INTRODUCTION AND AIM OF WORK

The genus candida belongs to a group of yeast-like fungi, naturally present in the mucous membranes in the respiratory, gastro-intestinal and female genital tract. Candida albicans, C.tropicalis, C.glabrata and parapsilosis are present as commensal organisms in the female genital tract, usually present during reproductive life and rarely found before puberty or after the menopause (Mead, 1974).

Candida albicans is the most common species pathogenic to humanbeing as compared to other types of Candida. Many of the predisposing conditions which favour the virulence of Candida albicans in the vagina are well known, the most important is pregnancy, diabetes mellitus, antibiotic therapy, steroid therapy and the use of oral contraceptive pills (Caterrall, 1971). Glucose content of the vaginal mucosa is a factor which promotes the growth of this fungi. There is a good correlation between the occureance of inflammatory reactions and the presence of Candida albicans which can be demonstrated in every case of severe vulvitis and vulvovaginitis specially during pregnancy (Dwyer, 1975).

Candida albicans, is capable of pathogenicity when host defense mechanisms become impaired (Dorothy and Warnook, 1977).

The infectivity of Candida albicans and most other fungi, however, is thought to be suppressed predominantly by cellualr immunity rather than the circulating antibodies (Valdimarsson et al., 1973). Recent evidence suggests that recurrence of vaginal Candidiasis can arise as a sequence of a transient and localized inhibition of cell-mediated immunnity (Witkin, 1987). So, the forecoming study is intended to evaluate the T-cell function in cases of recurrent vaginal candidiasis in pregnant and non pregnant women.

MICROBIOLOGY OF CANDIDA ALBICANS

Candida albicans is the commonest Candida species isolated from man, it accounts for 90% of all Candida species. Its presence as a commensal or pathogen depends on the conditions of its environment or substrate (Honeim et al., 1983)

Superficial infection of the mucous membrane and skin is the most common and important forms, but less serious than involvement of internal organs as endocardium and meninges or even blood provoking septicaemia which may also occur (Roberts and Mackenzie, 1979).

The condition of the skin is variable in different sites Candida are more commonly found in mucous membranes around orifices, on fingers and in moist intertriginous areas. The isolation of the yeast from any site is only of epidemiologic interest unless clinical state is present associated with the presence of mycelia obtained from the lesion and seen by direct smear (Arendorf and Walker, 1980).

Morphology:

Candida albicans is a small oval, unicellular yeast like fungus, measuring 2-4 micron in diameter, it appears as

gram positive, reproduce by budding, characterized by pseudomycelia formation and chlamydospore formation on special media (Lodder, 1970).

Candida albicans consists of a cell wall, cell membrane and cytoplasm. The cytoplasm contains endoplasmic reticulum, nucleus, nucleoli, storage granules, mitochondria and other organelles. The cell wall is a thick rigid structure. The polysaccharides present in the cell wall are chitin, glucan and mannan. The cell wall stain violet with gram stain (Anita et al., 1975).

The growth of Candida albicans on solid media is not different from other Candida species and appear as white creamy in colour, pasty, heaped, smooth or corrugated at the center, with a mycelial fringe in the depth of the agar along the edge of the colony as shown in Fig. (2). But on fluid media the growth is of little value in the identification of the species and produce different types of growth either as turbidity alone or turbidity with sediment, or turbidity with a surface pellicle (Lodder, 1970).

At temperature below 26 C in nutritionally poor media, candida produces thick walled, resting cell called

chlamydospores, which is large as 8 to 12 micron, and rounded. They are particularly adopted for maintaining viability during starvation and other adverse conditions. The large size is due to storage of reserve nuritional substances and the thick wall protects them from unfavourable environment (Rippon, 1982).

Germ Tube Production:

The most characteristic feature to differentiate Candida albicans from other Candida species is the germ tube formation test (Roberts and Mackenzie, 1979).

The germ tube test, based on the rapid formation of filamentous out growths by blastospores of Candida albicans when placed in human serum at 37 C, it is widely used to distinguish Candida albicans from other species of candida which do not form germ tubes (Joshi and Gavin, 1974).

Dolan and Ihrke (1971) observed the rapid formation of filaments by the cells of Candida albicans when this yeast was incubated in tubes containing either plasma, serum, CSF egg albumin or oleic acid albumin. These filaments were called "germ tubes" and are described as narrow filaments 1-15 microns wide and up to 20 microns long depending upon the strain, time and temperature of incubation.

The germ tubes are cylindrical and lacking any constriction at the point of origin from the mother cell, this character clearly distinguishes them from normal buds or pseudohyphae even at a very early stage of development. The optimum temperature for their development is between 37 C - 42 C and the time needed depends upon the strain tested, ranging from 2-4 hour (Joshi and Gavin, 1974).

Cultural Characteristics:

Candida albicans grows at both room temperature and incubator temperature (37 C) on sabouraud's dextrose agar medium. The colonies are of moderate size usually reach 0.5mm in diameter after 18 hours, smooth, pasty and have a specific yeast odour after 2 days the colonies become highly convex 1.5mm in diameter. The giant colonies may be honey coombed in the center and produce radial furrows (Cruickshank et al., 1973).

Biochemical Properties:

Candida albicans can ferment various sugars as glucose, galactose, sucrose and maltose and assimilate, glucose, galactose, sucrose and maltose (Rippon, 1982) (Table A).

Vaginal pH

The acid pH of the vagina, specially in adult women, makes the vagina resist infections. This is due to the glycogen content of the epithelium, which depends on ovarian endocrine activity, specially oestrogens. The normal pH of the vagina in the adult ranges between 4 and 4.5 due to the breakdown of glycogen by the action of Doderlein's bacilli, with production of lactic acid. This acidity prevents the growth of the essentially pathogenic bacteria like Streptococcus faecalis, and anaerobic streptococci, but fungi, can live in an acidic vagina (Peel and Patts, 1969).

The vaginal pH of the newly born ranges from 5.7-7 then after 24 hours of birth it becomes 4-5 due to the production of lactic acid by the action of Doderleins bacilli on the glycogen of the desquamated epithelial cells. At the 10th day of life, the Doderlein's bacillus is replaced by other organisms and the pH approaches neutrality. The neutral pH continues from the 10th day of life until puberty. The Doderlein's bacilli are flourished under the effect of endogenous oestrogens of the ovary. These steroids are responsible for glycogen formation and the appearance of Doderlein's bacilli and an acid pH (Sumner et al., 1971).

During pregnancy the vaginal epithelium is very rich in glycogen and so the vaginal acidity is marked. The growth of pathogenic organisms diminishes the glycogen content in the vagina which in turn inhibits the growth of Doderlein's bacilli that live on the glycogen and flourish in a low pH, which constitutes a vicious circle with progressive disturbance until nature's efforts at restoration are aided by appropriate treatment. It appears from these studies on the pH that there is a definite cyclic pattern, dependent on the ovarian function and accordingly the vaginal discharage tends to be alkaline in girls before puberty as well as in women after menopause reaching an average pH of 7.2 (Peeters et al., 1972).

The degree of vaginal acidity depends on the production of lactic acid from the glycogen in the vaginal epithelium by the action of Doderlein's bacillus. The organisms which produce lactic acid in the vagina lie in the genus acidophillus which is the most important vaginal inhabitant. This organism splits glycogen of the vaginal epithelium during female reproductive life and flourishes in a highly acidic medium. During reproductive life, the female oestrogens aid the maintenance of vaginal epithelial cells, which become filled with glycogen granules and huge number

of Doderlein's bacilli. After menopause, oestrogens are withdrawn and the epithelial cells of the vagina become atrophied due to drop in the oestrogen level in the blood, and Doderlein's bacilli are diminished and other micro-organisms become in abundance (Peel and Patts, 1969).

Louria (1965) stated that the normal vaginal flora consists of many commensals and some pathogenic organisms like:

* Staphyloccus albus.
* Diphtheroid bacilli.
* Non-haemolytic streptococci.
* Streptococcus viridans.
* Minute streptococci.
* Bacteroids and anaerobic streptococci.
* Haemolytic streptococci.
* Escherichia coli.
* Proteus vulgaris.
* Micrococci.
* Mycoplasma.
* Candida albicans.

Influence of Vaginal pH on Vaginal Flora: (Ribbon, 1982)

The fluid content of the vagina is derived from:

1- Mucous secretions of the cervical columnar cells.

2- Transudation through the vaginal walls.

3- Vulval secretions from sebaceous sweat glands.

4- Bartholin glands, which produce an "acid mucous secretion".

Vaginal acidity is thought to depend on the presence and 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 has 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.

Average Vaginal pH Values (Feo, 1958):

New born	:	5.7
Children	:	6 to 8
Puberty	:	4.0
Pregnancy	:	4.0
Menopause	:	7.0
Mean pH in the child bearing age	:	5.5 falling during ovulation.

Optimal Growth pH of Some Common Organisms (Rippon, 1982):

Trichomonas vaginalis.	4.9 to 7.5 3.5 to 4.7 5.8 to 6.4
Candida albicans.	5.4
N.gonorrhoeae.	7.5
B.proteus.	7.4
Streptococci.	7.4
Diphtheroids.	7.2

IMMUNOLOGY OF CANDIDA ALBICANS

Antigenic Structure of Candida Albicans:

Many investigators have found that Candida albicans possess several antigens as components of the cell wall and the cytoplasm (Axelsen, 1973). Candida albicans has a soluble polysaccharide which consists of galactomannan linked to a peptide. The mannan fraction is the major immunogen for immunoglobulin production. It is not serologically specific and can cross-react with other Candida species (Hasenclever and Mitchell, 1960).

Cytoplasmic antigens of Candida albicans are mainly protein in nature in contrast to the polysaccharide cell wall antigen (Buckley et al., 1970). It has been claimed that antibodies to cytoplasmic antigen occur only in systemic diseases (Merz et al., 1977).

Immunity to Candida Albicans:

Opportunistic fungi as Candida albicans are normally non-pathogenic in healthy human but may have a virulent effects in individuals with depressed immune function. This includes the lymphoreticular malignancy, immunosuppressive therapy, acquired immunodefeciency syndrome (AIDS),

prolonged use of broad-spectrum antibiotics, other clinical conditions as diabetes, or anaemias. Candida may establish a variety of localized acute or chronic types of infection. The pattern of disease can not be described merely as a function of the pathogen but rather must be viewed in total perspective with the host response (Joseph and Bellanti, 1978).

Immunity to Candida albicans in general may be natural or acquired:

I. Natural Immunity:

It includes intact skin, mucous membranes (Taschdjian et al., 1970), peripheral leucocytes, reticuloendothelial cells, tissue phagocytes and the inflammatory reaction (Frisk, 1978). According to Frisk (1978) and Kuttin et al., (1983), localized loss or interference with natural immunity is the basis of occurrance of pathogenic infection.

Macrophages play a role in the resistance against Candida albicans. The process of phagocytosis, ingestion of particles, is a part of the non-specific immuneresponse representing the host's initial response to foreignes, this is achieved by the phagocytic cells. The basic functional

property of a macrophage is its ability to engulf and remove foreign materials. Phagocytosis is facilitated by the presence of antigen coated with its specific antibody, a process called "opsonization". Macrophages process, and present the antigens to both T and B lymphocytes. An additional function attributed to macrophages is the production of factors which influence the activity of lymphocytes (Joseph and Bellanti, 1978).

It is known that macrophages participate actively in the elimination of Candida cells in infected individuals and it seems likely that the processing of Candida cells and cell fragments by macrophages results in the elaboration of potent antigens capable of eliciting production of high amounts of antibody (JanGoudswaard, 1978). It is possible that impairment of phagocytic activity may be responsible for the increased susceptibility to Candida albicans. Phagocytosis involves a stepwise removal of cell wall, probably followed by rupture of the cell membrane, extrusion and disintegration of the cytoplasm and death of the organism (Mildred and Seelig, 1966).

As the cell membrane of the phagocytosed organism finally ruptures, the cytoplasmic contents are released.

These may be the source of the soluble cytoplasmic antigens that stimulate the formation of precipitins characterising systemic Candidiasis. It is interesting to note that Candida albicans invading keratotic epithelial cells appear to retain an intact cell wall, and presumbly cytoplasmic integrity. This may account for the absence of precipitins in uncomplicated cutaneous and mucocutaneous candidiasis (Claire et al., 1971). Reduced phagocytic activity of leucocytes of approximately one-fourth of a group of pregnant women is of interest in view of the increase incidence of vaginal candidiasis during pregnancy. It was demonstrated that in vitro phagocytic ingestion of particles is almost entirely dependent on energy from glycolysis, this provides a new basis of understanding the impaired phagocytosis seen in diabetes and possibly in glucocorticoid treated patients (Mildred and Seelig, 1966).

II- Acquired Immunity:

Micro-organisms which overcome the innate or non-specific mechanisms will face the host's second line of defense, that is the specific immune response. This response entails two forms, which usually develop in parallel and are closely related as both depend upon cells of the lymphoid system:

1) Humoral immunity, this represents the appearance in the blood of antibodies. These combine specifically with the antigen which stimulates their production and can lead to some remarkable consequences (Valdimarrson et al., 1973).
2) Cell-mediated immunity (CMI). T-lymphocytes are sensitized by prior exposure to antigen (Weir, 1977). The infectivity of Candida and most other fungi, is thought to be suppressed predominantly by cellualr immunity rather than circulating antibodies. If, however, the fungus has succeeded in getting into the tissues, a complex defense response is initiated, the final step of which is killing and digestion, therefore defects in immune response might be responsible for Candida persisting in human tissues (Helgi et al., 1970).

Candida infections are common among patients with severe defects in cellualr immunity. Kirkpatrick et al., (1971), reviewed the immune status of patients with chronic mucocutaneous candidiasis and reported that the studies had demonstrated anti-Candida agglutinins and precipitins, however, circulating antibodies were not protective. Some investigators concluded that both CMI and innate defense were involved in host resistance to candidiasis and that

antibody does not enhacne this response (Hadfield and Stanley, 1982).

It has been assumed that antibodies play a minor role in the defense against fungal infections and the fact that agammaglobulinemic patients with intact cellular immunity are not reported to be abnormally susceptible to such infections supports this view. However, this may merely reflect the subsidary role the antibodies may have in the primary defense against fungi. This event which determines whether or not they succeed in starting an infection, it does not exclude that the behaviour and severity of the infective process once it has been established in the presence of defective cellualr immunity, might be influenced by the character and quality of the humoral immune response (Valdimarsson et al., 1973).

Precipitins to Candida Albicans:

Candida albicans may produce superficial infections of the skin or mucous membranes. Inspite of its superficial location, infection with this organism, seems to produce systemic sensitization to the antigens of the infecting fungus (Sohnle et al., 1983). In fact, uninfecetd persons

show such sensitivity to Candida albicans; presumably due either to previous subclinical infections or absorption of fungal antigens since these organisms are normal inhabitants (Sohnle et al., 1983). Many serological tests have been devised, including agglutination, complement fixation, direct and indirect fluorescent antibody staining tests, to detect anticandida antibodies (Valerie et al., 1972).

The detection of precipitating antibody to cytoplasmic antigens in Ouchterlony plates, has been considered as reliable evidence for systemic Candidiasis. Serum from healthy controls and patients with mucocutaneous candidiasis did not contain precipitating antibodies to cytoplasmic antigens (Nancy and Esterly, 1968).

PATHOGENESIS OF VAGINAL CANDIDIASIS

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 vaginal candidiasis commonly exhibit a significant local leukocytic response to the infection, the discharge is made up of clots of epithelial cells with interwined mycelia. Vaginal candidiasis represents primarily an infection of the vaginal secretions rather than an inflammation of the vaginal wall, and so it occurs when vaginal secretions are either normally or exogenously increased. Thus, the conditions is rare in premenarchal and postmenopausal women. Large numbers of Doderlein bacilli result in the production of an acid medium, which favours the growth of candida (Mead, 1974).

Alteration of vaginal pH in both normal and diseased states are frequently mentioned in discussion of pathophysiology of vaginal candidiasis. The belief that vaginal pH has a significant influence on vaginal candidiasis is based on the misconception that these organisms flourish only within a narrow pH range. Actually,

in the laboratory, Candida albicans grows well at a pH 5.4. This contrast sharply with Trichomoniasis, which will not grow below pH 5.0 or above pH 7.5 (Rippon, 1982).

Little data exist to explain the intense pruritus associated with vaginal candidiasis. Hesseltine (1959), has noted that 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 provoke pruritus when placed on mucous membranes. It is suggested that, these are the agents that give rise to the patients extreme discomfort, pruritus associated with vaginal candidiasis is produced as a result of the production of an undefined mycotoxins which can be both locally irritating and even allerginic (Mead, 1974).

Joklik et al., (1980), pointed out that, the physiologically normal epithelium is usually resistant to Candida invasion. If, there is a marked increase in the number of Candida present or if the skin and mucosa are traumatized or hormonally altered, these barriers are susceptible to Candida invasion, Iatrognic candidaemia and candiduria, induced by catheters or surgery, are often successfully managed by the normal host defense mechanisms.

However the ability of patients with hormonal imblance, immunodeficiencies, and malignancies to control invasion of the deeper tissues by Candida is limited. They added that, host defence against candidiasis were both specific and non-specific, cellular and humoral. Serum components, such as opsonins, complements 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 yeast. It also affect yeast morphogenesis or respiration and mediate antibody dependant cellualr cytotoxicity. Cellular host defenses against Candida involve neutrophils, which kill 30% to 40% of the ingested yeast. The host factors involved in chronic muco-cutaneous candidiasis were associated with the thymus-dependant immune system.

Source of Infection:

The reason for the presence or appearance of clinical symptoms and signs of yeast infection, as opposed to the carriage of the organisms as commensals, are often obscure (Oriel et al., 1972).

Rohatiner, (1966), reported that, it has been suggested that the intestinal tract is the most important reservoir of

infection in vaginal candidiasis. They added that, a high proportion (71.4%) of patients with vaginal infection were harbouring the same species in the ano-rectal tract. Although, the intestinal tract may be the source of infection to the ano-rectal tract, it is possible that in patients with profuse vaginal discharge, contamination from the genital tract can be the source of ano-rectal infection.

Teokharove (1969), added that, Candida is very persistent when present in the genital organs, and can be transmitted by various ways. Candida urethritis usually arise from sexual transmission, but the female genitalia are usually infected in other ways, often from the rectum.

Many men develop Candidal infection of the genitalia, which usually appears as a balanitis. The source of such an infection is normally a female sex partner, and the incubation period is two or three days. Host factors are less important in men than in women. The infectivity of Candida albicans to the male has been estimated at about 10% (Oriel et al., 1972).

Carrol et al., (1973), reported that, since Candida albicans is found in the mouth and intestinal tract of a

high percentage of human, the fungus may spread from such locations to cause skin and nail infections. Aspiration of material may lead to broncho-pulmonary infections which eventually, may initiate generalized infection. Such types of infection can be assumed to be endogenous in origin. Infections due to Candida albicans can be transmitted, balanoposthitis has been observed in the husbands of women with vaginitis, and cutaneous candidiasis about the nipples of nursing mothers has been caused by infants who become infected by passage through the birth canal of a mother with vaginitis. He added that, since candidiasis usually is endogenous in origin, it can be prevented best by personal hygenic measures.

Stewart and Benswick (1977), pointed out that, Candida infection is an opportunistic one, where the organism normally present as a commensal, when patient resistance has been lowered either by the administration of immunosu-pressives drug or broad-spectrum antibiotics, the fungus flourishes and cause Candidiasis.

PREDISPOSING CONDITIONS TO VAGINAL CANDIDIASIS

Predisposing Factors: (Mougahed, 1984)

There are two factors predisposing to vaginal candidiasis.

A) Intrinsic Factors:

1- Physiological conditions: pregnant women are more susceptibe to infection which is primarly a hormonal effect (Von Maillot et al., 1978).

2- Pathological conditions, this diminish immunity of the individual with the result of increased susceptibility to acquire vaginal candidiasis (Roberts and Mackenzie, 1979). These include:

a- Blood dyscriasis, agranulocytosis, and aplastic anaemia. (Mougahed, 1984).

b- Post-operative states and debilitation (Mitchell et al., 1982).

c- Malabsorption and malnutrition syndromes (Mitchell et al., 1982).

d- Malignancies especially leukaemia, Hodjkin and metastatic carcinoma as lung metastasis (Mougahed, 1984).

e- Endocrinopathies as diabetes mellitus, cushing syndrome, Addison's disease, hypo and hyper-thyroidism and obesity (Mougahed, 1984).

f- Iron deficiency anaemia.

g- Deficiences of T-lymphocyte or phagocytic function (Hurley, 1979).

3- Age.

B) Extrinsic Factors:

1- Prolonged and repeated exposure to radiation causing suppression of immunity (Mougahed, 1984).

2- Drugs affecting vaginal milleau either by changing the flora, affecting pH, or immunity of the host:

a- Oral contraceptives, which induce vaginal candidiasis by changing vaginal mucosal glycogen increasing the available carbohydrate source for the yeast (Mougahed, 1984).

b- Long term administration of broad spectrum antibiotics which act through killing competing bacterial flora so diminishing the inhibiting factors produced by these flora that inhibit Candida (Janssen, 1979).

c- Antimitotic drugs enhance candidiasis through depression of immunity of the host (Janssen, 1979).

d- Corticosteroids act through interfering with acute tissue inflammatory responses and the ability to produce antibodies.

Mead, (1974) reported that 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 defneses, 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.

The Intrinsic Factors:

1- Pregnancy:

It has been known for more than 50 years ago that the incidence of Candidal vaginitis is much higher in pregnant than in non-pregnant women although the figures given by various authors showed a great variability in incidence. For example Seelig (1966) reported an incidence of 11.5% in pregnant patients as compared to 2.85% in non-pregnant

women. In pregnant women, the increased incidence of Candidal vulvovaginitis is due to the action of oestrogen secreted from the placenta leading to increased glycogen deposition in the cells, this increases the vaginal acidity, so the growth of the pathogenic bacteria diminishes (Peeters et al., 1972).

It was found that vaginal pH of the pregnant women, who yielded Candida species in cultures ranged from 3.4 - 3.8 with an average of 3.6. These observations indicate that Candida could flourish in an acid medium with a good supply of sugar and this optimum vaginal environment would be available during pregnancy for the fungus to cause vaginal infection (Nagesha and Ananthakrishna, 1970). The incidence is highest in the 3rd trimester but during pregnancy there is no seasonal incidence. In the non-pregnant women, the incidence is higher in the summer months and exacerbations occur in the pre-menstrual period (Elliot et al., 1972).

Candida albicans is a cause of leukorrhea and pruritus in pregnant and also in non-pregnant women, causing thick curdy discharge and white patches on vaginal wall called "thrush" (Aleck et al., 1965). During pregnancy hyperaemia of internal genitalia leads to an increase in the vaginal

secretions in a consequenc with a hypersecretion of the cervical glands. This increased discharge is not pathologic except if infection by Candida occurs (Rafael Bonfante - Carrido et al., 1969).

As a matter of fact, cell mediated immunity is reduced in pregnant women to help in protecting the fetus from maternal immunologic rejection. An unfortunate consequence may be that the maternal immunologic surveillance becomes impaired and this permits certain micro-organisms to flourish and disseminate in pregnant women (David and Purilo, 1975). The presence of a glycogen rich vaginal epithelium due to increased oestrogen production or alterations in carbohydrate metabolism during pregnancy may also play a role in the transition of the organism from a saprophyte to a pathogen. Candida albicans is the most common cause of vulvovaginitis in pregnancy and on occasion taxes the ingenuity of the physician in attempting to relieve the patient from her symptoms (Charles, 1980).

Jackson, (1956) pointed out that, in pregnant patients with symptoms, the incidence of fungus infections ranged form 0.74 to 66.7%, while in pregnant patients without symptoms, the incidence ranged from 4 to 38%. They added

that, one factor which probably influences the reported incidence of infection is the method of determining the presence of fungi.

Winner, (1966), quoted typical british figures of the occurrance of Candida albicans in vaginal swabs as 16% in non pregnant and 25% in pregannt women. Amin (1983), screened 38 pregnant females, attending antenatal clinic, Benha University Hospital for Candida albicans. He found that, 12 (31.3%) were harbouring Candida.

Catterrall, (1966), stated that, an increased incidence of vaginal candidiasis in pregnancy has been widely recognized for many years. They demonstrated that the amount of mycelia and yeasts in the vagina increased greately during pregnancy. 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.

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). The cleaning effect of locia in puerperium contributes to the decline of the incidence of the disease (Toppozada and Rizk, 1977).

2- Diabetes Mellitus:

Plass et al., (1930) pointed out that, diabetes mellitus is a definite predisposing factor for infection by Candida vulvovaginitis, they added that it would seem very probable that, in patient with so called diabetic pruritus vulvae, the irritation may be due to the presence and growth of monilia with the resulting increased vaginal acidity rather than chemical action of the glucose itself, as has been usually thought.

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

Mead (1974) reported that there is an associating increased incidence of vulvovaginal candidiasis among diabetics and the presence of such infection had led to the discovery of previously undetected diabetes mellitus. However, after application of glucose solution to the vagina of 23 patients all patients who did not harbour vaginal Candida remained asymptomatic, whereas those with Candida either develop clinical vaginitis or their existing

vaginitis become more severe. After discontinuing the application, symptoms in all instances reverted to their previous state.

Glucosuria predispose to vulvovaginitis due to Candida albicans and this is common in diabetics who are poorly controlled (Browne and Dixon, 1978).

Fleury, (1981) pointed out that 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 oestrogenised 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 infection is a significant indicator of diabetes in the patient who has not recently received 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 of such source. Patients with renal glycosuria may also have Candida vulvovaginitis because the urinary carbohydrate bathes the vulva and distal vagina providing the necessary carbohydrates.

Plass et al., (1930), stated that, the availability of suitable food material is also a factor in determining the rate of growth of organism and may explain directly the predisposition of diabetics to the infection. They added that, the actual presence of dextrose in urine is a determining factor in the growth of the organisms and in the development of symptoms in diabetic individuals. This is suggested by the fact that treatment of diabetes with insulin and diet management was followed by relief of the vaginitis without the aid of local applications.

3- Age:

Plass et al., (1930) pointed out that, paruous women are more infected with Candida than children. However, virginal adults and senile women may also show the organisms.

Davis, (1969) after an extensive study concluded that, vaginal moniliasis was found but rarely among prepubertal girls and menopausal women. Meanwhile Oriel et al., (1972), in their study of genital yeast infection in 533 women, found no evidence that presence of yeast was related to age. Amin (1983), in his study of genital yeast infection in 100 women, also found that, age had no efefct in the incidence of candidiasis in his study.

Timonen et al. (1966), found that, analysis of age distribution of the patients with mycosis showed that this condition was most frequent in age group 28-35 years.

The Extrinsic Factors:

1- Oral Contraceptives:

The use of contraceptive pills has increased rapidly in the past decade and the World Health Organisation estimates that they are being taken by more than 20 million women throughout the world (Catterrall, 1971). It has been stated that there is a higher incidence of candidal vulvovaginitis in women utilising oral contraceptives than in those who do not (Rohatiner and Grimble, 1970).

Porter and Lyle (1966), stated that oral contraceptive therapy should be added to the list of those predisposing factors known to enhance fungal growth in the vagina. It is significant that the incidence of genital candidiasis in pill-users was especially high with either a high oestrogen content or a combination of oestrogen with a high quantity of convertible progestogen (Rohatiner and Grimble, 1970).

Catteral (1966) found that vaginal candidiasis started to appear in pill users after an average period of nine

months from the start of oral contraceptives, while Diddle et al., (1969) found that clinical candidiasis progessively increased as the duration of oral contraceptives medication was prolonged.

It was believed that the hormonal changes induced by contraceptive pills corresponded closely to those of pregnancy and regarded the resulting state of the patient as "pseudopregnancy". As in a true pregnancy the glycogen content of vaginal mucosa was increased. This led to the growth of lactobacilli, which converted the glycogen into lactic acid. The increased glycogen content, the presence of lactic acid and the change in the pH of the vagina, was thought to promote the growth of Candida albicans and the vagina was considered to become the ideal culture medium for Candida. Women with low vaginal pH were suggested to be more liable to infection due to excessive desquamation of the epithelium causing a break in the continuity of the vaginal mucosa (Catterrall, 1971).

It has been postulated that the responsible mechanisms in breaking the integrity of immune response in women using oestrogen-progestogen combinations may be similar to those depressing cell-mediated immune function during the third

trimester of pregnancy. Factors which supress the function of the T-cell; which mediates CMI, were important clinically since they led to higher susceptibility to cancer and less adequate response to fungal and viral infections (Dwyer, 1975).

De Costa (1969), speculated that, the increase in candidiasis in patients using oral contraceptive is based on non-specific factors, rather than on any endocrinologically mediated mechanisms. Such factors in patient using birth control pills might include, increased frequency of intercorse with increased local trauma to the vagina, failure to use a condum, thus promoting spreade of infection from male to female and failure to use contraceptive creams and jellies which may be fungistatic.

Fleury (1981), pointed out that, the role of oral contraceptives in causing Candida vulvovaginitis has been over estimated. Many investigators have actually found no difference in the incidence of Candida infections in patients using oral contraceptives or intrauterine devices. He added that, oestrogen therapy in postmenopausal women may precipitate a Candida infection, for the reasons mentioned above. The atrophic postmenopausal vagina does not support

the growth of Candida, but when oestrogens are given, the cells mature and glycogen content is restored as in younger women. Meanwhile Howkins and Bourne (1971), stressed upon the fact that, the progesterone content of contraceptive pills is a predisposing factor for monilial vulvovaginitis, this is brought about through the same mechanism as that in pregnancy, mainly increased vaginal acidity and glycogen content. Most studies indicated a statistically significant increase in patients using combination oral contraceptives (Mead, 1974). 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 (Gardner, 1967).

2- Antibiotics:

The mechanism by which antibiotics increased the prevalence of candidiasis were explained by Kashkin et al., (1961), as 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.

Diddle et al. (1969), reviewed the increased incidence of candidiasis in past years. They stated that, it is clealry related to the increased use of antibiotics. In particular, the increased incidence of disseminated candidiasis and localized visceral candidal infection, has been correlated with the administration of combined or broad spectrum antibitocs, with or without corticosteroid treatment. Candida in the intestinal lumen were readily attacked by the antifungal antibiotics. To prevent the intestinal overgrowth of Candida, especially in patients receiving broad spectrum antibiotics, the concomitant use of an antifungal agent is to be considered.

White and Timbury, (1973), pointed out that, since the introduction of antibiotics, it has been repeatedly observed that patients under therapy develop Candidiasis. Sometimes the use of penicillin lozengens precipitates oral thrush, or heavy oral dosage of various antibiotics, especially the tetracyclines, results in monilial enteritis or pneumonitis which may be fatal. The micro-organism in the mouth or gut is the result of competing of bacteria, protozoa, spirochaetes and fungi. The elimination of the bacteria sensitive to a given antibiotic may get rid of the successful competitors of yeast which then multiplies and

overwhelms the natural resistance of the tissue. The exact details of the mechanism involved are unknown, but candidiasis following antibiotic therapy may be controlled to some extent by adequate intake of vit. B complex.

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

Jeffcoate (1975), had not doubt that, there had been an appreciable incresae in the prevalence of vaginal candidiasis due to use of antibiotics. He added that wrote in non pregnant women the commonest cause in receiving antibiotics is responsible for the widespread manifestation of candidiasis".

DIAGNOSIS OF VAGINAL CANDIDOSIS

I) Clinical Diagnosis:

Candida species are the most common cause of primary vaginitis, a fact that has been existed since the advent of antibiotic therapy (Pickhardt and Breen, 1957). The presence of one or more factors that predispose to Candida infection is commonly elicited in the history (Mead, 1974). Percival (1980), reported that vaginal thrush (monilial vulvovaginitis) is the commonest infection found during pregnancy.

SYMPTOMS:

1- Pruritus:

Teokharove, (1969), stated that the patients may complain of external itching or burning. Catterrall (1970), stated that, vaginal discharge is variable and when present it is preceeded by pruritus. Pruritus vulvae is the most prominent symptom of the patient with vulvovaginal candidiasis. Itching may be intense, although features of infection are minimal (Peeters et al., 1972).

Browne and Dixon (1980), pointed out that, sometimes there is mild or severe pruritus, generally worse at night.

Fleury, (1981), pointed that, intense pruritus vulvae is a cardinal symptom in mycotic vulvovaginitis. This symptom causes a great problem to the patients, since it leads to insomnia and irritability.

Charles (1980), pointed out that, pruritus vulvae is the cardinal symptom of candidiasis and is present in about 90 percent in patients with monilial infection.

2- Discharge:

The character of the vaginal discharge is variable, during acute infection with Candida albicans, vaginal discharge is generally thick and highly acidic, but not especially profuse, while in cases of prolonged infection, it is thick and yellowish. The discharge is usually acidic, often thin and watery, but at times yellow and thick, sometimes it contain white curds (Browne and Dixon, 1980).

3- Dysuria:

Slight dysuria is not frequent (Browne and Dixon, 1980). Itching along the urethra or pain with simple wiping or voiding is a symptom known as vulval dysuria and it must be differentiated from urethral and cystic dysuria. Many

patients with burning micturation had been treated with oral antibiotics for persumed urinary tract infection, and these antibiotics made candidiasis worse (Flury, 1981).

4- Dyspareunia:

Mead, (1974), pointed out that, digital, speculum examination and coitus are extremly painful. Dyspareunia is a common complaint, Steven et al. (1977), reported that, dyspareunia is one of the cardinal symptoms of candidal vulvovaginitis that often bring a women to her gynaecologist or family physician.

The hallmark symptoms of Candida infection is pruritus. It is important to emphasize that this is vulvar symptom, since the vagina does not possess the necessary nerve endings to dected itching. It is not surprising therefore that examination of vaginal discharges often fails to reveal the organism infecting the vulva. As such, many cases of candidiasis can be classified as a vulvovaginitis (Mead, 1974).

In addition to the clinical history, there are several findings quite characteristic of Candida infection. The most common are erythema, excoriations (from scratching), and

small red spots called satellite lesions. As the disease progresses, vulvar oedema and fissuring of the skin may occur. Occasionally, a patient will manifest actual pustules, which are colonies of candidal organism within the squamous epithelium. Some patients manifest a greyish sheet, which is not noted with other infections, but that can mimic a vulvar dystrophy (lichen sclerosis or hypertrophic dystrophy) (Hilton and Warnock, 1975).

The vaginal secretion in patients with Candida is often described as "cottage cheese" in character. While this may be true in third trimester pregnant patients and others who have recently received antibiotics, the majority of patients with Candida vulvovaginitis do not demonstrate these classic curds, rather there may be a thick paste-like vaginal discharge with varying degrees of texture or curdiness. Since many patients without Candida infection also have curd-like and pasty vaginal secretions, this finding cannot be used as the sole basis for making a diagnosis. The confirmation of candida infection depends upon the microscopic examination or culture (Fleury, 1981).

II- Laboratory investigation:

A) Microscopic examination of vaginal smears by the following procedures:

a- Wet smear examination by low and high power.

b- Methylene blue stained smear.

c- Gram stain.

d- Ink solution stained smear.

B) Cultures:

The swabs were cultured on:

a- Sabouraud's medium.

b- Potato-carrot-bile medium.

c- Corn meal agar.

C) Specific identification of candida (by chlamydospore formation and germ tube test).

D) Serological methods of diagnosis.

Eddie, (1968), pointed out that, although provisional diagnosis of vaginal infection caused by Candida albicans may be made on clinical observation alone, but in many cases the cause of vaginal discharge is not clear until laboratory investigation has been performed. Thus a discharge that attributed to a minor degree of cervical errosion or visible lesions may be associated with unsuspected candidal

infection and simple treatment of the lesion will not ensure relief of symptoms. In many cases therefore, microscopic evidence of an infecting organism is an ensurance against incomplete diagnosis. They added that the most common method used to aid clinical diagnosis in this way is microscopical examination of a wet smear preparation, in which Candida can be seen. Cultural methods can also be used, and the increased use of vaginal cytology provides further diagnostic aid. Eddie, (1968) studied women at gynaecological out patient clinic over a period of one year and in whom laboratory diagnosis of Candida albicans in vaginal infection was made, Candida infection was detected in only 52% of all yeast positive cases by direct microscopy using methylene blue, whereas 93% of cases were proven by culture. They suggested that, wet smears of vaginal exudate should be examined microscopically from all women with excessive vaginal discharge or with symptoms of vaginal infection. If the wet smear is microscopically negative; then the vaginal discharge should be cultured for yeast; even if another lesion is believed to be responsible for the discharge.

Fleury (1981), pointed out that, the diagnosis of Candida vulvovaginitis begins with a clinical suspetion but

must be confirmed using laboratory methods. In most cases wet smear of the vaginal or vulval secretions, is necessary but occasinoally the microscopic examination is negative and a culture is indicated. Several Candida-specific cultures are available for office use. They are rapid, easy to use, and relatively inexpensive.

A- Microscopic Examination:

a) Wet smear:

In using this technique, one should be aware of saprophytic fungi that closely simulate pathogens but incapable of initiating disease (Rhobinson and Mirchandani, 1965).

Candida appear as oval budding cells and pseudomycelia whereas non pathogenic genera are present as yeast cells alone without accompanying pseudomycelia (Timonen et al., 1966).

Vaginal speculum should not be lubricated prior to specimen intake (McLennan et al., 1972). It should also be noted that vaginal douches and intra vaginal suppositories must be withheld for 72 hours prior to obtaining material for smear and culture. Direct microscopic examination of

the vaginal discharge is the most common method of diagnosing candidial vaginitis (Mead, 1974).

Material that found in the vagina is diluted with either saline or 10% potassium hydroxide and covered with a cover slide. Microscopic examination under low or high power is generally necessary for easy recognition of the yeast cells or budding stage, the Candida appears as oval budding cells and in the pseudomycelia stage. When there is no obvious discharge for examination, specimen are taken from lateral vaginal walls at the muco-cutaneous border of the entroitus (Mead, 1974).

Burrow and Ferris, (1977), pointed out that the use of wet mount preparation to detect Candida is cnosiderably less accurate than cultural diagnosis, wet mount preparation are examined with 10% solution of KOH which causes lysis of other cellular elements if present and thus aids in visualization of blastospores and pseudomycelia. Joklik et al. (1980), pointed out that, the appearance of Candida albicans in fresh preparation as psudohyphae along with budding cells of yeast is pathogenomonic.

b) Methylene blue stained smear:

Of the many preparations of this dye, Loffler's Methylene Blue is generally the most useful stain (Cruickshank et al., 1975)

c) Gram's stained smear:

Eddie (1968), considered that, the sensitivity of the gram stain for Candida species range from 70 to 100 percent. Baily and Scott (1974), reported that, the specimen obtained from the vaginal mucosa should be crushed under a cover glass and stained by gram stain. Candida appears as small (2-4micron), oval or budding, yeast like cells, along with mycelial fragments of varying thickness and length. The yeast-like cells and mycelial elements are strongly gram positive.

Mead (1974), pointed out that on staining by Gram's method, Candida cells are approximately at the size of R.B.C's and are round or oval and have small capsule that may be seen on routine examination. Oval cells, elongated buds and pseudomycelia are usually present on the slide.

Charles (1980), reported that, Gram's stain, will reveal Candida species. The Candida appears by Gram stain as

dense, gram positive, ovoid bodies of approximately 2-4 micron in their greatest diameter. The psuedomycelia are seen as elongated Gram-positive tubes.

d) Ink solution stained smear:

Cruickshank et al. (1975), pointed out that parker ink can be added for the selective staining of the agent particularly if Candida is being sought.

B- Cultural Methods:

Oriel et al. (1972), on writing about the importance of laboratory mycotic investigations pointed out that, clinical diagnosis of vaginal mycosis can not be made with accuracy without cultural method which is the most satisfactory. Hurley et al. (1973), reported that, the media used in bacteriological laboratories are adequate for the diagnosis of Candida from clinical specimens.

Culture Media:

a) Sabouraud's Medium:

White and Timbury (1973), cultured specimens of vaginal discharge for Candida albicans on sabouraud's medium. The incubation was done at room temperature and left for 48 hours. The colonies produced were smooth, cream-coloured and lenticualr with a definite yeast smell.

Mead (1974), pointed out that simple culture on sabouraud's glucose agar is adequate for routine vaginal swabs. Primary growth is moderately rapid and appears after 2 or 3 days. They appear as medium sized, cream coloured, smooth, pasty colonies with a characteristic yeast-like appearance.

Anti microbial agents are added to the media to prevent bacterial growth, as the later inhibit the recovery of the pathogenic fungi. Combination of penicillin (40 units/ml) with streptomycin (40 units/ml) and gentamycin (5 mg/ml) with chloramphenicol (16 mg/ml) have been found to be satisfactory for this purpose. The swabs obtained from the vagina and cervix should be inoculated on sabouraud's glucose peptone agar containing the combination of antibiotics (Roberts, 1976).

b) Potato-Carrot-Bile Medium (Paralrou and Marcelou, 1956):

This medium is selective for chlamydospore formation of Candida albicnas.

c) Corn Meal Agar (Cruickshank et al., 1973):

This medium is also selective for detection of chlamydospores which are diagnostic for Candida albicans.

C- Specific Identification of Candida Albicans:

The laboratory diagnostic procedures described before can only help the technician to decide whether or not the fungus isolated is a member of the genus Candida. Further tests are necessary to prove quite conclusively that the organism isolated is the pathogenic species Candida albicans, and not some other commensal species (Stewart and Benswick, 1977):

These Tests Include:

1- Demonstration of chlamydospores which considered as the most distinguishing feature of Candida albicans.
2- A specific pattern of sugar fermentation tests.
3- A specific pattern of sugar assimilation tests.
4- Germ tube test.

The usual results obtained with these four groups of tests for species of Candida likely to be isolated form clinical material are shown in tables (A & B).

Chlamydospore Formation:

Chlamydospore formation is considered as the most important distinguishing feature of Candida albicans (Table B), species of Candida except occasionally (Candida stellatoidea), do not form chlamydospores. The chlamydospores have

been defined as thick walled, spherical or elepsoidal, asexual spores, formed by the rounding of a cell or cells and usually formed terminally or in lateral positions. They are rich in lipid material and so are well adopted for maintaining vitality through period of dormacy they are 7-17 micron in diameter (Lodder et al., 1970).

When Candida is cultured on potato carrot bile medium and incubated at 27 C for 24-48 hours, chlamydospores are produced by Candida albicans strains (Al-Doory, 1980).

Germ Tube Test:

Al-Doory, (1980), stated that one of the most valuable tests for rapid identification of Candida albicans is the germ tube test. This test is done by dilution of a yeast colony in 0.5 to 1 ml of rabbit or human serum then incubated at 37 C for 2 to 4 hours, then a drop of the inocubated serum is examined microscopically for germ tube formation. A narrow filamentous tubes originating from yeast cells will be observed.

D) Serological Diagnosis:

The results of serological tests for Candida are often inconsistent with the disease. Numerous authors (Mead, 1974 and Wier, 1977), have measured agglutinating antibody levels against Candida albicans in the serum of normal patients and found that as many as 89% of apparently healthy individuals have such antibodies. Thus titres of agglutinating antibody are not usually diagnostically meaningful. However, a significant rise in agglutinating antibody titre is a reliable indication of visceral candidiasis. All patient groups at high risk with respect to disseminated fungal disease should be followed with serial determination of agglutinating antibody.

The detection of precipitating antibody has been considered more reliable evidence of disseminated candidiasis. By using an antigen obtained by disrupting Candida albicans cells, one can demonstrate precipitating antibody by agar gel diffusion and immunoelectrophoresis techniques in most patients with evidence of invasive

candidiasis and in few without disseminated disease. Serial determination of agglutinating antibodies as an aid to detecting systemic disease (Weir, 1977).

In summary, skin tests are of no help in diagnosing systemic or vulvovaginal candidiasis. detection of agglutinating and precipitating antibody is similarly not helpful in the diagnosis of vulvovaginal candidiasis. However, serial determination of agglutinating antibody may be of some value in the diagnosis of disseminated candidiasis.

Immunofluorescent techniques are useful for measuring serum antibodies to Candida and for the study of the pathogenesis of Candida infection, but their value in the clinical diagnosis of candidiasis is unproved (Miller, 1971).

Cruickshank et al. (1975), pointed out that, serology and skin testing are not yet of established value in diagnosis of vulvovaginal candidiasis. Whereas patients with chronic Candidal infections and healthy members of the general population may have similar levels of agglutinin and precipitins in their sera.

Jawetz and Melnick, (1978), reported that a cabohydrate extract of Candida albicans gives positive precipitin reactions with the sera of 50% of normal persons and of 70% of persons with mucocutaneous candidiasis, in systemic candidiasis, the titre of antibodies to Candida (agglutination, indirect immunofluorescence, precipitation) may rise. The presence of high antibody titres detected by immunodiffusion test suggest continuing activity of a deep infection. Skin testing for diagnosis of Candida albicans has almost universly positive in normal adults. It is therefore used as an indicator of competent cellualr immunity.

Table (A): Carbohydrate fermentation and assimilation reactions of different candida species (Rippon, 1982).

Species	Fermentation						Assimilation							
	G1	Ga1	M	S	L	T	G1	Ga1	L	M	R	S	C	T
C.albicans	+	+	+	V	O	V	+	+	O	+	O	+	O	+
C.stellatoidea	+	O	+	O	O	O	+	+	O	+	O	O	O	+
C.tropicalis	+	V	+	+	O	V	+	+	O	+	O	+	+	+
C.krusei	+	O	O	O	O	O	+	O	O	O	O	O	O	O
C.Pseudotropicalis	+	+	O	+	+	O	+	+	+	O	+	+	+	O
C.guilliermondii	+	V	O	+	O	V	+	+	O	+	+	+	+	+
C.parapsilosis	+	V	O	O	O	O	+	+	O	+	O	+	O	+

G1 = Glucose; Ga1 = Galactose; M = Maltose;
S = Sucorse; L = Lactose; T = Trehalose;
R = Raffinose; C = Cellobiose; (+) = Utilized;
O = Not utilized; V = Variable.

Table (B): Gross and microscopic appearance of the different specific strains of Candida (Al-Doory, 1980).

Species	Colonies * on agar	Yeast cell morphology	Germ tube	Chlamy-dospores
C.albicans.	Creamy	Ovoid	+	+
C.stellatoidea.	Creamy	Ovoid	±	rare
C.tropicalis.	Creamy	Ovoid	0	0
C.krusei	Flat, dull, dry.	Cylindrical & crossed.	0	0
C.pseudotropicalis	Soft, smooth, white.	Elongated & parallel	0	0
C.guilliermondii	Thin, flat, glassy.	Ovoid or cylindrical	0	0
C.parapsilosis	Creamy	Ovoid	0	0

* 3 days growth on sabouraud's dextrose agar at 25 C.

RECURRENT VAGINAL CANDIDIASIS

Vaginitis caused by C.albicans has become one of the most troublesome forms of vaginitis because it is frequently a recurrent problem. The reasons that some persons present with repeated episodes of vaginitis and other forms of mucocutaneous candidiasis are not clearly known although precipitating events are recognised. These include pregnancy, oral combined contraceptions, diabetes mellitus, use of tight restricted clothing and use of antibiotics (Sobel, 1982). Nevertheless most women with recurrent vulvovaginal candidiasis have no identifiable risk factor (Sobel, 1985).

Miles et al. (1977) reported that recurrent vaginal candidiasis orginate from a persistent rectal focus. The intestine acts as a reservoir for C.albicans, where it may live in harmony with the rest of the hosts faecal flora. Minor alterations in the milieu of the host (i.e. pregnancy and ingestion of broad spectrum antibiotics facilitates change from commensal to parasite on mucocutaneous surfaces). Miles et al., (1977), demonstrate that vaginal candidiasis does not occur naturally without the concomitant presence of C.albicans within the large bowel and that a "cure" is not likely as long as the vagina remains the only treatment target.

Odds and Aboutt (1980 & 1983) reported a method of typing isolates of C.albicans on the basis of differential growth on nine selected agar media. In an attempt to gain further epidemiological knowledge concerning its pathogenesis of recurrent vulvovaginal candidiasis. Badr et al. (1989) identified and typed clinical isolates of C.albicans from women suffering from recurrent vulvovaginitis. They reported that C.albicans was isolated from 84.7%, 71.7% and 32.9% of female vaginal, rectal and oral specimens respectively, and from 34.8% of penile and 26.1% of male oral swabs. Identical strains of Candida species, to those isolated from vagina, were detecetd in 86.4% rectal isolates, 60% oral isolates in women and in 100% penile isolates and 66.7% oral isolates from male partner. The data suggest endogenous and exogenous sources of infection to recurrent vulvovaginal candidiasis.

Cellualr and humoral immunologic data are rapidly accumulating (Taschdijian et al., 1973; Young et al., 1974 and Kozinn et al., 1976), but as yet, have contributed little knowledge in understanding the pathogenesis of recurrent vulvovaginal candidiasis.

CASES AND METHODS

I- Cases:

This prospective study was conducted in the Departments of Obstetrics, Gynaecology and Microbiology, Benha Faculty of Medicine during the period from August, to December, 1991.

A total of one hundred twenty women at reproductive age (25-45 years) were recruited. All women were subdivided into three groups:

Group (1): Forty five pregnant women suffering from recurrent vaginal candidiasis (recurrence of the symptomatology of the disease after it had been cured.

Group (2): Fifty five non-pregnant women, suffering from recurrent vaginal candidiasis.

Group (3): Twenty, healthy women (from family planning clinics) as a control group.

II- Methods:

Every women was subjected to:

a) A full history taking: including name, age, pregnant or not, number of pregnancy, history of oral combined pills,

diabetes mellitus, antibiotc therapy, complaint of itcting, discharge, sorness, dyspareunia and dysurea. They were asked about past history of similar condition and treatment by antimycotic therapy was noted.

b) Physical examination: general, abdominal and gynaecological examination was performed to verify the clinical diagnosis of vulvo-vaginal candidiasis by gynaecologist.

A) Mycological examination: for vaginal candidiasis was conducted as follows:

- Two vaginal swabs were taken with sterile swabs under direct illumination from the lateral vaginal fornices through sterilized vaginal speculum. One swab was used for inoculation on mycological culture media, and the second swab for making wet smear and gram's stained film, which were examined to detect the presence of yeast cells, hyphae, pus cells, epithelial cells and bacteria.

Media Used for Cultivation and Isolation of Candida Albicans:

Media used for isolation was prepared by the method described as follows:

1- Sabouraud glucose agar: (Cruickshank, 1975).

This medium consists of:

Peptone	10 gm.
Glucose	20 gm.
Agar	15 gm.
Distilled water up to	1 litre
pH was adjusted at	6 - 6.3.

2- Sabouraud chloramphenicol agar: (Cruickshank, 1975)

This medium consists of:

Peptone	10 gm.
Glucose	20 gm.
Chloramphenicol	0.5 gm.
Agar	15 gm.
Distilled water up to	1 litre
pH was adjusted at	6 - 6.3.

3- Media for sugar fermentation: (Marcelou & Vournus, 1961).

This medium consists of:

Peptone	10 gm.
Agar	6 gm.
Distilled water up to	1.000 ml.
Andrad's indicator	10 ml.
pH was adjusted to 7.2 with	1 N NaoH.

The medium appears pale pink when warm and colourless when cold.

4- Media for sugar assimilation: (Marcelou & Vournus, 1961)

This medium consists of:

Ammonium sulphate	5 gm.
Biotin	10^{-8}
Thiamin	10^{-6}
Pyridoxine	10^{-6}
Calcium pantothenate	10^{-6}
Nicotinic acid	10^{-6}
Inositol	10^{-5}
Histidine	10^{-5}
Methionin	2×10^{-5}
Tryptophan	2×10^{-5}
Potasium dihydrogen phosphate	1
Magnesium sulphate	0.5 gm.
Calcium chloride	0.1 gm.
Sodium chloride	0.1 gm.
Inorganic salt solution	10 drops.
Purified agar	18 gm.
Distilled water	1 litre.

5- Corn Meal Agar:(Cruickshank, 1975).

To one litre of distilled water, 40 grams of corn meal ground yellow maize was added. The corn meal was mixed and heated in a hot water bath for one hour after adjusting the

temperature of the bath at 60 C, then filteration was applied through cotton gauze or filter paper, the volume was adjusted to one litre distilled water, 20 grams bacteriological agar was added. Steaming was repeated to dissolve the agar.

The pH was 6.8, then poured in plates after autoclaving at 121 C for 15 minutes. This medium is used for detection of chlamydospores which are diagnostic of Candid albicans.

Methods of Isolation and Identification of Candida Albicans:

1- Two vaginal swabs were taken, one used for making a wet smear and gram stained film (to show epithelial cells, desquamated epithelium, some of the bacterial flora of the vagina and somtimes the Candida cells if present). The second swab was used for inoculation on sabouraud's agar plate incubated at 37 C aerobically and examined after 24 up to 72 hours for yeast colonies (Candida albicans), gram's stained film was examined to demonstrate the Candida albicans cells.

2- Sugar fermentation test. (Marcelou & Vournus, 1961).
The sugar fermentation medium was placed in tubes and boiled for 10 minutes. then cooled to 40-50 C and 20

drops of sterile sugar solutions (20%) were added, the six sugars used were; glucose, sucrose, maltose, galactose, lactose, raffinose. The organism from fresh culture 24-48 hours on sabouraud chloramphenicol agar was inoculated and incubated at 30-37 C for 24-48 hours.

Fermentation of the sugar is shown by the indicator turning red, indicating that acid has been produced, and by the formation of gas bubbles. Candida albicans ferment glucose and maltose with production of acid and gas while the other sugars are not fermented.

3- Sugar assimilation test (Marcelou and Vournus, 1961):
The medium of sugar utilization was poured into perti dishes, after solidification, 0.1ml of yeast suspension prepared by suspending 2 loopful of a fresh 24-48 hours culture in 1ml sterile water, 0.1 ml samples were spread on plates. Small discs of absorbant paper, 0.5 cm in diameter, each of them which contains standard amount (2 drops of 20% of sugar references dried in the oven were spread on the surface of the medium as for antibiotic sensitivity tests. The six sugar routinely used were: Glucose, sucrose, malitose, galactose, lactose and

raffinose. After 48 hours incubation at 30-37 C, sugar utilization was scored as a zone of growth arround the disc.

4- Chlamydospore formation: (Cruickshank, 1975)

One separate colony was picked from sabouraud's agar plate and inoculated deeply in the corn meal agar plate which was incubated at 25 C up to 14 days until colonies appear. Then microscopic examinations were applied to detect the chlamydospores which are diagnostic of Candida albicans as shown in Fig. (3).

5- Germ tube formation: (Chaffin and Sogin, 1976)

Inoculate 0.5ml of Human serum with a loopful of organisms and incubate for 2-4 hours at 37 C, then with a clean pipette, a drop of the sediment was placed on a slide with a drop of lactophenol, overlaid with a cover slip, and examined for germ tube, which appears as blue filament emerging from blastospore parent and gram stained film was done as shown in Fig. (4).

B) Immunological Examination:

* Evaluation of T-cell Function:

The materials used were:

1- Preservative-free heparin.

2- Hank's balanced salt solution.

3- Lymphocyte separation medium.

4- RPMI 1640 medium (Roswell Park Memorial Institute Medium).

5- Foetal calf serum (F.C.S.).

6- Phytohaemagglutinin (PHA).

All these materials from 1 to 6 from flow laboratories.

7- Screw capped sterile plastic tubes for cell culture reserch work, (Nunclon, Denmark).

8- Acetic-alcohol mixture (Glacial acetic acid: methyl alcohol = 1:3).

9- Trypan blue dye 1%.

10- Giemsa stain.

Method used were:

1) **Isolation of Lymphocytes from Whole Blood:**
(Gupta et al., 1975):

* Five ml of freshly drawn heparinized blood (using preservative free heparin) was diluted with an equal amount of Hank's balanced salt solution.
* Blood diluted with Hank's was layered on the top of 2ml lymphocyte separation media (L.S.M.) slowly by keeping the pipette against the tube wall.
* Centrifugation at 2000 rotation per minute (r.p.m.) for 20 minutes at room temperature, the lymphocytes form a

layer at the interface between an upper layer of Hank's solution and middle layer of lymphocyte separation media, the lower layer was formed of red blood cells and granulocytes.

* The lymphocyte layer was taken off with a Pasteur pipette after discarding the upper layer.
* The lymphocytes washed 2-3 times in Hank's solution i.e. lymphocyte layer +2 ml Hank's solution, then centrifugation at 2000 r.p.m. for 5 minutes, the supernatant was removed and the deposited lymphocytes were shaked by the palms of hands. Again Hank's solution was added and the process was repeated for 3 times.
* After the third step of washing, the deposited lymphocytes were resuspended in 1ml RPMI and the lymphocyte count was adjusted to $1x10^6$ cell/ml.

2) **Estimation of T-Lymphocytes Function by In Vitro Lymphocyte Transformation Test: (Camarasa et al., 1975).**

- **Principle:**

The estimulation of T-lymphocytes by a non specific mitogen like phytohaemagglutinin (PHA) results in a sequence of events called lymphocytes transformation

with the production of blast like cells which synthesize DNA de novo. The extent of transformation can be quantified morphologically on the basis of change of typical small lymphocytes to larger lymphoblasts as shown in Fig. (5) in which the nuclei become enlarged and euchromatic and develop one or more nucleoli. Also their is increase in cytoplasmic basophilia and vacuolization (Oppnheim and Schecter, 1980).

- **Method:**

The technique described by Gimenez camarasa, Carcia calderon and Morgan (1975).

* 10 ml venous blood was collected aspetically using asterile syring containing 50-100 units heparin per ml. After gentle mixing of the blood, a fresh disposable needle with it protective sheet in place is fixed to the syring and the syring placed in an up right position (with the needle pointing upward) in a 37 C in cubator for 1-2 hours.
* The needle was bent at an angle of 45 C and the supernatant plasma and white blood cells delivered into a sterile tube by upward displacement of the syringe plunger.

* Leucocytes were seperated from the plasma by centrifugation at 1000 r.p.m. for 10 minutes and washed twice in Hank's solution.
* After washing, the cell pellet resuspended in 1 ml RPMI and the lymphocyte count was adjusted to $1x10^6$ /ml.
* In a sterile screw capped tubes the following were added:
  - 1 ml of lymphocyte suspension ($1x10^6$ cell).
  - 3 ml RPMI.
  - 0.5 ml foetal calf serum (F.C.S.).
  - 0.1 ml phytohaemagglutinin (PHA).
* All culture tubes were closed and incubated at 37 C for 3 days (72 hours).
* On the third day, the cell cultures centrifused at 1000 r.p.m. for 10 minutes and the supernatant decanted, the cell button fixed in 2 ml acetic acid alchohol mixture for 10-20 minutes.
* The cells suspended by pipetting then centrifuged for 10 minutes at 1000 r.p.m. the supernatant decanted and the cells resuspended in the last drops of fixative.
* The cells then dropped on clean glass slide and spreaded by air blowing, the slide left to dry and stained by Giemsa's stain (dilution 1-10 for 10-20 minutes).

* The slides examined under the oil immersion lens. Blast like cells and unchanged lymphocytes were counted to a total of at least 200 cells and the percentage of blast cells was then deduced.

N.B.:

The mean of blastoid transformation in healthy normal individual is 80% (Bach and Hirshhorn, 1965).

Viability Test:(Ford and Hunt, 1973)

When lymphocytes have been manipulated in vitro, it is often necessary to make sure that they are a live before assessing their performance. The most common principle of these tests is the ability of the living cells to exclude trypan blue dye, the dead cells lack membrane integrity and therefore allow intracellular staining by the dye. The living lymphocytes are small refractile and the dead ones are large and stained blue. Trypan blue dye was made up as 1% solution in distilled water and filtered.

Steps of the viability test are as follows:

* Four drops of cell suspension were added in a steril tube, to one drop of 1% trypan blue dye and mixed well.
* The mixture was allowed to stand at room temperature for 5 minutes, then the haemocytometer counting chamber was filled and the stained and unstained cells were counted.
* Assuming those cells which were unstained to be viable, then the results were expressed as % viable cells.