INTRODUCTION

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The white patches and ulcers which appear in the mouth in course of sever and debilitating diseases have been observed for centures Hippocrates(1950) described two cases ; one occuring in the course of sever enteric fever, the other in the course of fatal erysipelas. Lagenbeck(1839) described vaguely the fungus. Gruby (1842) classified the fungus in genus sporotrichum.

Robin (1853) gave it the name "Oidium Albicans" Hansen (1888) called it Monilia Candida Bonorden, Berkhout (1923) introduced the generic name "Candida"., The third international congress of microbiology (1939) agreed to substitute the generic name "Candida" for monilia.

Lodder and kreger van-Rij (1952) classified and differentiated species of candida on morphological and biochemical basis.

Morphology :

Candida exists basically in 2 morphological forms are:

- Yeast form.
- Mycelial form.

. Yeast forms (Blastospores) :

These are cells, round or oval , ranging form 1.5 - 5 U in diameter. They are Gram +ve and they bud asexually.

. Mycelial form:

This is a mass of branching thread-like structures forming the vegetative portion of Fungus. Two types of mycelia are found.

(1) Pseudo-mycelium:

It is composed of branced filaments composed of elongated cells. It occurs in yeasts reproducing by budding.

(2) True mycelium :

It is composed of long thread- like branching structures or septate branched filaments. It occurs in yeasts which reproudce by fission.

. Calamydospores :

Thick walled, spherical, refractile spores containing oil droplets, they represent resistant stages of Candida albicans as they occur in poorely nutrient media, deficient in Proteins, (Lodder et al, 1958) and reducing sugars (Reid 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.

Culture characteristics:

Candida grows on organic substrates containing sugars, better under aerobic than anaerobic conditions(Johnson et al, 1954).

Biotin and thyamine are growth factors (Schopfer and Guilloud 1944). The optimum temperature for growth is about 30 °C (Catchings et al, 1973). The growth of the orgamism appears between 48 hours and few days.

On fluid media (Sabouraud's broth), the different species of candida produce different types of growth, ethier turbidity alone, turbidity with a sediment, or turbidity with surface pellicle.

These differences are of value only in identification of Candida Krusei which produces apellicle that creeps up against the glass wall (Lodder and Kregr Van-Rij. 1952).

On soild media colonies of candida are white or creamy in colour, their consistency is pasty; surface is usually smooth, sometimes corrugated; edge is usually

The morphological appearance of the colony is liable to a great variation and thus is not of much diagnostic value, except for Candida Krusei, which gives a flat, membraneous, sometines wrinkled colony.

CANDIDIASIS ;-

Predisposing factors :-

Yeasts, especially C. albicans are commonly present in healhy individuals (Schnidt, 1971). It is part of the resident microbiota of the oral covity, pharyrx, Large intestine and vagina (Hoeprich, 1977).

The progression of C. albicans from commensal to parasitic state is enhanced by a combination of increased yeast population and decreased host resistance.

Increased yeast population occurs when bacterial population is depleted by antibiotics. (Briody and Gillis, 1977).

Decreased host resistance occurs in obesity, diabetes, avitaminosis, corticosteroid therpay, extremes of life and in debilitated people. (Wistriech, 1976).

Infants and children are more liable to infections than adults.

Their skin and mucocuteneous membrones differ from that of the adult anatomically, physiologically chemically, physically and immunologically. It is less well developped than that of the adult. Thus, not only in absolute number are infections different, but initial or primary infections are more common and give norecharacteristic forms because they start upon immunologic immaturity (Leider, 1961). That is why monlial dermatitis in infants, especially the malnourised is extremely common. (Stowens, 1966).

Different factors accourding for diminution of host resistance include:

(A) Disturbance in Iron metabolism :

1. Iron deficiency:

It may predispose to candida infections as iron is important for mitosis and iron deficiency per se seems to be associated with reduced cell-mediated immunity, both in adults (Jonson et al, 1972) and in malnourished children (Bhaskaram and Reddy,1975)

Classification of candidiasis :-

The different chinical types of candidiasis are summarised in the flowing lines (Rook et al, 1972).

1. Infective :

   A. Mucosal candidiasis:

      i. Oral (Thrush):

         glossitis, denture stomatitis (denture sore mouth), angular stomatitis, chronic hyperplastic candidiasis, etc.

      ii. Vaginitis and balanitis.

      iii. Bronchopulmonary.

      iv. Alimentary : oesophageal,enteric.

   B. Cutaneous candidiasis:

      i. Intertrigo (toes, fingers, axillae, submammry folds, etc.)

      ii. Onychia, paronychia.

      iii. Napkin candidiasis.

      iv. Candidal granuloma.

The most commonly encountered disease caused by C. albicans is mucocutaneous candidosis (Goldsteir,E et al, 1977 Odds, F.C. 1979).

Because mucocutaneous candidial infections are endogenous in origin. (Ahearn, 1978 and Emmons 1977). The primary basis for the association of C. albicans with the host is its ability to colonize mucosal surfaces. Epidemological studies have shown that C. albicans is the yeast species which most commonly colonizes hospitalized parients, healthy adults, and children, although it is no more prevalent than other fungi in the enviroment (Arendorf and Walker 1980).

Adherence to mucosal surfaces is one mechanism which may allow. C.albicons to selectively colonize the human host, and it may play a key role in the pathogenesis of mucocutaneous candidosis.

Adherence has been shown to play a central role in the pathogenesis of many bacterial infection (Gibbons et al 1975). Less emphasis, however, has been placed on the role that adherence may play in fungal infection.

Since the initial observation that. C.albicans would adhere to human mucosal epithelial cells(King et al,1977).

Colonization and attachment to isolated rodent epitheial cells was markedley suppressed by the presence of salviary strains of streptococci.

We showed that seven different candida species raried in their in vitro adherence capabilities toward human vaginial and buccal epitheial cells (King et al,1980). C.albicans adhered in significanthy greater numbers than the other candida species, and this may explain its greater potential for colonizing and infecteting human hosts. Massch and calder one observed similar patterns of adherence of the candida spieces to fibrin-platelet dots formed in vitro (Maisch et al, 1980).

* Antibodies against antigens of C.albicans in patients with fungaemia and bacteramia:

The diagnosis of systemic mycoses is difficult. Blood cultures yield negative results in most cases and a positive finding may stem from transient frngaemia or contamination. A biopsy with histological evidence is needed for a decisive diagnosis (Kozinn et al,1978). and cases

with positive blood cultures alone should be considered presumptive. Because candida antibodies are found in subjects without active infection, serological diagnosis may be unreliable (Cobb and Parrat 1978 and Kozinn et al 1978).

In immunocompromised hosts, deep organ mycoses occur but are rare.

Septic episodes caused by yeasts are more often seen in paients with intravenous. Catheters, and form a potential source for dissemination.

* Ultrastuctural localiziation of intracellular immune gloublins in plasma cells and lymphoblasts by enzyme labeled antibodies:-

The localization of antigens by labeled antibodies at the ultrastructural level is an experimental method of possible wide applicability in biology and medicine. The first label employed successfully, ferritin (Singer, 1959), is excellent for extracellular antigens (Duc- Ngugan et al 1966 and Sternberger, 1967). and by special devices of tissue preparation also serves extremely well for some intracelluar antigens (Levinthal, et al 1967, and Rifkind et al, 1961).

However because of large size of the ferritin molecule (Mol. W. 650,000), its penetration into the interior of intact cells is difficult .

The use of enzymes as markers in antigen-antibody reaction was recently introduced (Avrameas et al, 1967 Avrameas and uriel 1966 and Nakame et al 1966).

Nakane and Piercebeing the first to use enzyme labeling for detection of tissue antigens at the eleetron microscopic level (Nakane and peierce, 1966).

A problem remains, however, even when a relatively small enzyme molecule like peroxidase (Mol wt 40,000) or a somenhat larger molecule like E. coli alk aline. Phostase (Mol wt, 80,000) is used as a marker, because 7 S antibodies themselves have an average molecular wieght of 150,000 and when coupled to enzymes they also penetrate into interior of cells with some difficulty.

This problem bas been attacked by 3 ways, first, simple methods for isolation of antibodies were developed (Avrameas, et al, 1967) together with a procedure for the coupling of proteins with enzymes using glutaralehyde as coupling agent with more regularly reprouducible results than was formerly possible (Avrameas, et al 1967).

second, extensive attempts to carry out the antiger antibody conjugate reaction directly on ultra thin sections of well fixed tissues, where penetration wauld not present aproblem.

Third, avariety of fixing schedules has been compared in an attempt to achieve penetration of conjugate into whole cells while permitting an adquate conservation of ultrastucture.

* A quantitaive immunofluorescence test for detection of anticandida antiboides :-

Considerable interest has been devoted to the serologic diagnosis of C.albicans infection over the past 10 years. One reason for this interest, has been the increasing prevalence of systemic infections with candida, particularly in debilitated or immunosuppressed patients (Glew et al, 1978).

Confirmation of the diagnosis of systemic candidiasis based on the growth of the infectious agent has met with difficulties some resulting from false negative cultures, others from the fact that the isolation of candida species from peripheral blood does not necessarily imply adiagnosis

of invasive or systemic camdidiasis (Goldsterin et and Hoeprich, 1972).

The detection of precipitins by double immunodiffusion (Taschdjian et al, 1969) has been widely used for several years.

Increased sensitivity has been achieved with agglutination tests using latex particles (Stickle et al,1972). or sensitized red blood cells (Mathur et al, 1977). and with counterimmunoelectrophoretic methods (Remington et al 1972 and Goudswaard and Virella, 1978). Which also decrease the time required for test.

More recently, crossed immunoelectrophoresis has been used to detect and characterize anti-Candida antibodies, providing detailed analysis of the different types of precipitins present in patient's sera (Axelson et al 1975).

Immunofluoresence has also been toied, using whole candida either in the blasto spore form (Esterly,1968) or in germ tube form (Ho et al, 1976), or using candid polysaccharide antigens engulfed by mouse peritoneal macrophages (Goudswaard and Virella, 1978) Finally, the most recent technique suggested for detection and quantition of anticandida antibodies has been enzyme

* Immunohistochemistry of glomerulonephritis using Horseradish peroxidase and fluorescein- labeled antibody:

The immunofluorescent method for the localization of antigens and antibodies in tissue sections (Coons and Kaplan, 1950). The method has been widely used, and is helpful in distinguishing between proposed mechanisns in the pathogensis of glomerulonephritis. Linear glomerular basement membrane (GBM) fluorescence suggests that the deposition of antibody directed against GBM antigens (nephrotoxic nephritis). Granular patterns imply deposition of antigen-antibody complexes (Dixon 1968).

The fluorescein labeled antibody method has several disadvantages:

a) The requirement for amicroscope equipped with an ultraviolet light source.

b) Rapid deterioration of fluorescence and tissue sections.

c) Difficulty in obtaining consistently satisfactory fluorescence photomicrographs.

Recently, an alternative method using enzyme labeled antibody has been developed (Nakane and Pierce, 1966) antibodies localize

at the sites of their specific antigens, and coupled horsradish peroxidase (HRP) reacts with its substrate to produce the histochemical stain. These sections can be examined and photographed with the ordinary light microscope, are permenent, and are avaible for review at any future time.

Advantages of the HRP method include:

a) Permanence of tissue sections.

b) Use of light microsope.

c) Easy of photomicroscopy.

As in the fluorescent technic, paraffin- embedded blocks can be used for HRP staining and multiple tissne antigens can be localised with HRP in asingle histologic section (Nakane 1968).

However, the HRP stain is electron- dense and the same antisera can also be used for ultrastructural localization of antigens or tissue bound antibody (Avraneas and Bouteille, 1968 and leduc et al, 1969). a property not shared by fluorescence conjugates.

With HRP, the GBM's tended to be more positvie and in some instances eshibited weak deposits of albumin which were not seen with FITC.

HRP accentuated granular GBM deposits and rendered interperetation of linear patterns more diffuclt. The HRP stain may be more sensitive than FITC because it more closely resembles the indirect immunofluorescent technic, since the HRP reaction product is the result of many molecules (antigen, antibody peroxidorse, substrate).

* Immunohistoperoxidase procedure in Defined antigen substrate spheres (DASS) system as serologic field test :-

For the epidemiological studies of several parasitic diseases it is important to have releable and simple serological field tests.

Although a serological field for schistosomiasis has been developed, the plasma card test (Sadun et al, 1963), this reaction not commonly used.

The indirect fluorescent antibody techniques. The indirect fluorescent techinques are now generally considered

as the most sensitive and specific tests for schistosomiasis and may be performed on natural substates : eggs, cercariae, frozen sections of adult parasite (Ambroise and thomas,1969).

Or an artifical substrates: Soluble Antigen fluorsecncent Antibody (SAFA) technique. (Taussaint and Anderson 1965), Defined Antigen substrate spheres (DASS) system (Deeler and Ploem, 1974).

A main disadvantage of the use of fluorescence reactions remains, i.e the necessary use of a fluorescence microscope (IFA, DASS) or a fluorometer (SAFA). For a field test it is necessary that the results of the test can be observed with simple equipment. At the monent enzyme labeled immunoglobulin are frequenly used as markers in immunohistochemical tests. One of the advantages of these immunohistoenzyme techniques is that the reaction products, indicating presence of antigen, are colored precipitates, which can be seen without the use of a fluorescence microscope.

It is found that when adding sera of individuals with schistosoma mansoni infections were examined with the DASS - system, using peroxidase labeled conjugates.

The results were compared with obtaimed with indirect fluorescencent antibody (IFA) technique on frozen sections of the adult parasit - the DASS- system proved to give specific positive reactions at higher serum dilulations than the IFA- technique. The results of test could readily be observed with naked eye (Deelder and streefkark, 1974).

* Enhanced antibody responses induced by candida albicans:-

When dead yeasts, cell walls or a water- soluble candidal polysaccharide were used, immunopotention of antibody responses to antigess unrelated to C.albicans was most dramatic when antigen and fungal materials were given concomitamtly via an intraperitoneal injection.

. However, mice infected with viable C.albicans several days before antigen administration also developed heightened responses to the antigen.

The presence of C. albicans or other inflammatory agents in the peritoneal cavity caused a more rapid uptake of particulate antigen by the liver.

* Laboratory diagnosis of Candidiasis

(1) Direct microscopical examination :

It usually reveals the characteristic budding cells of candida in smears prepared from clinical material, provided that the specimen is fresh i.e examined within 1-2 hours after sampling.

The number of yeast cells visible per field can provide an indication of the degree to which they ase implicated in the disease process.

The presence of pseudo- hyphal forms is a reliable indication of enteric candidiasis (Kozinn and Taschdjian, 1962 and Rogers 1957).

Also prescence of pseudophyphal forms in smears from the mouthes of infants is a reliable and valuable warning that clinical thrush is imminent (Anderson et al 1944).

Direct microscopical versus cultural method in sereeing for condidiasis among non gravid Nigerian women made by Elegbe and he found that the incidence of vaginal candida infection in 125 symptomless, non-gravid womer was determined by microseopic examination

of wet amount using 10% KoH, microscopic examination of Gram stained smears or by culture. The cultural method had the highest recovery rate with 75 (60%) of clutures positive for C.albicans. (Elegbne and Botu,1982).

(2) Culture Method :

Sabouraud's dextrose agar is the most commonly used. Mony modification was subjected to this medium pencillin is incorporated (Gohar and Elian, 1944).

Streptomycin was added (Thompson,1945) cyclohexamide (actidione) was added by fuentes et al, 1952.

Mycobiotic agar contains chloramplenical and actidione to isolate pathogenic fungi from contaminated specimens as sputum, stools.

Corn meal agar was first introduced in 1931 by Benham, as a medium that stimulates the development of mycelia and chlamydo spores.

Bakerspiegel (1954) recommended soil extract agar. Addtion of tween 80 to cornmeal agar was

recommended (Rosenthal and furnasi, 1959).

Medium containing a polysaccharider as the sole source of carbon (Nickerson and Mankowski, 1953).

It permits a growth of candida albicans rich in Addition of trypan blue to this medium gives the chlamydospores a blue colour.

Other media are advised, potato - carrot- bile agar (Walker and Huppert, 1959), malt extract agar (Crofet and Black, 1938) and sodium taurocholate agar (Backerspigel, 1962).

The aim of the culure is to determine whether the isolated yeast is candida or not, but it does not show to which species it belongs.

To determine this, other steps should be undertaken namely chlamydospore production, patterns of sugar fermentation and assimilation and germ tube production.

(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 specializied, rounded thick walled, refractile chlamydospores generally 9-18 U in diameter. They are differentiated from vegetative budding cells by their size, shape, refractility and thick wall. They arborn 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 protoporphyrns activity (Andrieu et al 1977).

(4) Carbohydrate fermentation :-

Carbohydrate fermentation tests are usuful to supplement carbohydrate utilization tests results when there is difficulty in making the definitive

Many methods have been developed for detecting carbohydrate utilizing carbohydrate - impregnated disks (Roberts 1976).

Or carbohydrate nutrient - impreganted disks (Huppert, 1975); agar slant utilization methods involving individual carbohydrate sources contained within yeast nitrogen base agar slants (Adams,1974) and broth tube methods containing individual carbohydrate sources within geast nitrogen base broth (Wickerham, 1948). Numerous commercially prepared systems which cantain carbohydiate utilization tests are avaible for yeast identification (Bowman 1976 and Buesching 1979) and are recommended for most laboratories(Elmer et al, 1984).

(6) Serum tube test (germ tube test) :

Reynaulds and Brank (1956) and Taschdjian et al, (1960) showed that candida albicans is apparently unique in undergoing a rapid transformation from blastospore (y) to psendohyphal (M) development, when inoculated into serum or ather serum substitutes. After inoculation and incubation at 37°C for ½ - 2 hours, numerous cylindrical outgrowth can be observed from the majority of cells.

(7) Animal pathogenicity test :

Injection of guinea - pigs intracutaneous will cause a mild inflammatory reaction.

Rabbits are killed by intravenous injection with a suspension of the organism.

Miliary absesses are produced in all parts of the animal, particulary in kidneys (lewis et al, 1958). However, some authors deny the value of the animal pathogencity tests in determining the pathological significance of Candida isolated (Ajello et al, 1966).

(8) Immunological diagnosis :

a) Complement fixing antibodies.

They are found in nomal human sera, They are of no value in the diagnosis.

In systemic candidiasis, shivanada found that when complent fixation tests with human sera were carreid out on 4 groups of patients: those with non - candida systemic infections, those with superfical candidosis, normal healty indviduals, and those with systemic candidiasis.

The latter was the only test group to show positive complement fixation tests with a titre of >1:5 Mycelial C.albicans antigen was shown to be better for the determination of complement fixation titre than yeast antign (Shivanada et al, 1981).

b) Precipitating antibodies :

They are found in patients with systemic candidiasis (Akiba et al, 1961 and aschdjian 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 infection. Cell free, dialysed and concentrated culture filtrates of C.albicans were used as antigens for the detechion of precipitating antibodies in the serm of patients with candida by an immunodiffusion test (staib et al, 1977).

c) Haemagglutation inhibition assay :

This method used to detect the surface antigen mannan is an early and specific signal of invasive disease (Winner and yount, 1976). Warren advised the use of enzyme immunoassay as a diagnostic test for invasive candidiasis (Warren et al, 1977).

d) Skin Test :

The intradermal injection of an extract of C.albicans mny be followed by a tuberculin type of reaction which indicates present or previous exposure to the organism. the test has little or no diagnostic value (Lewis et al, 1937).

Immediate anaphylactic reaction can be also worked (Kerr et al, 1934).

e) Quantitaive immunofluorescence test for the detection of anti-candida antibodies .

A quantitative I.F assay for anticandida antibodies has been developed using a recently introduced system that includes antomatic fluorometer and special immunoadsorbent for antigen coating. A commercially avaiable cytoplasmic antigen preparation was adsorded into substrate, and after incubation with sera from patients with systemic candidiasis or from normal controls, the antibodies bound to the antigen coated immunoadsorbent were revealed by the use of fluorescein labeled to human immunoglobulins Using doubling dilutions of a high titre serum, a positive relation was found between antibody concentration and the logarithm of the intensity of fluorescence. Quantitative assays of unknown samples were performed using a calibration curve constructed from dilutions of that strongly positive sample; the results of antibody determinations were expressed as percentages of the control. Seven of nine sera from patients with systemic candidiasis and only 2 of 42 from a symptomatic individuals, had antibody levels considered significant in this

assay which made by Gene,B. Perciptating antibodies were detedted by counterimmunoelectrophoresis in all patients and in 18 of the a symptomatic controls; measurable antibody levels were also found in 14 controls showing no perciptating antibodies.

This method is simple and sensitive and its quantitative nature makes it useful in investigntion of immune response to C.albicans. (Gene et al, 1980).

It has been shown that horseradish peroxidase can be coupled to antibody by a simple procedure to give stable conjugates which retain immunological reactivity and can be used for immunotracing in asimilar manner to fluorescence labelled antibody.

In tissues treated with peroxidase conjugate the sites of uptake are made visible by a simple histochemical procedure. Peroxidase catalyses the oxidation by hydrogen peroxide of a number of substrates, in some cases producing a highly coloured insoluble reaction product. (Nakane and Pierce, 1966).

The work of Nakane and perce (1966) and (Davey and Busch (1970) suggested that the horseradish peroxidase method is at least as sensitive as the immunofluorsenee technigue.

Nakane and pierce (1966) stated that "this might be expected because enzyme is not consumed in the reaction with substrate, and each molecule of enzyme- labelled antibody bound to antigenic site deposits many moleucles of reaction product".

Since immunofluorsecence was first used for detecting antinuclear factor (Holborow et al,1957) the method has undergone considerable development (Johnson et al 1967 and Holborow et al, 1969) to improve its usefulness in diagnosis and is increasingly used in clinical laboratories.

Difficulties, however, are still encountered, especially those associated with the special requirements of fluorescence microscopy. These would be avoided by use of a labe such as horseradish peroxidase which could be rendered visible by conventional microscopy.