CHAPTER II

REVIEW OF LITERATURE

Candida as the normal flora

Candida, which is yeastlike fungi in the family Cryptococcaceae, is widely scattered in nature and frequently isolated from other animal sources. The human body is a natural but not exclusive habitat for these yeastlike forms. *Candida* is a member of the indigenous microbial flora of humans. In a high proportion of healthy persons, it is found in the gastrointestinal tract, the upper respiratory tract, and the oral cavity. It is also harbored in the vaginal tract by a high proportion of healthy women. Infection with *Candida* is a relatively frequent mycosis of worldwide distribution. (1)

Of the several different species of fungi that have been cultivated from the human mouth, the *Candida* species have attracted the greatest amount of study because they are frequently present in substantial numbers and because they are opportunistic pathogens capable of causing very serious deep infections as well as quite annoying superficial infections. Among the

Candida species that inhabit the mouth are *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. stellatoidea*. (3, 4)

Candida species with the same potentialities as those in the mouth occur on the other exposed mucosal surfaces of the body as well as on the skin. *C. albicans* has been isolated from clinical specimens, carriers, soil, vegetation, and food. The most pronounced pathogenic species among the parasitic forms is *C. albicans*, although there is good experimental evidence for the pathogenicity of *C. tropicalis*, *C. stellatoidea*, *C. pseudotropicalis*, and *C. viswanathii*. There is also reason to suspect that *C. parapsilosis*, *C. guilliermondi*, and *C. krusei* may be pathogenic for humans. (4)

C. albicans is unicellular and reproduces by budding. The blastoconidia are oval to round, varying from 2 to 4 µm in diameter, gram positive staining. *C. albicans*, in contrast to the other *Candida* species, has a marked tendency to form large, thick-walled spores, referred to as chlamydoconidia, when cultured on special media such as cornmeal agar and glutinous rice agar. (1, 5)

Candida is found as an oral commensal in up to 11 - 85 per cent of healthy adults without any sign of

inflammation, called candidal carrier. (6 - 11) This disparity may reflect differences in sampling technique, the site sampled, and the selection of subjects.

Transition from commensalism to parasitism. (2, 3, 12-16)

Candida albicans, which normally inhabits the gut, mucous membranes, skin, respiratory tract, genital organ, and elsewhere in man, is in balance with its host. No one knows whether its presence is useful to human beings or not. It is possible for the change from commensalism to parasitism by mutation, which is not infrequent. In parasitic life the organisms often undergo an initial multiplication in the yeast phase, and transform themselves into the mycelial phase when they start to invade the host tissues.

The alteration from commensalism to parasitism in *Candida* is conditioned by modifications of the human host rather than the parasite. Here is a list of changes in the host which bring about such an alteration:

1. Physiologic change

Pregnancy appears to affect the carbohydrate content of the vagina and leads to an increase in the population of *Candida*. This overgrowth may be sufficient

to cause a clinically apparent vaginitis. In newborn the source of *Candida* is the vaginal tract of the mother during the birth process.

2. Iatrogenic medical factors

Prolonged administration of antibiotics has evidently associated with clinical disease. The most important effect is the elimination and alteration of the bacterial flora that holds the population of *Candida* in check. The anti-inflammatory and immunosuppressive properties of steroids together with their tendency to raise the blood and tissue glucose concentrations make this steroid-induced enhancement of candidiasis. Immunosuppressive drugs can also cause a suppression of both cell-mediated and humoral immunity to *Candida*.

3. Endocrine disorders

Diabetics and patients suffering from hypoparathyroidism and hypoadrenocorticism are prone to candidal infections.

4. Malnutrition and malabsorption syndrome

Deficiency of vitamin B12, folic acid, or, especially, iron predisposes to candidiasis, possibly because of the dystrophic effect of these deficiencies on oral epithelium.

5. Malignancy

Systemic candidiasis is frequently seen in advanced malignant diseases, particularly lymphoreticular malignancy.

6. Immunologic defects

Defects of cell-mediated immunity, whether congenital or acquired, predispose to candidiasis.

7. Chronic local irritants or trauma

Candida infections may also result from accidental barrier breaks such as burns, wounds, chemical damage, or injection.

Types of Diseases

It is doubtful whether any other microorganism produces as diverse a spectrum of disease in human as does *Candida albicans*. All tissues and organs are susceptible to invasion by this parasite provided that the appropriate condition exist. Rippon (1) has conveniently classified the clinical manifestations of *C. albicans* infections as follows:

I. Infectious Diseases

A. Mucocutaneous involvement

1) Oral : glossitis, stomatitis, cheilitis, perleche

2) Vaginitis and balanitis

3) Bronchial and pulmonary infections

4) Alimentary : esophagitis, enteritis, and perianal disease

5) Chronic mucocutaneous candidiasis

B. Cutaneous involvement

1) Intertriginous and generalized candidiasis

2) Paronychia and onychomycosis

3) Diaper disease (napkin candidiasis)

4) Candidal granuloma

C. Systemic involvement

1) Urinary tract

2) Endocarditis

3) Meningitis

4) Septicemia

5) Iatrogenic candidemia

II. Allergic Diseases

A. Candidids

B. Eczema

C. Asthma

D. Gastritis

Candidal infections of the oral mucous membrane are characterized distinctly by both the clinical and histological features as the following: (3, 13)

Common forms of oral candidiasis

A. Acute pseudomembranous candidiasis (thrush)
B. Acute atrophic candidiasis (antibiotic candidiasis)
C. Chronic atrophic candidiasis (denture sore mouth)
D. Candidal cheilitis (perlèche)

Rare forms of oral candidiasis

A. Chronic mucocutanous candidiasis
    1) Familial chronic mucocutaneous candidiasis (autosomal recessive)
    2) Candidal endocrinopathy (autosomal recessive)
    3) Late-onset chronic mucocutaneous candidiasis
    4) Diffuse chronic mucocutaneous candidiasis

B. Candida leukoplakia (chronic hyperplastic oral candidiasis)

C. Profound immunodeficiency mucocutaneous candidiasis

1) DiGeorge syndrome

2) Hereditary thymic dysplasia

3) Swiss-type agammaglobulinemia

Thrush (acute pseudomembranous candidiasis)

Thrush is probably a disease still predominantly affecting the newborn, elderly patients, and patients with debilitating diseases. Thrush in the newborn has been result of direct infection from the vagina during birth. The disease becomes clinically evident on the fifth or sixth postnatal day. Thrush is found in debilitated patients, such as cancer, leukemia, hospitalized, or diabetic patients and patients under long-term treatment with antibiotics, corticosteroids, and cytotoxic agents. The major cause of thrush is an overgrowth of endogenous oral candidal organisms.

Thrush is manifested by the formation of superficial strandlike growths that become confluent and form pearly-white elevated patches that resemble milk curds. The typical white adherent pseudomembrane on the mucosal surface is composed of desquamated epithelial cells, leukocytes, microorganisms, keratin, necrotic

tissue, and food debris. Forcible removal leaves a raw, bleeding and painful ulcerated or eroded surface. A potassium hydroxide smear or Gram's stain of the exudate discloses masses of pseudohyphae and yeast forms. The most prevalent locations are the sides and dorsum of the tongue, buccal mucosa, gingiva, and oropharynx. When disturbed, they are painless with little or no swelling. Focal lesions heal within a week after treatment with antifungal agent such as nystatin, clotrimazole or ketoconazole. (13, 17)

Acute atrophic candidiasis

(antibiotic candidiasis, antibiotic sore mouth, glossitis and glossodynia)

Acute atrophic candidiasis occurs as a sequel to the shedding of the fungal plaque in thrush or arises de novo during oral antibiotic therapy. The disease is manifested by a painful erosion of the oral mucosa and patchy depapillation of the dorsum of the tongue. The condition has been ascribed to an overgrowth of yeasts following antibiotic-induced depression of the oral bacterial flora. In theory, all antibacterial antibiotics have the ability to eliminate bacteria from the mouth that normally compete with *Candida* species for nutrients. In practice, only broad-spectrum antibiotics or multiple narrow-spectrum antibiotics appear to produce

this effect to a significant degree. The symptoms of the disease usually subside spontaneously following withdrawal of the offending antibiotics. (13)

Chronic atrophic candidiasis

(denture stomatitis, denture sore mouth)

Denture stomatitis can be regarded as iatrogenic disease in that its development is dependent on the prolonged occlusion of the oral mucous membrane by a closefitting denture. Enclosure of mucous membrane by the denture provides a protected environment which favors growth of this fungus. Any irritant metabolic products produced by the organism are also kept in close contact with the mucous membrane by this means. It is perhaps more surprising that so many patients can wear a denture, literally, day and night for period of 20 or more years without ill-defects. (18, 19)

Some experimental work has suggested that acrylic dentures themselves favor the growth of *Candida albicans* and that the fungus may grow in the surface of the denture in microscopic pores of irregularities rather than on the oral mucous membrane itself. Smears taken from either the denture or the denture-bearing mucous membrane sometimes show very long coiled masses of candidal hyphae, thus suggesting that the organism

proliferates in the interface between denture and mucous membrane. (19)

According to Newton and Budtz-Jorgensen (20, 21), denture stomatitis may be graded clinically in three types.

Type I : Simple localized inflammation, showing pin-point hyperemia and diffuse inflammation of a limited area of the palatal mucosa.

Type II : Simple diffuse (generalized) inflammation, which subsequently was designated diffuse erythema of the entire or amost entire denture bearing mucosa of the maxilla.

Type III : Granular inflammation (papillary hyperplasia) which most often localized to the central part of the hard palate.

Denture stomatitis is virtually only seen under an upper denture; and this is presumably because the lower denture usually fits very much less closely and is continually being lifted by the muscles of mastication allowing a freer flow of saliva between the denture and the denture-bearing area. (3, 19)

Clinical features of denture stomatitis is usually symptomless and the alternative name "denture sore mouth" is inappropriate. But some patients will complain of swelling, sensivity, and pain in the affected areas. (13, 21) The feature which often draws attention to denture stomatitis is associated angular stomatitis. The characteristic appearance of denture stomatitis is an area of bright, uniform erythema exactly corresponding to and precisely limited by the upper denture-bearing area of mucous membrane. A sharp line of demarcation between the redness of denture stomatitis and the normal pallor of the mucosa is seen at the posterior border of the denture. In more severe cases, there is edema of the upper denture-bearing mucosa and the palate is indented along the line of the posterior border of the denture. (19, 21, 22)

Smears from the palate show hyphae of Candida. Culture of the saliva is also positive. (18)

Angular cheilitis is often associated but is remittant and may not be present when the patient is first seen. A history of cracks or sores at the angle of the mouth should therefore be enquired into. A deep fold at the angles of the mouth caused by sagging of the facial muscles after middle age is often concurrent, but it is not an essential predisposing factor.

A few of these patients are suffering from iron deficiency; and this should be looked for by

haematological examination especially when angular cheilitis is present. (19, 23)

Riboflavin deficiency can also cause angular cheilitis but is hardly ever seen in healthy patients except in those with malnutrition and malabsorption syndrome. (3, 19) It is concluded that deficiency of iron, folic acid, or vitamin B_{12} alone does not promote growth of *Candida* on the oral mucous membrane but that in some susceptible individuals, iron or folic acid deficiency may facilitate epithelial invasion by hyphae of *Candida*. (24)

Denture stomatitis is sometimes said to be due to denture trauma or to hypersensitivity to denture base material. The evidence for these is unconvincing; and there is certainly no evidence at all to support the idea that denture materials such as polymethylmethacrylate can produce a contact hypersensitivity reaction in the mouth. Positive patch tests on the skin reported in the past have been due to faulty testing procedure. (19)

Candidal cheilitis

Candidal cheilitis is characterized by soreness, redness and cracks at the corners of the mouth, reflected by shiny red commissural erosions with desquamation of

the epithelium and surrounding hyperkeratosis. The granular type is usually accompanied by swelling of the lips. Antecedents to candidal cheilitis are perlèche and pseudocheilitis. Perlèche is created by habitual licking of the corners of the mouth and results in local excoriations that become infected with *C. albicans*. Pseudocheilitis emanates from a loss of vertical height in the lower third of the face due to edentia or to poorly fitting dentures that permit a collapse of the circumoral tissues and folding of the skin at the angles of the mouth. The macerated fissures are readily infected by drooled saliva that is colonized by *Candida* species. The disease responds in part to topical antifungal agents and in full to restoration of the vertical dimension and canine eminences by appropriately constructed dentures. (13, 18)

Chronic mucocutaneous candidiasis

Four rare clinically diverse types of chronic candidal infections have been grouped together into an entity termed "chronic mucocutaneous candidiasis." The disease is featured by a protracted and persistent candidiasis of the mouth, nails, skin, and vagina that is fairly resistant to treatment. Antifungal therapy alone produces only partial or temporary remissions. Histopathologically, there is epithelial hyperplasia with

superficial penetration by *Candida* hyphae and diffuse infiltration of lymphocytes, plasma cells, and monocytes at the lamina propria or dermis.

All forms of chronic mucocutaneous candidiasis, except for the late-onset variety, begin either in infancy or in the first two decades of life. The initial manifestation is usually thrush followed by nail and skin involvement. There is a wide spectrum of severity ranging from chronic involvement of a single nail to severe disfigurement by granulomatous skin lesions.

In about 20 per cent of the cases, a familial history of a similar disorder can be elicited. The disease is transmitted as an autosomal recessive trait (familial chronic mucocutaneous candidiasis). Almost 50 per cent of the cases have an associated endocrinopathy, usually hypoparathyroidism or Addison's disease, or occasionally hypothyroidism or diabetes mellitus. This veriety is also genetic in origin and autosomal recessive in transmission. In patients with candida endocrinopathy, *C. albicans* may invade the tissue to produce tuberculoid-like granulomas. Deep granulomatous candidiasis usually occurs in the mouths of newborn infants and children and spreads to the face and head, resulting in infiltrated crusty plaques and severe granulomatous reactions. (4, 13)

Candida leukoplakia

Oral leukoplakia is a precancerous condition composed of chronic, discrete raised white mucosal patches ranging from small translucent areas to large dense opaque plaques. The most common sites are the buccal mucosa, tongue, lips, and floor of the mouth. Histologically, the patches are composed of hyperplastic and hyperkeratotic epithelium. The disease occurs in the mouth as either the common homogeneous variety that is usually asymptomatic or as the speckled variety that is marked by stinging and burning during food intake. *Candida* has been isolated from 34 per cent of cases of common leukoplakia and from 91 per cent of cases of speckled leukoplakia, both requires long-term antifungal therapy and surgical resection of persistent lesions. (13, 25)

Profound immunodeficiency mucocutaneous candidiasis

Oral thrush is a common concomitant of several immunodeficiency states, such as DiGeorge syndrome, Glanzmann-Riniker syndrome, and acquired immune deficiency syndrome (AIDS). The presence of oral candidiasis, and sometimes of esophageal candidiasis as well, is a premonitory sign for the future development of AIDS. (1 3)

Development of techniques to detect oral *Candida*

Detection of Candida depends on the method of examination. It is important to select detecting techniques for correct diagnosis. Lilienthal obtained a 10-per-cent increase in yield by using salivary samples compared with mucosal swabs. (26) Subsequent workers (23, 27, 29) largely relied upon the salivary sample technique as the best available indicator of the presence of intra-oral yeasts. The other techniques are epithelial smear (24, 28, 30, 31) and impression culture, (7, 21, 28, 32) both of these techniques yielded the most sensitivity at that time.

In 1970 Davenport (30) found out the distribution of oral *Candida* in denture stomatitis by replica culture or imprint culture technique to compare with the epithelial smear, salivary sample technique. He concluded that epithelial smear could give 60% positive result but could not investigate further to *Candida* species, whereas imprint culture could do so. He also found that patients with lower salivary pH and patients who were cigarette smokers were also more frequently positive. Intra-oral temperature and patient age seemed to have no effect. Finally, he further proved that growth of *Candida* occurs in large number on denture than on the mucosa.

In 1977 Berdicevsky et al. (33) brought strip test that used for detection of *Candida* in vaginal specimens to investigate oral *Candida*, called "Microstix." The test was a simple and yielded reliable results after 24 hours' incubation. In the clinically apparent infections, the results corresponded fully with those obtained with the conventional methods. As a screening device for healthy populations, the results showed some discrepancy that was explained by the limit of sensitivity of the strip (500 yeasts per milliliter)

In 1979 and 1980, Arendorf and Walker (34,35) used impression culture, imprint culture, epithelial smear, and salivary sample techniques to investigate oral candidal populations in health and disease. Quantitative imprint cultures appeared to be more sensitive than other techniques for detecting candidal carriers and might be useful for distinguishing between the healthy carrier state and oral candidal infection. The imprint culture also demonstrated the intra-oral distribution of *Candida* rather than merely determining whether or not the organism could be isolated from a subject. The isolated yeast could finally be identified as *Candida* species.

Diagnosis

Diagnosis of candidal infection is frequently difficult. The difficulty arises from the fact that this microorganism is a frequent secondary invader in other disease processes, and a mixed microbial flora may be found in the diseased tissue. It may be very difficult, even though candidal cells are isolated and identified, to signify the exact role that these parasites play in the patient's disease. (36)

Clinical specimens may include swabs, skin or mucosal scrapings, sputum, blood, or urine. Microscopic examination of original material from a lesion, however, should be done only with fresh specimens. A more reliable diagnosis obtained by microscopic examination of periodic acid schiff-stained smears or by direct mounting in 10% potassium hydroxide (10% KOH) on a slide should show a tangled mass of pseudohyphae, as well as a variable number of blastoconidia, inflammatory cells, since the pseudohyphal phase is the invasive state of this fungus. This is an important point even if cultures are positive for *Candida*. (1,5,13,32)

A portion of the tissue specimen also should be cultured and the yeast identified by its typical morphology and growth pattern and by other tests that are

available. A reliable and rapid laboratory procedure useful for identification consists of determining whether so-called germ tubes are formed (37) after exposure to blood serum for 2 hours at 37 °C. The production of chlamydoconidia on a suitable medium is a property useful for presumptive identification also. A quite rapid presumptive test for the identification of *C. albicans* is slide agglutination of the cultured by adsorbed *C. albicans* antiserum (Difco). (4)

Growth of *C. albicans* or of another *Candida* species from cultures of tissue specimens does not imply in any way that the isolated fungus is responsible for the patient's disease. Therefore, it is necessary to assess the microbiological culture data in the context of the overall clinical setting. (36)

Prevention

Since *Candida albicans* is a normal inhabitant of so many areas of the human body, the only preventive procedures available would be to avoid or prevent any of the resistance-lowering conditions that have already mentioned. (36)

Therapy for oral candidiasis

Candidal disease essentially is a consequence of opportunistic advantage taken by commonly occuring organisms. The circumstances of the predisposing factors have an important role in the outcome of the infection. All cases should arouse suspicion of some underlying systemic disorder, for which a thorough search should be undertaken. Oral factors that may need attention include correction of ill fitting dentures and avoidance of irritating factors such as spicy or irritating foods. (4) Any underlying disorders which are predisposing factors must be treated. Occasionally such treatment is followed by clearance of the candidal infection without any direct antifungal measures. (19)

Specific therapy

Superficial lesions of the oral mucosa or skin were, in the past, treated successfully with 1% gentian violet solution. Today, however, nystatin topically applied in the form of a troche, oral suspension, or tablet, three or four times a day for one or two weeks, is the agent of choice for oral lesions, since in some cases gentian violet may produce a superficial necrosis (so-called gentian violet burn) of unsightly staining. Amphotericin B also may be used, expecially if the

patient has difficulty with the strong and unpleasantly bitter flavor of nystatin, but amphotericin B is generally more irritating than nystatin. In the case of denture stomatitis *Candida albicans* is harbored beneath dentures, especially beneath upper dentures; and therefore it is advisable that dentures be kept out of the mouth as much as possible. Certainly, dentures should be kept out of the mouth during the sleeping hours and kept scrupulously clean by scrubbing the fitting surface with a proprietary denture cleaner and stored overnight in a preporation having antimycotic activity such as 1% sodium hypochlorite and 0.2% chlorhexidine gluconate solution. (38)

Before insertion the dentures should again be cleaned, rinsed in water; and antimycotic suspension such as nystatin or miconazole should be applied to the fitting surface several times a day. (19)

When both *C. albicans* and gram-positive cocci are found in the fissures of angular cheilitis, neomycin ointment should be prescribed in addition to nystatin tablet or oral suspension. (4)

Miconazole, ketoconazole, and clotrimazole, which are synthetic imidazole, are recently developed broad-spectrum antimycotic agents that appear to be a

relatively safe and promising alternative to nystatin or amphotericin B in the treatment of chronic mucocutaneous candidiasis; parenteral amphotericin B has been successful in the treatment of disseminated candidiasis. (19)

Prescription for oral candidiasis (39)

Nystatin:

Tablet (500,000 IU)	1 tab sucked four times a day
Oral suspension (100,000 IU/ml)	1-5 ml held in the mouth every six hours
Vaginal tablet (100,000 IU)	1 tab dissolved in the mouth four times a day

Trade name - Mycostatin, Nystatin, Nilstat

Amphotericin B:

Tablet (100 mg)	1 tab sucked four times a day
Suspension (100 mg/ml)	1 ml held in the mouth every four hours
Orabase (20 mg/gm)	applied to the lesion every four hours

Trade name - Fungizone

Miconazole:

Gel (25 mg/ml)	5 - 10 ml	held in the mouth four times a day
Tablet (25 mg)	one tab	sucked every four hours

Trade name - Daktarin oral gel

Ketoconazole:

Tablet (200 mg)	1 tab	a day (parenterally)

Trade name - Nizoral

Clotrimazole:

Vaginal tablet (100 mg)		1 tab dissolved in the mouth three times a day

Trade name - Canesten

Denture stomatitis treatment (39)

- topical antifungal agent on inflammed palatal mucosa
- remove and disinfect denture as often as possible

- 0.2% chlorhexidine gluconate is recommended for denture disinfectant
- use topical antifungal agent on the inner surface of denture before inserting into the mouth
- apply denture liner or tissue conditioners.

Removal of *Candida* from denture

- Sodium hypochlorite (for denture cleaning)
  1 % sodium hypochlorite solution
  (suitable for normal acrylic dentures but discoloration of chrom - cobalt prostheses)
- Chlorhexidine
  Chlorhexidine gluconate 0.2% w/v solution
- Mycostatin oral suspension
- Daktarin oral gel