STAGE II B - With parametrial invasion

STAGE III - Tumor extends to the pelvic wall and /or involves lower third of vagina and /or causes hydronephrosis or nonfunctioning kidney

STAGE IIIA - Tumor involves lower third of vagina with no extension to pelvic wall

STAGE IIIB - Tumor extends to pelvic wall and/or causes hydronephrosis or nonfunctioning kidney

STAGE IV - The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema , as such, does not permit a case to be allotted to stage IV

STAGE IV A - Spread of the growth to adjacent organs

STAGE IV B - Spread to distant organs.

IMMUNE SYSTEM:

The immune system is the specific defense mechanism.[24] It comprises of humoral immune system (B cell mediated) and cell mediated immune system (T cell mediated).[13] Both the systems are responsible for elimination of a virus and virus induced lesions.

Immunological surviellance is performed by CTLs which are CD8 + T lymphocytes,which destroy tumor cells expressing HLA class 1 molecules over their surface.[14] HLA class 1 molecules are virtually expressed on all cells.

The Major Histocompatability Complex (MHC) or Human Leukocyte Antigen (HLA) is located on the short arm of chromosome 6 at 6p21.3 and comprises about 240 different gene loci of which may encode for HLA molecules.[15,25.] The MHC can be subdivided into three closely related families - Class 1 (the classical 1A genes, HLA- A, -B, and-C and the non classical 1B genes, HLA- E, -H, -G and -F) , Class 2 (HLA- DR, -DP and - DQ genes), Class 3 (includes genes encoding complement and tumor necrosis factor). The HLA class 1 and 2 molecules are encoded by HLA - Class 1 and 2 genes respectively. On each chromosome 6, the genes in the class 1 to 3 regions compose a combination which is called a

haplotype. The two haplotypes on the chromosome 6 pair combined are called the HLA genotype.

The HLA genotype is expressed as HLA Class 1 and 2 molecules on the cell surface and this is called the HLA phenotype.

The endogenously processed antigens (tumor or viral associated products) are processed and presented to the circulating cytotoxic T lymphocytes.

ROLE OF CD 8 IN CANCER CERVIX:

The cervix has the largest concentration of lymphocytes in the female genital tract. Also as a component of mucosal immune system, T lymphocytes guards against the ascending infection from the vagina. Two populations of T cells have specific cytotoxicity against virus infected cells : the CD 4 expressing T helper cell (T h) which is restricted to MHC class 2 expressing targets and the CD 8 expressing cytotoxic / suppressor T cell (T c/s) which is MHC class 1 restricted [16]. The CD 8 cells are believed to be the principal effectors against HPV infected epithelium and epithelial tumors.

IMMUNE EVASION IN CANCER CERVIX

Loss of HLA class 1 cell surface expression occurs in cancer cervix. [26] In 1976, the first description of MHC class 1 loss was done in a mouse model (Gardener Lymphoma) in Dr. Festenstein laboratory. It is thought to result in escape from the cytotoxic CTL attack. It is caused predominantly by losses at chromosome 6p21.3 , the region where the HLA genes are localised. Loss of heterozygosity (haplotype loss) is the most frequent alteration of class 1 expression. [12,27] This is caused by various defects in HLA genomic region including chromosomal dysfunction, mitotic recombination and genetic conversion. The accurate detection of HLA complex on tumor cell surface is fundamental as it predetermines the tumor cell recognition by both cytotoxic T lymphocytes and NK cells. [28]

Tumor cells with complete HLA loss are not recognised by CTL but NK cells able to target them for elimination. Tumors with partial loss may evade both NK cell as well as T cell mediated immune surviellance.

Ekaterina S. Jordanova et al[9] studied Human Leukocyte Antigen class 1 chain related molecule A and CD 8+ / Regulatory T

cell ratio: Which variable determines survival of cervical cancer patients ?

They have concluded Weak HLA- A expression combined with low CD 8+ ratio reveals a patient group with the poorest survival in cancer cervix. As a single variable , low CD 8+ / Treg (regulatory T cell) was a significant independant unfavourable prognostic factor.

N. Aptisiauri et al.,[12] MHC class 1 antigens in malignant cells: Immune escape and response to immunotherapy. Expression of HLA class 1 alterations in tumor cells is a key factor to be considered during selection of immunotherapy strategy and is a biomarker to be monitored during treatment. [12,29]

Akash M Mehta et al [15] studied association of antigen processing machinery and HLA class 1 defects with clinicopathological outcome in cervical carcinoma.

A complete understanding of the mechanisms and relevance of HLA class 1 downregulation and immune evasion may contribute to the rational design of tumor vaccines and T cell based immunotherapies.

Jiang Tao Fan et al[16] studied the expression of HLA- 1, CD 8 and CD 4 and their clinical significance in cervical cancer.

It showed the expression of HLA-1, CD 8 and CD 4 are downregulated or deleted in CIN and cervical cancer and they may play important roles in the development and progression of CIN to invasive cancer.

M J Arends et al [17] studied the aetiology, pathogenesis and pathology of cervical neoplasia.

Swati Patel and Shubhada Chiplunkar [18]studied the host immune responses to cervical cancer.

It showed the HPV uses different strategies to evade immune recognition. Understanding the immune evasion mechanisms used by HPV will help in designing newer therapeutic strategies for cervical cancer.

Robert P Edwards et al[19] studied T lymphocytes infiltrating advanced grades of cervical neoplasia.

The invasive lesion and its association with CD 8 T cells locally strongly suggests that the immune response is a clinical cofactor in the progression of cervical neoplasia.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study design

Prospective study

Study period

July 2016 to July 2017

Study place

Coimbatore Medical College Hospital, Coimbatore.

Sample size

A total number of 30 cases. From case records brief clinical data were collected , which included age, clinical diagnosis and surgical procedure.

The following inclusion and exclusion criteria were adopted.

Inclusion criteria:

1. Hysterectomy and small cervical biopsy specimens with cervical cancer.

2. Patients in all age groups (20 - 60 years) of abnormal uterine bleeding with suspected carcinoma cervix.

3. VIA / VILI positive cases.

Exclusion criteria:

1. Inadequate specimens.

2. Chronic cervicitis.

3. Squamous Intraepithelial Lesion.

4. In Treated patients before surgery.

5. Specimen not sent in formalin.

Methods:

Among the total cases received in the Department of Pathology in our hospital during the study period, 30 cases were taken into study as per inclusion criteria and further evaluated.

All those 30 small biopsies and hysterectomy specimens are selected , then fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin.

HEMATOXYLIN AND EOSIN STAINING:

REAGENTS USED:

1. Hematoxylin solution - Ehrlich' s Hematoxylin
2. Eosin 1% solution
3. Acid alcohol 1% solution

PROCEDURE:

1. Deparaffinize the sections by immersion in xylene for 30 seconds.
2. Then place the sections in isopropyl alcohol for 15 minutes.
3. Wash then in running tap water.
4. Stain for 10 to 15 minutes in Ehrlich's hematoxylin.
5. Differentiation with 1% acid alcohol is done two to three dips.
6. Blueing for 10 minutes is carried out.
7. Counterstain with Eosin 1% solution three to four dips.
8. Wash in running tap water done.
9. Air dried.
10. Mounting with DPX done.

IMMUNOHISTOCHEMISTRY

METHOD

Two step indirect technique

PRINCIPLES OF THE PROCEDURE

Using a two stage processes , antigens were detected in cells and tissues. The first was the binding to specific epitopes of the primary antibody. Second was a calorimetric reaction to detect the bindings.

Sections of the tissues were fixed and attached to the coated slides.

The paraffin embedded sections were then dewaxed. Antigen retrieval was done. This consists of treating the formalin fixed sections in a microwave oven in an aqueous solution. It recovered full antigenicity with most of the antibodies. These also included cases which were formerly unreactive with formalin fixed tissue. Subsequently , the tissue sections were treated with Peroxide block and Power block for blocking of endogenous peroxidases and non specific protein- protein interactions , respectively.

REAGENTS USED:

1. Peroxide block : 3% Hydrogen peroxide in water
2. Power block reagent : A highly effective universal protein blocking reagent. It contains casein and propriety additives in PBS with 15 mM sodium azide.
3. Chromogen : DAB- Diaminobenzidine.
4. Liquid DAB substrate : comprises Tris- buffer containing the peroxide and stabilizer.
5. Super enhancer reagent.
6. Poly- HRP reagent.
7. Counter stain: Ehrlich' s hematoxylin.

BUFFER SOLUTIONS:

TRIS BUFFER (pH - 7.6) :

Tris buffer salt : 0. 605 gms

Sodium chloride : 8 gms

Distilled water : 1000 ml

1 N Hydrochloric acid : 3 ml

CITRATE BUFFER (pH -6.0):

Trisodium citrate : 2.94 gms

Distilled water : 1000 ml

1 N Hydrochloric acid : 5 ml

TRIS - EDTA (pH -9.0):

Tris buffer salt : 6.05 gms

Disodium EDTA: 0. 744 gm

Distilled water : 1000 ml

PROCEDURE :

1. Sections are deparaffinised with xylene for 30 minutes as 2 changes 15 minutes each.
2. Sections are dehydrated in absolute alcohol for 5 minutes with 2 changes.
3. Sections are washed in tap water for 10 minutes.
4. Slides are then rinsed in distilled water for 5 minutes.

5. Antigen retrieval is done by placing the slides with appropriate buffer solution in microwave : medium temperature - 10 minutes, high temperature -10 minutes.
6. They are then cooled to room temperature and then rinsed in distilled water.
7. Sections are then washed in TBS buffer for 5 minutes with 2 changes.
8. Then the slides are treated with Peroxide block for 10 minutes.
9. Sections are again washed in TBS buffer for 5 minutes with 2 changes.
10. Treated with Power block for 10 minutes.
11. Slides are then drained and covered with primary antibody for 2 hours.
12. Wash in TBS buffer for 5 minutes with 2 changes.
13. Sections are then covered with super enhancer for 30 minutes.
14. Wash in TBS buffer for 5 minutes with 2 changes.

15. Poly - HRP reagent was applied and left for 30 minutes.
16. Wash in TBS buffer for 5 minutes with 2 changes.
17. Treat with DAB chromogen with substrate buffer for 5 to 8 minutes.
18. Wash in TBS buffer for 5 minutes with 2 changes.
19. They are then washed in tap water for 5 minutes.
20. Sections are then counterstained with Ehrlich's hematoxylin for 30 seconds.
21. Wash in tap water for 5 minutes.
22. Slides are then air dried and mounted with DPX.

Tumor cells were scored positive if there was golden brown membranous, cytoplasmic or nuclear staining in the neoplastic cells.

Negative diagnosis was obtained when no golden brown staining was observed.

Interpretation of IHC:

HLA - 1:

Positive staining refers to cytoplasmic or cytoplasmic membrane staining of cells [16]. Intensity of positive staining is graded between 0 to 3+.

CD 8:

Positive staining refers to staining of cytoplasm or cytoplasmic membrane [16]. Intensity of positive staining is graded between 0 to 3+ as below :

Sl. No	Grading	Percentage of cells expressing HLA - 1 and CD 8 positivity
1.	0 (negative)	No positively staining cells
2.	1+ (focally positive)	< 25 % of positively staining cells
3.	2+ (positive)	25 - 50 % of positively staining cells
4.	3+ (diffusely positive)	> 50 % of positively staining cells

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Of 2542 gynaecological specimens received at Pathology Department Coimbatore Medical College Hospital during the period of August 2016 - July 2017, malignancy was reported in 215 cases (8.45 %).

This prospective study included routinely processed biopsies from 30 patients with malignant lesions of the cervix diagnosed histologically during the period of August 2016 - July 2017.

AGE INCIDENCE:

The patients diagnosed as cervical carcinoma were divided into 6 groups according to age i.e 31 - 40 , 41 - 50 , 51 - 60 , 61 - 70 , > 71 years of age.

The age wise distribution of cervical cancer is given in the following Table 1 and Chart 1.

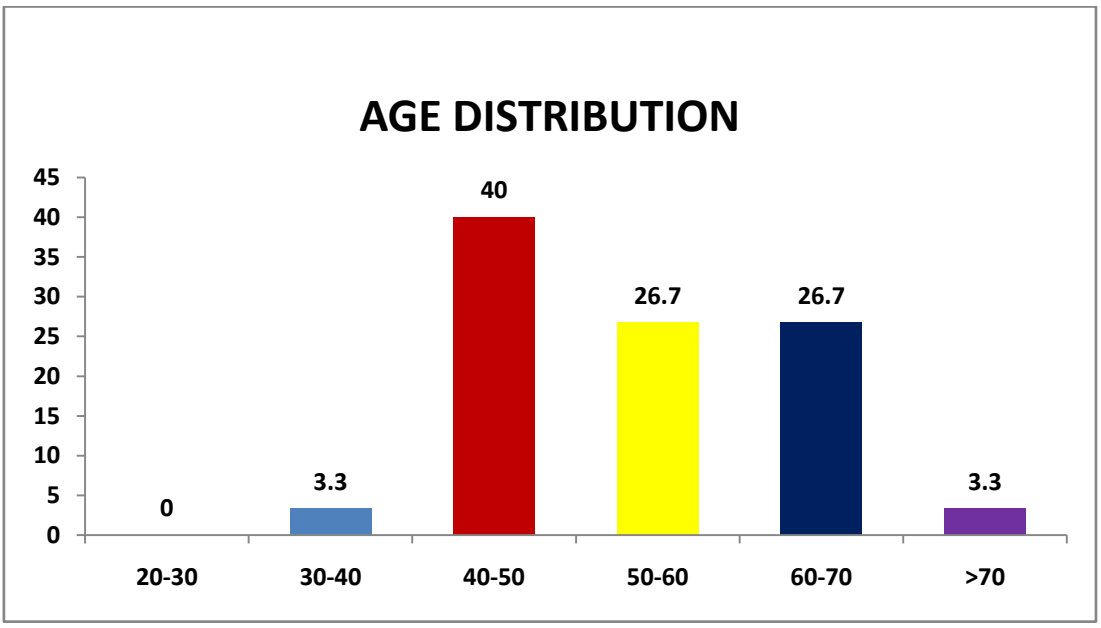
TABLE 1

AGE WISE DISTRIBUTION OF CANCER CERVIX CASES

AGE GROUP	NO. OF CASES	PERCENTAGE (%)
31 – 40	1	3.3
41 – 50	12	40
51 – 60	8	26.7
61 – 70	10	33.3
> 71	1	3.3
TOTAL	30	100

There was increased incidence of cervical cancer observed in the age group of 41- 50 years (40 %) followed by 51-60 years (26.7%) and 61 - 70 years (26.7 %).

CHART 1



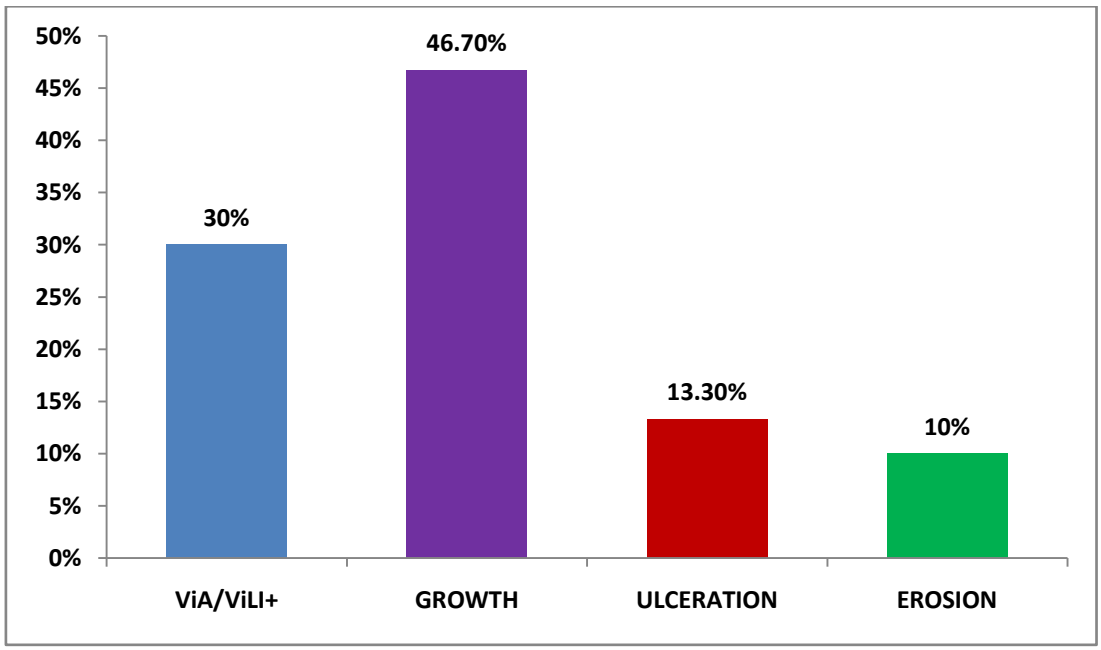
The following table 2 and chart 2 shows the colposcopic findings in carcinoma cervix cases clinically :

TABLE 2

COLPOSCOPY FINDINGS IN CANCER CERVIX CASES

FINDINGS	NO. OF CASES	PERCENTAGE (%)
ViA / ViLI +	9	30
GROWTH	14	46.7
ULCERATION	4	13.3
EROSION	3	10
TOTAL	30	100

CHART 2



It includes ViA / ViLI (Visual inspection with Acetic acid and visual inspection with Lugol ' s Iodine), clinically obvious growth, cervical ulceration and erosion cases. Most common clinical finding was growth (46.7%).

The following Table 3 and Chart 3 shows the type of carcinoma cervix cases diagnosed histologically as Squamous cell carcinoma, Adenocarcinoma, Adenosquamous carcinoma and Clear cell carcinoma.

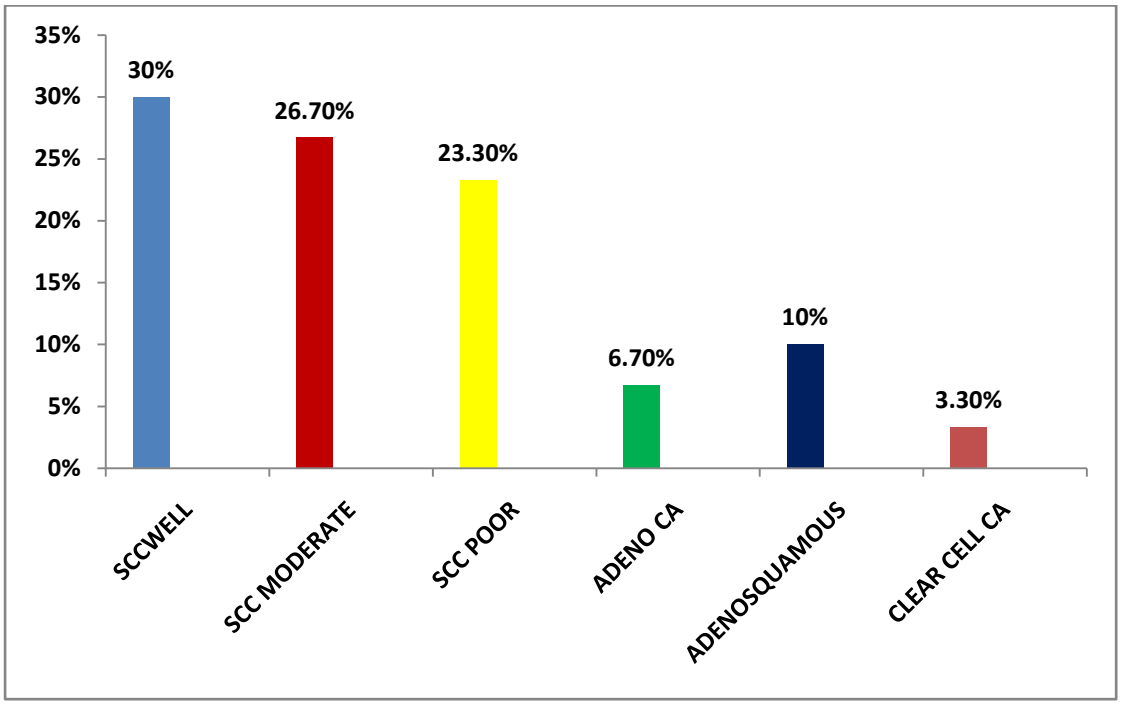
TABLE 3

TYPE OF CARCINOMA CERVIX CASES

TYPE	NO.OF CASES	PERCENTAGE
SCC- Well differentiated	9	30%
SCC- Moderately differentiated	8	26.7%
SCC- Poorly differentiated	7	23.3%
Adenocarcinoma	2	6.7%
Adenosquamous carcinoma	3	10%
Clear cell Ca	1	3.3%
TOTAL	30	100%

CHART 3

TYPES OF CARCINOMA CERVIX CASES



Squamous cell carcinoma is the most common type constituting 80% followed by Adenosquamous carcinoma and Adenocarcinoma.

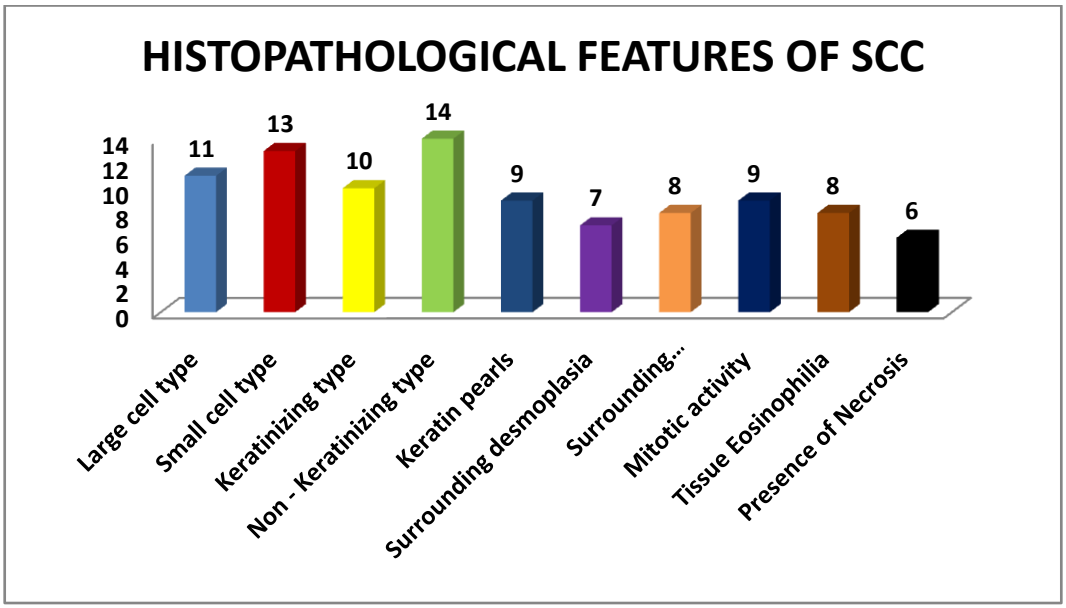
Table 4 and Chart 4 includes the various histopathological features seen in Squamous cell carcinoma.

TABLE 4

HISTOPATHOLOGICAL FEATURES OF SCC

FEATURES	NO.OF CASES
Large cell type	11
Small cell type	13
Keratinizing type	10
Non - Keratinizing type	14
Keratin pearls	9
Surrounding desmoplasia	7
Surrounding Inflammation	8
Mitotic activity	9
Tissue Eosinophilia	8
Presence of Necrosis	6

CHART 4



Non keratinizing and small cell types are the common features that are identified. These types are seen in both moderately differentiated as well as poorly differentiated squamous cell carcinoma.

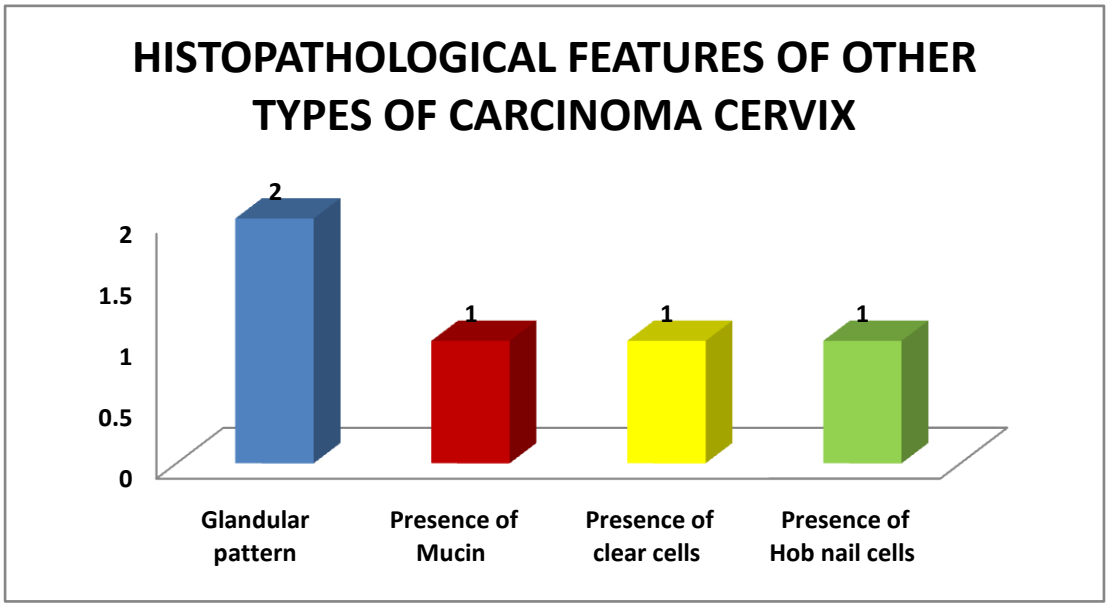
Table 5 and Chart 5 includes the histopathological features seen in other types of carcinoma cervix.

TABLE 5

HISTOPATHOLOGICAL FEATURES OF OTHER TYPES OF CARCINOMA CERVIX

FEATURES	NO.OF CASES
Glandular pattern	2
Presence of Mucin	1
Presence of clear cells	1
Presence of Hob nail cells	1

CHART 5



Glandular pattern and the presence of mucin are observed in Adenocarcinoma. Clear cells and Hob nail cells are observed in Clear cell carcinoma.

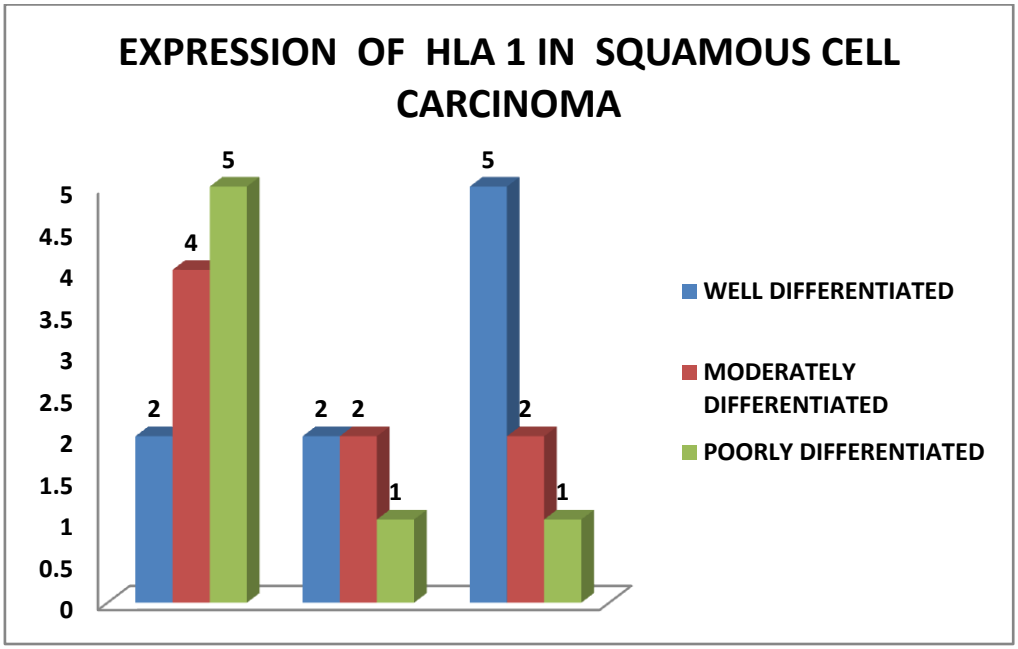
The following Table 6 and Chart 6 shows the expression of HLA 1 in different grades of carcinoma cervix .

TABLE 6

EXPRESSION OF HLA - 1 IN SQUAMOUS CELL CARCINOMA

TYPE	1+ (< 25%)	2+(25 -50%)	3+(>50%)
SCC - Well differentiated carcinoma	2	2	5
SCC- Moderately differentiated carcinoma	4	2	2
SCC-Poorly differentiated carcinoma	5	1	1

CHART 6



1+(25%) 2+(25-50%) 3+(>50%)

HLA 1 expression with less intensity is observed in 2 well differentiated and 5 poorly differentiated SCC. The intensity is more in 7 well differentiated and 2 poorly differentiated SCC.

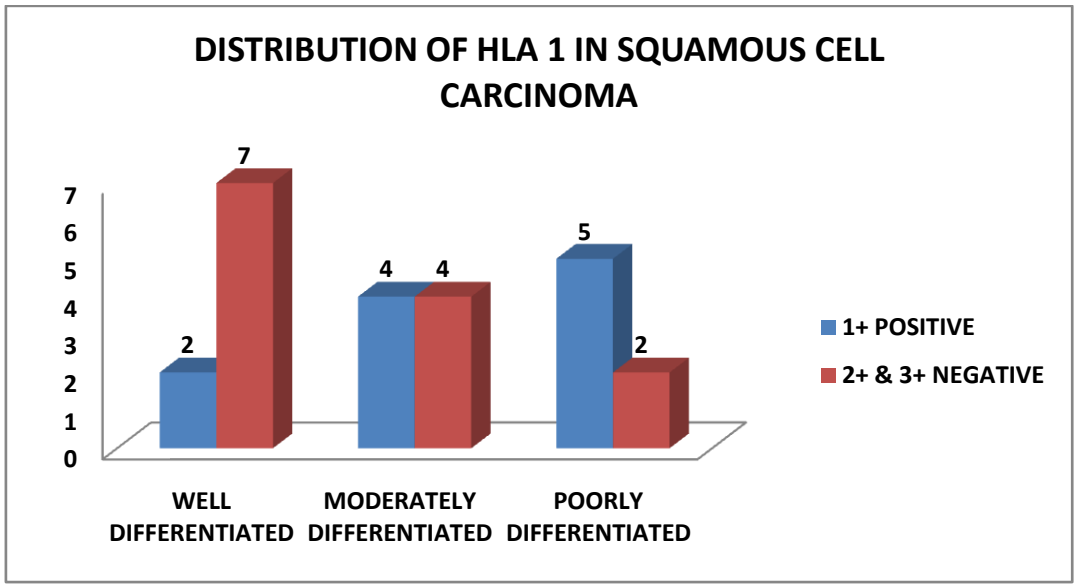
The following Table 7 and Chart 7 shows the distribution of HLA 1 in different grades of Squamous cell carcinoma of cervix.

TABLE 7

DISTRIBUTION OF HLA 1 IN SCC CERVIX

TYPE	1+ (negative)	2+& 3+(positive)	TOTAL
SCC - Well differentiated carcinoma	2	7	9
SCC- Moderately differentiated carcinoma	4	4	8
SCC-Poorly differentiated carcinoma	5	2	7
TOTAL	11	13	24

CHART 7



According to this , the positive expression of HLA 1 is seen in 9 out of 24 squamous cell carcinoma cases which are of well differentiated grade. It is negative in 7 out of 24 SCC cases which are of poorly differentiated grade. It is positive in 4 out of 8 moderately differentiated grade of Squamous cell carcinoma.

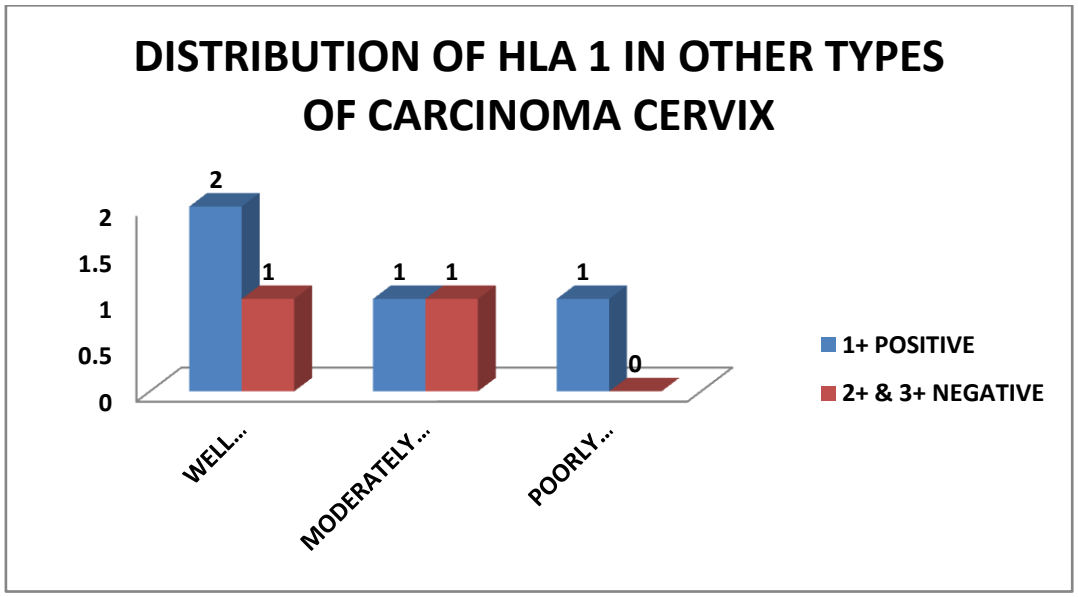
The following Table 8 and Chart 8 shows the distribution of HLA 1 in other types of Carcinoma cervix.

TABLE 8

DISTRIBUTION OF HLA 1 IN OTHER TYPES OF CARCINOMA CERVIX

TYPE	1+ (Negative)	2+& 3+(Positive)
Adenocarcinoma	2	1
Adenosquamous carcinoma	1	1
Clear cell carcinoma	1	0

CHART 8



The following Table 9 and Chart 9 shows the expression of CD 8 in different grades of Squamous cell carcinoma cervix.

TABLE 9

EXPRESSION OF CD 8 IN SCC - CERVIX

TYPE	1+(< 25%)	2+(25 -50%)	3+(>50%)
SCC -Well differentiated carcinoma	2	2	5
SCC - moderately differentiated carcinoma	4	2	2
SCC -Poorly differentiated carcinoma	5	1	1

CHART 9

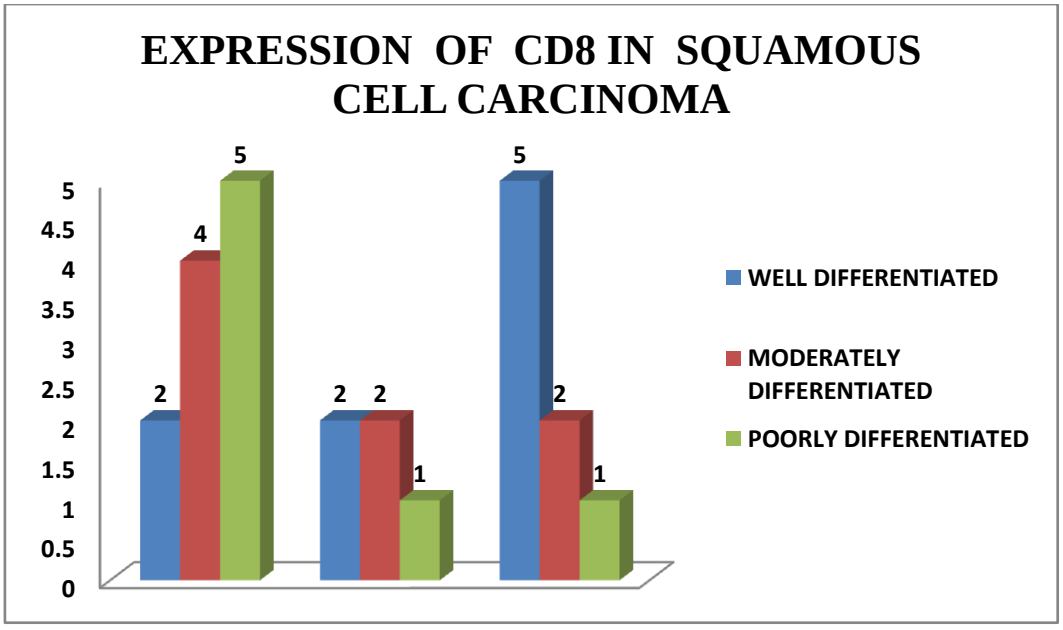


TABLE 10

DISTRIBUTION OF CD 8 IN SCC- CERVIX

TYPE	1+ (negative)	2+& 3+(positive)	TOTAL
SCC - well differentiated carcinoma	2	7	9
SCC- moderately differentiated carcinoma	4	4	8
SCC -poorly differentiated carcinoma	5	2	7
TOTAL	11	13	24

CHART 10

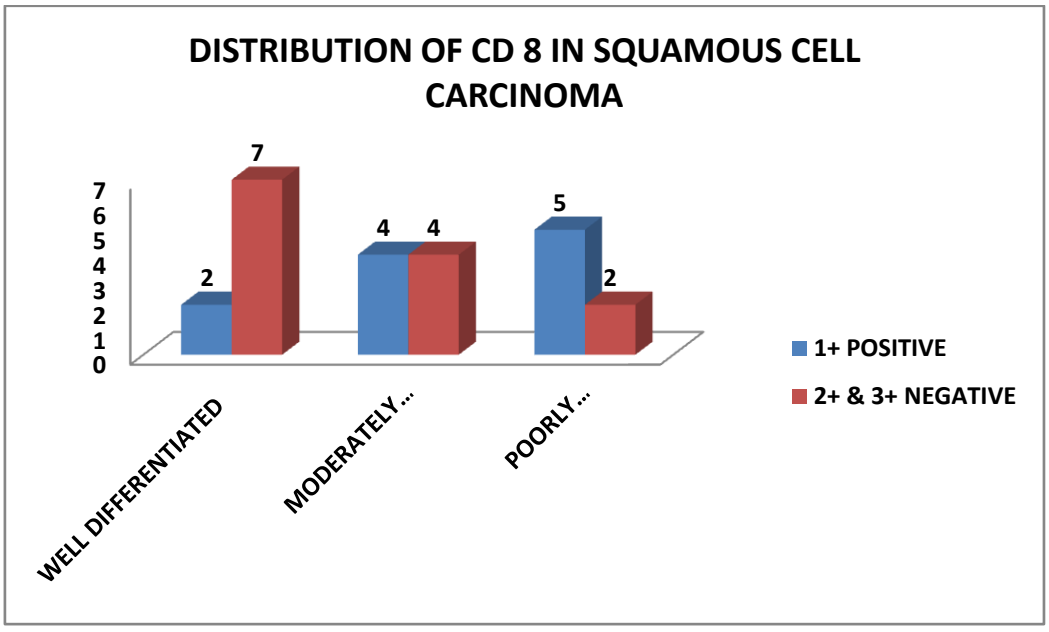


TABLE 11

DISTRIBUTION OF CD 8 IN OTHER TYPES OF CANCER CERVIX

TYPE	1+ (Negative)	2+& 3+(Positive)
Adenocarcinoma	2	1
Adenosquamous carcinoma	1	1
Clear cell carcinoma	1	0

CHART 11

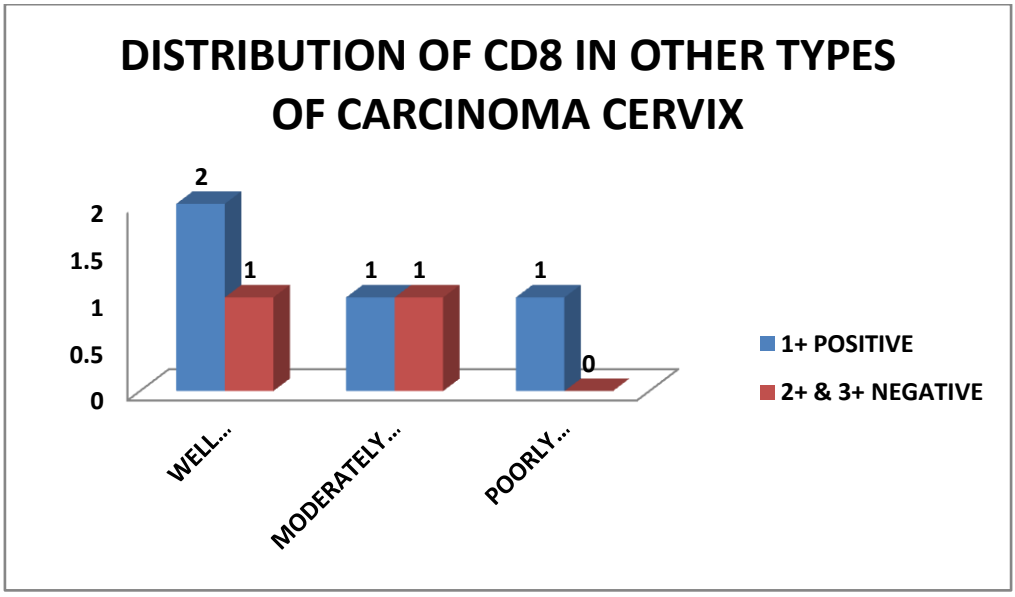


TABLE 12

CORRELATION OF HLA 1 AND CD 8 IN SQUAMOUS CELL CARINOMA

TYPE	HLA 1 +	HLA 1 -	CD8 +	CD 8 -
SCC -well differentiated carcinoma	7	2	7	2
SCC- moderately differentiated carcinoma	4	4	4	4
SCC- poorly differentiated carcinoma	2	5	2	5

TABLE 13

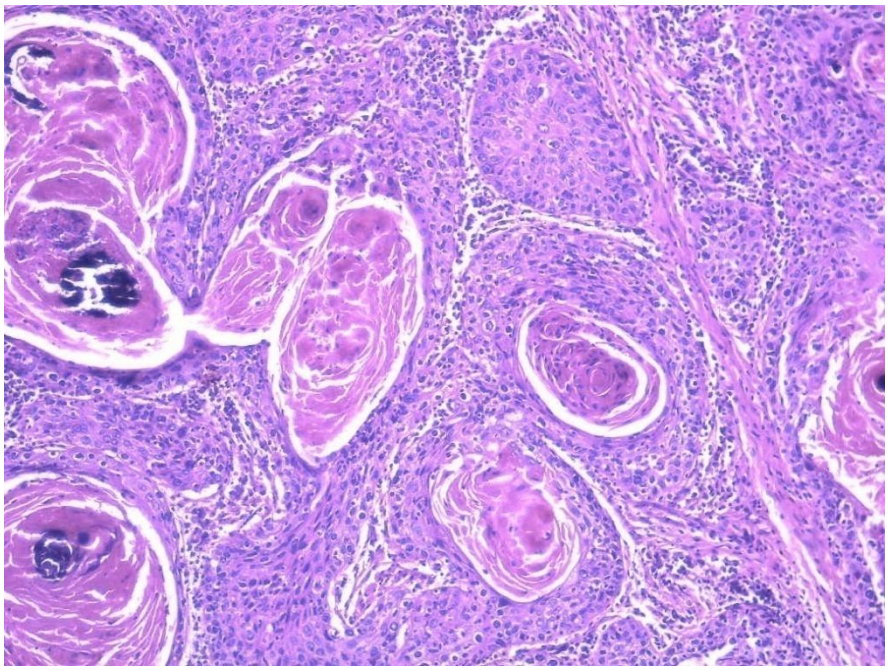
CORRELATION OF HLA 1 AND CD 8 IN OTHER TYPES OF CARCINOMA CERVIX

TYPE	HLA 1+	HLA -	CD 8+	CD 8-
Adenocarcinoma	1	2	1	2
Adenosquamous carcinoma	1	1	1	1
Clear cell carcinoma	0	1	0	1

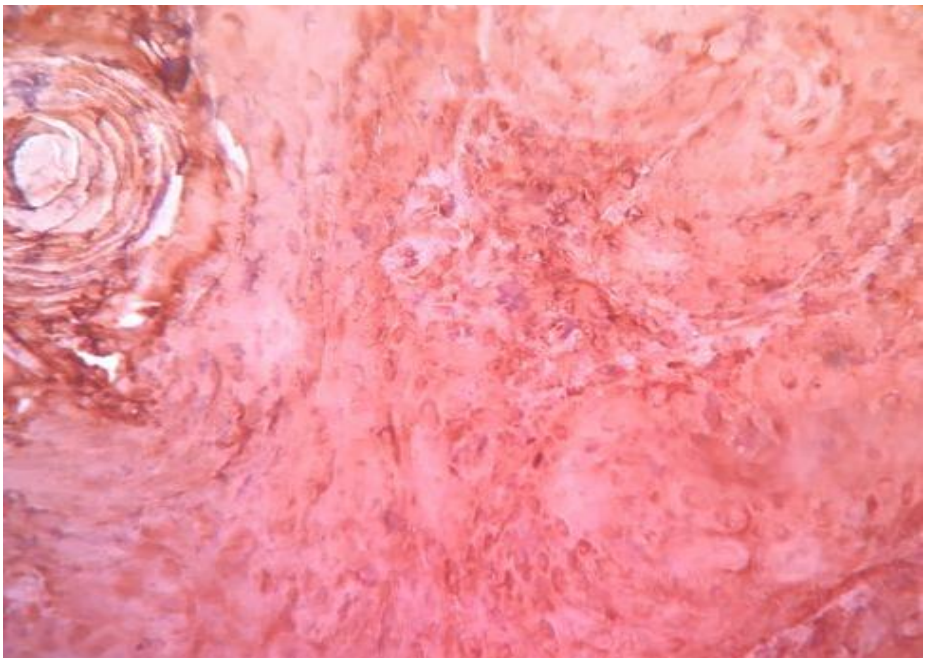
COLOUR PLATES

COLOUR PLATES

H&E - WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA (10X)

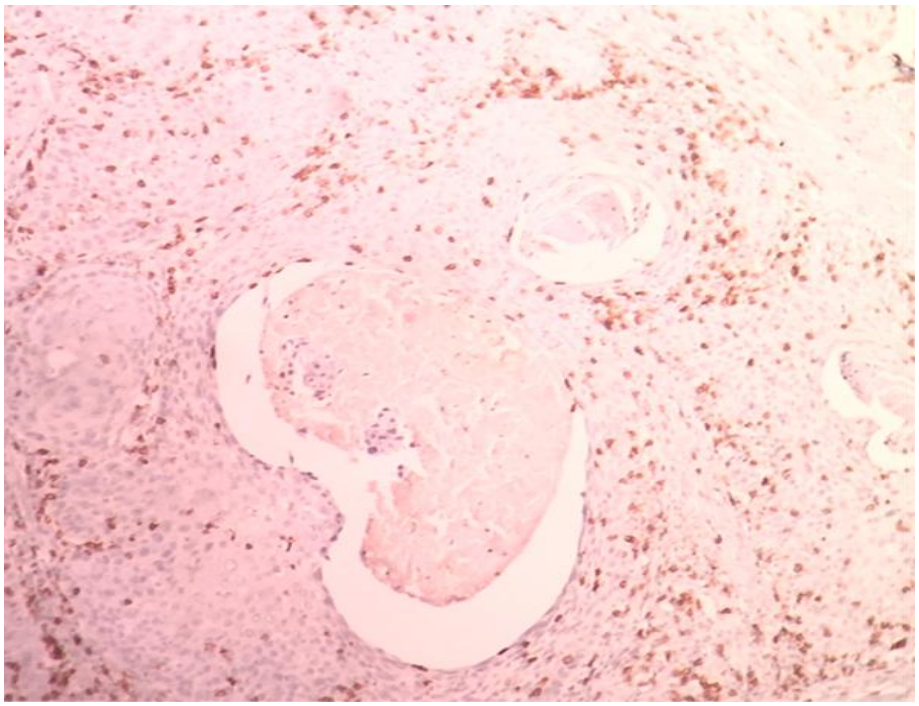


IHC- STRONG EXPRESSION OF HLA 1 IN WELL DIFFERENTIATED SCC(40X)

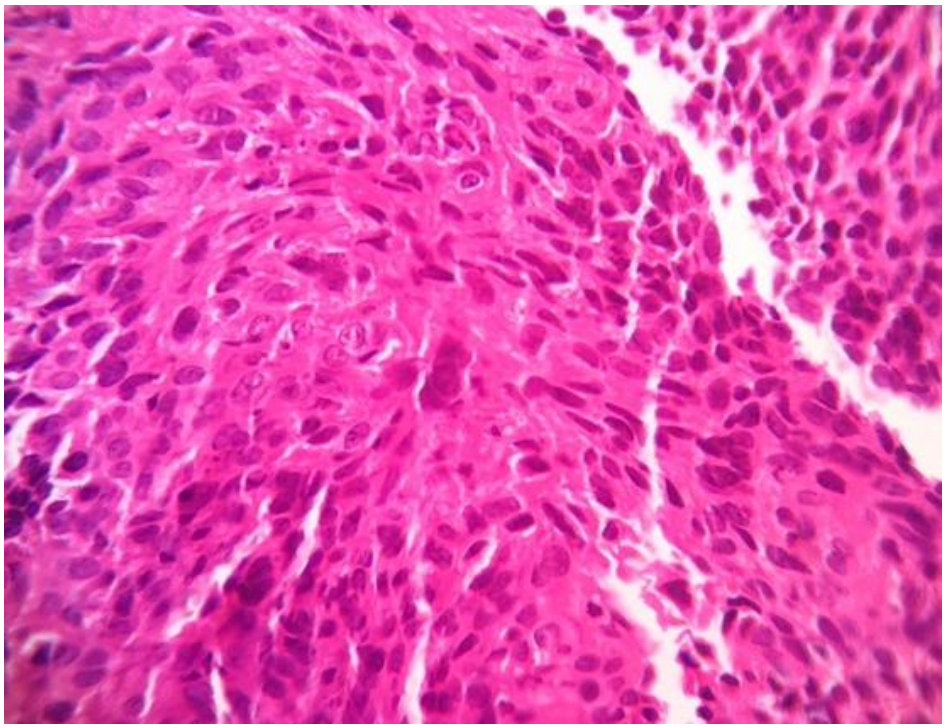


IHC - HIGH EXPRESSION OF CD 8 IN WELL

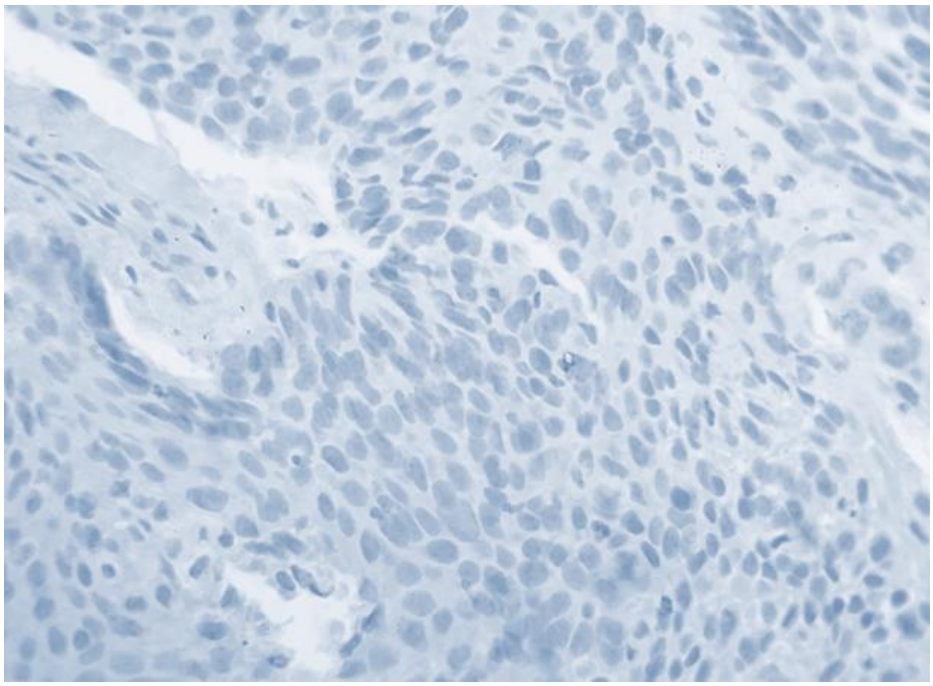
DIFFERENTIATED SCC (10X)



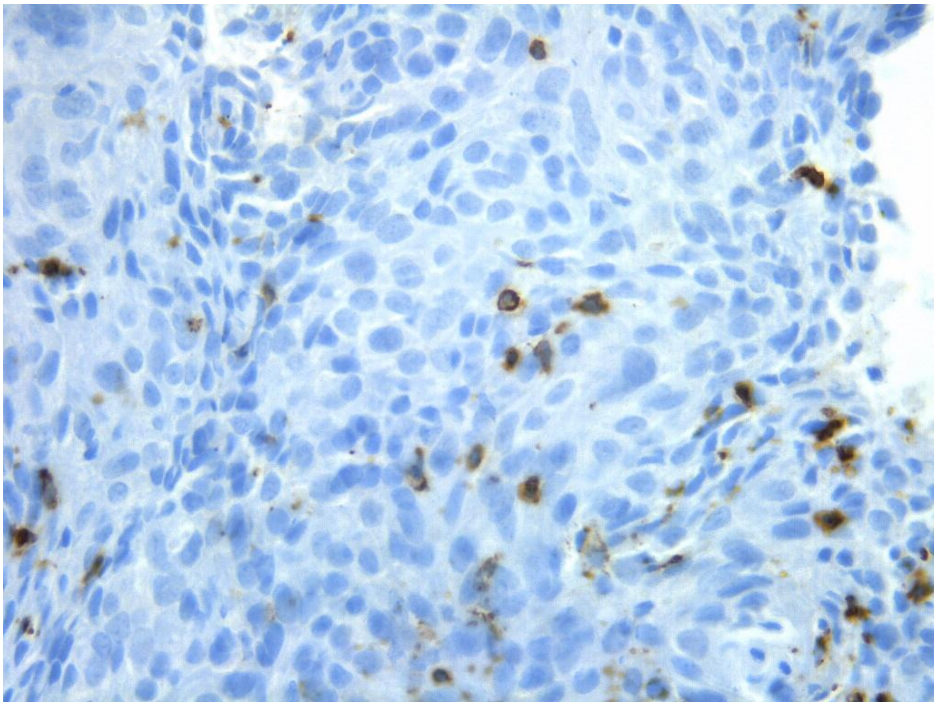
H & E - POORLY DIFFERENTIATED SCC (40X)



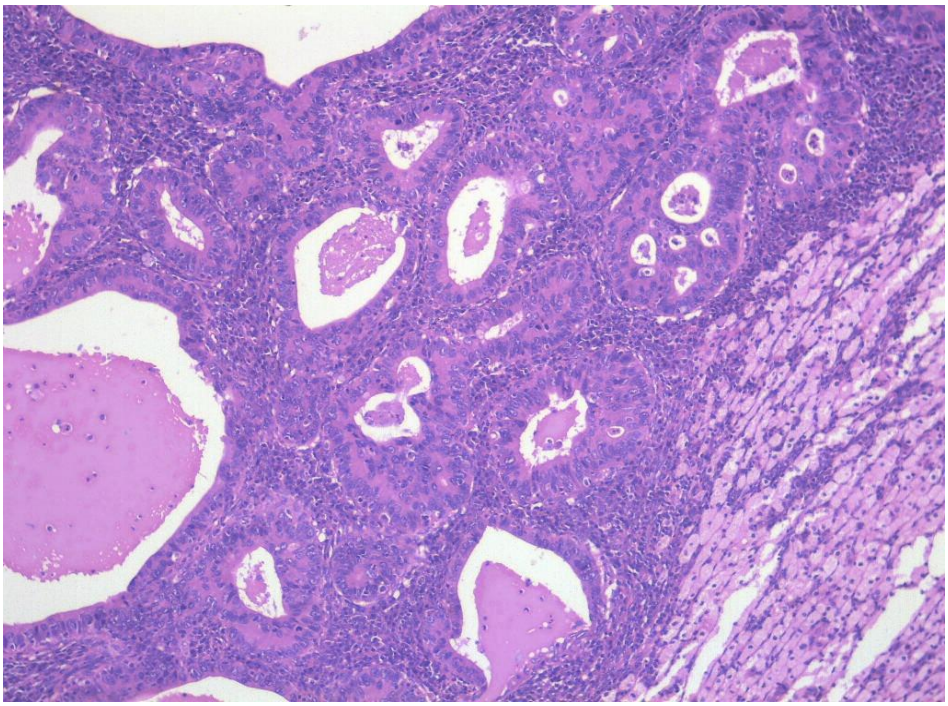
IHC - WEAK EXPRESSION OF HLA 1 IN POORLY DIFFERENTIATED SCC (40X)



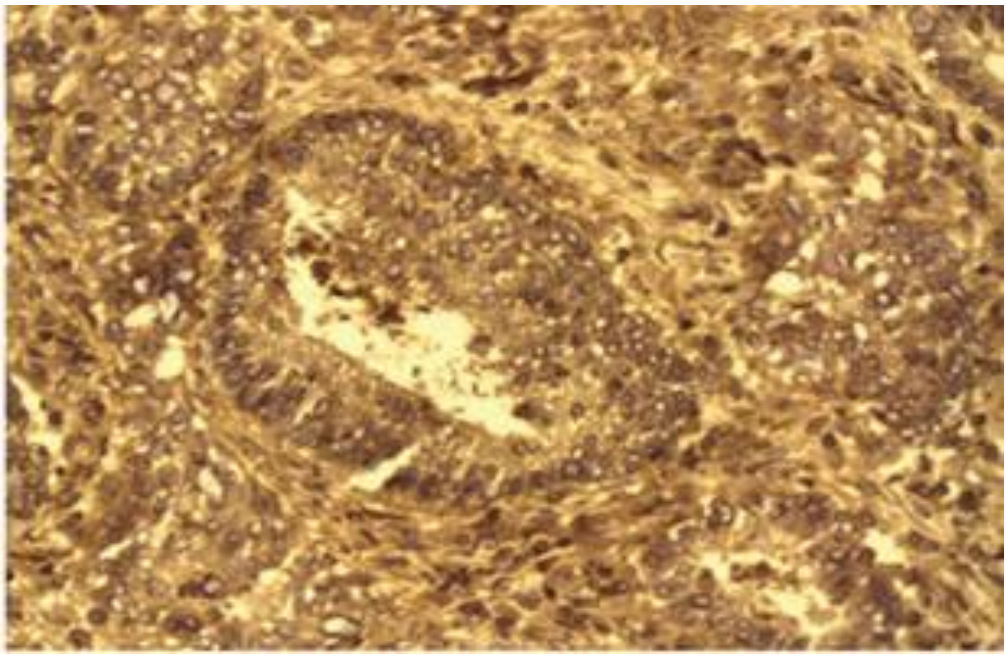
IHC - LOW EXPRESSION OF CD 8 IN POORLY DIFF. SCC(40X)



H&E -ADENOCARCINOMA - WELL DIFFERENTIATED CARCINOMA (10X)

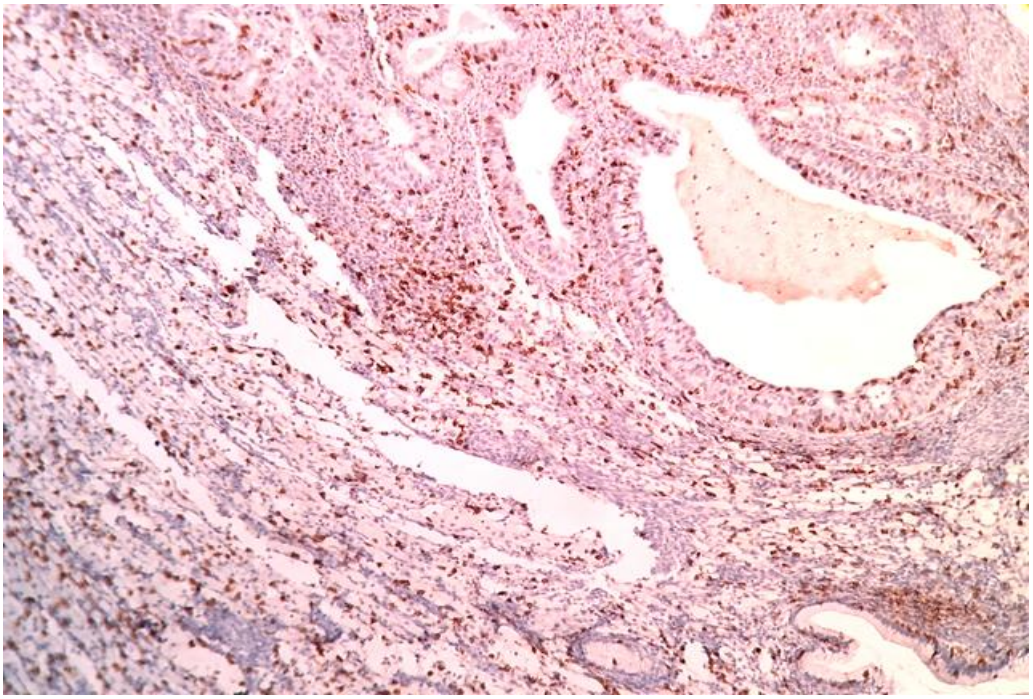


IHC - STRONG EXPRESSION OF HLA 1 IN WELL DIFFERENTIATED ADENOCARCINOMA (40X)

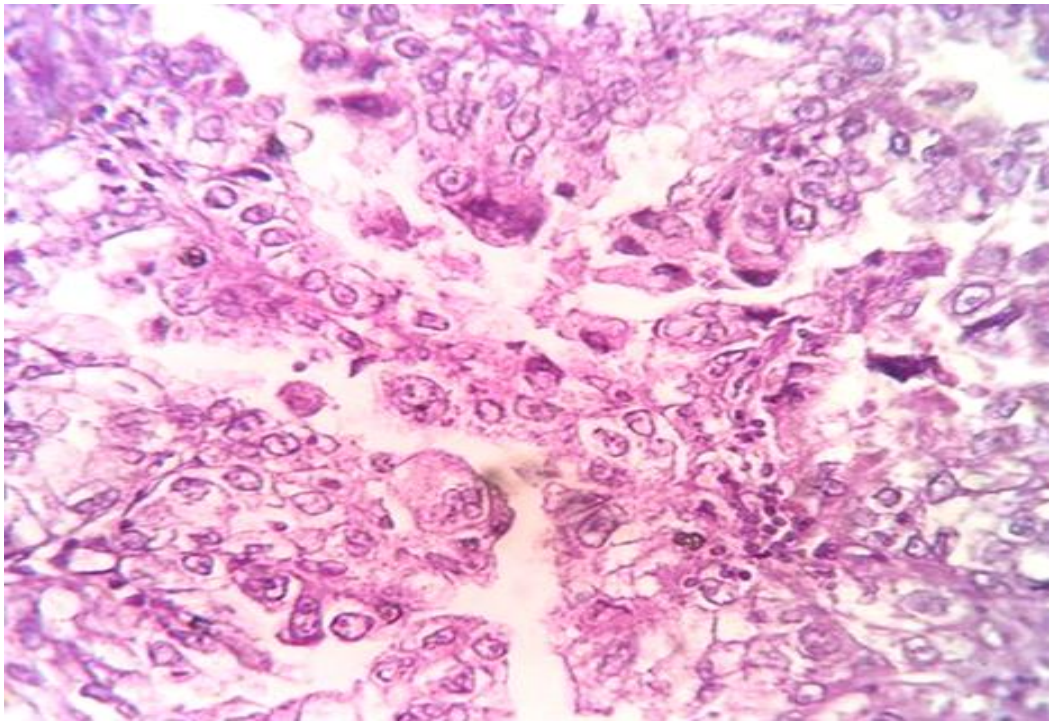


IHC - HIGH EXPRESSION OF CD 8 IN ADENOCARCINOMA

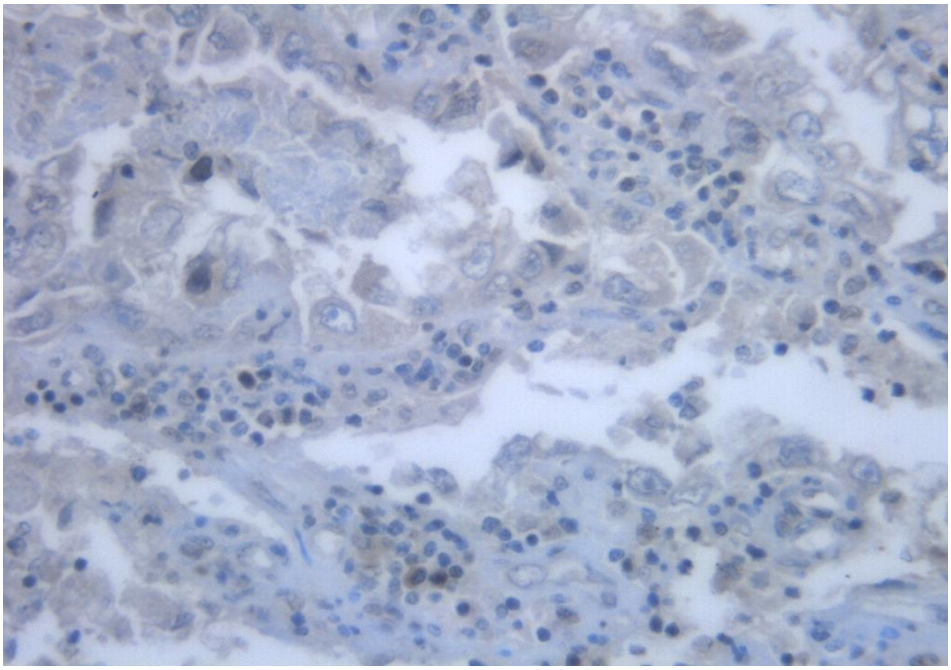
(10X)



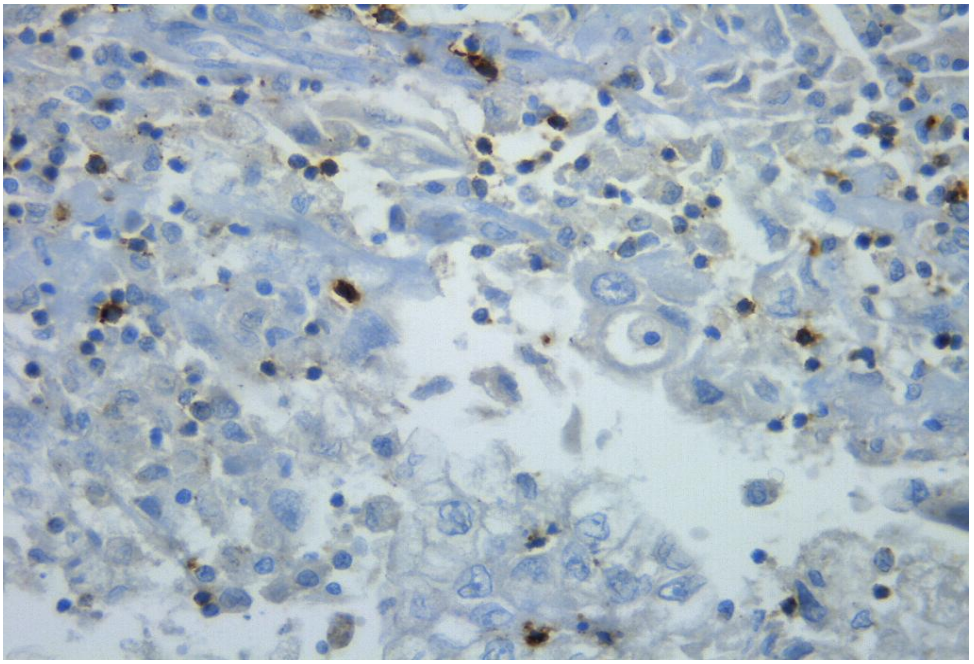
H&E -CLEAR CELL CARCINOMA (40X)



IHC - WEAK EXPRESSION OF HLA 1 IN CLEAR CELL CARCINOMA (40X)



IHC - LOW EXPRESSION OF CD 8 IN CLEAR CELL CARCINOMA (40X)



DISCUSSION

DISCUSSION

Carcinoma cervix is the second most common cancer among the world [30,26] with increased mortality in around half of the new cases diagnosed every year.

There is increasing incidence of invasive cancer cervix and deaths related to it according to a recent estimate in America.

Greater than 80% of all the cases occur in developing countries.

The risk of cancer cervix increases with increase in age. Because of the high prevalence of HPV infection around 25 years of age, the carcinoma incidence increases with age. Due to the persistent HPV infection , the risk of developing carcinoma increases with age. [53] It constitutes about 20% of cancer cervix at the age of > 65 years.

In the present study, the youngest age group at which carcinoma cervix was reported is 39 years. Its incidence is common between 40 to 50 years.

Data from the cancer registries indicate that > 75% of cervical carcinomas develop in women more than 35 years of age.

Epidemiological studies suggested that high risk HPV infection as the primary risk factor for cancer cervix.[32,35] It is well established that persisting infections were the most significant risk factor for cancer cervix.[36] Almost all cervical cancers contain the genes of most common types 16, 18, 31 and 45 which are high risk groups.[11,35] HPV 16 was found to be associated with tumors expressing HLA 1 antigen.[56] Enormous lines of evidence suggest that cell - mediated immune responses are very important in controlling established HPV infections and HPV associated neoplasms, especially in tumor cell clearance.[34,37]

In patients with impaired cell mediated immunity, including HIV infected patients and transplant recipients, the prevalence of HPV related diseases are higher in incidence.[50] Infiltrating CD 4^+ and CD 8^+ cells have been observed in spontaneously regressing warts. Being an important component of immune system, HLA system is able to present antigenic peptides to antigen - specific T lymphocytes[59,61], they then trigger the immune response to eliminate tumor specific proteins.

The main function of HLA 1 is to combine with antigen and forming HLA -antigen complex and presenting them to the cell

surface.[60] CD 8 $^{+}$ T cells can identify the complex and then be activated and differentiate into cytotoxic T cells.[38]

These cytotoxic cells can directly kill target cells via secreting substances like perforin or by inducing target cell apoptosis through Fas / Fas L way resulting in a special lethality for target cells. Hence any modification in cellular antigen - presenting system can correlate with the escape of CIN and carcinoma in situ from immunological control and then progress to invasive carcinoma.[39]

Immune - surveillance escape mechanisms emerges as an important step in the progression of HPV associated tumors.[40,41,42] Low HLA 1 expression cannot present antigenic peptides to the cytotoxic cells.[43,44] The tumor cells can avoid the specific killing effects of CTL cells , continue to progress and metastasize. Strong tumor immunity is present in patients without lymph node metastasis.[45]

Our immunohistochemical study demonstrated that HLA 1 expression is statistically low in poorly differentiated carcinomas and CD 8 infiltration is also low in poorly differentiated carcinomas. The low expression of HLA 1[62,63] and CD 8 T cells in cervical cancer tissue[57] may indicate that tumor cells exhibit HLA1downregulation, [23,33]

resultant loss of antigen presentation capability and downregulation of endogenous antigen processing machinery for MHC presentation. [46]

Michael Campol, et al[13] studied HLA class 1 antigen loss, tumor immune escape and immune selection.

They have concluded that the outgrowth of a patient's tumor reflects immune selection of tumor cells which have acquired escape mechanisms from immune recognition.

Louise A . Koopman et al[14] studied about Human Leukocyte Antigen class I gene mutations in cervical cancer.

In that they have concluded the nature of nucleotide insertions and single - base substitutions responsible for the complete absence of HLA class I molecules in cervical cancer in vitro and ex vivo. Such tumor - specific mutations may permit the cell to escape from HLA class 1 restricted cytotoxic T - cell responses.

The present study also states that there is complete loss of expression of HLA - 1 in poorly differentiated cervical squamous cell carcinoma.

Jiang Tao Fan, Yan Liao et al[16] evaluated the expression of HLA - 1, CD 8 and CD 4 and their clinical significance in cervical cancer.

They observed that the diminished local expression of HLA - 1, CD 8 and CD 4 in cervical tissue may be involved in the occurence and progression of cervical carcinoma.

The present study also observed that the diminished expression of HLA - 1 and CD 8 seen in the cervical cancer tissue.

Ekaterina S. Jordanova et al[9] studied Human Leukocyte antigen class 1, MHC class I chain - related molecule A, and $CD\,8^{+}$ / Regulatory T cell ratio: which variable determines survival of cervical cancer patients?

They have concluded that weak HLA-A-MICA expression combined with low $CD\,8^{+}$ / Treg ratio reveals a patient group with the poorest survival in cervical cancer.

The present study shows that the expression of HLA - 1 and CD 8 is low in poorly differentiated carcinoma cervix.

Robert P Edwards, M.D., et al[19] studied about T lymphocytes infiltrating advanced grades of cervical neoplasia. They have concluded that the CD 8 positive T cell infiltrate far exceeded the CD 4 positive cells in the invasive , but not in the preinvasive lesions, a finding that suggests that CD 8 cells are recruited preferentially to cervical lesions with progression to invasion.

The present study inferred that CD 8 T cells are more recruited to the invasive cancer tissue site and it is lost in more advanced cases.

Vivian M Spaans et al[22] evaluated HLA - E expression in cervical adenocarcinomas : association with improved long - term survival.

They have concluded that high expression of HLA - E occured in the majority of all histopathological subtypes of cancer cervix ; especially in cervical adenocarcinomas. High HLA - E expression in cervical adenocarcinoma was associated with improved patient survival.

The present study also demonstrated that HLA - 1 is expressed in all histopathological subtypes and it is more intensely expressed in well differentiated carcinomas.

Debbie M Ferns et al [17] (2016) evaluated the Classical and non - classical HLA class I aberrations in primary cervical squamous and adenocarcinomas and paired lymph node metastases.

They have concluded that the tumor immune escape variants leads to metastasis. Moreover , SCC tumors shows downregulation of HLA - 1 A or total classical HLA in combination with HLA - G expression had poor prognosis. HLA expression is used as a biomarker for patient selection for CTL - and NK - cell based immunotherapeutic intervention.

Sine Hadrup et al[31] studied effector CD 4 and CD 8 T cells and their role in the tumor microenvironment.

Sytse J . Piersma [41] (2011) studied Immunosuppressive tumor microenvironment in cervical cancer patients.

The final remarks of the study is that the tumor microenvironment in cervical cancer patients has similar character to other types of cancer. The immune escape variants can be targeted by alternative

approaches , such as vaccination against epitopes that are associated with impaired antigen processing.

Bethwaite PB et al [51] studied infiltration by immunocompetent cells in early stage invasive carcinoma of the uterine cervix : a prognostic study.

Present study also concluded that there is infiltration of CD 8 T lymphocytes in the early or well differentiated form of invasive cervical carcinoma of squamous cell type.

Hildes C et al[66] studied the association between HLA expression and infiltration of immune cells in cervical carcinoma.

They have concluded that the tumor infiltrating CD 8 + T lymphocytes correlated with monomorphic HLA class I expression. This stresses the existence of HLA - restricted immune response of T - lymphocytes in cervical carcinoma.

This is in accordance with the present study.

SUMMARY

SUMMARY

The normal cervix expresses MHC class 1 antigen on the lower one third to one half of squamous epithelium. [64] All cells express MHC class 1 molecules over their cell surfaces including the cervical lining epithelium.

In the present study, well differentiated squamous cell carcinoma recapitulates partly the normal expression of HLA 1 . Thus in well differentiated squamous cell carcinoma, HLA 1 has been expressed in 7 cases.

As the grade of malignancy increases, the expression of HLA 1 is lost .This is exemplified by our study in which 5 poorly differentiated squamous cell carcinoma cases have only < 25% positivity (1+).

In the present study, CD 8 expression was found in 7 cases of well differentiated squamous cell carcinoma. Thus it explains that the antigen HLA 1 is expressed in well differentiated squamous cell carcinoma for destruction by CD8 + T lymphocytes. In parallel, in poorly differentiated squamous cell carcinoma, HLA 1 expression was low in 5 cases . In these 5 cases of poorly differentiated squamous cell carcinoma , CD 8 + lymphocytes expression was also low (<25% positivity).

CONCLUSION

CONCLUSION

Thus in our study , we found that well differentiated carcinomas express HLA 1 more efficiently and also we found that good number of CD 8 + lymphocytes in well differentiated carcinomas. Also in poorly differentiated carcinomas, HLA 1 expression was low with diminished number of CD 8 + lymphocytes in the tumor environment and this scientifically reconfirms good prognosis in well differentiated carcinomas due to HLA 1 expression and cytotoxic destruction of HLA 1 + tumor cells by CD 8 + lymphocytes.

Also we noticed that in our study, even the HLA 1 expression was cytoplasmic and not at the membrane level in all carcinomas and further studies are required to study the mechanisms involved in the lack of expression of HLA 1 at the cell membrane level. Also further studies are required for targeting HLA 1 expression and CD 8+ lymphocytes for treatment strategies.

ANNEXURES

ANNEXURE I

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

COIMBATORE

Particulars of the patient:

Name : IP/OP NUMBER:

Age: Ward number:

Sex: occupation:

Address:

Presenting complaints:

Change in bowel habits

Blood in the stool

Loss of weight, loss of appetite

Family history:

Malignancy +/-

Personal history:smoker

GENERAL PHYSICAL EXAMINATION:

Built: Febrile :

Nourishment : Pallor:

Conscious : Jaundice:

Weight : Cyanosis:

Pulse rate: Clubbing:

Respiratory rate : Lymphadenopathy:

SYSTEMIC EXAMINATION:

RS: P/A:

CVS: CNS:

CLINICAL DIAGNOSIS:

RADIOLOGICAL FINDINGS:

USG:

CT:

MRI:

MICROSCOPIC FINDINGS:

Histopathological diagnosis:

FINAL DIAGNOSIS:

ANNEXURE II

ABBREVIATIONS

1. HLA - Human Leukocyte Antigen
2. MHC - Major Histocompatability Complex
3. CD - Cluster Differentiation
4. PAP – Papanicolaou
5. HPV -Human Papilloma Virus
6. HSV - Herpes S implex Virus
7. IUCD - Intrauterine Contraceptive device
8. DPX - Di- N-Butyle Phthalate in Xylene
9. WHO- World Health Organisation
10. CIN - Cervical Intraepithelial Neoplasia
11. ISGYP - International Society of Gynaecological Pathologist
12. LSIL - Low grade Squamous Intraepithelial Lesion
13. HSIL - High grade Squamous Intraepithelial Lesion
14. HIV - Human Immunodeficiency Virus

15. NOS - Not Otherwise Specified

16. CTL - Cytotoxic Lymphocytes

17. NK - Natural Killer cells

18. SCC -Squamous Cell Carcinoma

19. AIS - Adenocarcinoma in Situ.

ANNEXURE III

MASTER CHART

Sl. No	AGE	HPE NO	IP NO	CLINICALDIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	GRADE	TYPE	IHC - HLA1		IHC - CD8	
								1+ (negative)	2+ &3+ positive	1+ (negative)	2+ & 3+ (positive)
1	48/ F	2175/16	88369	?carcinoma cervix	Squamous cell carcinoma	Well differentiated	Large cell keratinising	-	+	-	+
2	60/F	4273/16	78384	Bleeding P/V	Clear cell carcinoma			+	-	+	-
3	45/F	30/17	886465	?carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
4	51/F	31/17	7303	?carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
5	65/F	95/17	909437	?carcinoma cervix	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
6	48/F	121/17	910926	ViA/ ViLI positive	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
7	60/F	138/17	858680	?carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
8	59/F	261/17	941077	?Carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
9	39/F	291/17	951955	ViA/ ViLI positive	Squamous cell carcinoma	Well differentiated	Keratinizing	-	-	-	+
10	85/F	410/17	16830	Bleeding P/V	Squamous cell carcinoma	Moderately diff.	Large cell Keratinising	-	+	-	+
11	45/F	423/17	981215	ViA/ ViLI positive	Squamous cell carcinoma	Moderately diff.	Keratinizing	-	+	-	+
12	56/F	344/17	13113	Bleeding P/V	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
13	41/F	553/17	1008875	?carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
14	60/F	683/17	1030105	?carcinoma cervix	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
15	55/F	751/17	24819	Bleeding P/V	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	+	-	+	-
16	45/F	752/17	1041548	ViA/ ViLI positive	Adenosquamous Ca	Moderately diff.		-	+	-	+

Sl. No	AGE	HPE NO	IP NO	CLINICALDIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	GRADE	TYPE	IHC - HLA1		IHC - CD8	
17	51/F	822/17	30762	ViA/ ViLI positive	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	-	+	-	+
18	70/F	901/17	1071573	Bleeding P/V	Squamous cell carcinoma	Poorly differentiated	Non Keratinizing	+	-	+	-
19	68/F	60/17	901542	?carcinoma cervix	Adenosquamous Ca	Moderately diff.		-	+	-	+
20	46/F	139/17	918914	ViA/ ViLI positive	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	+	-	+	-
21	47/F	717/17	24819	ViA/ ViLI positive	Adenocarcinoma	Well differentiated		-	+	-	+
22	42/F	46/17	360	ViA/ ViLI positive	Adenocarcinoma	Moderately diff.		+	-	+	-
23	55/F	1055/17	1099715	?carcinoma cervix	Adenosquamous Ca			-	+	-	+
24	46/F	1056/17	44873	ViA/ ViLI positive	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	-	+	-	+
25	70/F	1089/17	46651	Bleeding P/V	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	+	-	+	-
26	68/F	1106/17	442127	Bleeding P/V	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
27	50/F	1108/17	1108575	?carcinoma cervix	Squamous cell carcinoma	Moderately diff.	Non Keratinizing	+	-	+	-
28	65/F	1294/17	52282	?carcinoma cervix	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
29	70/F	1306/17	81122	?carcinoma cervix	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+
30	55/F	1435/17	87007	Growth cervix	Squamous cell carcinoma	Well differentiated	Keratinizing	-	+	-	+

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