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# CHRONIC HYPERPLASTIC CANDIDOSIS/CANDIDIASIS (CANDIDAL LEUKOPLAKIA)

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**ABSTRACT:** Chronic hyperplastic candidosis/candidiasis (CHC; syn. candidal leukoplakia) is a variant of oral candidosis that typically presents as a white patch on the commissures of the oral mucosa. The major etiologic agent of the disease is the oral fungal pathogen *Candida* predominantly belonging to *Candida albicans*, although other systemic co-factors, such as vitamin deficiency and generalized immune suppression, may play a contributory role. Clinically, the lesions are symptomless and regress after appropriate antifungal therapy and correction of underlying nutritional or other deficiencies. If the lesions are untreated, a minor proportion may demonstrate dysplasia and develop into carcinomas. This review outlines the demographic features, etiopathogenesis, immunological features, histopathology, and the role of *Candida* in the disease process. In the final part of the review, newer molecular biological aspects of the disease are considered together with the management protocols that are currently available, and directions for future research.

**Key words.** *Candida*, hyperplastic candidosis/candidiasis, review.

## (1) Introduction

### (1.1) DEFINITION, HISTORICAL PERSPECTIVES, AND CLASSIFICATION

The fact that many oral leukoplakias are associated with *Candida* infections was first reported by Cernea *et al.* (1965) and Jepsen and Winther (1965). However, Lehner (1964, 1967) recognized the presentation of chronic candidal infection in the form of leukoplakia and introduced the term "candidal leukoplakia". The terms "chronic hyperplastic candidosis" (CHC) and "candidal leukoplakia" (CL) appear to have been synonymously used until the mid-1980s (Cawson, 1966a,b; Cawson and Lehner, 1968), but confusion prevailed, since chronic mucocutaneous candidal lesions, encountered in patients with endocrine and immune defects, and affecting the skin and other mucosae, were also described by some as chronic hyperplastic candidosis. Several authors therefore preferred the term "candidal leukoplakia" to describe lesions confined to the mouth alone. In recent times, however, the term "candidal leukoplakia" appears to have lost currency, and most histopathologists prefer the term "chronic hyperplastic candidosis/candidiasis".

To minimize this confusion, Samaranayake (1991) proposed a revised classification where the oral candidosis lesions were subdivided into two main groups: Group I, or primary oral candidoses confined to lesions localized to the oral cavity with no involvement of skin or other mucosae; and Group II or secondary oral candidoses, where the lesions are present in the oral as well as extra-oral sites such as skin (Table 1). The Group

I lesions consist of the classic triad—pseudomembranous, erythematous, and hyperplastic variants—and some have suggested further subdivision of the latter into plaque-like and nodular types (Holmstrup and Besserman, 1983).

One distinct clinical difference between the CHC of Group I and that of Group II rests in the fact that the onset of the former is in adulthood, while the latter is almost always first seen in childhood, secondary to relatively uncommon, inherited immune defects (*e.g.*, Di George syndrome). This review pertains to Group I CHC or primary oral candidoses in the above classification. It should be noted, however, that the term "candidal leukoplakia" is still used by some, especially in the medical world. This is not strictly correct, since leukoplakia in an oral context is defined as a white patch that cannot be characterized clinically or pathologically as any other disease (WHO Collaborating Reference Centre for Oral Precancerous Lesions, 1978). Notwithstanding this caveat, we use the terms CHC and candidal leukoplakia (CL) synonymously throughout this review, to facilitate referral to the previous literature that commonly uses the latter terminology.

Interest in CHC appears to have waned globally, as evident from the paucity of recent articles published on the subject. For instance, a search of the Web-based archives of the US National Library of Medicine for publications on "chronic hyperplastic candidosis/candidiasis" during the last decade yielded only 12 publications, of which seven had only indirect reference to the subject. A single paper in Chinese was the only relevant non-English-language publication. A similar search on "candidal leukoplakia" produced only nine papers, six of which made only indirect reference to the topic.

**TABLE 1**  
**Classification of Oral Candidosis**

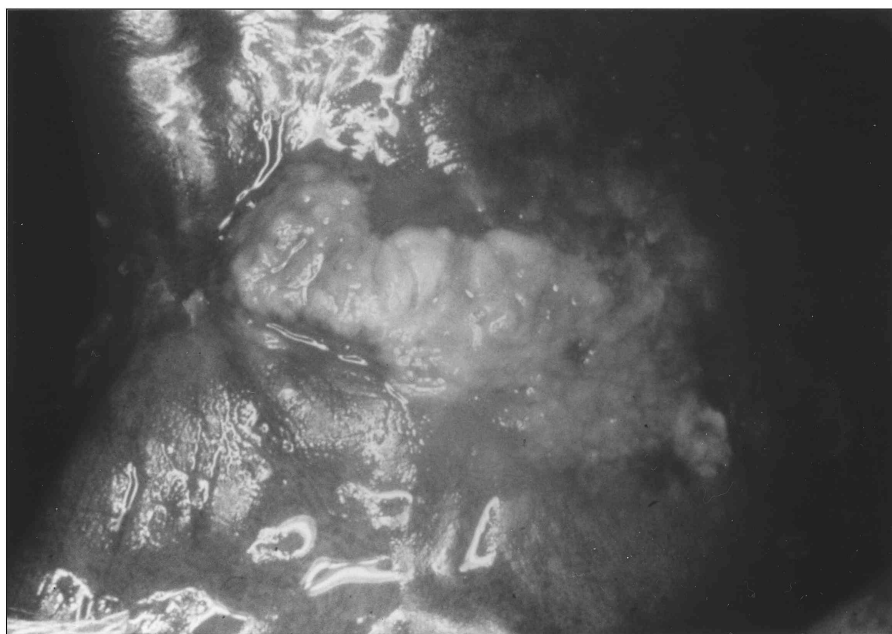
Oral Candidosis	
Primary Oral Candidosis (Group I)	Secondary Oral Candidosis (Group II)
<b>The 'Primary Triad':</b>	<b>Condition</b>
Pseudomembranous (mainly acute)	Familial chronic mucocutaneous candidosis
Erythematous (acute/chronic)	Diffuse chronic mucocutaneous candidosis
Hyperplastic (mainly chronic)	Candidosis endocrinopathy syndrome
- Plaque-like	Familial mucocutaneous candidosis
- Nodular/speckled	Severe combined immunodeficiency
<b>Candida-associated lesions</b>	Di George syndrome
Denture stomatitis	Chronic granulomatous disease
Angular cheilitis	Acquired immunodeficiency syndrome
Median rhomboid glossitis	
Linear gingival erythema	
	<b>Subgroup</b>
	1
	2
	3
	4
	5a
	5b
	5c
	6

Modified from Samaranyake (1991).

### (1.2) EPIDEMIOLOGICAL AND DEMOGRAPHIC ASPECTS OF CHC: INCIDENCE, AGE, GENDER, AND SITE VARIATIONS

The most common and arguably the classic clinical presentation of CHC is a white plaque that cannot be rubbed off and presenting most frequently in the commissural regions of the oral mucosa (Fig. 1). However, other oral sites can be infrequently affected. The lesion can be differentiated from oral leukoplakia of idiopathic origin, since appropriate antifungal therapy usually leads to resolution of the condition.

The epidemiological data on CL are linked to those of oral



**Figure 1.** The classic presentation of chronic hyperplastic candidosis at the commissures of the oral mucosa.

leukoplakia in general. At this point, it is appropriate to review the definition of oral leukoplakia. After considerable confusion in the preceding decades, leukoplakia was defined in 1978 under the auspices of the World Health Organization as a white patch or plaque that cannot be (rubbed off or) characterized clinically or pathologically as any other disease (World Health Organization, 1997).

The prevalence of oral leukoplakia as a whole in unselected population samples has been reported to range from 0.2-4.9% in rural India (Mehta *et al.*, 1969, 1972), 3.6% in rural Hungary (Bruszt, 1962), and 11.6% in Sweden (Axéll, 1976). According to several reports, the leukoplakias that harbor candidal hyphae appear to constitute from 7 to 50% of all leukoplakias (Jepsen and

Winther, 1965; Roed-Petersen *et al.*, 1970; Daftary *et al.*, 1972; Roed-Petersen and Pindborg, 1973; Bánóczy, 1982; Krogh *et al.*, 1987b). Lehner (1971) has suggested that differential counts of pyroninophilic monocytes be carried out to distinguish between candidal and non-candidal leukoplakias. The clinical and histological criteria laid down by Cawson and Lehner (1968) in their original description of CL appear to be fulfilled by only 10% of leukoplakias encountered in the mouth (Burkhardt and Seifert, 1977; Arendorf, 1984).

It has already been mentioned that CHC of Group I candidosis is a disease of adulthood. The age range of a cohort studied by Arendorf *et al.* (1983) in Cardiff was from 31 to 81 years, and the majority of the patients were more than 50 years old.

Gender distribution among oral leukoplakias varies, with M:F ratios ranging from 4:1 to 85:1 in different parts of India (Mehta *et al.*, 1969) and 20:1 in Sweden (Axéll, 1976). Not surprisingly, this preponderance of the male gender among affected individuals is reflected in the case of CL as well. Cawson and Lehner (1968) showed a M:F ratio of 2:1 among those affected by CL. Walker and Arendorf (1990) pointed out that the latter ratio was in conformity with the gender ratio found among UK tobacco smokers in a study by Arendorf *et al.* (1983).

CL affects the following oral sites in decreasing order of frequency: the buccal commissures, cheeks, palate, and the tongue (Williams, 1969).

### (1.3) PATHOGENIC ATTRIBUTES OF CANDIDA

Regardless of the type of candidosis, the ability of *Candida* species to persist on mucosal surfaces of healthy individuals is an important

factor contributing to its virulence. This is particularly important in the mouth, where the organism has to resist the mechanical washing action of a relatively constant flow of saliva toward the esophagus. It is of course true that infection by an opportunistic pathogen such as *Candida* is dependent not only on virulence factors of the organism but also equally, or indeed more, on host factors. Such host factors, both local and systemic, will be discussed below. However, a brief review of the virulence factors of *Candida* species in general is relevant at this juncture.

The adherence of *Candida* to oral mucosal surfaces is critical to its persistence in the mouth and thus can be a prelude to the study of its virulence (Kennedy, 1988). The ability of the organism to adhere to mucosal and artificial surfaces in the mouth has been extensively studied (for a recent review, see Samaranayake and Ellepola, 2000). The factors that contribute to the adhesion of *Candida* fall into three major groups, as shown in Table 2.

Seen from a general perspective, candidal adherence appears to be a complex process, with no single factor independently contributing to adherence. Several surface components on the yeast cell wall appear to interact with oral epithelial cells initially through a variety of physical forces such as van der Waals' interactions, hydrophobicity, and electrostatic bonding. Subsequent intimate host-cell interactions are mediated through lectin-like proteins of the yeast cell wall that interact with a terminal sugar of the glycoproteins of the human epithelial cell, which acts as a receptor for the former (Critchley and Douglas, 1987a). A fibrillar surface component of the yeast cell wall, a strain-specific mannoprotein named adhesin, has been identified by Critchley and Douglas (1987b) and subsequently demonstrated electron microscopically (Marrie and Costerton, 1981; McCourtie and Douglas, 1981). Recent studies have also uncovered a gene family termed ALS (Agglutinin-like substances) that plays a critical role in candidal adhesion (Hoyer, 2001).

From the standpoint of CL where hyphal forms of *Candida* are seen invading the superficial layers in histopathological specimens, it is of interest that the adherence of the hyphal phase of *C. albicans* is greater than that of blastospore-phase cells (Samaranayake and MacFarlane, 1982; Tronchin *et al.*, 1988). Kimura and Pearsall (1978) and Tronchin *et al.* (1988) have suggested that structural or biochemical changes in surface components such as adhesins may account for this phenomenon. However, Kennedy and Sandin (1988), on the basis of their study of candidal adhesion in a variety of media and growth conditions, have proposed that fungal adhesins and hydrophobicity play a negligible role in candidal adhesion to epithelia.

Most strains of *C. albicans* and *C. glabrata* display a phenomenon known as 'switching', which enables the organism to change phenotype and display various colonial morphology and physiological properties (Di Menna, 1952; Slutsky *et al.*, 1985; Soll *et al.*, 1987; Soll, 2002). The biological significance of this phenomenon is poorly understood. There is some evidence, however, to imply that switching systems may contribute to the pathogenicity of *Candida* by enabling the organism to survive in adverse host environments and alter its surface antigenicity, thus evading the host immune mechanisms and even escaping antifungal treatment. It has also been proposed that switching may selectively augment candidal adhesion and increase the capacity for tissue penetration and secretion of proteinases and phospholipases (for a recent review, see Soll, 2002).

Another major virulent attribute of *Candida* is its ability to invade superficial layers of the epithelium, aided, in particular,

**TABLE 2**

**Factors Affecting Candidal Adhesion to Host Tissues or Prostheses**

**Factors related to yeast cells**

- Medium/cultivation
- Phenotype
- Germ tubes/hyphae
- Extracellular polymeric material (EP)
- Floccular/fibrillar surface layers
- Mannan
- Chitin
- Hydrophobicity
- Cellular lipids
- ALS (agglutinin-like substances; up to 9 are known)

**Factors related to host cells**

- Cell source
- Mucosal cell size and viability
- Fibronectin
- Fibrin
- Sex hormones
- Yeast carriers vs. patients with overt candidosis

**Environmental factors affecting adhesion**

- Cations
- pH
- Sugars (especially galactose)
- Saliva
- Humoral antibody and serum
- Low concentrations of antifungal agents
- Bacteria
- Lectins

Modified from Samaranayake and Ellepola (2002).

by their hyphal appendages. Early workers have shown that the candidal proteinases (secreted aspartyl proteinases [SAP]) and lipases are particularly concentrated at the tips of these hyphal elements (for a recent review, see Ghannoum, 2000). Since adherence of *C. albicans* to epithelial cells is optimal at acidic pH (Samaranayake and MacFarlane, 1982), and such an environment is conducive for SAP production, acidic conditions prevalent intra-orally, especially in diseased states, appear to promote candidal virulence (Samaranayake and MacFarlane, 1985). Extracellular proteolytic activity of *C. albicans* was first discovered by Staib (1965), who first pointed out the role of SAPs in the pathogenesis of candidosis (Staib, 1969). Borg and Ruechel (1988) found a strong association between fungal adherence to the buccal mucosa and the activity of the SAPs. Borg *et al.* (1984) found that proteolytic candidal cells were highly cytotoxic for human monocyte-like cells, whereas non-proteolytic blastospores caused little toxicity. Several studies have shown that many protective salivary proteins such as lactoferrin, lactoperoxidase, and immunoglobulins, especially IgA1 and IgA2, are susceptible to degradation by SAPs of *Candida*. The same is true for hemoglobin (Ruechel, 1986, 1990). Since *Candida* SAPs have keratinase-like activity *in vitro* (Negi *et al.*, 1984), it is likely that this property would enable the organism to invade orthokeratinized mucosa. Zimmermann (1986) has demonstrated the ability of the fungus to invade even the blood vessels in the submucosal tissues.

As has been mentioned, candidal SAPs require a low pH (*circa* 5.5) to act optimally. Ruechel (1990) points out that suit-

able acidic conditions may exist in sites of poor blood supply, while others have shown that *Candida* can secrete organic acids into its micro-environment, thus creating a favorable milieu for proteolytic activity (Samaranayake *et al.*, 1983). The same authors have shown that such acidification is particularly prominent in budding yeasts and in the tips of growing filaments, a feature which is significant in the context of CL. Secretory lipases of *Candida* species were identified by Werner (1966), who described it among strains of *C. albicans* (*C. stellatoidea*, now *C. albicans*) and *C. tropicalis*. Samaranayake *et al.* (1984b,c) and Kantarcioglu and Yucl (2002) have shown that the phospholipase activity differed significantly among isolates of *C. albicans*. Previous workers have shown that the phospholipase activity was highest where the hyphae were in direct contact with the cell membranes at the invading front of the lesion (Pugh and Cawson, 1977). In conclusion, it is tempting to speculate that the tandem activity of both phospholipases and SAPs of *Candida* is likely to play a key role in host invasion by this ubiquitous fungus. Unfortunately, however, there is a scarcity of data on this front and also on the qualitative differences of SAPs and phospholipases among different *Candida* species.

## (2) Host Factors in Candidal Leukoplakia/Chronic Hyperplastic Candidosis

It is well-known that *Candida* species are normal commensals in up to 50% of the healthy population (Samaranayake and MacFarlane, 1990). It is therefore conceivable that many local and systemic host factors may operate *in vivo* to facilitate the conversion of this harmless commensal to a pathogenic organism. These are adequately dealt with elsewhere in relation to the many clinical variants of oral candidosis (Samaranayake, 1990). In the following section, we attempt to clarify the role of these factors in the pathogenesis of CHC.

### (2.1) LOCAL PREDISPOSING FACTORS

It is likely that a major element leading to the initiation of CHC is a breach of the integrity of the host oral mucosa. Oral mucosa is an intrinsically thick, impervious integument with a multiplicity of defense mechanisms, including the relatively recently discovered antibiotic peptides in epithelial cells, termed "defensins" (Zasloff, 1992). However, these defenses are not uncommonly breached by a variety of factors. Trauma to the mucosa from natural as well as artificial teeth is relatively common. A denture, especially a maxillary appliance, poses an additional threat, since it acts as a potent reservoir of *Candida* in comparison with the prosthesis-free oral cavity (Budtz-Jørgensen, 1990). Walker and Arendorf (1990) have proposed a schematic model for the pathogenesis of candidal leukoplakia wherein occlusal friction acts as a co-factor leading to the initial oral keratosis. Since it is a common observation that most commissural leukoplakias, predominantly characterized as CHC, occur along the occlusal line, it is tempting to speculate that occlusal trauma or friction is a prime protagonist of CHC. However, we did not find any direct evidence in the literature to suggest that occlusal friction or trauma from normal or artificial dentition is significantly high in cases of CHC. There is only a single report (Arendorf *et al.*, 1983) that provides some indirect support for this hypothesis, where a significantly higher proportion of candidal leukoplakia patients had dentures and wore them continuously day and night. Interestingly, though, in angular cheilitis, which often accompanies CHC of

the adjacent commissural mucosa, maceration at the commissural angle of the mouth has been recognized as a contributory factor leading to the condition (Scully and Cawson, 2001).

Epithelial changes of the oral mucosa, such as atrophy, hyperplasia, and dysplasia, may compromise the mucosal barrier and may facilitate candidal invasion, especially in the event of epithelial atrophy (Samaranayake, 1990). However, there are no studies that have compared the thickness of epithelium in relation to candidal invasion. On the contrary, there is a considerable volume of evidence that candidal invasion itself may provoke a hyperplastic response (*vide infra*).

Reduced salivary flow rate in diseased states such as Sjögren's syndrome, or during cytotoxic therapy and radiation therapy, has been shown to favor increased oral carriage of *Candida* and a concomitant increase in candidal infection (MacFarlane and Mason, 1973; Martin *et al.*, 1981; Samaranayake *et al.*, 1984a, 1988). Although no definite link between xerostomia and CHC has been reported, it is conceivable that CHC may be precipitated with other variants of candidosis in xerostomic patients, provided that conducive factors co-exist.

Apart from the quantitative changes in salivary flow discussed above, qualitative changes of saliva, such as its glucose content and pH, are also factors that may influence oral candidal colonization and probably indirectly affect the genesis of CHC (Samaranayake, 1990). High glucose content of saliva and its low pH have been shown by several workers to favor oral candidal colonization (Knight and Fletcher, 1971; Shipman, 1979; Arendorf and Walker, 1980; Samaranayake *et al.*, 1984b, 1986).

### (2.2) TOBACCO SMOKING AND CHEWING HABITS: EPIDEMIOLOGICAL EVIDENCE OF RELATIONSHIP TO TOBACCO USAGE

Bánóczy *et al.* (2001) have provided strong evidence for the role of smoking in the development of both oral cancer and oral leukoplakia in Hungarian cohorts. Cross-sectional studies show a higher prevalence of leukoplakia among smokers, with a direct dose-response relationship between tobacco use and oral leukoplakia in general.

In one study, Arendorf *et al.* (1983) have reported that all of their 53 candidal leukoplakia patients were smokers with a very high degree of statistically significant prevalence ( $P < 0.001$ ) than the non-smoking controls. They also pointed out that areas protected from the direct insult of the tobacco smoke, such as the denture-bearing mucosa of the palate, were relatively unaffected in general.

In a study involving Indian villagers, it has been reported that 98% (48 out of 49) of those with *Candida*-infected leukoplakias smoked or chewed tobacco (Daftary *et al.*, 1972). Previously, Pindborg *et al.* (1967) found a strong correlation between bidi smoking and commissural leukoplakia in Indians. Pindborg (1980) asserted, citing evidence from Hoffmann *et al.* (1974), that bidi smoke has a higher content of noxious agents than does cigarette smoke.

There is contradictory evidence, too, with regard to the relationship between tobacco smoking and oral candidosis in general. Several researchers claim to have found no correlation between smoking habit and oral candidosis (Gergely and Uri, 1966; Coleman *et al.*, 1976; Bastiaan and Reade, 1982; Oliver and Shillito, 1984). Indeed, Beasley (1969) has reported three iso-

lated cases where the onset of oral thrush coincided with the cessation of the smoking habits of the patients.

Although it might appear that evidence for a direct relationship between oral candidosis as a whole and smoking is far from irrefutable, the studies cited above show conclusively that smoking habit has a direct link at least to commissural leukoplakia and, by inference, to CHC. Furthermore, Arendorf and Walker (1980) have shown that *C. albicans* was isolated more frequently from the mouths of smokers than from non-smokers. So why does tobacco smoking favor oral candidal colonization? Analysis of the available data indicates that this could be due to: (a) induction of increased epithelial keratinization (Zimmermann and Zimmermann, 1965; Mosadomi *et al.*, 1978; Bánóczy, 1982); (b) reduction in salivary immunoglobulin A levels (Bennet and Reade, 1982); and (c) possible depression of polymorphonuclear leukocyte function (Kenney *et al.*, 1977).

### **(2.3) SYSTEMIC PREDISPOSING FACTORS (DIABETES, IMMUNOLOGICAL DEFECTS, NUTRITIONAL FACTORS)**

#### **(2.3.1) Diabetes mellitus and candidal leukoplakia**

Only a minority of patients with candidal leukoplakia have associated medical conditions, including diabetes mellitus (Walker and Arendorf, 1990). Furthermore, an analysis by the present authors of a large number of reports on oral candidal infections among diabetics reveals that candidal leukoplakia is fairly uncommon in this patient group (unpublished data). Nevertheless, an appraisal of the general relationship between oral candidosis and diabetes mellitus cannot be considered irrelevant in the context of this review.

Experiments on animal models for oral candidosis have shown that diabetes increases the susceptibility to candidal infestation (Andriole and Hasenclever, 1962; Hurley, 1966; van Cutsem and Thienpont, 1971; for a recent review, see Samaranyake and Samaranyake, 2001).

Willis *et al.* (1999) have recently provided comprehensive data on the subject of oral candidosis in diabetics. Their study, involving 414 insulin-dependent diabetes mellitus patients, has revealed that 77% of diabetics carried *Candida* species in their oral cavity, with *C. albicans* being the species most frequently isolated, and that 40% patients colonized with candidal species had no clinical signs of oral candidosis. They further showed that where oral candidosis was present, erythematous candidosis was the most common clinical presentation, and candidal load was not associated with age, sex, or glycemic control. On the contrary, they found candidal load to be significantly increased in smokers but not in denture-wearers or those with clinical signs of oral candidosis. However, there had been some reports contradictory to some of these findings. Arendorf and Walker (1979) and Tapper-Jones *et al.* (1981) have previously shown that the candidal carriage rate in diabetics who wear dentures is higher than that in dentate diabetics. Farman, in 1976, observed that poorly controlled diabetic individuals have a higher oral carriage of *Candida* than do better-controlled patients. But Tapper-Jones *et al.* (1981) and Fisher *et al.* (1987) were unable to show any correlation between poor glycemic control and oral candidal carriage.

Several reasons for the high oral candidal carriage in diabetics have been suggested. First, the high salivary glucose levels in diabetics may favor the growth of yeasts (Knight and Fletcher, 1971). However, the studies cited above which demonstrated a

lack of correlation between glycemic control and oral candidal carriage fail to substantiate this. Another possible mechanism that has been proposed is that the adhesion of *Candida* to buccal epithelial cells of diabetic patients is significantly greater than that of cells obtained from non-diabetic controls, implying that there are intrinsic qualitative changes in the cell-surface receptors of diabetics that modulate yeast adhesion (Darwazeh *et al.*, 1990).

Kumar *et al.* (1980) have shown that germ tube formation of *C. albicans* is higher in sera of diabetics. Yet, the same authors did not observe a similar finding with regard to saliva of diabetic patients (Kumar *et al.*, 1982).

Finally, Wilson and Reeves (1986) have shown that candidal activity of neutrophils may be defective, particularly in the presence of glucose—a further mechanism that may aid candidal infestation in diabetics.

#### **(2.3.2) Immunological aspects of chronic hyperplastic candidosis**

Researchers in oral candidosis and clinicians interested in the condition have always been intrigued by the existence of the several clinical variants of the entity, despite the single etiologic agent causing the disease. Reichart *et al.* (2000) have attempted to address this issue in the light of currently available information on the histopathological and immunocytochemical aspects of the disease, as well as the pathogenic characteristics of the fungus. They surmise that known and still-unknown differences in the virulent attributes of the fungus, such as the production of extracellular proteinases, within and between species may play a contributory role in the genesis of the clinical variants. They also suggest that hyperplastic candidosis could be considered a superficial cellular reaction to the pathogen, which cannot entirely be eradicated by the systemic or local host immune response. Similar hypotheses have been proposed by others with regard to cutaneous candidosis (Sohnle and Kirkpatrick, 1978).

At this juncture, it is appropriate to review the immune mechanisms that operate in candidal infections in general with particular relevance to chronic hyperplastic candidosis.

As with any other infection, both specific as well as non-specific immune mechanisms are involved in the defenses against human candidal infections. The role of some of the non-specific and local host factors has already been alluded to. These are summarized in Table 3.

As far as specific immunity against oral candidosis is concerned, both secretory IgA and cellular immunity might play a role in the protection of the oral mucosal surfaces against candidal infection. Indeed, a markedly increased prevalence of candidal infection can be seen in IgA-deficient individuals. Among patients with chronic mucocutaneous candidosis, which is a systemic disease with widespread chronic hyperplastic candidosis lesions, over 50% appear to have reduced IgA antibodies (Lehner *et al.*, 1972b).

Specific antibodies of IgG and IgM types against whole cell or antigens of *Candida* are found in most people (Winner, 1955; Lehner *et al.*, 1972a), even in those who carry *Candida* only as a commensal in their mouths or other mucosal surfaces, such as the vagina. However, serum antibodies against whole cells of *Candida* appear to vary between patients with different types of candidosis (Lehner, 1971). In the latter study, it was shown that antibody titers in chronic hyperplastic candidosis patients were not as high as in patients with *Candida*-associated den-

**TABLE 3****Non-specific Factors in the Defense against Candidal Infections**

Non-specific Factors	Possible Mechanisms
Skin and mucous membranes	Shedding of epithelial cell; antibiotic peptides (defensins)?
Secretions	Salivary flushing action
Antimicrobial factors in secretions	Histatins, lactoferrin, lactoperoxidase system, lysozyme
Commensal bacteria	Inhibition of candidal colonization
Phagocytosis	Polymorphonuclear leukocytes and macrophages
Natural killer cells	Direct cytotoxicity
Complement binding	Inhibition of complement activation

ture stomatitis. It had been suggested that this observation perhaps reflects the greater mucosal area involved in the latter condition with the concurrent transmucosal penetration of antigens compared with most cases of chronic hyperplastic candidosis.

Serum antibodies are generally not capable of killing *Candida*, even in concert with complement. However, they appear to act as opsonins for polymorphonuclear leukocytes and macrophages. They are also believed to act as chemotactic agents for these cells, thereby attracting them to the site of infection. As with other infections, the fixation of complement by antibody leads to the release of C3a and C5a, which are chemotactic (Challacombe, 1990).

Lehner (1966) showed, using immunofluorescent assays, that salivary antibody titers were raised in patients with candidosis, although not stated specifically as candidal leukoplakia, compared with carriers and non-infected controls. Epstein *et al.* (1982) confirmed this, using similar techniques, by demonstrating that the rise in titer of both IgG and IgA antibodies, mainly the IgA antibodies, was able to inhibit the adherence of *Candida albicans* to buccal epithelial cells.

Budtz-Jørgensen (1973) demonstrated the role of cellular immunity in resisting chronic candidal infections in the rhesus monkey. Evidence for the significant role played by cellular immunity against *Candida* infection can be found in the observation that infection by the fungus is a widespread problem among patients with severe T-cell defects and not in those with B-cell defects, unless the latter also have concomitant T-cell defects (Challacombe, 1994). Williams *et al.* (1997), who characterized the inflammatory cell infiltrate in CHC using immunocytochemical techniques, concluded, on the basis of their findings (*vide infra*), that mucosal defense against *Candida* infection involves a cell-mediated reaction. This consists of recruitment of macrophages and local production of immunoglobulin with a prominent IgA component.

Finally, in another study on the role of Langerhans cells in candidosis, Daniels *et al.* (1985) found them distributed in a fairly patchy manner, whereas control tissue sections showed even distribution. The difference in the numbers of cells, however, was not statistically significant. Although candidal anti-

gens themselves were not detectable, these workers found ATPase-positive Langerhans cells among, or at least near, intra-epithelial hyphae and T6-positive cells separated from the hyphae by epithelial cells. They postulated that this difference in distribution of ATPase-positive and T6-positive Langerhans cells may indicate locations of two cell subtype, or a change in T6 antigen expression by the Langerhans cells closest to the candidal hyphae.

### (2.3.3) Nutritional factors in the pathogenesis of candidal leukoplakia

Samaranayake (1986) has reviewed the extensive literature on the role of nutritional factors in the pathogenesis of oral candidosis. A summary of the available data on the role of iron deficiency shows that oral candidosis may be caused in the deficient individual by at least four mechanisms that render the oral mucosa susceptible to infection by the fungus. Iron deficiency can cause epithelial abnormalities such as hyperkeratosis and atrophy through alterations in the kinetics of the rapidly dividing cells of the oral mucosa, which, in turn, result from an impairment of iron-dependent enzyme systems (Jacobs, 1961; Rennie *et al.*, 1984; Ranasinghe *et al.*, 1989). Iron deficiency has also been shown to cause depression of cell-mediated immunity both in vivo and in vitro (Joynson *et al.*, 1972) and may also cause defects in phagocytosis and inadequate antibody production (Wilton and Lehner, 1981). Jenkins *et al.* (1977) have shown that a significant proportion of patients with chronic hyperplastic candidosis suffered from a deficiency in folic acid. Challacombe (1986) showed significant hematological abnormalities in patients with non-ulcerative diseases of the oral mucosa, including leukoplakia. Deficiencies of Vitamins A (Montes *et al.*, 1973) and B1 and B2 (De Greciansky *et al.*, 1957) are generally implicated in the causation of oral candidosis on the basis of animal experiments. There are also isolated reports of a link between deficiencies of Vitamins C and K and zinc and the presence of oral candidosis (Samaranayake, 1986).

It is probable that deficiencies in the above-mentioned micronutrients act not only alone but also in concert, through their direct effect on the nutrition and kinetics of the oral epithelium as well as the systemic effects they may cause. Carbohydrate-rich diets are particularly implicated in oral candidal infections, although not necessarily in relation to chronic hyperplastic candidosis.

### (2.3.4) Blood group antigen secretor status as risk factor

ABO blood group antigens, which are essential components of red blood cells, are secreted in saliva and other body fluids of most individuals, who are thus referred to as secretors (Mourant *et al.*, 1978). These antigens are also found in all human tissues, including epithelial cells (Bird and Tovey, 1982). It is already an established fact that the secretion of these antigens reduces susceptibility to many infections (Cruz-Coke *et al.*, 1965; Barua and Pagvio, 1977; Kinane *et al.*, 1983). May *et al.* (1986) were the first to report that, when compared with the saliva of non-secretors, saliva from secretors reduced the adhesion of *C. albicans* to buccal epithelial cells. They postulated that the lectin-like adhesins found in the yeast cell wall, which would otherwise bind with receptors on buccal epithelial cells, are prevented from doing so by the blood group antigens secreted in saliva by binding to the receptors on the yeast as well as to the host cells.

Burford-Mason *et al.* (1988) subsequently demonstrated that non-secretion of blood group substances in saliva significantly increases oral candidal carriage. The same authors also showed that the blood group H antigen functions as a *C. albicans* receptor, and therefore individuals who are of the blood group O (who possess a large amount of H antigen on their cell surfaces) are very susceptible to candidal colonization and subsequent infection.

The relationship between the inherited ability to secrete blood group antigens in saliva and chronic hyperplastic candidosis was investigated by Lamey *et al.* (1991). The proportion of non-secretors of blood group antigens was significantly higher in patients with chronic hyperplastic candidosis (68%) compared with control subjects (38%). On this basis, Lamey *et al.* (1994) concluded that the inability of an individual to secrete blood group antigens in saliva might be a risk factor in the development, or persistence, of chronic hyperplastic candidosis, which some workers consider as a pre-cancerous lesion (Roed-Petersen and Pindborg, 1973; Bánóczy, 1977).

### (3) Clinical Features of Chronic Hyperplastic Candidosis

CHC presenting as leukoplakia appears as well-demarcated, palpable, raised lesions that may vary from small translucent whitish areas to large opaque plaques that cannot be rubbed off. Some or all areas of the plaque may have a smooth, homogeneously white surface, and if this feature predominates, the lesion is referred to as a homogenous leukoplakia. However, the surface often has erythematous areas intermingled with white areas that, more often than not, possess a nodular characteristic. Such lesions are referred to as nodular or speckled leukoplakia. It was Pindborg *et al.* (1963) who pioneered the concept of nodular (speckled) leukoplakia, based mostly on their findings in the labial commissure. They found 35 nodular leukoplakias in a sample of 185 mostly commissural leukoplakias (18.9%). Pindborg (1980) deplored the tendency among certain workers to consider candidal leukoplakias as florid oral papillomatosis, or Bowen's disease (Nater *et al.*, 1977). Although many homogenous lesions are asymptomatic, speckled leukoplakias cause intermittent soreness or discomfort.

As mentioned earlier, the most common site for these lesions is the buccal mucosa, especially the commissural areas (Fig. 1). The palate and tongue may also be involved, although less frequently, with the former being affected relatively more often. Not uncommonly, the commissural lesions of CL tend to be associated with angular cheilitis. Indeed, in about one-third of candidal leukoplakias, other forms of oral candidosis are found to co-exist (Arendorf *et al.*, 1983). These are *Candida*-associated denture stomatitis, angular cheilitis, median rhomboid glossitis (MRG), and an oval or circular erythematous lesion on the palate in the area corresponding to that of MRG. The term "chronic multifocal candidosis" has been used to describe this tetrad, the constant member of the group being commissural candidal leukoplakia (Cernea *et al.*, 1965; Holmstrup and Besserman, 1983).

## (4) Pathology

### (4.1) HISTOPATHOLOGY OF CHRONIC HYPERPLASTIC CANDIDOSIS

The histopathology of chronic hyperplastic candidosis was first described by Cawson (1966a) and Cawson and Lehner (1968). The features of the lesion may vary according to the clinical

subtype, whether homogenous or speckled, and the degree of dysplasia present in the lesion. Homogenous leukoplakia may be either hyperorthokeratinized or hyperparakeratinized (Pindborg, 1980). Epithelial dysplasia is generally absent in homogenous leukoplakia but is more commonly noted in nodular leukoplakias. Various degrees of a chronic inflammatory cell infiltrate are seen in the lamina propria; the parakeratinized surface epithelium may show irregular separation. *Candidal* hyphae may be seen invading the epithelium at right angles to the surface. In some cases, the organisms may be so sparse that several periodic acid-Schiff (PAS)-stained specimens have to be examined for candidal hyphae to be detected.

Nodular leukoplakia may show marked variations in the thickness of the epithelium. Parakeratosis is invariably present, but hyperkeratosis is rarely seen in the infected part of the specimen. The parakeratotic layer is of variable thickness, sometimes about 12 or more cells deep, generally corresponding to the depth of invasion of the hyphae. Thus, the candidal invasion always stops short of penetrating beyond the junction between the parakeratotic layer and the stratum spinosum (Cawson and Lehner, 1968). Interestingly, the reasons for this abrupt cessation of hyphal invasion beyond the so-called "glycogen-rich" cells of the epithelium remains unknown. It is likely that the cellular defenses of the host invariably overcome the hyphal penetration at this juncture. Yet, full-thickness invasion of the epithelium is not seen in compromised patients totally devoid of cellular defenses, such as those suffering from AIDS.

Another characteristic histological feature of CHC is the collections of polymorphonuclear leukocytes forming "micro-abscesses" associated with candidal hyphae. Indeed these are considered to be diagnostic markers. The stratum spinosum itself shows acanthosis with hyperplasia of the rete ridges.

Jepsen and Winther (1965) isolated yeasts from smears of 34% of homogenous leukoplakias and *C. albicans* alone from 91% of speckled leukoplakias. These authors observed aggregates of candidal hyphae only in smears from the latter. This finding of a strong association between candidal invasion and speckled leukoplakia was confirmed by several subsequent publications (Renstrup, 1970; Roed-Petersen *et al.*, 1970; Daftary *et al.*, 1972; Bánóczy, 1982).

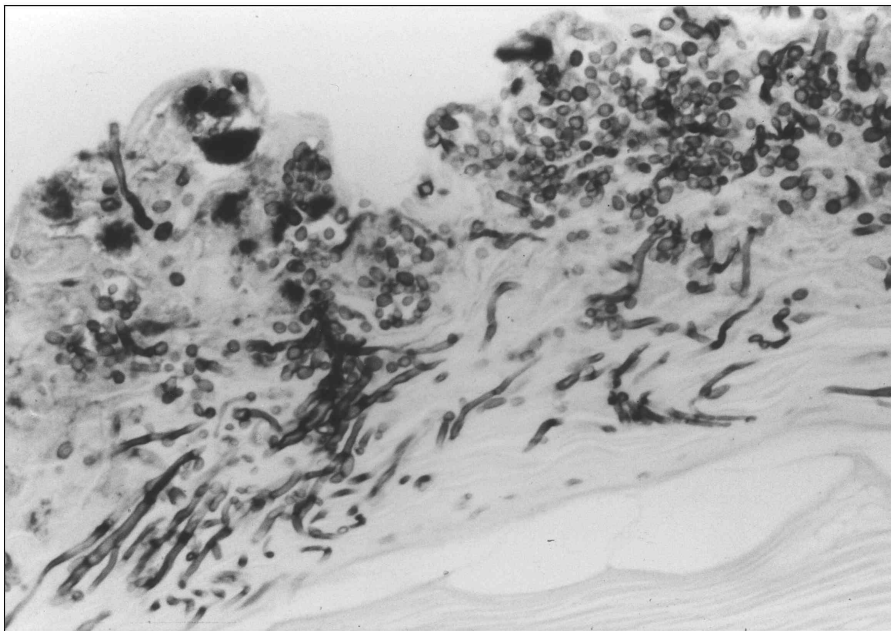
### (4.2) THE ROLE OF *CANDIDA* IN THE CAUSATION OF CHRONIC HYPERPLASIA AND EPITHELIAL DYSPLASIA

#### (4.2.1) Chronic hyperplasia

Jepsen and Winther (1965) suggested that when candidal hyphae are seen in association with epithelial hyperplasia, it is due to candidal invasion of an existing hyperplastic lesion rather than to *Candida* directly causing the epithelial hyperplasia. In an attempt to refute this hypothesis, Cawson (1973) elegantly demonstrated, using a chick chorioallantoic membrane model, that *Candida albicans* is indeed able to elicit hyperplasia. Several subsequent studies have confirmed that if *Candida* does begin to invade the superficial layers of the oral mucosa, a hyperplastic response of the epithelium ensues (Sohnle and Kirkpatrick, 1978; Odds, 1988). This has been further confirmed by Jennings and MacDonald (1990), who observed a 66% increase in the average epithelial thickness in 10 patients with chronic atrophic candidosis when compared with a normal population.

Partridge *et al.* (1971) contend that such a hyperplastic response, resulting in the formation of a plaque, is directly related to the virulence of the *Candida* isolates. Corroborative





**Figure 2.** Grocott's methenamine silver-stained histopathological section of hyperplastic candidosis. This stain clearly demonstrates the blastospores and filamentous fungal elements (in black), although it poorly visualizes the inflammatory exudates and other host cells (x200).

data in Wistar rats were obtained by Shakir *et al.* (1986), who demonstrated that an acrylic appliance laced with *Candida* induced histological changes, including increased mitotic activity and thickening of the palatal epithelium.

Citing clinical data, Samaranayake (1990) surmised that the regression of a significant proportion of chronic hyperplastic lesions as a result of antifungal therapy is an indication that hyperplasia is a protective response of the host mucosa against a disseminated infection by *Candida*. Nagai *et al.* (1992) have presented ultrastructural evidence to support this view. In biopsies of CL, these authors demonstrated, ultrastructurally, the presence of numerous small desmosomes and the interdigitation of cytoplasmic membranes between spinous cells in both epithelial hyperplasia and epithelial dysplasia. Moreover, eosinophilic spinous cells, observed predominantly in epithelial hyperplasia, showed an intricate arrangement of dense tonofibrils. They suggested that the findings indicate provision of mechanical strength between spinous cells in *Candida*-infested oral mucous epithelium and conclude that the hyperplastic reaction is a protective response.

#### **(4.2.2) The role of *Candida* in the induction of epithelial dysplasia**

According to several authors, *Candida* infection not only causes epithelial hyperplasia but may also induce epithelial atypia, leading to malignant change. Renstrup (1970) reported epithelial atypia in 71% cases of speckled leukoplakia, with 40% of all candidal leukoplakias demonstrating epithelial atypia. In animal models, *Candida* infection can induce epithelial dysplasia when inoculated into non-dysplastic hyperplastic lesions (Zhang *et al.*, 1994).

Barrett *et al.* (1998) reviewed 223 biopsies and reported that there is a statistically significant positive association between fungal infection and moderate and severe epithelial dysplasia.

An analysis of subsequent biopsies showed that epithelial dysplasia associated with fungal infection significantly worsened over time in comparison with non-infected epithelial dysplasias. In a very recent study, McCullough *et al.* (2002) (using the oral rinse technique) also described a significant correlation between epithelial dysplasia and the overall degree of oral yeast carriage in 223 patients who underwent biopsy sampling for investigations of an oral mucosal lesion. Intriguingly, in 44.6% of patients who had a histopathological diagnosis of either epithelial dysplasia or oral squamous cell carcinoma, the frequency of oral yeast carriage was significantly greater ( $p < 0.001$ ) than that in those without such histopathologically demonstrable lesions. The foregoing, along with the clinical evidence, suggests that up to 15% of the CHC lesions may progress into dysplastic lesions (Samaranayake and MacFarlane, 1990) and underscores the importance of close monitoring of recalcitrant lesions that do not resolve after appropriate therapy.

The probable role of yeast in oral carcinogenesis is unclear. It could be argued that the increased colonization and infestation associated with dysplasia are entirely coincidental, reflecting a change in the environmental conditions conducive to the proliferation of these ubiquitous commensals. If chronic candidal infestation were to be an important co-factor in carcinogenesis, then many more patients with chronic mucocutaneous candidiasis syndromes should develop oral carcinomas. Perhaps the mystery may lie in the genotypic attributes of the colonizing yeasts, which may be more virulent, in this respect, than their counterparts. Further research is warranted on both the phenotypes and the genotypes of species of *Candida* isolated from patients with and without oral dysplasias or carcinomas.

#### **(4.3) DETECTION OF *CANDIDA* IN BIOPSY SPECIMENS —PAS AND GMS TECHNIQUES**

Histopathological examination of a suspected lesion is essential for the diagnosis of CHC. Because candidal hyphae tend to be poorly stained by the routine hematoxylin/eosin stain, there is a risk that they may not be adequately visualized. Therefore, special staining techniques may be required to demonstrate their presence. The periodic acid-Schiff (PAS) and Gridley's or Grocott's methenamine silver (GMS) stains are suitable for the demonstration of fungal elements within tissues, which are dyed deeply by these stains. In the latter techniques, the adjacent hydroxyl groups of the complex polysaccharides of the yeast cell wall are oxidized to aldehydes in the presence of chromic or periodic acid. In the Gridley's and PAS techniques, the aldehydes react with the Schiff reagent, and the fungi then appear a pinkish red, whereas in the GMS method the aldehydes reduce the methenamine silver nitrate complex, producing a brown-black staining as a result of the deposition of reduced silver (Fig. 2).

The presence of blastospores and hyphae or pseudohyphae may enable the histopathologist to identify the fungus as a species of *Candida* and, given the presence of other histopathological features, make a diagnosis of chronic hyper-

plastic candidosis. However, speciation of *Candida* in chronic hyperplastic candidosis would require cultural studies to complement the biopsy procedure, although molecular biology techniques with PCR tools have recently helped speciate *Candida* directly from biopsy specimens in archival, formalin-fixed, paraffin-wax-embedded oral mucosal tissues, by means of ribosomal DNA sequencing (Williams *et al.*, 1995, 1996).

It is important that a swab of the lesion and a smear be taken prior to the biopsy procedure. It is also important that the biopsy site be an area that has not been previously swabbed or scraped, since fungal elements may be absent from such regions, thus reducing the usefulness of the procedure (Silverman *et al.*, 1990).

#### **(4.4) CHARACTERIZATION OF THE INFLAMMATORY CELL INFILTRATE IN CANDIDAL LEUKOPLAKIA**

Williams *et al.* (1997) characterized the inflammatory cell infiltrate in biopsy material of CHC using immunocytochemical techniques to stain different cellular antigens, immunoglobulins, and lysozyme. They found that the density of the infiltrate varied considerably between cases, and that the lymphocytes were predominantly T-cells (53.9%), with macrophages constituting about 14% and the B-cells 8.2%. They also found, overall, that about 61% cells contained immunoglobulins. A high proportion of cells (36.7%) contained IgA, and a smaller proportion IgM (2.5%).

#### **(4.5) IMMUNOCYTOCHEMICAL DETECTION OF CANDIDA IN FORMALIN-FIXED HISTOPATHOLOGICAL SPECIMENS**

Although the detection of *Candida* in tissues, by direct and indirect immunofluorescent and ELISA techniques, was possible for several years after Lehner (1966) pioneered the application of these techniques, these methods failed to find wider use. Indeed, Silverman *et al.* (1990) questioned the usefulness of such techniques when relatively simpler and more sensitive methods were available. Furthermore, these immunofluorescent methods required frozen specimens and were not feasible with formalin-fixed tissue specimens. In recent years, with the advent of sophisticated immunocytochemical techniques involving mono- and polyclonal antibodies, it is now feasible to detect *Candida* in formalin-fixed specimens. Williams *et al.* (1998) have demonstrated the usefulness of this technique using a commercially available monoclonal antibody to detect the presence of *Candida albicans* in formalin-fixed biopsy material.

### **(5) *Candida* and Oncogenesis**

#### **(5.1) PURPORTED ROLE OF CANDIDA IN ORAL CARCINOGENESIS; CANDIDA ALBICANS AND THE CARCINOGENIC NITROSAMINES**

There are several reports of carcinomas developing in candidal leukoplakias or similar lesions (Robinson and Tasker, 1947; Kugelmann *et al.*, 1963; Cawson, 1966a; Williamson, 1969; Eyre and Nally, 1971; Hornstein *et al.*, 1979; Cawson and Binnie, 1980). These reports claimed that candidal infection was the direct cause of the malignant transformation. Skeptics, however, have claimed that these reports do not necessarily prove a causal relationship, but merely an association, between chronic candidosis and carcinoma. It was not clear whether the host cell

proliferation, secondary to candidal invasion, leads to uncontrolled epithelial growth or whether these events, together with other as-yet-unidentified factors, initiate a neoplastic change (Pindborg *et al.*, 1980).

In an early study, Russell and Jones (1975) found that the consistent colonization of the lingual mucosa of rats by artificial inoculation of candidal organisms could lead to a histological appearance similar to that of candidal leukoplakia in humans. They also observed that long-term *Candida* infection of the rat tongue resulted in epithelial hyperplasia and some features of epithelial dysplasia, but further progression to carcinoma *in situ* or squamous cell carcinoma did not take place. In a related study, Shakir *et al.* (1986) found that fungal infestation of the oral cavity leads to an initial decreased mitotic activity and a subsequent sharp and significant rise in mitotic activity. At about the same time, Franklin and Martin (1986) showed that painting the hamster cheek pouch mucosa with 50% (v/v) turpentine and liquid paraffin, followed by *Candida* inoculation and suturing of the pouches, resulted in hyperplastic changes resembling those seen in human leukoplakias.

The notion of the carcinogenic potential of *Candida* has received further support from the report of Krogh *et al.* (1987b), who demonstrated that certain *Candida albicans* biotypes are capable of producing carcinogenic nitrosamine *N*-nitrosobenzylmethylamine, from its precursors. They also showed that strains with high nitrosation potential were associated with lesions showing more advanced precancerous changes. Jepsen *et al.* (1988) implanted nitrosamine-treated epithelial cells into the oral cavities of rats and demonstrated that the animals that received treated cells developed carcinoma within three weeks. These investigators concluded that nitrosamines produced by *Candida* appear to play a role in the causation of oral cancer. In a separate study, O'Grady and Reade (1992) showed that *Candida* can act as a promoter of oral carcinogenesis in the rat tongue when a known carcinogen, 4-nitroquinoline-1-oxide, was repeatedly applied. It is interesting to note, however, that Lacey *et al.* (1995) have ruled out a possible role for *Candida* in the pathogenesis of uterine cervical cancer.

Field *et al.* (1989), after reviewing the evidence for a role of *Candida* in oral epithelial neoplasia, postulated that nitrosamine compounds produced by *Candida* species may directly, or in concert with other chemical carcinogens, activate specific proto-oncogenes and thus initiate the development of a malignant lesion. They further suggested that the progression of the activated cell into a tumorigenic cell might well be linked to the amplification and over-expression of oncogenes, a phenomenon observed in many other human malignancies.

#### **(5.2) p53 EXPRESSION IN CANDIDAL LEUKOPLAKIC LESIONS**

Mutations in the tumor suppressor gene p53 have been observed by many investigators in almost half of head and neck lesions, including oral leukoplakias. In one immunohistochemical study, Gao (1996) elegantly demonstrated p53 expression in all 17 CL (100%) cases compared with 78% of the controls. They found that the number of p53-positive cells was significantly higher in lesions exhibiting epithelial dysplasia than in those without dysplasia ( $P < 0.01$ ) and concluded that p53 expression may be considered as a biological marker for CL. However, Crosthwaite *et al.* (1996) reported equivocal results, in that p53 expression was demonstrable only in CHC of the lip but not when the lesion involved the buccal/commissural

**TABLE 4****Candida Isolation in the Clinic and Quantification from Oral Samples**

Method	Main Steps	Advantages	Disadvantages
Smear	scraping, smearing directly onto slide	simple and quick	low sensitivity
Swab	taken by rubbing cotton-tipped swabs over lesional tissue	relatively simple	selecting sampling sites critical
Imprint culture	sterile plastic foam pads dipped into Sabouraud's (Sab) broth, placed on lesion for 60 sec; pad pressed on Sab agar plate and incubated; colony-counter used	sensitive and reliable; can discriminate between infected and carrier states	Reading above 50 cfu/cm <sup>2</sup> can be inaccurate; selection of sites difficult if no clinical signs present
Impression culture	Maxillary and mandibular alginate impressions; casting in agar fortified with Sab broth; incubation	useful to determine relative distributions of the yeasts on oral surfaces	useful mostly as a research tool
Salivary culture	Patient expectorates 2 mL saliva into sterile container; vibration; culture on Sab agar by spiral plating; counting	as useful as imprint culture	considerable chair-side time; not useful for xerostomics; cannot identify site of infection
Oral rinse	Subject rinses for 60 sec with PBS at pH 7.2, 0.1 M, and returns it to the original container; concentrated by centrifugation; cultured and counted as in previous methods	comparable in sensitivity with imprint method; better results if cfu > 50/cm <sup>2</sup> ; simple method	Recommended for surveillance cultures in the absence of focal lesions; cannot identify site of infection

mucosa, which are usually shielded from exposure to sunlight. Warnakulasuriya (2000), in contrast, asserts that p53 overexpression in oral leukoplakia has been misinterpreted as a marker of impending malignant transformation. The latter author emphasizes that, when used as a single marker, p53 is unsuitable as a predictor of malignant transformation of oral pre-cancerous lesions, and further, that there is no method sufficiently superior to conventional histopathology for the prediction of the behavior of oral pre-cancerous lesions.

## (6) Mycology of Candida in Candidal Leukoplakia

### (6.1) CANDIDA ISOLATION IN THE CLINIC AND IDENTIFICATION OF YEASTS FROM ORAL SAMPLES

The laboratory identification of *Candida* species is an important requirement for the diagnosis of oral candidosis in general, and this applies to CHC as well. Identification of the yeast in histopathological specimens is discussed elsewhere in this review. However, identification from oral samples obtained from lesions other than by biopsy is also necessary, particularly in epidemiological studies. Recently, Williams and Lewis (2000) and Williams *et al.* (2000) have reviewed this subject, and diagnostic methods that are available are listed in Table 4.

### (6.2) IDENTIFICATION OF CANDIDA SPECIES

It has been shown by several workers that *C. albicans* is the predominant species associated with CL. Jepsen and Winther (1965) isolated *C. albicans* alone in 91% of speckled leukoplakias. Roed-Petersen *et al.* (1970) succeeded in culturing *C.*

*albicans* from all leukoplakias in which hyphae were detected. Lipperheide *et al.* (1996) showed that *C. albicans* constituted 76% of the yeast isolated from oral leukoplakias.

Krogh *et al.* (1987a), who found association of *C. albicans* with 82% of leukoplakic lesions, identified the following species of yeasts as well: *Candida tropicalis*, *Candida pintolopesii*, *Candida glabrata*, and *Saccharomyces cerevisiae*. A study by Bartie *et al.* (2001), referred to in detail below, has shown the association of *C. dubliniensis* in three patients among 17 cases of CHC.

In view of the possibility that specific sub-strains of *Candida albicans* may be associated with CHC, Krogh *et al.* (1987b) biotyped the *C. albicans* isolates from these lesions and found 18 biotypes, with the most frequently occurring biotypes being 355 and 177. More interestingly, the same workers claimed that nodular leukoplakias were found to be associated with the rarely encountered biotypes 145, 175, and 575. However, other recent studies with DNA fingerprinting have cast doubt on this assertion (Lischewski *et al.*, 1999).

### (6.3) STRAIN DIFFERENTIATION WITHIN CANDIDA SPECIES

The advent of genotyping methods based on analysis of nucleic acid sequences has made it possible for the strains involved in CHC to be identified more accurately than ever before. (For a recent review, see Dasanayake and Samaranayake, 2003.) In a recent study, Bartie *et al.* (2001), who genotyped *C. albicans* strains from patients with CHC and other disease variants using the random amplification of polymorphic DNA (RAPD) technique, demonstrated that the CHC strains are not restricted to any individual clone or cluster of clones. This study fur-

ther demonstrated the genetic similarity of isolates from clinical variants of oral candidosis, but failed to show a correlation between the genotypes identified and the clinical condition of the patient. In conclusion, these genotypic and the older phenotypic findings imply that host factors are likely to be more important in the etiology of CHC than the virulence of specific strains of *C. albicans*.

## (7) Treatment and Prognosis of Chronic Hyperplastic Candidosis

### (7.1) GENERAL MEASURES

#### TO ELIMINATE PREDISPOSING FACTORS

Since tobacco smoking has been clearly shown to be linked to the causation of many/most leukoplakias, and to candidal leukoplakias in particular, elimination of this habit is an important step in the management of CL. This applies equally to the tobacco chewing habit that is prevalent in some parts of the world. Prostheses, particularly maxillary dentures, act as reservoirs of *Candida* and have been shown to be linked to a high prevalence of CL. Although requesting patients to abandon their dentures is not a realistic option, preventive measures such as not wearing the prosthesis at night, together with stringent denture hygiene, should be encouraged to prevent candidal colonization of the denture surface. Patients using a steroid inhaler for medical reasons should be advised to rinse their mouths or drink water soon after using the inhaler (Spector *et al.*, 1982; Ellepola and Samaranyake, 2001).

Detection of anemia and deficiencies of hematinics and vitamins by appropriate investigations must be undertaken and necessary remedies prescribed.

### (7.2) SPECIFIC TREATMENT OF CHRONIC HYPERPLASTIC CANDIDOSIS/CANDIDAL LEUKOPLAKIA

Various modalities of treatment for CL have been used and diverse claims made regarding their success. These include medical management in the form of antifungal therapy or topical application of retinoids, bleomycin, beta carotene or mixed tea, and surgical methods (Lodi *et al.*, 2002).

Surgical methods that include cold-knife surgery, laser therapy, and cryosurgery have been in use for the treatment of oral leukoplakias. The lesion is usually excised and the wound closed, primarily if it is small, and with a split-skin graft in the case of an extensive lesion. This may or may not be combined with carbon dioxide laser therapy or cryotherapy. Many clinicians prefer to treat the lesions with a period of topical and/or systemic antifungal therapy prior to embarking on surgical management. There is a paucity of information or indeed any controlled clinical trials with regard to these management methods and no overall clinical consensus as to the best approach.

#### (7.2.1) Antifungal therapy

Cawson and Lehner (1968), in their first report on CL, claimed improvement and disappearance of a significant number of their cases of leukoplakia with polyene-nystatin (tablets) dissolved slowly in the mouth. Lamey *et al.* (1989) reported a case of CL with a significant degree of epithelial dysplasia that resolved within 11 days of systemic treatment with the triazole antifungal, fluconazole. Holmstrup and Bessermann (1983) reported that, following antifungal therapy, nodular lesions of the commissur-

al areas showed the highest recurrence rate after 12 months.

#### (7.2.2) Medical, surgical, laser, and cryo-therapies

The available information on evaluation of the different forms of treatment of oral leukoplakia is not specific to candidal leukoplakia. However, it is useful to review the successes and failures of the different methods as they are applied to oral leukoplakias in general. Lodi *et al.* (2001) have reviewed a large number of publications on the different methods of treatment of oral leukoplakia. They analyzed reports of randomized clinical trials related to topical application of vitamin A, retinoids, bleomycin, mixed tea, or beta-carotene, individually. However, none of these treatment methods showed any significant benefit with regard to reduction of malignant transformation rates in comparison with the placebo treatment. Nevertheless, Vitamin A/retinoids and beta-carotene were associated with better rates of clinical remission. They concluded that none of the available methods could be considered as very effective. They found that there is a dearth of randomized controlled trials that have assessed the efficacy of surgical methods in the management of candidal leukoplakia.

Schoelch *et al.* (1999) analyzed the efficacy of laser treatment in the management of oral leukoplakias and claimed successful control of the lesions in 48 of 70 total cases.

Saito *et al.* (2001) have studied the effectiveness of surgical methods of treatment of oral leukoplakia in a controlled trial involving a total of 142 patients. They found that malignant transformation rates were lower in patients who underwent conventional surgical treatment *vs.* those subjected to cryotherapy.

## (8) Conclusions and Future Directions

It would appear that during the nearly three and a half decades since Cawson and Lehner (1968) introduced the terms "candidal leukoplakia" and "chronic hyperplastic candidosis", the subject has attracted only sporadic attention from researchers. There appears to be some revival of recent interest, particularly in the molecular biologic features of the disease, especially in relation to diagnostics. Nevertheless, it is clear that, at present, the overall database on the etiology, pathogenesis, and management of the disease is still in its infancy. The characteristics of *Candida albicans* that enable it to produce epithelial dysplasia and eventually malignant transformation still remain far from thoroughly elucidated. The ultrastructural biochemical and molecular features of the invasive phase of the organism in relation to the hyperplastic response it evokes, and the inter- and intra-species differences that might exist, and the related yeast biology are aspects worthy of further study.

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## REFERENCES

- Andriole VT, Hasenclever HF (1962). Factors influencing experimental candidiasis in mice. I. Alloxan diabetes. *Yale J Biol Med* 35:96-142.
- Arendorf TM (1984). Factors affecting oral candidal colonisation in health and disease. *Br Dent J* 147:267-272.

- Arendorf TM, Walker DM (1979). Oral candidal populations in health and disease. *Br Dent J* 147:267-272.
- Arendorf TM, Walker DM (1980). The prevalence and distribution of *Candida albicans* in man. *Arch Oral Biol* 15:1-10.
- Arendorf TM, Walker DM, Kingdom RJ, Roll JRS, Newcombe RG (1983). Tobacco smoking and denture wearing in oral candidal leukoplakia. *Br Dent J* 155:340-343.
- Axéll T (1976). A prevalence study of oral mucosal lesions in an adult Swedish population. *Odontol Dinamica Suppl* 36:1-103.
- Bánóczy J (1977). Follow-up studies in oral leukoplakia. *J Maxillofac Surg* 5:69-75.
- Bánóczy J (1982). Oral leukoplakia. The Hague: Martinus Nijhoff, pp. 53-59.
- Bánóczy J, Gintner Z, Dombi C (2001). Tobacco use and oral leukoplakia. *J Dent Educ* 65:322-327.
- Barrett AW, Kingsmill VJ, Speight PM (1998). The frequency of fungal infection in biopsies of oral mucosal lesions. *Oral Dis* 4:26-31.
- Bartie KL, Williams DW, Wilson MJ, Potts AJ, Lewis MA (2001). PCR fingerprinting of *Candida albicans* associated with chronic hyperplastic candidosis and other oral conditions. *J Clin Microbiol* 39:4066-4075.
- Barua D, Pagvio AS (1977). ABO blood groups and cholera. *Ann Human Biol* 4:489-492.
- Bastiaan RJ, Reade RC (1982). The prevalence of *Candida albicans* in the mouths of tobacco smokers with and without oral mucous membrane keratoses. *Oral Surg Oral Med Oral Pathol* 53:148-151.
- Beasley JD III (1969). Smoking and oral moniliasis. *J Oral Med* 24:83-86.
- Bennet KR, Reade PC (1982). Salivary immunoglobulin A levels in tobacco smokers and patients with minor aphthous ulceration. *Oral Surg Oral Med Oral Pathol* 53:461-465.
- Bird GW, Tovey GH (1982). The blood groups. In: Blood and its disorders. Hardisty RM, Weatherall DJ, editors. Oxford: Blackwell Scientific, pp. 1339-1431.
- Borg M, Ruechel R (1988). Expression of extracellular acid protease by proteolytic *Candida* spp. during experimental infection of oral mucosa. *Infect Immun* 56:626-631.
- Borg M, Kirk D, Baumgarten H, Ruechel R (1984). A colorimetric assay for the assessment of cytotoxicity of yeasts. *Sabouraudia* 22:357-367.
- Bruszt Von P (1962). Stomato-onkologische Reihenuntersuchungen in sieben Gemeinden. *Sud Schweiz Monats Zahnheilkund* 72:758-766.
- Budtz-Jørgensen E (1973). Immune response to *C. albicans* in monkeys with experimental candidiasis in the palate. *Scand J Dent Res* 81:360-371.
- Budtz-Jørgensen E (1990). *Candida*-associated denture stomatitis and angular cheilitis. In: Oral candidosis. Chap. 9. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Burford-Mason AP, Weber JCP, Willoughby JMJ (1988). Oral carriage of *Candida albicans*, ABO blood group and secretor status in healthy subjects. *J Med Vet Mycol* 26:49-56.
- Burkhardt A, Seifert G (1977). Morphologische Klassifikation den oralen leukoplakien. *Dtsch Med Wochenschr* 102:223-227.
- Cawson RA (1966a). Chronic oral candidiasis and leukoplakia. *Oral Surg Oral Med Oral Pathol* 22:582-591.
- Cawson RA (1966b). Chronic oral candidosis, denture stomatitis and chronic hyperplastic candidosis. In: Symposium on *Candida* infections. Winner HI, Hurley R, editors. London: Livingstone, pp. 138-53.
- Cawson RA (1973). Induction of epithelial hyperplasia by *Candida albicans*. *Br J Dermatol* 89:497-503.
- Cawson RA, Binnie WH (1980). *Candidal* leukoplakia and carcinoma. A possible relationship. In: Oral premalignancy. Mackenzie IA, Dabelsteen E, Squier C, editors. Iowa City: University of Iowa Press, pp. 59-66.
- Cawson RA, Lehner T (1968). Chronic hyperplastic candidosis-candidal leukoplakia. *Br J Dermatol* 80:9-16.
- Cernea P, Crepy C, Kuffer R, Mascaro JM, Badillet G, Marie JL (1965). Aspects peu connus de candidosis buccales. Les *Candidases* à foyers multiples de la cavité buccale. *Rev Stomatol (Paris)* 66:103-138.
- Challacombe SJ (1986). Haematological abnormalities in oral lichen planus, candidiasis, leukoplakia and non-specific stomatitis. *Int J Oral Maxillofac Surg* 15:72-80.
- Challacombe S (1990). Immunology of oral candidosis. In: Oral candidosis. Chap. 6. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Challacombe SJ (1994). Immunologic aspects of oral candidiasis. *Oral Surg Oral Med Oral Pathol* 78:202-210.
- Coleman G, Beighton D, Chalk A, Wake S (1976). Cigarette smoking and the microbial flora of the mouth. *Aust Dent J* 21:111-118.
- Critchley IA, Douglas LJ (1987a). Isolation and partial characterization of an adhesin from *Candida albicans*. *J Gen Microbiol* 133:629-636.
- Critchley IA, Douglas LJ (1987b). Role of glycosides as epithelial cell receptors for *Candida albicans*. *J Gen Microbiol* 133:637-643.
- Crosthwaite N, Teale D, Franklin C, Foster GA, Stringer BM (1996). p53 protein expression in malignant, premalignant and non-malignant lesions of the lip. *J Clin Pathol* 49:648-653.
- Cruz-Coke R, Parades L, Montenegro A (1965). Blood groups and urinary micro-organisms. *J Med Genet* 2:185-188.
- Daftary DK, Mehta FS, Gupta PC, Pindborg JJ (1972). The presence of *Candida* in 723 oral leukoplakias among Indian villagers. *Scand J Dent Res* 80:75-79.
- Daniels TE, Schwartz O, Larsen V, Dabelsteen E, Pindborg J (1985). Langerhans cells in candidal leukoplakia. *J Oral Pathol* 14:733-739.
- Darwazeh A, Lamey P-J, Samaranayake LP, MacFarlane TW (1990). The relationship between oral yeast colonization and in vitro adhesion of *Candida* to buccal cells of diabetics. *J Med Microbiol* 33:43-49.
- Dasanayake RS, Samaranayake LP (2003). Amplification based nucleic acid scanning techniques to assess genetic polymorphism in *Candida*: a review. *CRC Crit Rev Microbiol* 29:1-24.
- De Greciansky O, Leclerc R, Delaporte J, Gouin De Roumilly P (1957). Influence des carences (deficiencies) sur l'infection experimentale a *Candida albicans* sur rat blanc. *Sem hôpitaux de Paris* 33:273-276.
- Di Menna ME (1952). Natural occurrence of rough variant of a yeast. *Nature* 196:550-551.
- Ellepola ANB, Samaranayake LP (2001). Inhalational and topical steroids, and oral candidosis: a mini review. *Oral Dis* 7:211-216.
- Epstein JB, Kimura LH, Menard TW, Truelove EL, Pearsall NN (1982). Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. *Arch Oral Biol* 27:469-474.
- Eyre J, Nally FF (1971). Oral candidosis and carcinoma. *Br J Dermatol* 85:73-75.
- Farman AG (1976). Atrophic lesions of the tongue. A prevalence study among 175 diabetic patients. *J Oral Pathol* 5:225-264.
- Field EA, Field JK, Martin MV (1989). Does *Candida* have a role in oral epithelial neoplasia? *J Med Vet Mycol* 27:277-294.
- Fisher BM, Lamey P-J, Samaranayake LP, MacFarlane TW, Frier BM (1987). Carriage of *Candida* species in the oral cavity in diabetic patients: relationship of glycaemic control. *J Oral Pathol* 16:282-284.
- Franklin CD, Martin MV (1986). The effect of *Candida albicans* on

- turpentine induced hyperplasia of hamster cheek pouch. *J Med Vet Mycol* 24:281-287.
- Gao Y (1996). Cell proliferation in oral candidal leukoplakia. *Zhonghua Kou Qiang Yi Xue Za Zhi* 28:35-37, 64 (in Chinese).
- Gergely L, Uri J (1966). Day-by-day variation in the mycotic flora of the mouth. *Arch Oral Biol* 11:15-19.
- Ghannoum MA (2000). Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microbiol Rev* 13:122-143.
- Hoffmann D, Sanghvi LD, Wynder EL (1974). Comparative chemical analysis of Indian bidi and American cigarette smoke. *Int J Cancer* 14:49-53.
- Holmstrup P, Besserman M (1983). Clinical, therapeutic and pathogenic aspects of chronic oral multifocal candidiasis. *Oral Surg Oral Med Oral Pathol* 56:388-395.
- Hornstein PO, Grabel R, Schuner E, Shell H (1979). Oral *Candida*—Besiedlung bei leukoplakien und Karzinomen der Mundhohle. *Deutsche Medizinische Wochenschrift* 104:1033-1036.
- Hoyer LL (2001). The ALS gene family of *Candida albicans*. *Trends Microbiol* 9:176-180.
- Hurley R (1966). Experimental infection with *Candida albicans* in modified hosts. *J Pathol Bacteriol* 92:57-67.
- Jacobs A (1961). Iron containing enzymes in the buccal epithelium (letter). *Lancet* ii:1331.
- Jenkins WMM, MacFarlane TW, Ferguson MM, Mason DK (1977). Nutritional deficiency in oral candidosis. *Int J Oral Surg* 6:204-210.
- Jennings KJ, MacDonald DG (1990). Histological, microbiological and haematological investigations in denture induced stomatitis. *J Dentistry* 5:34-37.
- Jepsen A, Winther JE (1965). Mycotic infection in oral leukoplakia. *Acta Odontol Scand* 23:239-256.
- Jepsen A, Arenholdt-Bindslev D, Krogh P, Holmstrup P (1988). *In vitro* effect of *Candida* related nitrosamines (abstract). *J Dent Res* 67:752.
- Joynson DHM, Walker DM, Jacobs A, Dolby AE (1972). Defects of cell mediated immunity in patients with iron deficient anaemia. *Lancet* ii:1058-1059.
- Kantarcioglu AS, Yucel A (2002). Phospholipase and protease activities in clinical *Candida* isolates with reference to the sources of strains. *Mycoses* 45:160-165.
- Kennedy MJ (1988). Adhesion and association mechanisms of *Candida albicans*. *Curr Top Med Mycol* 2:73-169.
- Kennedy MJ, Sandin RL (1988). Influence of growth conditions on *Candida albicans* adhesion, hydrophobicity and cell wall structure. *J Med Vet Mycol* 26:79-92.
- Kenney EB, Kraal JH, Saxe SR, Jones J (1977). The effect of cigarette smoke on human oral polymorphonuclear leukocytes. *J Periodontal Res* 12:227-234.
- Kimura LH, Pearsall NN (1978). Adherence of *Candida albicans* to human buccal epithelial cells. *Infect Immun* 21:64-68.
- Kinane DF, Blackwell CC, Winstanley FP, Weir DM (1983). Blood group secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br J Venereal Dis* 59:44-46.
- Knight L, Fletcher J (1971). Growth of *Candida albicans* in saliva: stimulation by glucose associated with antibiotics, corticosteroids and diabetes mellitus. *J Infect Dis* 123:371-377.
- Krogh P, Hald B, Holmstrup P (1987a). Possible mycologic aetiology of oral mucosal cancer: catalytic potential of infecting *Candida albicans* and other yeasts in production of N-nitrosobenzylmethylamine. *Carcinogenesis* 8:1543-1548.
- Krogh P, Holmstrup P, Thorn JJ, Vedtofte P, Pindborg JJ (1987b). Yeast species and biotypes associated with oral leukoplakia and lichen planus. *Oral Surg Oral Med Oral Pathol* 63:48-54.
- Kugelman TP, Cripps DJ, Harrell ER Jr (1963). *Candida* granuloma with epidermophytosis: report of a case and review of the literature. *Arch Dermatol* 88:150-155.
- Kumar S, Joshi KR, Mathur JS, Mathur DR (1980). A study of germ tube formation by *Candida albicans* in biological fluids of diabetic patients. *Indian J Pathol Microbiol* 23:45-49.
- Kumar S, Mathur JS, Chaudhary SK, Chawla SN (1982). A comparative study of germ tube formation by *Candida albicans* in serum and saliva of healthy individuals and diabetic patients. *J Postgrad Med* 28:84-87.
- Lacey CJ, Dutton S, Smith RA, Walmsley RM, Wilkinson BM, Evans EG, et al. (1995). The oncogenic potential of *Candida* in the female genital tract. *Int J Gynecol Cancer* 5:8-14.
- Lamey PJ, Lewis MA, MacDonald DG (1989). Treatment of candidal leukoplakia with fluconazole. *Br Dent J* 166:296-298.
- Lamey PJ, Darwazeh AM, Muirhead J, Rennie JS, Samaranayake LP, MacFarlane TW (1991). Chronic hyperplastic candidosis and secretor status. *J Oral Pathol Med* 20:64-67.
- Lamey PJ, Douglas PS, Napier SS (1994). Secretor status and oral cancer. *Br J Oral Maxillofac Surg* 32:214-217.
- Lehner T (1964). Chronic candidiasis. *Br Dent J* 116:539-545.
- Lehner T (1966). Classification and clinico-pathological features of *Candida* infections in the mouth. In: Symposium on *Candida* infections. Winner HI, Hurley R, editors. Edinburgh: Livingstone Ltd., pp. 119-136.
- Lehner T (1967). Oral candidosis. *Dent Pract* 17:209-216.
- Lehner T (1971). Quantitative assessment of lymphocytes and plasma cells in leukoplakia, candidiasis and lichen planus. *J Dent Res* 50:1661-1665.
- Lehner T, Buckley HR, Murray IG (1972a). The relationship between fluorescent, agglutinating, and precipitating antibodies to *Candida albicans* and their immunoglobulin classes. *J Clin Pathol* 25:344-348.
- Lehner T, Wilton JMA, Ivanyi L (1972b). Immuno-deficiencies in chronic mucocutaneous candidosis. *Immunology* 22:755-787.
- Lipperheide V, Quindos G, Jimenez Y, Ponton J, Bagan-Sebastian JV, Aguirre JM (1996). *Candida* biotypes in patients with oral leukoplakia and lichen planus. *Mycopathologia* 134:75-82.
- Lischewski A, Harmsen D, Wilms K, Baier G, Gunzer U, Klinker H, et al. (1999). Molecular epidemiology of *Candida albicans* isolates from AIDS and cancer patients using a novel standardized CARE-2 DNA fingerprinting technique. *Mycoses* 42:371-383.
- Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A (2001). Interventions for treating oral leukoplakia (Cochrane review). *Cochrane Database Syst Rev* Vol 4, CD001829.
- Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A (2002). Systematic review of randomized trials for the treatment of oral leukoplakia. *J Dent Educ* 66:896-902.
- MacFarlane TW, Mason DK (1973). Changes in the oral flora in Sjögren's syndrome. *J Clin Pathol* 27:416-421.
- Marrie TJ, Costerton JU (1981). The ultrastructure of *Candida albicans* infections. *Can J Microbiol* 27:1156-1164.
- Martin MV, Al-Tikriti V, Bramley P (1981). Yeast flora of the mouth and skin during and after irradiation for oral and laryngeal cancer. *J Med Microbiol* 14:457-467.
- May SJ, Blackwell CC, Weir DM (1986). Nonsecretion of blood group antigens and susceptibility to *Candida albicans*: the role of Lewis blood group antigens (abstract). *J Dent Res* 65:503.
- McCourtie J, Douglas LJ (1981). Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. *Infect Immun* 32:1236-1241.
- McCullough M, Jaber M, Barrett AW, Bain L, Speight P, Porter SR (2002). Oral yeast carriage correlates with presence of oral epithelial dysplasia. *Oral Oncol* 38:391-393.
- Mehta FS, Pindborg JJ, Gupta PC, Daftary DK (1969). Epidemiological and histological study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer* 24:832-849.

- Mehta FS, Gupta PC, Daftary DK, Pindborg JJ, Choksi SK (1972). An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra. *Int J Cancer* 10:134-141.
- Montes LF, Krumdiech C, Cornwell PE (1973). Hypovitaminosis A in patients with mucocutaneous candidiasis. *J Infect Dis* 128:227-230.
- Mosadomi A, Shklar G, Loftus ER, Chauncey HH (1978). Effects of tobacco smoking and age on the keratinization of palatal mucosa: a cytologic study. *Oral Surg Oral Med Oral Pathol* 46:413-417.
- Mourant AK, Kopee AC, Domaniewski-Sobezak K (1978). Blood groups and disease: a study of associations of diseases with blood groups and other polymorphisms. Oxford: Oxford University Press.
- Nagai Y, Takeshita N, Saku T (1992). Histopathologic and ultrastructural studies of oral mucosa with *Candida* infection. *J Oral Pathol Med* 21:171-175.
- Nater JP, Zecha JJ, Heida F (1977). Floride orale papillomatose oder "speckled Leukoplakie". *Hautarzt* 28:18-22.
- Negi M, Tsuboi R, Matsui T, Ogawa H (1984). Isolation and characterization of proteinase from *Candida albicans*: substrate specificity. *J Invest Dermatol* 83:32-36.
- O'Grady JF, Reade PC (1992). *Candida albicans* as a promoter of oral mucosal neoplasia. *Carcinogenesis* 13:786-786.
- Odds FC (1988). *Candida* and candidosis. 2nd ed. London: Baillière Tindall.
- Oliver DE, Shillitoe EJ (1984). Effects of smoking on the prevalence and intraoral distribution of *Candida albicans*. *J Oral Pathol* 13:265-270.
- Partridge BM, Athar MA, Winner HI (1971). Chick embryo and inoculation as a pathogenicity test for *Candida* species. *J Clin Pathol* 24:645-648.
- Pindborg JJ (1980). Oral cancer and precancer. Bristol: Wright.
- Pindborg JJ, Renstrup G, Poulsen HF, Silverman S (1963). Studies in oral leukoplakia. V. Clinical and histological signs of malignancy. *Acta Odontol Scand* 21:407-414.
- Pindborg JJ, Kjaer J, Gupta PC (1967). Studies in oral leukoplakia. Prevalence of leukoplakia among 10,000 persons in Lucknow, India, with special reference to use of tobacco and betel nut. *Bull WHO* 37:109-116.
- Pindborg JJ, Reibel J, Roed-Petersen B, Mehta FS (1980). Tobacco-induced changes in oral leukoplakic epithelium. *Cancer* 45:2330-2336.
- Pugh D, Cawson RA (1977). The cytochemical localization of phospholipase in *Candida albicans* infecting the chick chorio-allantoic membrane. *Sabouraudia* 15:29-35.
- Ranasinghe AW, Johnson NW, Scragg MA, Williams RA (1989). Iron deficiency reduces cytochrome concentrations of mitochondria isolated from hamster cheek pouch epithelium. *J Oral Pathol Med* 18:582-585.
- Reichart PA, Samaranayake LP, Philipsen HP (2000). Pathology and clinical correlates in oral candidiasis and its variants: a review. *Oral Diseases* 6:85-91.
- Rennie JS, MacDonald DG, Dagg JH (1984). Iron and the oral epithelium: a review. *J R Soc Med* 77:602-607.
- Renstrup G (1970). Occurrence of *Candida* in oral leukoplakia. *Acta Pathol Microbiol Scand* 78:421-424.
- Robinson SS, Tasker S (1947). Chronic latent oral moniliasis (thrush): report a case of twelve years duration in which the disease was resistant to treatment. *Arch Dermatol Syphilol* 55:85-90.
- Roed-Petersen B, Pindborg JJ (1973). A study of Danish snuff-induced oral leukoplakias. *J Oral Pathol* 2:301-313.
- Roed-Petersen B, Renstrup G, Pindborg JJ (1970). *Candida* in oral leukoplakia. A histological and exfoliative cytologic study. *Scand J Dent Res* 78:323-328.
- Ruechel R (1986). Cleavage of immunoglobulins by pathogenic yeasts of the genus *Candida*. *Microbiolog Sci* 3:316-319.
- Ruechel R (1990). Virulence factors of *Candida* species. In: Oral candidosis. Chap. 4. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Russell C, Jones JH (1975). The histology of prolonged candidal infection of the rat's tongue. *J Oral Pathol* 4:330-339.
- Saito T, Sugiura C, Hirai A, Notani K, Totsuka Y, Shindoh M, et al. (2001). Development of squamous cell carcinoma from pre-existent oral leukoplakia: with respect to treatment modality. *Int J Oral Maxillofac Surg* 30:49-53.
- Samaranayake LP (1986). Nutritional factors and oral candidosis. *J Oral Pathol* 15:61-65.
- Samaranayake LP (1990). Host factors and oral candidosis. In: Oral candidosis. Chap. 5. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Samaranayake LP (1991). Superficial fungal infections. In: Current opinions in dentistry. Scully C, editor. Philadelphia, PA: Current Science, pp. 415-422.
- Samaranayake LP, Ellepola ANB (2000). Studying *Candida albicans* adhesion. In: Handbook of bacterial adhesion: principles, methods and applications. An Y, Friedman RJ, editors. New Jersey: Humana Press, pp. 527-540.
- Samaranayake LP, MacFarlane TW (1982). Factors affecting the in-vitro adherence of the fungal oral pathogen *Candida albicans* to epithelial cells of human origin. *Arch Oral Biol* 27:869-873.
- Samaranayake LP, MacFarlane TW (1985). Hypothesis: on the role of dietary carbohydrates in the pathogenesis of oral candidosis. *FEMS Microbiol Lett* 527:1-5.
- Samaranayake LP, MacFarlane TW, editors (1990). Oral candidosis. London: Butterworth.
- Samaranayake LP, Geddes DAM, Weetman DA, MacFarlane TW (1983). Growth and acid production of *Candida albicans* in carbohydrate supplemented media. *Microbios* 37:105-115.
- Samaranayake LP, Calman KC, Ferguson MM (1984a). The oral carriage of yeasts and coliforms in patients on cytotoxic therapy. *J Oral Pathol* 13:390-393.
- Samaranayake LP, Hughes A, MacFarlane TW (1984b). The proteolytic potential of *Candida albicans* in human saliva supplemented with glucose. *J Med Microbiol* 17:13-22.
- Samaranayake LP, Reaside J, MacFarlane TW (1984c). Factors affecting the phospholipase activity of *Candida* species *in vitro*. *Sabouraudia* 22:201-204.
- Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM (1986). A comparison of rinse culture and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. *J Oral Pathol* 15:386-388.
- Samaranayake LP, Robertson AG, MacFarlane TW (1988). The effect of chlorhexidine gluconate and benzylamine mouthwashes on mucositis induced by therapeutic irradiation. *Clin Radiol* 39:291-294.
- Samaranayake YH, Samaranayake LP (2001). Experimental oral candidiasis in animal models. *Clin Microbiol Rev* 14:398-429.
- Schoelch ML, Sekandari N, Regezi JA, Silverman S Jr (1999). Laser management of oral leukoplakias: a follow-up study of 70 patients. *Laryngoscope* 109:949-953.
- Scully C, Cawson RA (2001). Medical problems in dentistry. Bristol: Wright.
- Scully C, ElKabir M, Samaranayake LP (1994). *Candida* and oral candidosis: a review. *Crit Rev Oral Biol Med* 5:125-157.
- Shakir BS, Martin MV, Smith CJ (1986). Epithelial mitotic activity during the induction of palatal candidosis in the Wistar rat.

- J Oral Pathol* 15:375-380.
- Shipman B (1979). Clinical evaluation of oral *Candida* in cancer chemotherapy patients. *J Prosthet Dent* 41:63-67.
- Silverman S Jr, Migliorati J, Epstein L, Samaranayake LP (1990). Laboratory diagnosis of oral candidosis. In: Oral candidosis. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Slutsky B, Buffo J, Soll DR (1985). High frequency switching of colony morphology in *Candida albicans*. *Science* 230:666-669.
- Sohnle PG, Kirkpatrick CH (1978). Epidermal proliferation in the defence against experimental cutaneous candidiasis. *J Invest Dermatol* 70:130-133.
- Soll DR (2002). Phenotypic switching. In: *Candida* and candidiasis. Calderone RA, editor. Washington, DC: ASM Press, pp. 123-142.
- Soll DR, Slutsky B, MacKenzie S, Langtimm C, Staebell M (1987). Switching systems in *Candida albicans* and their possible roles in oral candidiasis. In: Oral mucosal diseases: biology, etiology and therapy. Mackenzie IC, Squier CA, Dabelsteen E, editors. Denmark: Laegeforenings-forlag, pp. 52-59.
- Spector SL, Wangaard C, Bardana EJ (1982). The use of cultures and immunologic procedures to predict oropharyngeal candidiasis in patients on steroid aerosols. *Clin Allergy* 12:269-278.
- Staib F (1965). Metabolism of human serum proteins by strains of *Candida albicans* and *Cryptococcus neoformans*. *Mycopathol Mycol Appl* 26:209-224.
- Staib F (1969). Proteolysis and pathogenicity of *Candida albicans* strains. *Mycopathol Mycol Appl* 37:345-348.
- Tapper-Jones LM, Aldred MJ, Walker DM, Hayes TM (1981). Candidal infections and populations of *Candida albicans* in mouths of diabetics. *J Clin Pathol* 34:706-711.
- Tronchin G, Bauchara JP, Robert R, Senet JM (1988). Adherence of *Candida albicans* germ tubes to plastic: ultrastructural and molecular studies of fibrillar adhesion. *Infect Immun* 56:1987-1993.
- van Cutsem J, Thienpont D (1971). Experimental cutaneous *Candida albicans* infections in guinea-pigs. *Sabouraudia* 9:17-20.
- Walker DM, Arendorf TM (1990). Candidal leukoplakia. Chronic multifocal candidosis and median rhomboid glossitis. In: Oral candidosis. Chap. 10. Samaranayake LP, MacFarlane TW, editors. London: Butterworth.
- Warnakulasuriya S (2000). Lack of molecular markers to predict malignant potential of oral precancer. *J Pathol* 190:407-409.
- Werner H (1966). Untersuchungen über die Lipaseaktivität bei Hefen und hefeähnlichen Pilzen. Zentralblatt für Bakteriologie und Hygiene I. *Abt Orig* 200:113-124.
- WHO Collaborating Reference Centre for Oral Precancerous Lesions (1978). Definition of leukoplakia and related lesions; an aid to studies on oral pre-cancer. *Oral Surg Oral Med Oral Pathol* 46:517-539.
- Williams DM (1969). Chronic hyperplastic candidiasis and squamous carcinoma. *Br J Dermatol* 81:125-127.
- Williams DW, Lewis MAO (2000). Isolation and identification of candida from oral cavity. *Oral Dis* 6:3-11.
- Williams DW, Wilson MJ, Lewis MAO (1995). Identification of *Candida* species by PCR and restriction fragment length polymorphism analysis of intergenic spacer regions of ribosomal DNA. *J Clin Microbiol* 33:2476-2479.
- Williams DW, Wilson MJ, Lewis MAO (1996). Identification of *Candida* species in formalin fixed, paraffin wax embedded oral mucosal by sequencing of ribosomal DNA. *J Clin Pathol Mol Pathol* 49:M23-M28.
- Williams DW, Potts AJ, Wilson MJ, Matthews JB, Lewis MA (1997). Characterisation of the inflammatory cell infiltrate in chronic hyperplastic candidosis of the oral mucosa (abstract). *J Dent Res* 75:1144.
- Williams DW, Jones HS, Allison RT, Potts AJ, Lewis MA (1998). Immunocytochemical detection of *Candida albicans* in formalin fixed, paraffin embedded material. *J Clin Pathol* 51:857-859.
- Williams DW, Wilson MJ, Potts AJ, Lewis MA (2000). Phenotypic characterisation of *Candida albicans* isolated from chronic hyperplastic candidosis. *J Med Microbiol* 49:199-202.
- Williamson DM (1969). Chronic hyperplastic candidiasis and squamous carcinoma. *Br J Dermatol* 81:125-127.
- Willis AM, Coulter WA, Fulton CR, Hayes JR, Bell PM, Lamey PJ (1999). Oral candidal carriage and infection in insulin-treated diabetic patients. *Diabet Med* 16:675-679.
- Wilson RM, Reeves WG (1986). Neutrophil phagocytosis and killing in insulin-dependent diabetes. *Clin Exp Immunol* 63:478-484.
- Wilton JM, Lehner T (1981). Immunology of candidiasis. In: Immunology of human infection. Nahmias EJ, O'Reilly RJ, editors. New York: Plenum Medical, pp. 525-559.
- Winner HI (1955). A study of *Candida albicans* agglutinins in human sera. *J Hygiene* 53:509-512.
- World Health Organization (1997). Histological typing of cancer and precancer of the oral mucosa. 2nd ed. Pindborg JJ, Reichart PA, Smith CJ, van der Wall I, editors. Berlin: Springer-Verlag, pp. 21-31.
- Zasloff M (1992). Antibiotic peptides as mediators of innate immunity. *Curr Opin Immunol* 4:3-7.
- Zhang KH, Wang HJ, Qin JX (1994). Effect of candidal infection on the hyperplastic oral epithelium. *Zhonghua Kou Qiang Yi Xue Za Zhi* 29:339-341.
- Zimmermann CF (1986). Immunohistochemischer Nachweis von candida Protease bei verschiedenen Manifestationen der *Candida*-Mykose (DDS Thesis). Göttingen, Germany: Faculty of Medicine, University of Göttingen.
- Zimmermann E, Zimmermann A (1965). Effect of race, age, smoking habits and oral systemic disease on oral exfoliative cytology. *J Dent Res* 44:627-631.