# INTRODUCTION

Candidiasis is a primary or secondary infection, caused by any of several species of the yeast Candida (Rippon, 1982). In most instance Candida albicans is the prime etiologic agent of Candidiasis (Blumer, 1969). The clinical manifestations of the disease are extremely varied, ranging from acute, sub-acute and chronic to episodic. Involvement may be localised to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs or the gastrointestinal tract or become systemic as in septicemia, endocarditis and meningitis.

Candida vaginitis (thrush) is one of the most frequently encountered forms of superficial candidosis (Zinsser, 1985).

Usually C.V.* arises due to alterations in the normal physiological state of the host and is a common infection in, for example, poorly controlled diabetics (Peats et al., 1961), women taking oral contraceptives (Catterall RD. 1971) and in those in the third trimester of pregnancy (Pedersen GT. 1964).

A presumptive diagnosis of candida vaginitis may be based on clinical features such as a pruritis, milky white discharge, erythema and oedema, some of these features are also seen in other forms of vaginitis, particularly in infection due to Trichomonas or gonorrhoea

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* C.V. = Candida vaginitis.

and therefore an unequivocal diagnosis of candidosis requires the demonestration of yeasts in material from lesions by both microscopy and culture (Odds FC. 1979).

# AIM OF THE WORK

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The aim of this work is the detection of candida antigens in vaginal secretions by slide latex agglutination test and comparing the efficiency of this rapid new test with the clinical and conventional laboratory diagnosis.

# REVIEW OF LITERATURE

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## History :

Hippocrates in his "Epidemics" described the thrush (white patches) in debilitated patients and the presence of this clinical condition has been recognized for centuries. In 1839 Gruby classified the fungus in genus Sporotrichum (Gruby D. 1842). By 1853 Robin recognized that the thrush fungus could become systemic as a terminal event of other illnesses, he named the organism Odium albicans. Hansen in 1868 described it as a yeast and mycelial fungus, and named it Monilia Candida. Reess in 1877 redefined it as saccharomyces albicans. Zopf in 1890 described the disease as Monilia albicans. Berkhout 1923 introduced the generic name "Candida". This name was accepted as a nomen conservandum by the Eighth Botanical Congress at Paris in 1954. Novak and Zsolt (1964) classified all the species of candida under two genera:

1) Genus Candida: Pseudo-mycelia are only found.
2) Genus Procandida: True and pseudo-mycelia are found (Rippon, 1982).

From that we find that various names were applied to the organism, but Monilia albicans is the one most commonly used (Jones and Jones, 1978).

## OUTLINE OF CANDIDA

Of more than a hundred species of Candida, several are part of the normal flora and are potential pathogens. Most candida infections are caused by candida albicans, although at least seven other candida species have also been encountered in vaginitis (Joklike et al., 1984).

Table (1) shows the points of differences between the common species of candida causing vaginitis.

While it is well known that many candida species cause candidiasis, Candida albicans is still the most important etiologic agent (Schmidt, 1971) and for that it is explained herebelow in detailes.

**Candida albicans :**

* Morphology :

As indicated in figure (1) C. albicans is capable of producing yeast cells, pseudo-hyphae and true hyphae. As part of the normal flora,the organism grows as a budding yeast, which are cells round or oval ranging from 2-3 X 4-6 µm. in diameter, Gram positive and bud asexually. Hyphal forms are produced only during tissue invasion. It is either pseudo-hyphae or true hyphae. The differential features of the two forms are compared in table (2).

Table ( 1 ): Morphologic and physiologic characteristics of the common species of candida ( Joklik et al., 1984 ).

| Species | Property | | | | | Assimilation | | | | Fermentation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Growth at 37°C | Pseudo/ true Hyphae | chlamy- dospores | Germ tube | Cyclo- heximide | Glucose | Maltose | Sucrose | Lactose | Glucose | Maltose | Sucrose | Lactose |
| Candida albicans | + | + | + | + | + | + | + | + | - | F | F | - | - |
| " glabrata | + | - | - | - | + | + | - | - | - | F* | - | - | - |
| " Krusei | + | + | - | - | - | + | - | - | - | F | - | - | - |
| " parapsilosis | + | + | - | - | - | + | + | + | - | F | - | - | - |
| " pseudotropicalis | + | + | - | - | + | + | - | + | + | F | - | F | F |
| " stellatoidea | + | + | + | + | + | + | + | - | - | F | F | - | - |
| " tropicalis | + | + | - | - | - | + | + | + | - | F | F | F | - |

Cycloheximide : (+) Candida not inhibited.
(-) Candida inhibited.

F = Fermentation with acid and gas.

F* = Fermentation with acid only.

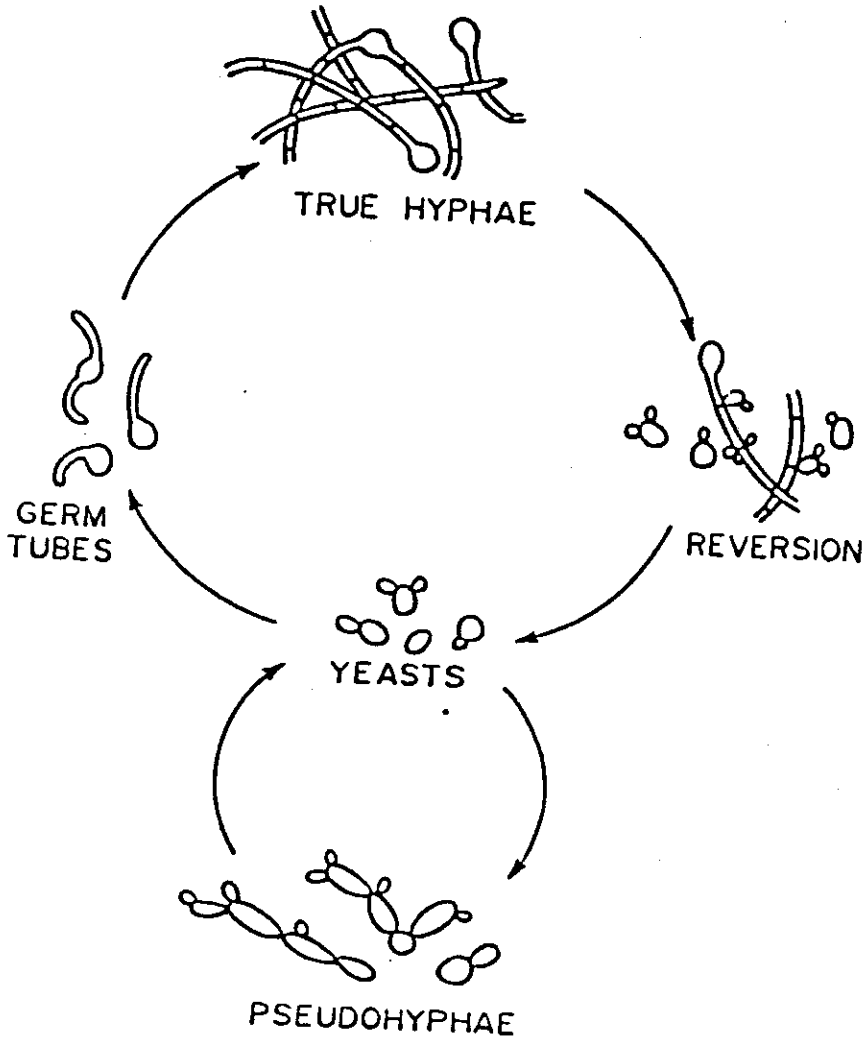


Fig. (1): Morphogenesis of Candida albicans (Joklike et at., 1984).

**Table (2) :** Comparison between the appearance of hyphae and pseudo hyphae.

| Feature | Hyphae | Pseudohyphae |
|---|---|---|
| 1. Growth process | Apical elongation | Budding |
| 2. Terminal cell | longer; cylindrical | shorter; spherical |
| 3. Cell wall | parallel | constricted at septa |
| 4. Septa | straight, perpendicular | curved or pinched |
| 5. Branches | No constriction or septum at origin | constricted and septated at origin |

Although a number of environmental stimuli are known to tigger or block conversion in vitro from yeast to hyphal growth, regulation of morphogenesis in candida albicans remains inconclusive. Both hyphae and pseudohyphae may revert to a yeast growth and it is not uncommon to see all three forms in vitro or in tissue.

The fine structure of Candida albicans includes a cell wall, cell membrane and cytoplasm containing an endoplasmic reticulum, nuclei, nucleoli, stoarge vacuoles, mitochondria and other organelles (Joklike et al., 1984).

Chlamydospores :

They represent resistant stages of Candida albicans as they occur in poorly nutrient media, deficient in proteins, (Lodder et al., 1958) and reducing sugars (Raid et al., 1953), they occur also in cases of Low $O_2$ tension, at a temperature between 21°C - 30°C, at pH (5-6 and 7.4-9).

Chlamydospores are large 8-12 µm, spherical, refractile spores of thick wall containing oil droplets, they are adopted for maintaining viability during starvation and other adverse conditions, their large size due to storage of reserve nutritional substances, and their thick wall protect them from unfavourable environment ( Reiss et al., 1974).

* Culture characteristics :

Candida grow on most bacteriological (blood and/or chocolate agar) and mycological media particularly on sugar rich peptone media such as sabouraud glucose agar with anti-bacterial antibiotics (Johnson et al., 1954).

Growth is aerobic with the formation of creamy, smooth, pasty, globrous colonies about 1.5-2 mm in diameter. Biotin and thyamine are growth factors (Schopfer and Guilloud, 1964).

Pure culture grow rapidly (24 hours or less), colonies may emerge slowly (after 36 hours to 1 week or longer) from the clinical specimens, possibly because of the host inhibition factor, drug, or therapeutic agents present in clinical specimens. The optimal temperature for growth is 25 - 30°C.

Candida albicans grow well at 37°C (Catchings et al., 1973) Filamentous forms and chlamydospores are produced on cornmeal agar with or without tween 80.

Joklik et al., (1984) pointed out that candida albicans may grow as yeast forms (i.e. basic morphological forms) and reproduce by budding, although a few undergo division by binnary fission, on the other hand, hyphal growth occurs by apical elongation i.e. extension in length from the tip of the filament. Rapid identification of Candida albicans sometimes may be effected by the formation of these germ tubes "Reynolds-Braude phenomenon".

Although some strains of Candida stellatoidea may form germ tubes , there is a 98 % chance of the yeast being C. albicans if the incubation is not extended beyond 2 hours (Haley, 1971).

Germ tube test :

The yeast is lightly inoculated into 0.5 ml of pooled human sera or commercially available product .

Other protein fluids as plasma, cerebro-spinal fluid and raw egg white can be used (Buckley and Van Uden, 1963) and then incubated for 2 hours at 37°C, the reaction is manifested by the appearance of a germ tube, an elongated appendage, growing out from and about half as wide and twice as long as the yeast cell (Joklik et al., 1984). Owing to the strain variation some species of C. albicans will not form germ tubes.

In smears of exudates, candida appears as a gram positive, oval, budding yeast, measuring 2-3 X 4-6 µm in diameter and gram positive elongated budding cells resembling hyphae "pseudo-hyphae" (Jawatz et al., 1978).

Microscopic appearance :

Of Gram stained films from colonies on sabouraud's glucose agar are short ovoid (5-7µ) sometimes elongate. On corn meal agar mycelium and pseudomycelium formed. Masses of blastoconidia at internodes, terminal thick walled chlamydospores are formed by most strains. Also as discussed before, all strains of C. albicans produce germ tubes in serum after 2 hours incubation at 37°C (Bulmer, 1969).

* Biochemical characteristics :

Fermentative or oxidative metabolism of sugars or both and assimilation of nitrates, occur in patterns useful for species differentiation (Table 1).

Antigenic structure :

Hasenclever and Mitchell (1961) have reported two antigen groups in C. albicans. They were separated by agglutination and agglutinin adsorption. The two antigenic groups were identical by chlamydospore formation, fermentation and carbohydrate assimilation reactions and by pathogenicity tests in animals (Norman et al., 1971).

The two groups are: group A, which shares antigenically with C. tropicalis and group B, which is shared by C. stellatoidea and isolated from more clinical specimens than C. albicans group A (Hasenclever et al., 1961) Nucleic acid-base composition studies (G-C ratios) indicate that C. albicans, C. tropicalis, C. clausenii, and C. stellatoidea are related (Stenderup et al., 1968). However, by DNA homology studies, C. albicans has close relationship to C. clausenii and C. stellatoidea but not C. tropicalis (Bak. et al., 1969). The various species of candida have been classified into six antigenic groups according to slide agglutination tests with mono-specific absorbed rabbit sera (Abraham, 1982).

Axelsen (1973) has demonstrated 78 soluble antigens associated with blastoconidia of C. albicans by using two-dimensional immunoelectrophoresis, but the two principal chemical types in serodiagnostic tests involved,

are proteins derived from the cell cytoplasm and cell wall polysaccharide "mannan" (Biquet et al., 1965).

The surface polysaccharides such as mannans and glucans appear to be the important antigenic determinants in candida species, mannans from the outer layer and glucan the inner layer of the cell wall of C. albicans the two sugars appear to occur naturally as complex of polysaccharide protein, linked together by N-acetyl-glucosamine. The antigenic specificity of the mannans depends on the length of the poly-saccharide side chains and the type of glycosidic linkages present in them (Abraham et al., 1982).

Determinants of pathogenicity :

No distinctive virulence factors for c. albicans have been discovered (Joklik et al., 1984).

Louria et al., (1977) stated that the sprouting of the yeast cell to form mycelium helped the organism to escape macrophage ingestion and was necessary for invasion of the tissue.

Thus the M (mycelial) phase of candida was considered the pathogenic or parasitic stage and the Y (yeast) stage the saprophytic form (Niekerson, 1954).

Recent investigations conclude that the yeast stage is necessary for initiation of a lesion and that

the mycelium is formed upon exposure to environmental factors which cause inhibition of cell division but not of growth. The result is elongate hyphae, the invasive capability of the yeast stage has been well documented (Montes et al., 1968).

The inflammatory, toxic and invasive abilities of C. albicans are due to cellular components and are characteristic of the species and not of a particular growth form (Maibuch et al., 1962).

Predominance of yeast forms indicates recent dissemination and an early lesion, whereas in old lesions, mycelium, blastoconidia and even thick walled macroconidia (chlamydoconidia) are found (Louria et al., 1977).

High doses of C. albicans extracts possess endotoxin like activity, but neither this nor any other antigen nor enzyme extracted from the organism correlates with pathogenetic potential. Recent studies indicate that cells of C. albicans are able to attach to epithelial cell membranes via a specific ligand-receptor interaction and that germ tubes appear to be more adhesive than yeast cells. Another provocative aspect of C. albicans is its immuno-modulating activity (Joklik et al., 1984).

Histopathology :

The pseudo-membranous lesion is seen to be composed of necrotic material, leukocytes and bacteria. Yeasts

are seen within vaginal epithelial cells, and the mycelium penetrates into and through these cells. The fungi are restricted to the vaginal stratum corneum. The rest of the tissue shows general inflammatory reaction, radial clumping, increased cellular turnover and haloing of squamous cells. No nuclear atypia is noted. Epidermal and subepidermal oedema is pronounced (Rippon, 1982).

Immunology :

The normal human adult has a high innate immunity to infection by candida (Rippon, 1982). Resistance is apparently based on cell-mediated immunity, since patients develop DTH reactions to fungal antigens, and the occurrence of chronic infections is associated with lack of these reactions. It is presumed that T cells release lymphokines which activate macrophages to produce destruction of the fungi. Disturbance of normal physiology by immunosuppresive drugs or of the normal flora by antibiotics can predispose to invasion by candida. Candida infections are also commonly seen in immunodeficiency diseases (Swiss Di George Syndrom) implying that the immune system is involved in confining the fungus to its normal commensal sites (Ivan et al., 1985).

The host defenses against candidiasis are both specific and non-specific, cellular and humoral serum components such as opsonins, complement and transferrin,

may inhibit either directly or indirectly the survival of candida specific antibodies to candida have a minimal direct effect but may inhibit the normal clumping of yeasts by serum, affect yeast morphogenesis or respiration, function as opsonins and mediate antibody dependent cellular cytotoxicity. Cellular host defenses against candida involve neutrophils which kill 30 - 40 percent of the ingested yeasts and the affector and effector functions of macrophages. The host factors involved in chronic muco-cutaneous candidiasis are associated with the thymo-dependent immune system (Zinsser, 1984).

Saturation of transferrin by iron has been noted to predispose to candidal infection in some cases (Caroline et al., 1969).

Gough et al., (1984) measured by enzyme - linked immunosorbent assay in serum and cervico-vaginal secretions the levels of anti-candida albicans immunoglobulin A (IgA) and G (IgG) from 64 non-pregnant women with vaginal candidosis and 158 uninfected non-pregnant women, specific IgA and IgG were detected in the serum and secretions of all 222 women, there was a significant correlation between secretions from women with candidosis and from uninfected women.

Specific anti-candida IgM antibodies have been detected in sera from women with a superficial (muco-cutaneous) form of candidiasis and with vaginitis (Lehrer,

1970 and Warren et al., 1978).

IgA deficiency in serum or body secretions does not appear to predispose to more sever forms of invasive candidiasis (Mathur et al., 1977) nor to vaginal candidiasis, however, high titers of IgG antibodies against candida albicans in serum may cause decreased filamentation of blastoconidia, associated with depression of fungal respiration (Grappel et al., 1976) this represents a depression in growth and metabolic activity of the tissue-invasive form of the organism.

In opsonization of candida, complement activation appears to play a greater role in phagocytosis by human neutrophils (Solomkin et al., 1978) than by monocytes (Cline M.J. et al., 1968).

Furthermore lesions from human patients with chronic mucocutaneous candidiasis often contain deposit of properdin or $C_3$, but not immunoglobulins or $C_4$ (Sohnle et al., 1976), it is suggested that generation of chemotactic factors might be responsible for the intense local inflammatory response seen in this disease, the cellular response may prevent deeper spread of the infection but is insufficient to clear all fungi from local lesions.

## CLINICAL INFECTION "Vaginal Candidiasis"

**Epidemiology :**

The interesting thing about C. albicans and several of the other candida species is that they are part of man's normal flora, Candida albicans can be isolated from the gastrointestinal tract, vagina, and oral areas in normal healthy individuals (Bulmer, 1969).

Candida albicans is a normal inhabitant of the alimentary tract and the muco-cutaneous regions (Marples, 1965 and Marples et al., 1968). It is regularly present in small numbers in the mouths of normal healthy adults, poor oral hygiene or even small amounts of antibiotics promote an increase in the number of organisms. The normal alimentary tract has a small but constant population of C. albicans, under normal conditions this is influenced by foods, since diet markedly affects the total number of organisms present, a high fruit diet appears to favor a rapid increase in the number of intestinal yeasts and propably explains the association of candida and tropical sprue (Mourad and Friedman, 1961).

Many surveys have been carried out which indicate that several factors influence colonization of the skin by C. albicans, whereas normal skin does not harbor a resident flora of C. albicans, almost any damage to the skin or environmental change leads to rapid colonization,

for this reason, candida is not infrequently isolated from a variety of dermatological conditions.

Most of the lesions are situated in moister areas, such as the inframammary folds, the perianal skin, and other intertriginous regions (Rippon, 1982).

Marples et al., (1968) stated that living in a tropical environment alone or having contact with infected patients increases the recovery of C. albicans from the skin.

Endocrine balance, the administration of steroids and other physiological factors also influence the rapidity and extent of C. albicans colonization (Rippon, 1982).

The incidence of C. albicans in the normal vagina of healthy, non-pregnant women is about 5 percent (Marples, 1965) and can be as high as 30 percent in pregnant women or women on oral contraceptives (Linares de et al., 1978).

In surveys of the normal vagina, it was found that between 11 and 30 percent of healthy women harbor C. albicans and up to 85 percent of gynecological patients have positive cultures (Andrussy and Horvath, 1979).

Elegbe, (1983) stated that C. albicans colony counts were far higher in patients with vaginitis wearing tight fitting clothing than in patients wearing loose fitting clothing, the tight fitting dresses, woolen and

corduroy jeans, coupled with nylon underwear, appear to create an environment favorable to candida albicans colonization.

The incidence of colonization in any site (especially: vagina, rectum, mouth) did not vary significally from 16 to 75 years of age (Current, 1983).

Negative rectal colonization was associated with lower vaginal colony counts and less frequent vaginal symptomatology, relatively high vaginal colony count was associated with symptomatic vaginal candidiasis (Bertholf et al., 1983).

Candida albicans is a saprophytic organism under normal conditions and a limited pathogen when present in large numbers, but is distinctly pathogenic in a host with depressed or otherwise compromised defense mechanisms (Seelig, 1966).

Generally candidiasis is a great mimic of other diseases i.e. all of the tissue and organ systems are subject to invasion (Current, 1983).

The main clinical conditions of candidiasis are:

I- Infectious diseases :

A) Muco-cutaneous involvement :

1. Oral: thrush, glossitis, stomatitis, cheilitis, perleche.

2. Vaginitis and balanitis.
3. Bronchial and pulmonary.
4. Alimentary: oesophagitis, enteric, and perianal disease.
5. Chronic mucocutaneous candidiasis.

B) Cutaneous involvement :

1. Intertriginous and generalized candidiasis.
2. Paronychia and onchomycosis.
3. Diaper disease (napkin candidiasis).
4. Candidal granuloma.

C) Systemic involvement :

1. Urinary tract.
2. Endocarditis.
3. Meningitis.
4. Septicemia.
5. Iatrogenic candidaemia (barrier break candidaemia).

II- Allergic diseases :

A) Candidids
B) Eczema
C) Asthma
D) Gastritis (Rippon, 1982).

* Predisposing Conditions :

The progression of c. albicans from the commensal to the parasitic state is enhanced by a combination of

increased yeast population and decreased host resistance, increased yeast population occurs when bacterial population is depleted in antibiotics therapy (Briody and Gillis, 1974), decreased host resistance occurs in obesity, diabetes, avitaminosis, corticosteroid therapy, extremes of life and in debilitated people (Current, 1983).

Joklik et al., (1984), pointed out that, there are many conditions that predispose individuals to opportunistic candida infection (Page 23), certain physiologic changes in otherwise healthy individuals provide the setting for opportunistic candidiasis.

In non pregnant women, the incidence of candidal vaginitis is between 10 and 17 percent, but this incidence approximately doubles during pregnancy (Mead, 1974).

The physiologic changes in the cervical and vaginal mucosa that result in overgrowth of candida have been correlated with :

1) An increase in moisture and carbohydrate substrates on the mucosal surface.

2) A local decrease in transferrin, which would lead to increased levels of available iron, an essential growth requirement for candida.

3) Increased secretion of steroids which might promote candidiasis indirectly by reducing local host defenses, such as phagocytosis.

4) A decrease in the concentration of specific IgA secretory component, although the protective value of this antibody has not been established (Mead, 1974).

Factors predisposing to Candida infections

(Joklik et al., 1984)

**Physiologic**

Pregnancy
Old age
Infancy

**Traumatic**

Maceration
Other infection

**Hematologic**

Cellular immunodeficiency
Aplastic anaemia
Agranulocytosis
Lymphoma, Hodgkin's disease, leukemia
Hypogamma globulinemia and agamma globulinemia

**Endocrine**

Diabetes mellitus
Hypoparathyroidism
Addison's disease

**Iatrogenic**

Immuno-supression
Transplantation
Post-operative
Steroid treatment
Antibacterial antibiotic
Birth control pill
Catheters
Vaccination
Hyperalimentation

**Miscellaneous**

Malignancy
Malnutrition
Malabsorption
Thymoma Heredity.

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1. <u>Pregnancy</u> :

An increased incidence of vaginal candidiasis in pregnancy has been widely recognized for many years, during pregnancy the amount of mycelia and yeasts in the vagina is increased greatly , fermentation of the increased glycogen content of the vaginal mucosa by lactobacilli leads to the production of lactic acid, which is said to favour the growth of yeast (Catteral et al., 1966).

Plass et al., (1930) pointed out that, pregnancy provides favourable conditions for the growth of monilia, and that in the majority of cases, no direct method of contamination can be detected.

They demonestrated that acidity of the vaginal secretions, in the gestational period favours the growth of monilia, and added that, the prevalence of the infection in pregnant women may be related to the known decreased sugar tolerance during that period.

Davis et al., (1969), conducted a survey of the occurrence of vaginal candidiasis, they found that pregnancy increased the incidence of vaginal candidiasis.

Amin, (1983), screened 38 pregnant female, attending antenatal clinic, Benha University Hospital for Candida albicans and he found that 12 (31.3%) were harbouring candida.

Throughout the course of normal pregnancy,large amount of oestrogen and progesterone are produced, at first by the corpus luteum, and latter by the placenta, these hormones, acting on the epithelial cells of the uterus and vagina cause typical changes which are recognizable in histological sections and vaginal smears (Mead, 1974).

Monif , (1974), were the first to emphasize that a high proportion of cases of leucorrhea in pregnancy are caused by Candida albicans. It seems probable that the change in reaction of the vaginal secretion during gestation is responsible for the high incidence of both moniliasis and trichomoniasis in pregnant women (Barnes, 1974).

Approximately 95% of all vaginal discharge or infections are caused by Gardnerella vaginalis, Candida albicans, Trichomonas vaginalis infections, cervicitis and excessive but otherwise normal vaginal secretions (Barclay, 1979).

Pregnancy is associated with reduction in cell-mediated immunity, which may explain why Candida albicans is more commonly present in the vagina during pregnancy.

The presence of a glycogen-rich vaginal epithelium due to increased oestrogen production or alteration in carbohydrate metabolism during pregnancy may also play a role in the transition of the organism from a saprophyte to a pathogen (Charles, 1980).

Jones and Jones (1981), pointed out that, it is estimated that 15% of non-pregnant females who complain of vaginal discharge harbour Candida albicans and that one third of pregnant females harbour it.

Following delivery, the regression of the factors enhancing fungal growth is reflected in a rapid disappearance of candida from the post partum vagina (Mead, 1974). Infants are especially at risk if they are heavily exposed to candida before the normal microbial flora of the gastro-intestinal tract and skin have been established. Normally, the attack rate of candidiasis among

infants is approximately 4 percent, but this is increased if the mother has candidal vaginitis. Infants usually develop oral thrush, perianal and genital infections, gastroenteritis with severe diarrhea, or prolonged and painful diaper rash (Joklik et al., 1984).

2. Diabetes mellitus :

As early as 1930, Plass and Barts, pointed out that diabetes mellitus is a definite predisposing factor in infection by candida vulvovaginitis. They added that, it would seem very probable that, in patient with so-called diabetic pruritus valve, the irritation may be due to the presence and growth of Monilia with the resulting increased vaginal acidity, rather than to chemical action. of the glucose itself, as has been usually thought.

There is an associating increased incidence of vulvovaginal candidiasis among diabetics and that the presence of such an infection has led to discovery of previously undetected diabetes mellitus. However, after application of glucose solutions to the vagina of 23 patients, all patients who did not harbour vaginal candida remained asymptomatic, whereas those with candida either developed clinical vaginitis or their existing vaginitis became more sever, after discontinuing the application, symptoms in all instances reverted to their previous state (Mead, 1974).

Glucosuria predisposes to vulvovaginitis due to Candida albicans and this is common in diabetics who are poorly controlled (Hopwood et al., 1985).

In the child bearing years, there is little point in testing for diabetes in women with recurrent candida infection. The vagina of these young women are well oestrogenized with abundant glycogen, providing a carbohydrate source for the growth of candida, thus there is little need to suspect diabetes. In post-menopausal women, breast feeding mothers or premenarchal children, however, candida infections are a significant indicator of diabetes in the patient who has not recently taken antibiotics or oestrogen, in these patients, the atrophic vagina has little or no glycogen and thus another carbohydrate source is required for the growth of candida. Diabetes is one such source, patients with renal glycosuria but without diabetes may also have candida vulvovaginitis because of the urinary carbohydrate bathes the vulva and distal vagina, providing the necessary carbohydrates (Fleury, 1981).

Not all studies, however, support the association between diabetes and candidiasis. In an extensive review, Louria et al., (1965), found that only 5 out of 95 pregnant females with candidiasis were diabetics i.e. in a percentage of 5.3% .

The treatment of diabetes with insulin and diet management was followed by relief of the vaginitis without the aid of local applications (Plass et al., 1930).

3. Antibiotics :

The increased incidence of candidiasis in recent years is clearly related to the increased use of antibiotics. In particular, the increased incidence of disseminated candidiasis and localized viseral candidal infections, has been convincingly correlated with the adminstration of combined or broad spectrum antibiotics, with or without corticosteroid treatment. Candida in the intestinal lumen are readily attacked by the antifungal antibiotics.To prevent the intestinal over growth of candida, especially in patients recieving broad spectrum antibiotics, the concomitant use of an antifungal agent is to be considered (Diddle et al., 1966).

The most important effect of antibiotics is the removal of bacterial flora which leads to simple disappearance of competitors and of some inhibitory factors for the growth of yeasts which may be produced by bacterial flora, antibiotics therapy may increase the virulence of candida and host resistance may be also depressed by antibiotics (Kashkin et al., 1961).

Winner, (1964) stated that the antibiotics itself may stimulate the growth of the candida.

Mead, (1974) pointed out that, the most likely explanation of increased incidence of candidiasis with antibiotic therapy appears to be the syppressive effect of antibiotic on susceptible bacteria, thus permitting over growth of resistant organisms including candida.

Jeffcoate, (1975) stated that, in non-pregnant women, the commonest cause is taking antibiotics indeed the irresponsible use of these is responsible for the widespread manifestation of candidiasis.

Sometimes the suking of penicillin lozengens precipitates oral thrush, or heavy oral dosage of various antibiotics, especially the tetracyclines, results in monilial enteritis or pneumonitis which may prove fatal. The population of microorganisms in the mouth or gut is the result of competing ranks of bacteria, protozoa, spirochaetes and fungi. The elimination of the bacteria sensitive to a given antibiotic may get rid of the successful competitors of a yeast which then multiplies and overwhelms the natural resistance of the tissues. The exact details of the mechanism involved are unknown, but candidiasis following antibiotic therapy may be controlled to certain extent by adequate intake of vitamin B complex (White et al., 1973).

4. Oral contraceptives :

Gardner, (1967) reported that, women given oral contraceptive medications had a five fold increase in the incidence of vulvovaginal candidiasis as contrasted to a similar group of women not given one of the pills.

Amin, (1984) screened 100 female to correlate the relation of contraceptive pills and prevalence of Candida albicans, he found that, out of 100 female, 35 were pill users, 16 (24.6%) were positive for candida. After statistical analysis of his results, he concluded that the use of contraceptive pills did not affect the prevalence of candidiasis in his study.

Davis et al., (1969) reported that, the incidence of candidiasis was higher among those patients using combined type of oral contraceptives compared to the sequential type, but the difference were small.

Also, it was found that the incidence of candida vulvovaginitis was more common in women using oral contraceptives for one or more years than in women taking the pills for less than one year (Diddle et al., 1969).

Other workers, however did not agree with these opinions (Morris, 1969, Rohatiner and Grimble, 1970).

With the combined type of oral contraceptives the dose of oestrogen or convertible progesteron is more

important than their relative amounts in contributing to the presence of candida. Also, if the oral contraceptive pills affect glycogen metabolism sufficiently, to result in a susceptibility to genital candidiasis, such an effect would depend either on the total amount of corticosteroid present or on the relative amount of oestrogen (Rohatiner and Grimble, 1979).

Meanwhile Howkins and Bourne, (1976), stressed upon the fact that, the progesterone content of contraceptive pills is a predisposing factor for monilial vulvovaginitis, this is brought about through increased vaginal acidity and glycogen content.

5. Age :

Plass and Borts, (1930) pointed out that, parous women are more infected with Candida and that children, virginal adults and senile women may likewise show the organism.

The pathologic physiology of leukorrhea due to vaginitis must be considered by age groups, because of the influence of endogenous and exogenous oestrogen and sexual activity.

Under the influence of oestrogen, the vaginal epithelium thickens and large quantities of glycogen are present in the epithelial cells, the collection of

intraepithelial glycogen results in the production of lactic acid, this acidic environment enhances the growth of a normal vaginal flora. Candida organisms may be present but in small numbers because of the preponderance of bacteria.

Estrogen depletion due to aging, causes atrophy of the vaginal mucosa, a reduction in glycogen content, and a decrease in the acidity of the vaginal fluid. A thin vaginal mucosa is susceptible to trauma. The bacterial population of the vagina changes from predominantly lactobacilli to a mixed flora consisting chiefly of pathogenic cocci (Barclay, 1969).

Amin, (1984) indicated that, age had no effect on the incidence of candidiasis in his study.

Patients who are treated with iron medications and whose host defenses are unduly compromised are at risk if they are iatrogenically exposed to candida. Immunosuppressive treatment, as an adjunct to transplanation or anticancer therapy, decreases resistance to candida. As many as 30 percent of leukemic patients acquire systemic candidiasis (Joklik et al., 1984). Many patients who develop systemic candidiasis present a history of having corticosteroids prior to the infection. Many blood dyscrasias predispose patients to systemic candidiasis, as does cellular or less frequently, humoral

immuno-deficiency. A decrease in numbers of functional capacity of neutrophils lowers resistance to candida, resulting in recurrent systemic infections. Defective T cell immunity is evident in most patients with chronic muco-cutaneous candidiasis. Among the opportunistic infections to which patients with aquired immune deficiency syndrom (AIDS) are highly susceptible are candidiasis. Especially involving the mucosal surface of the oesophagus, cryptococcal meningitis, and a myriad of other mycotic infections. The depression of cell-mediated immunity in these patients is often manifested as abnormally low numbers of T-helper/inducer cells and an inversion of the normal T-helper to T-suppressor/cytotoxic cell ratio. This immunologic defect in patients with AIDS is consistent with the evidence that competent cell-mediated immunity is essential for resistance to both muco-cutaneous candidiasis and cytococcosis (Joklike et al., 1984).

6-<u>Influence of pH</u> :

Vaginal acidity is thought to depend on the presence as well as the amount of lactic acid formed by the action of Doderlein's bacilli on the glycogen of the epithelial cells lining the vagina. Most pathogenic bacteria have a fairly restricted pH range and grow best at a pH of about 7.5. This may be a reflection of the fact that mammalian tissue and blood have a pH of this order. Considerably more epithelial cells are found in

those patients with a low vaginal pH. It is of importance to note that a low vaginal pH may exist in the relative absence or even total absence of Doderlein's bacilli, and it is suggested that the pH of the vagina depends on the degree of fermentation between the glycogen of the sheded epithelial cells and the cervical mucus secretion. This would explain the increased morbidity in patients with a low vaginal pH in the presence of primary vaginal infections. The cytological picture of the vaginal exudate of the patient with a low vaginal pH corresponds to that found in women taking oral contraceptives, in diabetes, in pregnant women, and in those taking oral steroids. It seems reasonable to assume that, if excessive epithelium is being shed, invasion of the deeper layers may occur by normally benign saprophytic organisms which then will become "pathogenic" (Koss and Durfee, 1961).

Charles, (1980) found that the women with a low vaginal pH are in the first instance relatively resistant to treatment of a vaginal discharge, conversely, the women with a high vaginal pH may harbour yeasts or trichomonads and yet remain symptomless.

Howkins and Bourne, (1976) reported that C. albicans thrives and flourishes in acid medium (pH 5.0-6.5) with an abundant supply of carbohydrate.

* Pathogenesis of vaginal candidiosis :

The intact or physiologically normal epithelium is usually resistant to candida invasion. If there is marked increase in the number of candida present, or if the skin and mucosa are traumatized or are hormonally altered, these barriers are susceptible to candida invasion. Iatrogenic candidemia and candiuria, induced by catheter, surgery or hyperalimentation, are often successfully managed by the normal host defense mechanisms. However, the ability of patients with hormonal imbalances, immunodeficiencies, and malignancies to control invasion of the deeper tissues is limited (Joklik et al., 1984).

Carcia et al., (1982) found that candida species can penetrate, invade, develop and proliferate within the deep layers of intact cells of the cervix and vaginal mucosal epithelium. The presence of mucopolysaccharides in the glycoproteinaceous coat of blastospores and pseudohyphae was demonestrated both outside and within squamous epithelial cells. The importance of the integrity of glycoprotein coat during the pathogenesis of human infection with C. albicans is therefore emphasized. Furthermore, the histoinvasive yeast apparently produces cytolytic enzymes during its growth with the human vaginal and cervical mucosal epithelium. The intracellular growth of candida organisms may represent a protective mechanism of the fungus against the host and a manner of resistance to antimycotic therapy.

The capacity of Candida albicans to produce hyphae appears to be an important but nonessential virulence factor in the pathogenesis of candidal vaginitis. (Sobel et al., 1984).

Segal et al., (1984) pointed to the increased Candida albicans adherence in situations where there is an increase in the number of intermediate epithelial cells: pregnancy, the first or fourth weeks of the menestrual cycle, or diabetes.

Lehrer et al., (1986) stated that adherence to vaginal mucosa may be an important determinant in the pathogenesis of vaginal infection caused by c. albicans.

Vaginal reinfections are frequently due to the associated intestinal colonization by Candida albicans and the infection of the vagina is from this reservoir (Meinhof, 1982).

Witkin et al., (1983), stated that women with recurrent C. albicans vaginitis appear to produce candida specific suppressor lymphocytes which block the cellular immune response to this organism.

* Clinical manifestations :

Vaginal thrush occurs more often in pregnant women, diabetics and women receiving antibacterial or hormonal treatment, including birth control pills.

Patches of gray white pseudomembrane develop on the vaginal mucosa, and a yellow white discharge may accompany the infection. From the mucous membranes, infection and inflammation may spread to the adjacent skin (Joklik et al., 1984).

The disease varies from a slight eczematoid reaction with minimal erythema to a severe disease process with pustules, excoriations, and ulcers. The whole area is greatly inflamed, and pruritis is usually intense. Popular and rarely ulcerative lesions may occur, and the condition may extend to involve the perineum, the vulva and the entire inguinal area (Rippon, 1982).

The vaginitis produced by Candida albicans differs significantly from that produced by other vaginal pathogens. The chief difference is the relative absence of purulent inflammation. Patients with severe candida vaginitis commonly exhibit a significant local leukocytic response to the infection. Instead the discharge is made up of clots of epithelial cells with intertwined mycelia (Mead, 1974) vaginal discharge tends to form plagues which are slightly adherent to the vaginal wall and that leave multiple haemorrhagic spots if removed (Fleury et al., 1981).

Catteral, (1970) and Charles (1980) stated that the amount of vaginal discharge if present, is variable

and it is not a marked feature of vulvo-vaginal candidiasis, while pruritus vulva is the most prominent symptom of the patient with vulvo-vaginal candidiasis. Itching may be intense leading to insomnia and irritability although features of infection are minimal (Peeters et al., 1977; Mead, 1974 and Fleury et al., 1981).

Browne and Dixon (1980) pointed out that, thrush like patches are seen on the vaginal wall, and even externally on the vulva, which are pathognomonic, also, there may be enormous vulval swelling.

The rate of vulvovaginitis is highest during the third trimester of pregnancy when vaginal pH is lowest. In non-pregnant women the discomfort may be particularly intense just prior to menestruation. Pruritis and pain in the introitus and labia minor can be aggravated by urination, sexual intercourse or gynecological examination (Rippon, 1982).

The condition can be mimicked or may coexist with trichomonas vaginitis. In the later disease, low level pruritus is constant and the crudlike patches are lacking (Felman and Nikitas, 1979). Significant difference between vaginal candidiasis and trichomonas vaginitis are outlined in table ( 3 ) (Mead, 1974).

Table ( 3 ): Differential characteristics of candida and trichomonas vaginitis.

| Profile | Candidiasis | Trichomoniasis |
|---|---|---|
| - Symptoms | - Severe itching | - Little itching |
| - Exacerbation | - Premenstrually | - Post menstrually |
| - History of Antibiotic, Steroid use and Diabetes | - Common | - No relation |
| - Discharge | - Variable amount, thick, white, crude like, odourless | - Profuse, frothy, green, and mal odorous. |
| - Vaginal pH | - Wide range (2.2 - 9.6) usually +4 | - Narrow range (5.0 - 7.55) usually +6 |

The dramatic change in comparative incidences of these common invaders is probably due to the introduction of metronidazole, so that, if present the fungus infection is more prevalent than trichomonas (Novak, 1979).

Bacterial vaginitis such as corynebacterium vaginale also mimic candida disease (Rippon, 1982)

Acetaldehyde, acetic acid, pyruvic acid and other products are formed and present in the discharge of patients with vaginal candidiasis, even dilute solutions of these substances cause pruritis when placed on mucous membranes, it is suggested that, perhaps these are the

agents that give rise to the patients extreme discomfort (Hesseltine, 1959).

Pruritis associated with candida vaginitis is produced as a result of the production of an undefined mycotoxins, which can be both locally irritating and even allerginic (Mead, 1974).

It is well established that candidal vaginitis during pregnancy contributes to thrush of the new born (Marples, 1965). Infants develop oral thrush or lesions in the perianal region which may persist as a diaper rash of the genital, perianal, and groin areas. By scratching, spread may occur to other skin sites.

Charles, (1980) pointed out that, neonatal candidal infections are common, and approximately 5% of newborn infants develop oral candidiasis. The predisposing factors include prematurity, intra-uterine growth retardation, low birth weight and resuscitative procedures. Whereas candida albicans may occur as an innocuous saprophyte on adult mucus membranes, its presence in the neonate is invariably associated with clinical manifestations. The newborn infant is incapable of resisting such infections because of the intrinsic lack of immunity, this is corrected by the age of 12 months, as evidenced by the fact that almost all children at that age have demonstrable candida agglutinating antibodies in their sera.

**Charles** also concluded that, infants born to women who are not colonized with the Candida albicans rarely develop thrush or fungal dermatitis.

Disseminated candidiasis in the neonate is usually encountered only in the presence of severe debility or immunosuppression. However, disseminated candidiasis in neonate was seen in a normal term infant of a mother who received corticosteroid therapy for ulcerative colitis throughout the pregnancy. Also, prolonged broad spectrum antibiotic therapy in late pregnancy also predisposes the neonate to sever candidal infection (Charles, 1980).

Systemic manifestation of candidiasis may follow introduction of candida into the blood stream. Candidemia may result from contamination of indwelling catheters, surgical procedures, trauma to skin or gastrointestinal tract, or aspiration. The extent and severity of the infection that follows, is determined by the inoculum size, the virulence of the organism and most importantly, the host defenses. The scope of systemic candidiasis is protean. Clinical indications of occult systemic candidiasis include candiduria (in the absence of catheterization and an imbalanced flora), candida endophthalmitis and maculonodular skin lesions. Although Candida albicans is the most common agent of candidiasis, C. guilliermondi, C. parapsilosis and C. tropicalis are frequent causes of endocarditis, overall C. tropicalis

is second to C. albicans in pathogenetic potential (Zinsser, 1984). In addition to Candida albicans, Candida tropicalis, Candida stellatoidea, and Candida pseudotropicalis also may cause vaginitis (Current, 1983).

* Prognosis :

As candidiasis is primarily an opportunistic infection, prognosis depends almost entirely on the type and severity of the predisposing conditions or diseases. Oral thrush in the newborn healthy child may clear uneventfully, but other forms of candida infection are much more difficult to treat and usually do not clear spontaneously. Control of cutaneous candidiasis in the diabetic depends on proper hygiene and regulation of the diabetes. In candidiasis associated with macerating conditions, prolonged exposure to moisture, and so forth, elimination of these factors will cause resolution of the disease even without treatment. Chronic disease in the constitutionally inadequate patient can be controlled with therapy, but the condition will return with cessation of therapy (Horsmanheimo et al., 1979).

In advanced systemic diseases, candidiasis is usually a terminal event which contributes to the ultimate demise of the patient (Rippon, 1982).

* Laboratory diagnosis of Candidiasis :

1) Direct microscopical examination :

Scrapings from cutaneous and muco-cutaneous lesions can be examined directly either in potassium hydroxide slide mounts or by Gram stain. The rather characteristic mixture of yeast and mycelial phase organisms permits a rapid diagnosis of such infections. Sputum, vaginal discharge, urine specimens, and fecal samples present more difficulty. The mere findings of yeast in such material is of no diagnostic importance, however mycelial form organisms usually cannot an established colonization of the involved area. Only fresh specimens should be examined in this manner, as candida multiplies rapidly in such milieu and often converts to a mycelial form in time. This would then give an erroneous impression to the number of organisms present and suggest the possibility of established colonization (Rippon, 1982).

In cases of iatrogenic septicemia especially, the organism can be seen in blood stream, Either Wright's stain or Giemsa stain can be used to demonstrate the organism (Anderson and Yardley, 1972).

Jeffcoate (1975) pointed out that the use of wet mount preparation to detect candida is considerably less accurate than cultural diagnosis.

Burrow and Ferris (1977), reported that wet mount preparation are examined with 10-20% solution of KOH which causes lysis of other cellular elements if present and thus aids in visualization of blastospores and pseudomycelia.

Joklik et al., (1984) pointed out that, the appearance of Candida albicans in fresh preparations as pseudohyphae or true hyphae along with budding cells of yeast is pathognomonic.

The use of methylene blue stain is also useful specially the Loffler's preparation of this dye:

Saturated solution of M.B. in alcohol ..... 300 ml
KOH, 0.01 percent in water .....1000 ml
(Kruickshank et al., 1975)
M.B. = Methylene blue

Timonen et al., (1960) stated that the morphology of fungi in vaginal smears stained by Papanicolou's stain (The specimen is spread on a glass slide and fixed with alcohol and stained by Papanicolou's stain with 24 hours and examined for any abnormal cells and for the presence of candida cells, which appears as bluish rods and the spore's membrane take up no stain, but it is readily distinguished from the environment by its strong refraction.) (Eddie et al., 1968) is very serotyped and either bands or balls are seen. As a rule, Candida albicans

forms pseudomycelia presenting as bands, but the absence of these,does not rule out the presence of this species.

Kruickshank et al., (1975) stated that, the ink solution stained smear can be used for the selective staining of the agent particularly if candida is being sought.

2. Culture Method :

For culture it is imperative that only freshly obtained specimens be examined.

The aim of the culture is to determine whether the isolated yeasts is candida or not, but it does not show to which species it belongs. Colonies of candida are identified by the colonial morphology and by microscopic examination. At room temperature, the organisms grow rapidly and will give a false impression of initial numbers present. As confirmation of the diagnosis of candidiasis often depends on quantitation of yeasts, this precaution is extremely important. Because of the inherent errors in culture methods, multiple specimens should be examined. Candida will grow on almost all common laboratory media, although for direct isolation sabouraud's agar with antibacterial antibiotics is recommended. Most species of candida are unaffected by the cycloheximide used in selective media for pathogenic fungi but some strains of C. tropicalis, C. krusei,

C. parapsilosis are sensitive to it. Optimal growth of all species occurs at room tempeature. A pasty yeast-like colony appears by 24 to 48 hours (Rippon, 1982).

As C. albicans is the most important yeast in human disease, many procedures have been proposed for its rapid identification, as the germ tube production test which is mentioned before (Reynolds et al., 1956). Furrowing inoculum into cornmeal agar with or without Tween 80 (Rippon, 1982).

3. Chlamydospore formation method :

This procedure is a diagnostic criterion of outstanding specifity and adequate in itself for the diagnosis of Candida albicans. On media of low nutritive value (e.g. corn meal) C. albicans produces pseudomycelium within 24-96 hours. The appearance of pseudohyphae and the disposition of the blastospores may be diagnostic, but greater importance should be attached to the specialized, rounded, thick walled, refractile chlamydospores generally 9-18 µ in diameter. They are differentiated from vegatative budding cells by their size, shape, refractility and thick wall. They are born singly or in pairs or in short chains on short pseudohyphal branches. It is advised by some authors to incubate the inoculated plates in the darkness as chlamydospore production is inhibited by adequate irradiation of light. This inhibition is explained by modulation of protoporphyrin activity (Andrieu et al., 1977).

## 4. Carbohydrate assimilation and fermentation :

The patterns of carbohydrate fermentation and assimilation are summarised in table ( 1 ).

a) Carbohydrate utilization (Assimilation): one of the most widely used methods for the definitive identification of clinically important yeasts and yeast-like organism. Most laboratories are prepared to perform these tests; however, little standardization of the method exists. Generally, all methods utilizing a basal medium (yeast-nitrogen base) which supports the growth of yeasts when an appropriate carbohydrate substrate is added. Many methods have been developed for detecting carbohydrate utilizing carbohydrate-impregnated disks (Roberts, 1976), or carbohydrate nutrient-impregnated disks (Huppert, 1975); agar slant utilization methods involving individual carbohydrate sources contained within yeast. Nitrogen base agar slants (Adams, 1974) and both tube methods containing individual carbohydrate sources within yeast nitrogen base broth (Wickerham, 1957). Numerous commercially prepared systems which contains carbohydrate utilization tests are available for yeast identification (Bowman, 1976 and Buesching, 1979) and are recommended for most laboratories ( Evans et al., 1986).

b) Carbohydrate fermentation tests are useful to supplement carbohydrate utilization tests results when there is difficulty in making the definitive identification of an organism. Fermentation tests are less reliable when used alone and are most commonly used as supplementary tests. Fermentation media contain peptone, beef or yeast extract, an indicator (Bromcresol purple), and individual carbohydrate sources, fermentation is detected by production of gas only, acid production (carbohydrate utilization), as indicated by a change in colour of the indicator, is not an indication of fermentation. Most fermentation tests require an extended incubation period of 6 to 10 days before final results can be reported (Evans et al., 1986).

5. Animal pathogenicity test :

Candida albicans is lethal to mice and rabbits when injected intravenously, causing miliary abscesses in the kidneys and sometimes in the spleen or liver. Injection of 0.2 to 0.8 ml of 1% suspension of C. albicans cells (calculated by volume of packed cells) in physiological salt solution into the marginal ear vein of 1- to 2- kg rabbit or of a similar volume of 0.2% suspension into the tail vein of a 20 to 30 gm mouse, causes death within 1 week (usually in 3 to 5 days), other species of candida may also produce lesions but they are seldom lethal to experimental animals ( Conant et al., 1971).

## 6. Immunological diagnosis :

### A- Agglutination :

Antibodies in human serum which cause specific agglutination of yeast cells were first detected in the early of this century (Winner and Hurley, 1964).

In practice, yeast cells are cultivated, washed, and killed by formalin or heat. Dilutions of test serum are mixed with a constant volume and concentration of yeast cells using rigid, molded plastic trays with wells, glass tubes or microscopic slides and allowed to react for a predetermined time. Agglutination is read against dark-field illumination.

Agglutination tests are almost always directed toward the detection of antibodies to C. albicans. Since this species accounts for an overwhelming majority of deep-seated candida infections. Different species of candida, can be shown to carry different antigenic determinants on their cell surface (Murray and Buckley, 1966).

The range of agglutinin titers to C. albicans obtained by different investigators is very wide. Agglutination will occur only within a defined range of antigen (cells) and antibody concentrations. The sensitivities of different agglutination procedures is dependent to

a large extent on the concentration of cells used in the test suspension. In the agglutination system in use at the author's laboratory, the majority of sera are negative at the initial serum dilution (1:4). Titers of 1:16 or greater are uncommon. In contrast, other systems, using fewer cells in the test suspension, have raised thresholds of possible significance (Tsuchiya et al., 1961).

Everett et al., (1975) for example, regard "high" levels of agglutinins to be > 1:640. In view of the widespread occurrence in humans of antibodies that react to C. albicans, sera from subjects with no evidence of current or previous infection with candida are frequently found to contain Candida agglutinins.

In a literature analysis made by Odds, (1979) the percentages of sera from healthy subjects containing Candida agglutinins ranged from 0 to 96%. IgG, IgA and IgM immunoglobulin classes have all been shown to agglutinate C. albicans blastoconidia (Lehner et al., 1972). In common with all other serological tests,inclusion of a control serum with a known titer is mandatory.

B- Inert particle agglutination :

a- Hemagglutination :

Erythrocytes coated with Candida antigens have commonly been used as a convenient means for detecting

and measuring antibodies. The antigen absorbed onto the surface of suitable and suitably prepared red cells is normally polysaccharide rather than protein. The test is sensitive, and titers up to 1:80 are often present in healthy subjects. Unlike the agglutination test using intact yeast cells, the immunoglobulin class involved appears to be primarily IgM Muller, (1974). Also, Muller has suggested that elevated levels of IgM antibodies to C. albicans are found exclusively or principally in sera of healthy subjects or in the early stages of systemic candidiasis. In later stages of infection IgG antibody classes predominate. For this reason, an indirect hemagglutination (IHA) test using polysaccharide-coated sheep erythrocytes has been made available commercially for the serodiagnosis of deep-seated candida infections. The dominant role for IgM antibody in IHA reactions was confirmed by Koñiger and Adam, (1973), their studies revealed, however, that IgG antibody also had hemagglutinating properties. Indirect hemagglutination is more rapid to perform than agglutination of intact yeast cells and the end point may be easier to read. A practical difficulty with this test is the apparent instability of anti-candida IgM antibody when maintained in the laboratory.

Muller, (1974) reported that 14 of 20 sera showed reduction in IHA titers of three or more doubling dilutions within 2 weeks of storage. If confirmed, the IHA test may have value only in the laboratory examination of freshly drawn sera. A haemagglutination inhibition assay to detect the surface antigen mannan is an early and specific signal of invasive disease (Winer and Yount, 1976).

Warren et al., (1978) advised the use of enzyme immunoassay as a diagnostic test for invasive candidiasis.

The rapid identification of candida species is a common problem in routine laboratories (Harley and Winner, 1964).

One of the most important methods of rapid identification is the:

b- Latex agglutination test :

First described in 1972 by Stickle et al. This procedure uses polystyrene latex particles coated with a soluble antigenic extract prepared by disintegration of formalin-killed blastoconidia of C. albicans. The resultant suspension of sensitized particles behave in a comparable manner to a suspension of intact yeast cells.

In one collaborative study by Merz et al., (1977), the suspension was shown to be more sensitive than an agglutination test using yeast cells (89% compared with 64%). In the slide latex agglutination test used at the CDC (Plamer et al., 1977), sensitized latex particles may be stored without loss of reactivity for up to 3 months at 4°C.

Hopwood et al., (1985) found out that the new slide latex particle agglutination test gave better results with 100% specificity and diagnostic efficiency of 95%. From the results of this preliminary study, slide latex particle agglutination looks a promising, rapid alternative to conventional laboratory methods for confirming a clinical diagnosis of vaginal candidosis.

Khan and Jones, (1986) have studied the utility of two latex agglutination tests in detecting antigenemia in patients with invasive candidiasis. To perform one test, they treated sera with protease and heat to free mannan from antibodies to mannan. Latex beads coated with antibodies to mannan detected mannan in supernatants. In the second test, untreated sera were tested for capacity to agglutinate a commercially available preparation of latex beads coated with antibody to Candida. They have found that, while latex agglutination tests for circulating candida antigens may be useful for diagnosing

invasive candidiasis, the transient nature of antigenemia requires frequent testing of patient's sera and limits the usefulness of the tests in diagnosing invasive candidiasis.

A rapid technique for the immunological identification of group B streptococci in vaginal swabs is reported. The investigators concluded that nitrous acid extraction of vaginal broth cultures followed by latex agglutination testing can significantly shorten the time needed to detect group B streptococci, resulting in the intrapartum detection of these organisms (Teti et al., 1985).

A new reverse passive latex agglutination test for the detection of serum antigen in systemic Candida albicans infection is reported, 1700 sera were examined from 91 patients who had either proven or suspected systemic candidosis, 183 patients who were colonized and 636 patients with no evidence of candidal infection. Thirty of the systemically infected patients had lymphoproliferative disorders and the rest a variety of surgical or medical diseases with no underlying neutropenia. The latex particles were sensitised with an antiserum raised in rabbits against a pressate of Candida albicans. The degree of antigenaemia was proportional to the likelihood of invasive disease such that a diagnostic cut-off point

of 1 in 8 produced a test for systemic candidosis with a sensitivity of 90% and specificity of 80.4% in patients with lymphoproliferatie disorders. In the remaining medical and surgical patients a diagnostic cut-off point of 1 in 10 produced a test with a sensitivity of 96.7% and specificity of 98.8%. The patients with lymphoproliferative disorders tended to produce lower serum antigen levels. The sera were also assayed for antibody using latex particles sensitised with pressate (Burnie, 1985).

Latex agglutination tests for Candida antigen in treated serum may prove to be a useful procedure for the rapid diagnosis of severe disseminated candidiasis (Bailey et al., 1985).

In all patients who recovered after antifungal therapy antigen levels returned to within the range found in normal controls. This is suggested by Gentry et al., (1983) in their study for detection of a circulating antigen in patients with systemic infection due to Candida albicans, Candida tropicalis and Candida parapsilosis by using a latex agglutination test.

Gentry et al., (1983) reported that, the latex agglutination test is quantitative and appears to have prognostic value.

## The methods of preparing antigens from C. albicans

1- Cultures :

The selection of cultures for antigen production is important. Candida albicans shares many antigens with other yeasts (Tsuchiya et al., 1974), but at least two serotypes among strains of C. albicans, types A and B, can be differentiated by agglutination and absorption techniques (Hansenclever and Mitchell, 1961).

Although reciprocal reactions occur with the two serotypes, type A appears to be the stronger and more complex immunogen. Antibodies to type A strains recognize all the antigenic determinants of type B strains, but the converse is not true. Hence, if a single-strain antigen preparation is to be produced, a type A isolate should be used. Whole cells have served as the source of antigens with few exceptions (Fischer et al., 1978 and Pepys et al., 1968).

Consequently, most investigators culture C. albicans on complex organic media with subsequent washings of harvested cells. The question of whether yeast cells, pseudohyphae, or hyphae are more significant in pathogenesis is not settled, but these morphological forms differ immunologically to some extent and this can

influence the results of immunological studies (Evans et al., 1973 and Ho et al., 1979).

Land et al., (1975) devised a glucose biotin phosphate buffer medium (pH 7.2) in which addition of proline favors filamentous growth and substitution of ammonium chloride for proline induces yeast cell formation.

2- Crude antigens :

Whole cells are used for both inducing (Hansen-clever / Mitchell, 1964 and Sweet / Kaufman, 1970) and eliciting (Gordon, 1975) immunological responses.

Immunological assays with whole cells presumably involve cell surface antigens, and some of these occur in other microorganisms. The ubiquity of C. albicans and other yeasts among humans and the widespread distribution of common antigens, even to bacteria (Kabat and Mayer, 1961), make it imperative that interpretation of positive reactions with whole cells be corroborated by clinical, pathological and cultural studies. In contrast to the qualified value of whole cells as serological aids for diagnosis, such preparations are useful for the identification and immunological typing of isolates.

Hasenclever and Mitchell, (1961) defined two antigenic groups among isolates of C. albicans by agglutination, demonstrating that serotype A is closely related

to C. tropicalis and serotype B to C. stellatoidea (Hasenclever et al., 1961).

Gordon et al., (1967) differentiated each species in these two pairs,and also the antigenically related Torulopsis globrata, with fluoresceinlabeled anti-C. albicans A and anti-T. glabrata immunoglobulins, the former absorbed with C. stellatoidea and the latter with C. albicans A. Tsuchiya et al., (1974) developed a kauffman-white type of system for identifying 88 species among 12 genera of yeasts based on heat-stable or heat-labile antigens differentiated by agglutination and reciprocal absorption of agglutinins. It is apparent that the yeast cell surface contains multiple antigens, some of which are distributed widely.

Solubilized antigens generally are obtained from cells rather than culture broths, although several reports indicate the presence of potentially significant antigens in culture filtrates (Pepys et al., 1968 and Staib et al., 1977).

Cells have been disrupted by sonic energy (Biquet et al., 1959; Lehmann/Reiss, 1978 and Stallybrass, 1964). pressure extrusion (Frisk et al., 1974; Negron; 1969 and Rogers / Balish, 1978), freeze-thaw cycles (Everett et al., 1975), shaking with glass beads (Hommel et al., 1976), grinding with sand (Laskownicka et al., 1969),

heating cells (Fukazawa et al., 1977), and by lysing protoplasts (Venezia / Robertson, 1974).

Fluids for extracting antigens include distilled water (Everett et al., 1975), physiological concentration of salts (Tran Van Ky et al., 1969), and hypertonic salt solution (Marconi et al., 1977).

3- Refined antigens :

Isolation of purified antigens has focused primarily on the cell wall mannans and peptidomannans. Several reasons provided the impetus for these studies. Taschdjian et al., (1964), based on their own studies and those of others (Biguet et al., 1962), postulated that antibody formation against cytoplasmic (somatic) antigens of C. albicans is induced by lysis of fungous cells in the infected host. Studies by other investigators have established that precipitins are found in sera from most persons without evident infection but that these are primarily reactions with cell wall mannan antigens (Chew / Theus, 1967). Hence, precipitins to purified cell wall mannan antigens need to be distinguished from antibodies to cytoplasmic antigens.

Second, the role of mannans or peptidomannans in the structure and plasticity of yeast cell walls (Gander, 1974) is of particular interest because of the question of whether hyphal or yeast forms are invasive.

Third, the mannans appear to be the principal cell wall antigens involved as antigenic determinants of C. albicans serotypes A and B and as the molecular components responsbile for cross-reactions with other species (Ballou, 1970).

Finally, mannan antigenemia is an early event of invasive candidiasis in experimental candidiasis (Poor / Cutler, 1979) and humans (Segal et al., 1979) and a potentially useful aid for early diagnosis.

Most investigators prepare mannans by the method of Peat et al., (1961) to avoid the degradation of the oligosaccharide that occured with the earlier methods of extraction with hot alkali.

Peat et al. method :

Yeasts, suspended in a neutral citrate buffer, are autoclaved, the extracted soluble materials concentrated under reduced pressure, and acetic acid added to normality. The resulting gelatinous material is removed by centrifugation, the solution neutralized with NaOH, and crude mannan precipitated with ethanol. After dissolving in water, addition of Fehling's solution separates mannan as an insoluble copper complex from glycogen.The precipitated mannan salt is suspended in water and solubilized with minimal amounts of concentrated HCl. Successive precipitations with alcohol in the presence of acetic

acid yield mannan with a trace of glucose, probably as a contaminant from cell wall glucan because acid hydrolisis produces no evidence that glucose is combined with mannose in the polysaccharide. This product can be separated into neutral and acidic mannans by treatment with cetavlon (Westphal / Kaben, 1977) or by chromatography on DEAE-Sephadex (acetate form), eluting first with water to obtain neutral mannan followed by gradient or stepwise elution up to 1 M NaCl, which yields the acidic mannan (Okubo et al., 1979). Both fractions contain mannose as the only sugar component and the neutral fraction is homogeneous by ultracentrifugation and moving boundary electrophoresis. The neutral mannan contains no phosphorus and insignificant amounts of nitrogen, but both phosphorus and nitrogen are present in the acidic mannan, a peptidomannan.

Although the structure of the mannans from C. albicans types A and B are essentially the same, type A mannan contains a higher proportion of long oligomers, averaging 5.0 mannopyranosyl units in chain length, compared to type B mannan, with an average of 3.3 residues (Sunayama, 1970).

Sunayama and Suzuki, (1970) postulated that the greater density of long-chain oligomers in type A mannan explained the fact that antibody to this mannan differentiated between cells of serotypes A and B whereas antibody

to type B mannan did not the greater size of the combining site on anti-type A mannan would accommodate all the antigenic determinants of both mannans and differentiate between them. In contrast, the smaller antibody combining site for type B mannan would accommodate only determinants consistent with those of type B mannan and would not combine with that portion containing the greater density of larger oligomers in type A mannan.

Although crude extracts from C. albicans contain many antigens (Axelsen, 1971), the proteinaceous components and the acidic peptidomannans have not been studied as intensively as the polysaccharides. Fractions enriched in protein composition are usually obtained from cells or cell walls by alkaline extraction (Domer / Moser, 1978) and from culture filtrates or cytosols by precipitation with ammonium sulfate (Ellsworth et al., 1977). In almost all of these reports, tests for homogeneity were not done and immunological assays revealed multiple components. The acid peptidomannan fractions eluted with NaCl from DEAE-Sephadex (acetate form) contain phosphorus and peptides.

Glycopeptides have been isolated from defatted cell walls by Reiss et al., (1974). Two soluble fractions were obtained, the first by extraction with cold 0.5 NNaOH and the second by sonicating the cell wall residue

suspended in the cold dilute alkali. The first fraction contained glucose and mannose in a ratio of 2:3, plus 1.47% nitrogen and 0.05% phosphorus (i.e., a peptidoglucomannan). The second fraction contained glucose and mannose in a ratio of 6:1, plus 0.94% nitrogen but no phosphorus. This fraction was considered to be a mannoglucan probably with a peptide moiety.

Nickerson (1963) pointed out the difficulty of separating peptide residues proximal to a polysaccharide moiety, and Phaff, (1971) agreed with Bishop et al., (1960) that criteria for homogeneity of the "glucomannan-protein complexes" have been insufficient.

C- Complement fixation test :

Complement fixing antibodies are also found in normal human sera, so they are of no value in the diagnosis.

D- Precipitation test :

Precipitating antibodies are found in patients with systemic candidiasis (Akiba et al., 1961; Taschdjian et al., 1964). They are not present in sera of healthy individuals or patients without disseminated forms of the disease or patients with superficial or subclinical infections. Cell free dialysed and concentrated culture

filtrates of C. albicans were used as antigens for the detection of precipitating antibodies in the serum of patients with Candida by an immunodiffusion test (Staib et al., 1977). As everyone is exposed to candida, serologic tests are limited to discriminating between normal and disease levels of antibodies. More specific tests for specific antigen are currently under development. Both Candida surface mannan and cytoplasmic proteins can be detected in sera by enzyme-linked immunosorbent assay, radioimmunoassay, or quantitative immunofluorescence. Mannan and arabinitol, a Candida metabolite, can also be measured in sera by gas-liquid chromatographic methods. The detection of circulating Candida antigens or metabolites appears to be diagnostic for systemic candidiasis (Joklik et al., 1984).

7-<u>Candida skin test</u> :

Is administered by intradermally injecting 0.1ml. of an appropriate dilution of antigen into the volar surface of the forearm. In sensitized persons, an area of induration and erythema develops at the injection site. An induration 5 mm or more in diameter is considered a positive reaction. The skin test is most useful in defining endemic areas for a disease. It has limited value as a diagnosis tool (Lewis et al., 1937). Delayed type skin test reactions are frequently negative in patients with chronic mucocutaneous candidiasis (Dexer, H. & Lois, H. 1983).

Treatment of vulvo-vaginitis :

There are many preparations available for the treatment of vulvovaginitis, such as creams, lotions, pessaries and foaming pessaries. The two major groups of pharmaceutical now in vogue are the inidazoles and the polyenes. Four polyenes-pimaricin, candicidin, amphotericin B, and nystatin have been used topically, nystatin being the most popular. Most recent studies, however, indicate the inidazoles give a higher cure rates than polyenes, clotrimazole, microazole, ketoconazole, and econazole have all had clinical trials (Dennerstein, 1979). Resistant infections and reinfections (Howat et al., 1979) are common lactobacilli have been suggested in recalcitrant disease (Sandler, 1979).

Although ketoconazole relieved symptoms and signs seven days after therapy, a high recurrence rate occurred by 28 days after therapy (Eschenbach et al., 1985).

Terconazole, a new triazle ketal is found to be highly active in vitro on a wide range of yeasts and mycelium-forming fungi. The in vitro activity depends largely on the medium used. In vitro it is a potent antifungal agent in preventing the morphogenetic transformation of the yeast into the (pseudo-) mycelium form of candida albicans. In vivo terconazole is highly active in topical treatment of various experimental models of

dermatophytosis and candidosis. It also possesses moderate oral broad spectrum activity. No side effects were observed (Van-Custem et al., 1983).

Butoconazole is a new imidazole, effective as therapy for vulvo vaginal candidiasis with no systemic side effects (Adamson et al., 1986).

# MATERIAL AND METHODS

## MATERIALS AND METHODS

### A) Materials :

* Case material :

This study was carried on 77 women, attending the outpatient clinics of the Department of Obstetrics and Gynaecology of Benha University Hospital. The age ranged from 16-45 years. The period of study extended over 4 months. All women were subjected to :

1- A full history.

2- Complete general, abdominal examination.

3- A full gynaecological examination in which the presence of vulvovaginitis and/or cervicitis was noted with the characteristics of any discharge or other symptoms.

4- Screening for diabetes mellitus was carried out by blood glucose tolerance test.

5- The patient were asked about different symptoms and if they are taking contraceptive pills or other drugs.

6- Recording of vaginal pH was done by a full range nitrazine paper (pH 1-14).

Accordingly, the studied cases were divided into different categories as follows :

a- Women with leucorrhoea (suspecting monitial vaginitis):

These were 19 cases, they complain of intensive pruritis, with moderate external discharge. The discharge is characteristically thick and nonoffensive, the vaginal wall is diffusely red and tender. The discharge appears as thick curdy white patches, slightly adherent to the vaginal wall.

b- Pregnant women :

These were 6 in the first trimester, 4 in the second trimester and 8 in the third trimester of pregnancy.

c- Diabetic women :

These were 8 proved to be diabetic by glucose testing in blood. Two were seeking advice for contraceptive method, and have no apparently gynaecological problem. The other 6 were complaining of symptoms suggesting vaginal candidiasis.

d- Women taking contraceptive pills :

These were 13. (5) of them with no clinical complaint, and the other 8 complaining of pruritis and discharge.

e- Healthy women as control :

These were 15 cases. None of them had any symptoms or signs relevant to vaginal infections. By examination,

a normal whitish, creamy vaginal content and normal mucus membrane of the vagina. Most of them were asked for contraceptive method.

f- Women with prolonged antibiotic therapy or corticosteroid therapy :

These were 4 cases complaining of symptoms suggesting vaginal candidosis.

* Media used :

1- Sabouraud's glucose agar with chloramphenicol : (Rippon, 1982).

| | |
|---|---|
| Glucose | 40 g |
| Peptone | 10 g |
| Agar | 20 g |
| Distilled water | 1000 ml |
| Chloramphenicol | 40 gm. |

The ingredients were dissolved by heat, and pH was adjusted to 5.6. Then autoclaved at 121°C for 15 minutes. Dispensed in the previously sterilized plates.

N.B. : Chloramphenicol was added by a sterile syringe, to inhibit nocardia, other actinomycetes and the growth of contaminating bacteria.

2- Corn meal agar (Benham, 1931) :

| | |
|---|---|
| Corn meal agar (oxoid) | 17 gm |
| Distilled water | 1000 ml |

The corn meal agar was dissolved in distilled water completely by heating, then sterilized by autoclaving at 121°C for 15 minutes and poured in sterilized petri dishes. This media was used for the detection of chlamydospores production by candida albicans.

3- Sugar media (Cruickshank, 1975) :

Glucose, maltose, sucrose and lactose in 3% concentration in peptone water 1% together with the indicator.

Inverted Durham's tube was immersed in each tube containing medium. Tubes were sterilized at 100°C for 20 mins. on 3 successive days. These sugars were used to identify the biochemical reactions of candida species.

4- Medium for carbohydrates assimilation :

(modified yeast nitrogen base for carbon atom assimilation) Rippon (1982).

| | |
|---|---|
| Yeast nitrogen base (difco) | 0.67 gm. |
| Washed agar | 20 gm. |
| Distilled water | 1000 ml. |

These ingredients were dissolved, poured into tubes 20 ml, each closed by cotton pool and autoclaved at 121°C for 10 minutes and stored at +4°C until used.

5- Latex agglutination-Candida antigen detection system*:

CAT./LC1001 (60 tests)

Latex agglutination system for the detection of the mannan antigens of candida albicans.

Reagents :

(All reagents are intended for in vitro diagnostic use only) :

1- Glycine buffered diluent with albumin (10X, GBDA) (10 ml) :
Concentrated glycin buffered saline (pH 8.6) containing bovine serum albumin, and sodium azide. Diluted 1:10 before use (Cat/GB0000).

2- Anti-Candida globulin Reagent (ACGR, 4 ml):
Contains standardized latex particles sensitized with rabbit anti-candida globulin (0.1% sodium azide, cat./LG0000).

3- Low Candida Mannan Antigen Control (LCAC, 2 ml) :
Contains freeze dried C. albicans mannan antigens isolated from cultures (0.1% sodium azide, Cat./Cd0000)

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From : * *(Alpha Laboratories Ltd. 40 parham Drive, Eastleigh, Hampshire 505 4NU, United Kingdom).*

Reconstituted by pipetting 1.9 ml of distilled water into the vial.

4- Detacher enzyme (DE, 2X1.5 ml) :

Contains a freeze dried preparation of proteolytic enzymes (0.005% sodium azide, stored at 4°C, Cat./ DE0000).

Reconstituted with 1.5 ml of distilled water, aliquoted into 12X75 mm tubes in 0.05 ml (50 ul) amounts and storedfrozen at -20°C or colder.

5- Negative Control (NC, 2 ml) :

Consist of normal goat serum (0.1% sodium azide, Cat./NB0000).

Reconstituted by adding 1.9 ml of distilled water into the vial (NOTE: when used this reagent for the first time, inactivated by heat at 56°C for 30 mins.).

6- Capillary tubes and Rubber Bulb :

Contains 100 capillaries and one bulb Cat./CQ0000).

7- Test Card :

The card was , removed from the kit during refrigeration storage to prevent absorption of moisture. (Cat./SC0000).

8- Enzyme Inhibitor: (EI, 7 ml).

Contains an inhibitor for the DE which shows enzyme activity and prevents digestion of the immunoglobulins of the latex particles.

Stability and Storage :

All reagents were preserved with 0.1% sodium azide, contamination and prolonged periods at room temperature were avoided after rehydration. All reagents (except DE after rehydration) were stored at 4-8°C (cards were removed and stored at room temp.). Freezing of latex suspensions were avoided. The detacher enzyme was stored at -20°C or colder after rehydration, it is stable for at least 3 months under these conditions.

Prior to rehydration, all reagents were stabled for at least one year after receipt and it continue to be used for as long as the controls (LCAC, and NC) continue to react properly.

B) Methods :

* Sampling :

Two sterile cotton tipped vaginal swabs were used for each case. The specimen was obtained from the posterior fornix of the vagina with the aid of sterile Cusco's speculum. Each swab was labelled with the name and number of case. 3rd sample was taken from the posterior fronix by dry wooden tongue depressor.

Certain precautions were taken into consideration:

1- The patient should not have any local vaginal medications prior to smear taking.

2- The patient should have refrained from intercourse or vaginal douching for at least 48 hours before examination.

3- The smear were taken before pelvic examination, and no lubricants were employed on any of the instruments used to obtain the specimen, since they interfere with staining reactions.

4- The glass slide and other instruments used in collecting the specimens were dry and clean.

5- The samples were spread immediately over the slides in a film, and in one direction by the use of the edge of another glass slide, or by the swab itself.

* Identification of the yeast :

A) Microscopic examination :

1- Wet smear examination:

The sample was taken from the posterior fornix by a dry wooden tongue depressor. The specimen spread on glass slide and a drop of 10% potassium hydroxide (10% wt/vol.) was put, and the slide was examined microscopically for the presence of yeast cells and mycelium or pseudomycelium.

2- Methylene blue stained smear :

One of the 2 swabes was spread on a glass slide and air dried. Few drops of aqueous methylene blue-solution

(1 in 4,000) were then added for one minute and washed gently by water, the slide was then examined microscopically under an oil immersion lens, for the presence of candida spores and hyphae which were lightly stained by the methylene blue. Of many preparations of this dye, Loffler's methylene blue is generally the most useful:

| | |
|---|---|
| Saturated slution of M.B. in alcohol | 300 ml |
| KOH,0.01 percent in water | 1000 ml |

**(Kruickshank et al., 1975)**

(M. = Methylene)

(B. = Blue )

B) Culture :

* The swab was cultured without delay on a plate of sabouraud glucose agar, containing chloramphenicol in petri dishes and incubated for 24-48 hours at 37°C. Cultures examined at 24 and 48 hours.

N.B. :

cycloheximide resistance :

Organisms were cultured on Saboraud's agar with cycloheximid (actidion) since some strains of yeast are characteristically inhibited in the presence of this drug, while other are resistant (Table 1).

Any yeasts that developed were identified as following :

a- Gross colonial appearance :

Colonies which were relatively small, reaching 2-3 mm in 24-48 hours, creamy, raised, moist and has yeasty odour, would be suspected to be candida.

b- Microscopic examination :

A small portion of the suspected colony was taken by sterile loop, spread and fixed on a clean slide. The film was stained by methylene blue and Gram's stains and examined microscopically.

c- Germ tube formation :

Part of the suspected colony was lightly inoculated into 0.5 ml of pooled human serum and incubated for 2 hours at 37°C (Haley, 1971). After that 1-2 drops were placed on a clean glass slide and mounted with a cover slipe and examined for germ tubes which are a true lateral hyphae extension of the yeast cell with no constriction at the base (Conant et al., 1971).

This is a screening test for the yeast candida albicans (Taschdjian et al., 1960).

* Culture on corn meal agar :

With a sterile needle, small amount of the yeast colony was removed by the needle tip and streak across the surface of the agar in parallel lines 1 cm apart, then circum-crossed several lines. A flammed cover slipe, is placed over the surface. Plates were incubated at room temperature 18-48 hours, then examined with the low power objective of microscope. The plate was examined after removing the lid of the dish (Moore and Jaciow, 1979). The site of inoculation was examined for presence of mycelium, pseudomycelium, large spherical thick walled chlamydospores at the tips of most branches, and clusters of smaller and oval thin walled blastospore at the junction of the filamentous cells.

C) The biochemical reactions :

* Sugar assimilation :

This was done by poor plate assimilation procedure. A tube of yeast nitrogen base medium was melted and allowed to cool to 47-55°C. In the same time a suspension of 20-72 hours old yeast culture in 3-4 ml sterile distilled water was prepared. Suspension was made dense enough to obscure Wicherham's lines, approximately $10^6$ colonies/ml. This suspension was poured into petri dish, and the yeast nitrogen medium prevously melted and cooled,

above them mixed and allowed to harden and to be dry. Traces of the sugars to be tested was inoculated at the periphery of the plates. Sugars used were glucose, lactose, maltose and sucrose. Plates were inoculated at room temperature for 48 hours and examined for the presence or absence of growth around the assimilated sugars, the size of the zone of growth is not significant (Rippon, 1982).

* Sugar fermentation :

Glucose, maltose, lactose and sucrose fermentation media were inoculated by 24 hours subcultured yeast cells. Incubated at room temperature for 24-48 hours and examined for acid production (red colour) and gas which collected in the tops of Durham's tubes.

Slide latex agglutination test :

Preparation of reagents :

An antiserum was raised in New Zealand white rabbits using a partially purified cell wall fraction (Reiss et al., 1974) of Candida albicans 3153, serotype A (ATCC NO 28367) as the immunogen. Purified immunoglobulins were obtained from this antiserum by caprylic acid precipitation (Steinbuch and Audran, 1969), followed by diethylaminoethyl sephadex chromatography. Immunoglobulins were lyophilised and stored under vacuum in sealed ampoules.

Extensive preliminary work was performed to determine the optimum concentration of immunoglobulins required to sensitize the latex particles so as to give maximum sensitivity compatible with stability. Polystyrene latex particles 0.8 µm diameter (Merrel-Dow Ltd, Hounslow, England) were adjusted to a 1% (vol/vol) suspension in distilled water. Ten millilitres of immunoglobulin, at a concentration of 100 µg/ml in glycine buffered saline, pH 8.2 (GBS), was added to 10 ml of latex suspension and the two were mixed vigorously for 2-3 mins. Ten millilitres of GBS containing 1% bovine serum albumin (GBS-BSA) was added and mixed thoroughly. The latex reagent was sonicated (12 µm amplitude) for 6 sec. to break up any aggregates and centrifuged (7000 g for 10 min). After washing twice with GBS-BSA (30 ml) the latex particles were resuspended in 10 ml of GBS-BSA and stored at 4°C. The sensitivity of the latex reagent for antigen detection was determined by reacting against C. albicans cell wall mannan (Peat et al., 1961) diluted in GBS, and its reactivity was checked every month to ensure that it had not deteriorated. The reagent used for the study could detect 500 ng/ml mannan in GBS.

Intended use :

The mannan antigens of C. albicans were produced in relatively high concentrations, both in culture and

in infected animals. The LA-Candida Antigen Test detects these antigens when present and is both qualitative (screening) and semi-quantitative (titration).

Explanation of the test :

Detection of candida antigens in specimens was first described in 1973 (Axelsen et al., 1973).

Using CIE "counter-immuno-electrophoresis" and immunodiffusion tests. Antigen and antibody detection have been shown to have value in distinguishing between invasive infection and colonization with C. albicans (Winer et al., 1976). The LA-Candida Test should be positive when the mannan antigens of C. albicans are present in specimens in concentrations of 5 ng/ml or greater. Previously, detection of these antigens was hampered by circulating antibodies binding the mannans in immune complexes which were not reactive in the test. An immune complex detaching enzyme (DE, a proteolytic enzyme) is now included in the system which has the dual property of clearing antibodies in immune complexes, which can mask the presence of antigens, and destroying rheumatoid factor (Stockman et al., 1969).

Destruction of the antibodies in the immune complexes makes the mannan antigen available for detection. Anti-coagulants [in cases of invasive candidiasis

using serum] such as nitrate or ethylenediaminetetra-acetic acid can cause interference with the test giving "false positive " reaction.

Principle of the test :

The LA-Candida Test is based upon the principle that latex particles sensitized with high titered purified globulin against candida mannan antigens will agglutinate with specimens containing the appropriate candida mannan antigens.

Test procedure :

* Specimen preparation : Swabs were agitated in 400 µl of GBS for 1-2 mins.

Then proceed as follow :

* For each specimen being tested, one tube containing 0.05 ml of Detacher enzyme (DE) was removed from the freezer (or a new vial was rehydrated).
* 0.3 ml of specimen was pipetted into the tube containing DE.
* Mixed well and the tube placed in 56°C water bath for 15 minutes.
* The tubes were removed from the bath and one (1) drop of enzyme inhibitor (EI) was added to each tube.

N.B. :

The specimen was tested immediately or stored at 4°C for up to one week. For longer storage, specimen was frozen at -20°C or colder.

Screening test (Qualitative) :

The prepared specimen was diluted 1:10 by adding 0.1 ml of specimen to 0.9 ml of GBDA (glycine buffered diluent albumin.).

* By using the test card and capillary tube.
* 2 drops ofundiluted specimen were placed onto a spot.
* 2 drops of diluted specimen were placed onto a second spot, by a new capillary tube (The capillary tube was discarded).
* One drop of ACGR was placed onto each spot and the capillary tube discarded.
* Separate segments of applicator stick were used, the contents on each spot mixed, covering the entire area.
* The card rocked by hand or placed on a rotator at 166 rpm for 5 mins.
* The reaction was read immediately and rate on a negative to 4+ positive scale.

Controls :

All controls were performed on the same day that specimens were tested, in order to determine the suitability of the reagents. The controls were run either before or simultaneously with the specimen test. However, if the control reading were not satisfactory, the test were invalid.

1- Capillary pipettes with the rubber bulb, were used held vertically for uniform drop size, 2 drops of the Low Candida Antigen Control (LCAC) were placed onto the indicated spot.

2- Step (1) was repeated with the Negative Control (NC). A separate capillary pipette for each reagent was used.

3- Mixed to resuspend the Anti-Candida Globulin Reagent (ACGR).

4- By a capillary pipette and bulb, one (1) drop of ACGR was placed into each of the spots.

5- Different applicator sticks were used for each spot.

* The contents ofthe 2 control spots were mixed thoroughly covering the entire spot.

6- The card was rocked by hand or placed on a rotator for 5 minutes to 10 minutes.

7- The reactions were read immediately to prevent drying.

Fig. (2): Colonies of Candida on Sabouraud's glucose agar.

Fig. (3): Microscopic appearance of Candida in Gram's stained film.

Fig. (4): Sugar assimilation by Candida albicans.

Fig. (5): Latex agglutination Candida antigen detection system.