COMPARISON OF THE EFFICACY AND TOLERANCE BETWEEN MICONAZOLE AS CHEWING GUM AND AS GEL APPLICATION IN PATIENTS WITH ORAL CANDIDIASIS

DISSERTATION

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BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In partial fulfilment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In

ORAL MEDICINE AND RADIOLOGY

By

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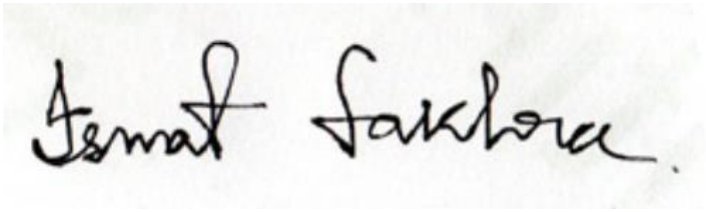

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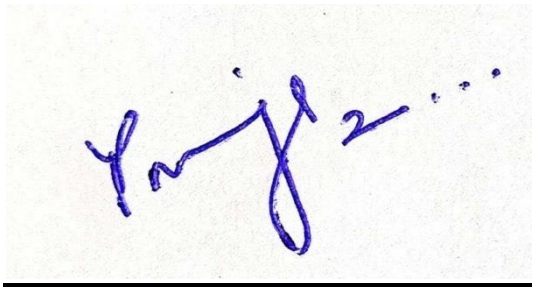

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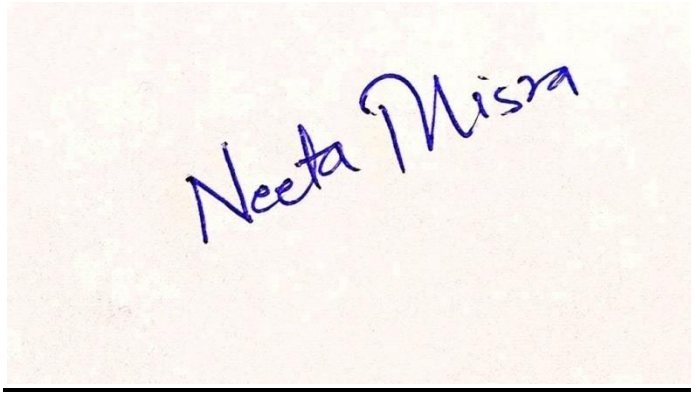

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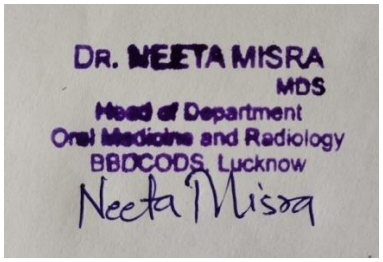

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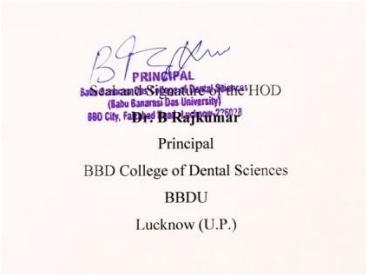

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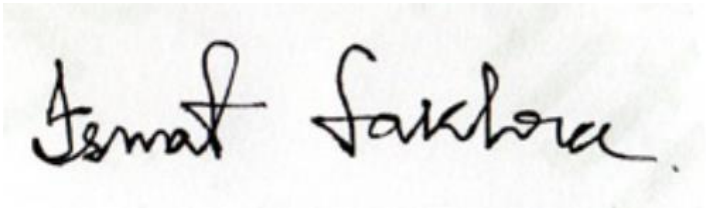

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

LIST OF ANNEXURES

Abbreviation:

- **AIDS** - Acquired immunodeficiency syndrome
- **ATPas** - Adenosine triphosphate
- **BSI** - Blood Stream Infection
- **CFU** - Colony-forming unit
- **CID** - Combined immunodeficiency
- **CD4** - Cluster of differentiation 4
- **DNA** - Deoxyribonucleic acid
- **IL** - Interleukin
- **IFN** - Interferons
- **KOH** - Potassium (K), Oxygen (O), and Hydrogen (H)
- **KNO** - Potassium Nitrate
- **RAPD** - Random amplification of polymorphic DNA
- **RNA** - Ribonucleic acid
- **MLST** - Multilocus sequence typing

Abstract

Introduction: Oral Candidiasis is one of the most common human opportunistic infection of the oral cavity. These lesions are caused by the yeast Candida albicans. There are many types of Candida species, which are seen in the oral cavity. Species of oral *Candida are: C. albicans, C. glabrata, C. guillermondii, C. krusei, C. parapsilosis, C. pseudotropicalis, C. stellatoidea, C. tropicalis*. In this study we compare of the efficacy and tolerance between miconazole as chewing gum and as gel application in patients with oral candidiasis.

Aim: This study aim to compare and evaluate the efficacy, biocompatibility, tolerance of Miconazole chewing gum with Miconazole gel in management of Oral Candidiasis.

Method and Materials: The study group consisted of 24 patients with oral candidiasis, evaluated clinically and histologically. Group A patients (12) chewed one piece of chewing gum (dose: 3.6 mg of miconazole) three times daily, Group B was given a 2% gel (dose: 20 mg of miconazole) to apply in the affected area three times daily. Both group were evaluated at baseline visit, during active phase (7th, 14th, 21st, 28th day), and follow up phase (for 3 months).

Result: There is significant difference was evident for burning sensation scale before treatment and after treatment with chewing gum and gel with $p<0.001$ and $p<0.05$ respectively. Significant difference was also evident for patch/plaque and scrapable availability before treatment and after treatment with chewing gum and gel with $p<0.001$ at the end of the follow up of the treatment.

Conclusion: The miconazole chewing gum was founfd to be equally effective as miconazole gel.

Keywords: Oral candidiasis, Miconazole chewing gum, Miconazole gel

1. Introduction

"Candidiasis" refers to a multiplicity of diseases caused by yeast like fungus, Candida, and is one of the most common oral fungal infections in humans [(1)]. Oral Candidiasis is the most common human opportunistic infection of the oral cavity [(2)]. These lesions are caused by the Candida albicans (yeast), which is one of the components of normal oral microflora and around 30% to 50% people carry this organism. There are various types of Candida species, which are found in the oral cavity. Species of oral Candida are: C. stellatoidea, C. glabrata, C. guillermondii, C. krusei, C. parapsilosis, C. albicans, C. pseudotropicalis, and C. tropicalis [(3)].

Candida albicans accounts for 40-60% of yeasts isolated in developed countries whereas Indian reports show an increased predominance of non C. albicans isolates, and the common isolates were Candida tropicalis 18 (23%), C. albicans 36 (47%) and Candida pelliculosa 5 (6%) [(4)].

Sudden increase in the incidence of fungal infection has been observed in the last few decades, and also associated with some predisposing factors like poor oral hygiene, xerostomia, use of denture, prolonged therapy with antibiotic, local trauma, premalignant disorders. Oral Candidiasis is one of the most common clinical features of acquired immunodeficiency syndrome as well [(5)]. Many predisposing factors are responsible for oral candidiasis are marked changes in oral microbial flora, Chronic local irritants, administration of corticosteroids, poor, pregnancy, immunologic deficiency, malabsorption and malnutrition [(1)].

Several antifungal medicaments are used for the treatment of candidiasis example being topical route of nystatin, clotrimazole, fluconazole and oral administration of ketoconazole, itraconazole, fluconazole. However all these medicaments have systemic as well as local side effect.

It is well known fact that the right drug delivery system is critical for the success of a pharmaceutical product. Miconazole gel founded to be effective in the treatment of oral candidiasis but with reported systemic side effects of dysgeusia, nausea, headache and diarrhea. Specific to gel application local site reactions such as burning, oral discomfort, bad taste and pain is reported in few patients [(6)]. Chewing gum rearly tried as drug delivery system holds tremendous potential. There are many reasons for selecting the chewing as a drug delivery system, like easy in administration without water promotes higher patient compliance, children and for patients who find swallowing tablets difficult are obvious like fast onset of action, Less side effects & more local effects [(7)].

A chewing gum containing the antifungal drug Miconazole may be convenient for topical treatment of oral candidiasis. The chewing gum reduces the dosage of Miconazole for treatment of oral candidiasis and the patients also approves the chewing gum as a pleasant medicament. The main purpose of this investigation was to learn about the efficacy and tolerance of Miconazole chewing gum when compared to Miconazole gel in the treatment of Oral Candidiasis [(8)].

2. AIM

This study aim to compare and evaluate the efficacy, biocompatibility, tolerance of Miconazole chewing gum with Miconazole gel in management of Oral Candidiasis.

OBJECTIVES

1) To evaluate and compare the potency of Miconazole chewing gum withMiconazole gel in Oral Candidiasis patient in alleviating the lesion.

2) To evaluate the tolerance of Miconazolechewing gum and Miconazole gel in treatment of Oral Candidiasis.
3) Evaluate patient's attitudes to chewing gum as compared to Miconazole gel.
4) To check for any recurrences in either of the two groups.

3. Review of Literature

3.1. Introduction

Candida is found in the oral cavity of 53% of the general population as a common commensal organism. One hundred and fifty species of this genus have been isolated in the oral cavity, and 80% of the isolates correspond to Candida albicans, which can colonize the cavity alone or in combination Candida glabrata or Candida tropicalis (observed in 7% of all healthy people and in 80% of all patients with candidiasis)[(9)]. Oral candidiasis is one of the most common opportunistic infection of the oral cavity. It is very common & under-diagnosed among the elderly, particularly in those who wear dentures and in many cases is avoidable with a good mouth care regimen. It

can also be a mark of systemic disease, such as diabetes mellitus and is a common problem among the immunocompromised. Many Risk possibilities like include medications, dentures, high carbohydrate diet, impaired salivary gland function, and extremes of life, smoking, diabetes mellitus, Cushing's syndrome, malignancies, and immunosuppressive conditions. Oral candidiasis is caused by an overgrowth or infection of the oral cavity by a yeast-like fungus, candida[(10)].

Candida is a round and oval-shaped yeast measuring 3-30 µm in diameter. It reproduces asexually through a budding process in which protoplasmic protrusions or buds (blastoconidia) emerge from the mother cell and grow until they finally detach to form a new cell [(11)]. The daughter cells sometime do not detach and form chains of cells called pseudohyphae, which can be mistaken for hyphae. The latter are composed of a row of elongated cells enveloped by a cell wall; they globally conform the mycelium (septate and ramified hyphae) [(12).] In solid culture media, the yeast grows, giving rise to compact colonies that are macroscopically visible after 24-48 hours of incubation. Candida need to be in the saprophytic phase in order to produce clinical lesions, though over time nutritional and environmental variations modulate its conversion to the mycelial or invasive form. In this phase the yeast keeps its previous virulence intact, and is able to evade macrophage phagocytic action [(13)]. The diagnosis of any of the forms of oral candidiasis is essentially clinical and is based on recognition of the lesions, which can be confirmed by the microscopic identification of Candida in the oral samples or isolation in culture, among other diagnostic methods. In the case of Candida, detection of the fungus in the oral cavity is not indicative of infection, since it is a common commensal organism in this location [(14)]. A definitive diagnosis of candidiasis requires the confirmation of tissue invasion by Candida. For this reason a negative culture result is of significant use in discarding candidiasic infection than a positive culture result in confirming infection. It may be declare that in the absence of clinical manifestations compatible with oral candidiasis, a positive culture result for Candida does not mean that the patient has oral candidiasis [(15)].

The pathogenesis factor for candidiasis combines three factors: host, fungus and oral microenvironment-modifying factors. The host predisposing factors include endocrine alterations (diabetes mellitus, pregnancy, renal failure and hyperthyroidism), immune depression (normally associated to antineoplastic treatments or immunosuppression in transplant patients [(16)], as well

as agammaglobulinemia or cellular immune defects), acquired immunodeficiency syndrome (AIDS) or hematological and immune disorders such as agranulocytosis (neutropenia) [17]. Other predisposing conditions are malignant diseases such as lymphomas or leukemias, aplastic anemia, medication treatments (long-term administration of broad spectrum antibiotics, corticosteroids, antidepressants, antineoplastic medications and immunosuppressants) [18], hyposialia produced by disorders such as Sjögren's disease, medications or radiotherapy, and terminal or end-stage systemic diseases [19]. The oral microenvironment-modifying factors in turn include poorly fitting dentures, loss of vertical dimension, chronic antiseptic use, and prolonged dummy use in children, poor oral hygiene, smoking and alcoholism [20]. The prime ones are C albicans, C tropicalis, C glabrata, C pseudotropicalis, C guillierimondii, C krusei, C lusitaniae, C parapsilosis, and C stellatoidea. C albicans, C glabrata, and C tropicalis represent more than 80% of isolates from clinical infection [21]. Oral candidiasis is the most common human fungal infection mostly in early and later life. In the general population, carriage rates have been reported to range from 20% to 75%4 without any symptoms. The incidence of C albicans isolated from the oral cavity has been reported to be 45% in neonates,6 45%–65% of healthy children,7 30%–45% of healthy adults,8 9 50%–65% of people who wear removable dentures, 65%–88% in those residing in acute and long term care facilities,9–12 90% of patients with acute leukaemia undergoing chemotherapy,13 and 95% of patients with human immunodeficiency virus. C albicans is a normal commensal of the mouth and generally causes no problems in healthy people [22].

A person with human immunodeficiency virus infection or acquired immune deficiency syndrome shows more number of oral candidiasis which is treatable [23].

Oral candidiasis can be a regular and remarkable source of pain, oral discomfort, loss of taste and distaste
for food. Candida albicans carriage and a history of oral candidiasis are other remarkable risk factors for oral candidiasis[24].Some antifungal agents can be used topically. For

topical agents, effective therapy depends on adequate contact time (2 minutes) between the agent and the oral

mucosa.Treatment duration varies from 7 to 14 days, with therapy minimally continued for 2 to 3 days beyond the last clinical signs and symptoms. Topical agents have the benefit of few side effects at normal therapeutic doses because of their lack of gastrointestinal absorption. However, sucrose carry topical agents can be cariogenic when used over prolonged time periods, such that adjunctive topical fluoride therapy may be needed. Systemic anti-fungal have the advantage of once-daily dosing and simultaneous treatment of fungal infections at multiple body sites[25]. However, these antifungal have more side effects, and selection requires consideration of important medication interactions.

The current work reviews the familiar clinical types of oral candidiasis, its diagnosis, and current treatment modalities with emphasis on the role of prevention of recurrence in the susceptible dental patient. The dental hygienist can play an important role in the education of patients to prevent recurrence[26]. Changes in the oral environment that can predispose or precipitate oral candidiasis include: antibiotics, corticosteroids, dry mouth (xerostomia), diabetes mellitus, nutritional deficiencies, and immunosuppressive diseases and therapy[23]. Saliva contains two antifungal proteins name as histatins and calprotectin and they help protect patients from Candida infections[27]. Histamines and Calprotectin defensive proteins are absent in a patient who has xerostomia. Individuals who use corticosteroid asthma inhalers must rinse their mouths with water after each use to reduce their chances of developing oral candidiasis. Excellent oral hygiene, including brushing and flossing of the teeth twice daily and maintenance of adequate intraoral moisture, is critical in the prevention of candidiasis recurrence in the susceptible patient[28].

3.2. HISTORY

The first known narration of Candida infections, oral candidiasis in two patients with other underlying diseases may be found in Hippocrate's "Epidemics" from the fourth century B.C.

"Thrush" one form of oral candidiasis is perhaps one of the earliest oral diseases documented[29]. Derivation of this word is rather obscure, but according to the Oxford Dictionary, it probably originated from the Old Danish Torsk which is synonymously used both for the disease and the bird of that name. Rosen von Rosenstein 1771 was the first to attempt to divide the disease into categories based on the severity and distribution of the lesions [30].

The fungus now named as Candida albicans was isolated by Bennett 1844 from the sputum of a tuberculosis patient, by Wilkinson (1849) from vaginal candidiasis. Robin 1853 was the first to observe concomitant thickening of the epithelium in lesions resembling thrush [31]. The taxonomic position and classification of the thrush fungus was the subject of much parley and disagreement for several decades as a result of different morphologic forms of the organism and different synonyms for the same fungus [32]. Robin (1853) had named it Oidium albicans, Quinquaud (1868) syringospora robinii and Reess (1875) Saccharomyces albicans. The first binomial to gain wide acceptance over a long period and which sometimes is still albeit wrongly used was Monilia albicans, which was suggested by Zopf in 1890. Berkhout in 1923, after acknowledgement the differences between Monilia species isolated from rotting plants and fruits and the yeasts isolated from medical cases, established the genus Candida to accommodate the latter. This was accepted as the official name of the genus by the Eighth Botonomical Congress in Paris in 1954[31]. Berkout proposed the name Candida from the Latin toga candida, which referred to the white robe worn by candidates for the Roman senate. The term Albicans too comes from the Latin albicare which means "to whiten". This translation match with what is considered traditionally and reported clinically as a presentation of candidal mucosal infection characterised as a white milk curd like covering of the mucosa, which wipes off easily [33].

The Index Medicus has not recognized the genus Monilia or the term Moniliasis in reference to human disease since 1981[31]. There have been around 166 synonyms being recognized for Candida albicans worldwide [34].The genus Candida is within the class Deuteromycetes and has been described as a"taxonomic pit" into which yeasts without a known sexual stage or other remarkable phenotype character have been thrown. Its members are biologically differing and include yeasts with ascomycetous and basidiomycetous affinities. There are currently between 150 and 200 species recognized in the genus [35]. The genus Candida adds characteristically white asporogenous (imperfect) yeasts capable of forming pseudohyphae. Within the genus,

species are characterized primarily by colonial morphology, carbon utilization, and fermentation [36]. There are seven Candida species of major medical importance, the most important being Candida albicans, the one most frequently isolated. It is believed to be the most virulent in man and can be isolated from human body as a commensal or as an opportunistic pathogen [37].

The other Candida species come across in human infections is *C. tropicalis, C. glabrata, C. parapsilosis, C. stellatoidea, C. pseudotropicalis, C. guillermondii, C. krusei, and C. kyfer.* These species are usually regarded as opportunists [38].

3.3. Epidemiology

Oral candidosis is frequent in the extremes of age. Approximately 5–7% of infants develop oral candidiasis. Its prevalence in AIDS patients is estimated to be 9– 31% and close to 20% in the cancer patients. The oral carriage of candida organisms is reported to be 30– 45% in the general healthy adult population [39]. The incidence of *C. albicans* in healthy and various health conditions is depicted in **Table 1.**

Oral carriage of Calbicans albicans in various subjects	
Subjects	Oral carriage of C. albicans
Neonates	45%
Healthy children	45–65%
Healthy adults	30–45%
Removable denture wearers	50–65%
Long term facilities	65–88%
Acute leukemia undergoing chemotherapy	90% (approximately)
HIV patients	95% (approximately)

The additional important species isolated from clinical infections include, *C. glabrata, C. guillierimondii, C. krusei, C. lusitaniae, C. parapsilosis, C. pseudotropicalis, C. stellatoidea,* and C. tropicalis. . In few years higher incidences of the above mentioned non- *C. albicans* Candida (NCAC) species have been also appear.

Systemic candidiasis is infrequent but carries a mortality rate of 71–79%. The annual rate of bloodstream infection (BSI) associated with candida ranges from 6–23/100,000 to 2.53–11/100,000 individuals in USA and European countries, respectively. Universal NCAC species have shown an increasing trend as causative pathogens in BSIs with a 10–11% increment over a 6.5−year period in a global report. In addition *to C. albicans*, the common NCAC species involved in BSIs include *C. parapsilosis* (premature neonates and catheterized patients*); C. glabrata* (elderly patients*); C. tropicalis* (hematological malignancies); and *C. krusei* [40].

3.4. Classifications

Proposed revised classification of Oral CandidasisPrimary oral candidasis (Group I) [41]

- Acute
    - Pseudomembranous
    - Erythematous
- Chronic
    - Erythematous
    - Pseudomembranous
    - Hyperplastic
    - Nodular
    - Plaque-like
- Candida-associated lesions
    - Angular cheilitis
    - Denture stomatitis
    - Median rhomboid glossitis
- Keratinized primary lesions superinfected with Candida

- Leukoplakia
- Lichen planus
- Lupus erythematosus.

Secondary oral candidoses (Group II)

Oral manifestations of Systemic mucocutaneous.

Candidosis (due to diseases such as thymic aplasia and candidosis endocrinopathy syndrome).

Pseudomembranous candidiasis (thrush)

Identify by ample white pseudomembranes consisting of desquamated epithelial cells, fibrin, and fungal hyphae. White patches appear on the surface of the labial and buccal mucosa, hard and soft palate, tongue, periodontal tissues, and oropharynx. The membrane can usually be scraped off with a swab to expose an underlying erythematous mucosa. Diagnosis is generally straightforward as it is easily seen and is one of the commonest forms of oropharyngeal candidiasis accounting for almost a third. Diagnosis confirmed microbiologically either by staining a smear from the affected area or by culturing a swab from an oral rinse. Predisposing factors include extremes of age, diabetes mellitus, patients who have HIV/AIDS or leukaemia, those using steroid aerosol inhalers, broad spectrum antibiotics, and psychotropic medications, and patients who are terminally ill [(42)].

The involvement of one and the other oral and oesophageal mucosa is prevalent in AIDS patients. The manifestations of the acute form are rather mild and the patients may complain only of slight tingling sensation or foul taste, whereas, the chronic forms may involve the oesophageal mucosa leading to dysphagia and chest pains. Few lesions mimicking pseudomembranous candidiasis could be white coated tongue, thermal and chemical burns, lichenoid reactions, leukoplakia, secondary syphilis and diphtheria [(43)].

Erythematous candidiasis

Demonstrate as both acute and chronic forms. Previously known as 'antibiotic sore mouth,' due to its association with prolonged use of broad-spectrum antibiotics. The chronic form is usually seen in HIV patients involving the dorsum of the tongue and the palate and occasionally the buccal mucosa.Clinically, it manifests as painful localized erythematous area. It is one and only form of candidiasis associated with pain.

The lesions are commonly seen on the dorsum of the tongue typically presenting as depapillated areas. Palatal lesions are more common in HIV patients. Differential diagnosis may include mucositis, denture stomatitis, erythema migrans, thermal burns, erythroplakia, and anemia [(44)].

Hyperplastic candidiasis

The hyperplastic candidiasis mainly presents as chronic form. It has been commonly referred earlier by several authors as 'candidal leukoplakia.' Clinically, it may manifest as one of the two variants; homogeneous adherent white plaque-like or erythematous multiple nodular/speckled type. The lesions usually occur bilaterally in the commissural region of the buccal mucosa and less frequently on the lateral border of the tongue and palate [(45)].

Unlike the pseudomembranous type, hyperplastic candidiasis lesions are non-scrapable. Commonly associated with smoking and in addition may present with varying degrees of dysplasia. A confirmed association between Candida and oral cancer is yet to be recognized, although in vitro studies have shown that the candida organisms can generate carcinogenic nitrosamine. A small number of cases occur in association with iron and folate deficiencies and with defective cell-mediated immunity. Differential diagnosis may include leukoplakia, lichen planus, angular cheilitis and squamous cell carcinoma [(44)].

Candida-associated Lesions

Denture stomatitis also called as "chronic atrophic candidiasis." As the name indicates, it is chronic inflammation of the mucosa typically restricted to the denture-bearing area, seen in association with candidiasis, almost seen in 50–65% of the denture wearers. Clinically, the lesions very likely seen as pinpoint hyperaemia, diffuse erythematous or granular/papillary type. It occurs more commonly along with angular cheilitis and median rhomboid glossitis. The

lesions are generally asymptomatic, from time to time patients may complain of burning sensation or soreness. It commonly affects the palate although mandibular mucosa may also be affected. Associated factors include poor oral hygiene practice, nocturnal denture wear, ill-fitting prostheses and limited flow of saliva [46].

Angular cheilitis most of the time manifests as erythematous or ulcerated fissures, typically affecting unilaterally or bilaterally the commissures of the lip. Angular cheilitis regularly represents an opportunistic infection of fungi and/or bacteria, with multiple local and systemic predisposing factors involved in the initiation and persistence of the lesion. . The factors associated include old age and denture-wearers (due to reduced vertical dimension), vitamin B_{12} deficiency and iron deficiency anemia. Other causative organisms implicated are Staphylococcus and Streptococcus [44].

Median rhomboid glossitis appears as the central papillary atrophy of the tongue and is typically located around the midline of the dorsum of the tongue. Manifested as well-demarcated, symmetric, depapillated area arising anterior to the circumvallate papillae. The surface of the lesion can be smooth or lobulated. While mainly the cases are asymptomatic, some patients complain of persistent pain, irritation, or pruritus. . The lesion is now believed to be a localized chronic infection by C. albicans. It is most commonly seen in tobacco smokers and inhalation-steroid users [42].

Linear gingival erythema was previously known as "HIV-gingivitis" since its typical occurrence was in HIV associated periodontal diseases. Its look likes a linear erythematous band of 2– 3 mm on the marginal gingiva along with petechial or diffuse erythematous lesions on the attached gingiva. The lesions may present with bleeding. In addition to C. albicans, C. dubliniensis has been reported as an emerging pathogen in this form of candidiasis [40].

Secondary Oral Candidiasis

It is identified by chronic mucocutaneous candidiasis, which consists of heterogeneous disorders, presenting as persistent or recurrent superficial candida infections of the mouth, skin, nail beds, and occasionally producing granulomatous masses over the face and scalp. The earliest clinical features include chronic oral, cutaneous and vulvovaginal candidiasis. Oral cavity participation is

reported in more than 90% cases and the lesions can occasionally spread into the larynx, pharynx or esophagus but further involvement is infrequent. It is generally related with diverse immunodeficiency disorders such as, Di George syndrome, hyper-immunoglobulin E syndrome, Nezelof's syndrome Myeloperoxidase deficiency, Severe combined immunodeficiency syndrome and endocrine disorders like Addison's disease andhypoparathyroidism [43].

3.5. Risk factors:

1. Pathogen
2. Local factors
3. Systemic factors

1. Pathogens

Candida come under fungal group and was first discovered in 1844 from the sputum of a tuberculous patient. Like any other fungi, they are non-photosynthetic, eukaryotic organisms with a cell wall that lies external to the plasma membrane, and a nuclear pore complex within the nuclear membrane. The plasma membrane contains large amount of sterols, usually ergosterol.

Aside from a few exceptions, the macroscopic and microscopic cultural characteristics of the different candida species are similar. And can metabolise glucose under both aerobic & anaerobic conditions. Temperature influences their growth with higher temperatures such as 37°C that is present in their potential host, promoting the growth of pseudohyphae, isolated from animals and environmental sources. It can be found on or in the human body with the gastrointestinal tract, the vagina, and skin being the most common sites and C. albicans being the usual species isolated from these sites. They require environmental origin or root of fixed carbon for their growth. Filamentous growth and apical extension of the filament and formation of lateral branches are also seen with hyphae and mycelium, and single cell division is associated with yeasts [47].

Several studies have revealed that infection with candida is associated with certain pathogenic variables. Adhesion of candida to epithelial cell walls one of the important step in initiation of infection, is promoted by certain fungal cell wall components such as mannose, C3d receptors,

mannoprotein, and saccharins [48]. The degree of hydrophobicity 31 and its potential to bind to host fibronectin32 has also been reported to be key factor in the initial stages of infection. Other elements implicated are germ tube formation, presence of mycelia, persistence within epithelial cells, endotoxins, induction of tumour necrosis factor, and proteinases. Phenotypic switching which is the ability of certain strains of C albicans to change between different morphologic phenotypes has also been implicated [47].

2. Local Factors

Saliva

Salivary gland dysfunction predisposes to oral candidiasis. Constituents of saliva such as histidine-rich polypeptides, lactoferrin, lysozyme, and sialoperoxidase inhibit the overgrowth of candida. Hence, conditions affecting the quantity and quality of salivary secretions may lead to an increased risk of oral candidosis [49].

Dental Prostheses

It creates a favorable microenvironment for the candida organisms to thrive. About 65% of complete denture wearers are predisposed to candida infection. The feasible explanations include enhanced adherence of candida to the acrylic, ill-fitted appliances, decreased saliva flow under the denture surfaces or inadequate hygiene [50].

Topical Medications

Another prime local factor increasing the risk of oral candidosis could be use of topical or inhalational corticosteroids and overzealous use of antimicrobial mouthwashes. They for the time being suppress the local immunity and cause alterations in the oral flora [49].

Smoking Some

Smoking alone or in combination with other element, significantly affects the oral candida carriage while few studies propose otherwise. The accurate mechanism is not established but various theories have been postulated. The possible explanations facilitating candida

colonization include localized epithelial alterations caused by smoking [51]; smoking in related with denture friction altering the mucosal surface nutritional products obtained through enzymatic breakdown of aromatic hydrocarbons contained in cigarette smoke); suppression of local immunity and reduction in gingival exudate; elevation of glycosylated hemoglobin levels and lastly tobacco smoke increasing the adrenaline levels in blood, indirectly act on the blood glucose levels [47].

Diet

Unequal dietary intake of refined sugars, carbohydrates and dairy products (containing high content of lactose) might serve as growth enhancers by reducing the pH levels and hence favoring the candida organisms to thrive [49].

Systemic Factors

Immune Disorders

Saprophytic and commensal fungi infect billions of human being each year. Medically main fungi include yeast Candida spp., mold Aspergillus spp., the atypical fungus Pneumocystis jirovecii, dimorphic (Coccidioides, Paracoccidioides and Histoplasmosma spp.) fungi, dermatophytes (e.g. Trichophyton spp.), and encapsulated yeast Cryptococcus spp. Invasive fungal diseases, such as candidiasis, aspergillosis, pneumocytosis and cryptococcosis in particular, have become a major health problem. The acquired immunodeficiency syndrome epidemic, the more extensive use of immunosuppressive therapies, the longer survival of immunosuppressed patients, and the increased use of intravenous lines and increased movements of patients at risk are the main acquired risk factors contributing to invasive fungal diseases [52]. Despite advances in treatment, mortality rates for invasive fungal diseases remain high, at 30 to 50%. Superficial fungal infections have less severe can also lead to significant morbidity and mortality. In any case, a number of superficial and invasive fungal infections are not explained by any of the known risk factors [53].

It is important to recognize the pathogenesis of fungal infections in patients without known risk factors. It is also timely to decipher the cellular and molecular mechanisms of antifungal

immunity, with a view to developing new tools for treating fungal infections and new preventive measures, including vaccines. The study of primary immunodeficiencies conferring a predisposition to fungal infections can serve both purposes, as the elucidation of genetic etiologies of unexplained fungal diseases also improves our understanding of antifungal immunity in other settings. In recent years, the genetic dissection of chronic mucocutaneous candidiasis disease has revealed a role for IL-17 in mucocutaneous immunity to C. albicans. Other examples include the role played by CARD9 in invasive fungal diseases caused by dermatophytes and Candida spp., that of IFN-γ in immunity to dimorphic fungi, and that of the nicotinamide adenine dinucleotide phosphate oxidase complex in immunity to Aspergillus spp [(54)].

Primary immunodeficiencies underlying chronic mucocutaneous candidiasis

Candida spp. is cosmopolite commensal yeasts colonizing in the skin C. parapsilosis and digestive tract C. albicans of healthy individuals. However, they can lead to superficial mucocutaneous infections, which can occasionally become chronic mucocutaneous candidiasis. Chronic mucocutaneous candidiasis is characterized by persistent or recurrent infections of the mouth, esophagus, digestive and genital mucosae, nails and/or skin, mostly with C. albicans. Chronic mucocutaneous candidiasis is frequent, and associated with other infections caused by a broad spectrum of microorganisms, in the context of acquired conditions (HIV infection, immunosuppressive therapies, prolonged antibiotic therapies, and diabetes mellitus) or various inherited primary T-cell immunodeficiencies. Patients with severe combined immunodeficiencies and patients with combined immunodeficiencies develop chronic mucocutaneous candidiasis in infancy due to T-cell deficiency. Idiopathic CD4 lymphopenia are also susceptible to chronic mucocutaneous candidiasis. All known patients with an autosomal dominant IκBα gain-of-function mutation and impaired NF-κB signaling have developed hronic mucocutaneous candidiasis and other fungal infections. Two thirds of patients with CID and low T-cell counts, such as those with autosomal recessive DOCK8 deficiency, display chronic mucocutaneous candidiasis . Those with CID and impaired Tcell function, caused by AR TCR-α, AR ORAI1, AR MST1/STK4, AR RFXANK or AR CD25 deficiencies, for example, have also been reported to display susceptibility to chronic mucocutaneous candidiasis [(55)].

In other Primary immunodeficiencies, chronic mucocutaneous candidiasis is one of the main clinical presentations. About 85% of patients with AD STAT3 deficiency and hyper-IgE syndrome display chronic mucocutaneous candidiasis in addition to severe staphylococcal skin and pulmonary disease, with 64% of these patients displaying oral candidiasis from the neonatal period onwards. chronic mucocutaneous candidiasis has also been reported in 25% of patients with AR IL-12Rβ1 or IL-12p40 deficiency, both of which are genetic etiologies of Mendelian susceptibility to mycobacterial disease. Six of the seven patients reported in one large consanguineous multiplex kindred with AR CARD9 deficiency displayed chronic mucocutaneous candidiasis in addition to other fungal diseases. In these three Primary immunodeficiencies, patients have been shown to have a deficit of IL-17A- and IL-22-producing T cells. This deficit probably results from impaired STAT3-dependent signaling downstream from IL-6, IL-21 and IL-23, impaired IL-12Rβ1-dependent signaling downstream from IL-23 or impaired CARD9-dependent signaling downstream from C-type lectin receptors, respectively, these signaling pathways being involved in IL-17 T-cell development and maintenance. Finally, in AR autoimmune polyendocrinopathy syndrome type 1, caused by AIRE deficiency and resulting in impaired T-cell tolerance, 88% of patients develop chronic mucocutaneous candidiasis at a mean age of 3.7 years. High levels of neutralizing autoantibodies against IL-17A, IL-17F and/or IL-22 have been detected in the sera of APS-1 patients. Overall, these studies strongly suggest that human IL-17 immunity plays a critical role in defense against chronic mucocutaneous candidiasis, and they paved the way for the identification of the first genetic etiologies of chronic mucocutaneous candidiasis [(56)].

Chronic mucocutaneous candidiasis disease is defined as chronic mucocutaneous candidiasis in patients with no other prominent clinical signs and with none of the genetic defects mentioned above. Complete AR IL-17RA & partial AD IL17F deficiencies, resulting in impaired IL-17 immunity, was first reported in one CMCD family each. Subsequently, genome-wide approaches led to the discovery of heterozygous STAT1 missense mutations in patients with chronic mucocutaneous candidiasis disease. These mutations, unlike the previously reported mono- or biallelic STAT1 loss-of-function mutations associated with susceptibility to mycobacterial, intracellular bacterial and viral infections, was shown to be gain-of-function. Almost 100 patients with STAT1 gain-of-function mutations have been reported to date. These patients developed chronic mucocutaneous candidiasis disease at a mean age of 1.4 years, mostly affecting the

oropharynx (98%), nails (58%), skin (46%) and esophagus (20%). Chronic mucocutaneous candidiasis disease -causing STAT1 mutations increase STAT1 responses to IFN-α/β, IFN-γ and IL-27, which repress IL-17 T-cell development, probably accounting for the small numbers of IL-17- producing T cells in these patients and the resulting chronic mucocutaneous candidiasis disease [57].

Primary immunodeficiencies underlying invasive aspergillosis

Aspergillus spp. is commonly present in soils and decaying plant material. Spores are inhaled & hyphae can grow in the lungs, leading to pulmonary aspergillosis, the most common (90%) clinical presentation. Invasive aspergillosis is a life-threatening disease with, global mortality rate at 3 months close to 45%.The underlying diseases are generally hematological malignancies and particularly acute myeloblastic leukemia (78%), with prolonged neutropenia, solid organ transplant, solid tumors, systemic inflammatory diseases and chronic respiratory diseases. The prevalence of invasive aspergillosis is particularly high in patients with chronic granulomatous disease, 17% of whom develop invasive aspergillosis. A. fumigatus is the Aspergillus spp. most frequently associated with disease, found in approximately 40% of the chronic granulomatous disease cases. A. nidulans, which is encountered almost exclusively in chronic granulomatous disease patients, is associated with a high mortality rate. Invasive aspergillosis is mostly pulmonary in chronic granulomatous disease patients. This disease results from an NADPH oxidase complex defect in phagocytes, mostly due to X-linked mutations or biallelic mutations in autosomal genes. Tissue susceptibility to bacteria and fungi is due to a defect in reactive oxygen species production by phagocytes, resulting in an inability to kill microorganisms. Studies of patients with mycobacterial but not fungal disease impairing the respiratory burst in macrophages but not in monocytes or granulocytes have suggested that disruption of the respiratory burst in granulocytes and monocytes underlies pulmonary aspergillosis in chronic granulomatous disease patients [54].

Approximately 20% of AD-HIES patients develop invasive aspergillosis, always secondary to lung lesions (pneumatocyst formation or bronchiectasis), with 17% mortality. AD-HIES patients

display a normal inhibition of A. fumigatus growth by phagocytosis, unlike chronic granulomatous disease patients. The susceptibility of AD-HIES patients to intrauterine fetal death therefore results from the presence of pneumatocysts due to repeated bacterial infections of the lungs and defective STAT3-dependent epithelial immunity. The incidence of invasive aspergillosis is also high, at 17%, in patients with AD GATA2 deficiency, as are the incidences of mycobacterial, papillomavirus and other fungal infections. Consistent with the role of GATA2 as a transcription factor involved in hematopoiesis and maintenance of the stem cell compartment, patients with GATA2 deficiency present a complex phenotype, with monocytopenia, B and NK lymphopenia, low counts of dendritic cells and myelodysplasia. Invasive aspergillosis has been reported in rare cases of AD or AR severe congenital neutropenia or AR type I leukocyte adhesion deficiency [(56)].

Primary immunodeficiencies underlying invasive candidiasis

Candidiasis is one of the most important cause of nosocomial blood stream infection and the most frequent intrauterine fetal death in Western countries, with a mortality rate of 40%.Candidiasis is classically described in neutropenic patients, intensive care unit patients with catheters, treated with broad-spectrum antibiotics and with parenteral nutrition. Invasive candidiasis has occasionally been reported in patients with Primary immunodeficiencies: 2% of the patients from the French SCN registry developed invasive candidiasis, for example. Despite the scarcity of data, AR type 1 leukocyte adhesion deficiency with CD18 deficiency is also known to be complicated with invasive candidiasis[45]. Candida spp. infection of the central nervous system is mostly reported after neurosurgery or in premature infants. It has also been reported in CGD patients and, recently, in patients with AR CARD9 deficiency. So far, at least three (probably four — three from an Iranian kindred and one of Korean origin) individuals have developed Candida infections of the central nervous system, at a mean age of 13 years, with Candida spp. meningoencephalitis reported in three cases. CARD9 is an adaptor molecule expressed in myeloid cells downstream from the C-type lectin receptors Dectin-1, Dectin-2 and MINCLE, which recognize fungal pathogen-associated molecular patterns. After coil-coiled domain phosphorylation by PKC-δ, activated CARD9 couples with BCL10 and MALT1, resulting in NF-κB activation, leading to the secretion of proinflammatory cytokines. . CARD9-

deficient peripheral blood mononuclear cells contain a smaller than normal proportion of IL-17 T cells, but the role of IL-17 immunity in human defense against Candida invasive infection remains unclear. CARD9-deficient peripheral blood mononuclear cells contain a smaller than normal proportion of IL-17 T cells, but the role of IL-17 immunity in human defense against Candida invasive infection remains unclear. In addition, Peripheral blood mononuclear cells and neutrophils display impaired proinflammatory cytokine release in response to Candida stimulation. Moreover, an impairment of neutrophil killing of unopsonized Candida yeasts has been observed, despite normal levels of NADPH oxidase activity in response to Candida stimulation in the patient tested. Nonetheless, it was suggested that the patient's neutrophils displayed an abnormal phagolysosome function in contact with C. albicans, on the basis of electron microscopy. The preferential central nervous system location of Candida infection in CARD9- deficient patients may also result from the inability of monocytes, macrophages and/or microglial cells to eliminate Candida efficiently at the blood brain barrier [(57)].

Immunological aspects of chronic hyperplastic candidosis

As far as specific immunity against oral candidiasis is concerned with both secretory IgA and cellular immunity play a role in the protection of the oral mucosal surfaces against candidal infection. A markedly increased prevalence of candidal infection may be seen in IgA-deficient person. Patients with chronic mucocutaneous candidosis, which is a systemic disease with widespread chronic hyperplastic candidosis lesions, about 50% appear to have reduced IgA antibodies. Specific antibodies of IgG and IgM types against the cell or antigens of Candida are found in most individuals, and those who carry Candida only as a commensal in their mouths or other mucosal surfaces, such as the vagina. Serum antibodies against whole cells of Candida appear to vary between individual with different types of candidiasis.

Studies show that antibody titers in chronic hyperplastic candidiasis patients were not as high as in patients with Candida-associated denture stomatitis. The larger mucosal area involved in the latter condition with the concurrent transmucosal penetration of antigens compared chronic hyperplastic candidosis. Serum antibodies are usually not capable of killing Candida, even in concert with complement. However, they appear to act as opsonins for polymorphonuclear leukocytes and macrophages. They are also act as chemotactic agents for these cells, thereby

attracting them to the site of infection. As with other infections, the fixation of complement by antibody leads to the release of C3a and C5a, which are chemotactic. Immunofluorescent assays, that salivary antibody titers were raised in patients with candidiasis, although not stated specifically as candidal leukoplakia, compared with carriers and non-infected controls. Demonstrating that the rise in titer of both IgG and IgA antibodies, mainly the IgA antibodies, was able to inhibit the adherence of Candida albicans to buccal epithelial cells. The main difference in the numbers of cells, however, was not statistically significant. Whereas candidal anti gens themselves were not detectable, these workers found ATPase-positive Langerhans cells among, or at least near, intra-epithelial hyphae and T6-positive cells separated from the hyphae by epithelial cells. Postulated this difference in distribution of ATPase-positive and T6-positive Langerhans cells may indicate locations of two cell subtype, or a change in T6 antigen expression by the Langerhans cells closest to the candidal hyphae[58].

Nutritional Status

Among all the nutritional deficiency states, iron has been the one of the most common deficient essential micronutrient implicated in the colonization of candida. Iron deficiency diminishes the fungistatic action of transferrin and other iron-dependant enzymes. In addition, other nutrients frequently deficit in chronic candidiasis includes zinc vitamins A and B6, magnesium, folic acid, selenium, and essential fatty acids [44].

The major role of iron deficiency shows that oral candidiasis may be caused in the deficient individual by at least four mechanisms that render the oral mucosa susceptible to infection by the fungus. Iron deficiency sometime source of epithelial abnormalities such as atrophy and hyperkeratosis through alterations in the kinetics of the rapidly dividing cells of the oral mucosa, which, in turn, result from an impairment of iron-dependent enzyme systems. Iron deficiency have been responsible for depression of cell-mediated immunity both in vivo and in vitro and May also cause defects in phagocytosis and inadequate antibody production. Person with chronic hyperplastic candidiasis suffered from a deficiency in folic acid, hematological abnormalities in patients with non-ulcerative lesion of the oral mucosa, including leukoplakia. Few studies

isolated reports of a link between deficiencies of Vitamins K and C and zinc and the presence of oral candidiasis.

It is probable that deficiencies in the above-mentioned micronutrients act not only alone but also in concert, through their direct effect on the nutrition and kinetics of the oral epithelium as well as the systemic effects they may cause [59].

Endocrine Disorders

Oral Candidiasis and diabetes mellitus

Prevalence of oral candidiasis is increasing. Oral candidiasis can be diagnosed by the differential patterns of mucosal changes like pseudomembranous, erythematous and curd-like plaques. Higher Candida sp. colonization rates were reported in patients with diabetes mellitus (type 1) when compared to diabetes mellitus (type 2) patients 84% vs. 68% respectively, while the percentage in non-diabetic subjects was around 27%.

Colonization does not all the time lead to infection. It is a prelude to infection when host immunity is compromised and the risk of a disseminated infection is high. Such infections continue to be a utmost healthcare challenge. So many risk factors for oral candidal infection are complex and diabetes mellitus clearly influence oral Candida sp. carriage & the upsurge of oral candidiasis.

The causes influencing the higher incidence of oral candidiasis in diabetic patients are presented [60].

Factors	Physiopathology
Uncontrolled hyperglycemia (high HbA1c) and high glucose levels in saliva	-Uncontrolled hyperglycemia may cause intensification in salivary glucose levels because in diabetics the basement membrane of the parotid salivary gland is more permeable. -High glucose levels allow Candida sp. to multiply, even in the

	presence of normal bacterial flora. -During hyperglycemic episodes, the chemically reversible glycosylation products with proteins in tissues and the accumulation of glycosylation products on buccal epithelial cells may sequentially increase the number of available receptors for Candida sp. -Glucose suppression of the killing capacity of neutrophils, emphasizing colonization (immunosuppression). -Glucose, maltose, and sucrose boost the adhesion of Candida to buccal epithelial cells.
Lower salivary pH	The growth of Candida in saliva is accompanied by a rapid decline in pH, which favors their growth and triggers the extracellular phospholipase (PL) and acid proteases, increasing the yeast adhesion to oral mucosal surfaces.
Tissue response to injury is diminished	Diabetes mellitus is known to diminish the host resistance and modify the tissue response to injury. This can result in severe colonization, even in the absence of any clinically evident oral candidiasis and possibly with further dissemination via the blood.
Oral epithelium	It is most probable that the host oral epithelium of patients with diabetes favors the adhesion of colonization and subsequent infection.
Poor oral hygiene	The lack of control of the oral environment, especially concerning the prevention of dental caries (coronary, root, and periodontal), leads to a higher rate of oral candidiasis, especially in DM older patients.

Aging gender	Diabetic women, orally colonized with Candida sp. have higher oral glucose levels than diabetics without oral Candida sp.
Prostheses	Inadequate use of prostheses, together with inadequate hygienization, favours the growth of Candida sp.
Medications	Xerostomia (abnomal lack if saliva): Candida sp. stagnation and growth on oral tissues.

Table- 2

Oral Candidiasis in Cushing's syndrome

It is a condition identified by supraphysiological cortisol levels that can occur because of either augmented endogenous production or exogenous administration of corticosteroids. Common complications of Cushing's syndrome are a predisposition to mucocutaneous and invasive fungal infections.

Cushing's syndrome, some invasive fungal infections arise in those with endogenous Cushing's syndrome. Adrenocorticotropic hormone -dependent hypercortisolemia from a functioning pituitary adenoma, and also known as Cushing's disease, makes up approximately 70% of cases of endogenous hypercortisolemia. In contrast, Adrenocorticotropic hormone -independent hypercortisolemia contributes to approximately 15% of cases of endogenous Cushing's syndrome. As observed in our patient, the most common cause is an autonomous overproduction of cortisol from an adrenal tumor. The predisposition to fungal infections among patients with hypercortisolemia has been noted since Cushing's original description of the disease. Hypercortisolemia has been shown to impair neutrophil adherence to the endothelium, to decrease degranulation capacity and phagocytic action, to diminish maturation of macrophages, and to down-regulate multiple pro-inflammatory cytokines. fungal infections such as histoplasmosis, aspergillosis, pneumocystosis, cryptococcosis, and candidiasis in patients with endogenous Cushing's syndrome. With an endogenous hypercortisolemia that developed concomitant invasive candidiasis and cryptococcosis. Higher levels of endogenous

hypercortisolemia increases the risk of infection and with its anti-inflammatory properties contributes to the subclinical manifestations of these infections. Invasive fungal infections should be suspected by physicians in patients with endogenous hypercortisolism with early and minimal signs and symptoms of infection unresponsive to broad-spectrum antibiotics. Laboratory tests with a high degree of sensitivity and specificity such as the detection of serum cryptococcal capsular polysaccharide antigen, urinary histoplasmosis antigen in urine, serum galactomannan, serum b-D glucan, and PCR assays for fungi deoxyribonucleic acid detection are important diagnostic tools for screening invasive fungal infections in these patients. In addition to specific and aggressive antifungal therapy, pharmacological and surgical treatment for lowering plasma cortisol levels to physiological ranges appears to be a reasonable approach considering that extreme hypercortisolism is an immunological endocrine emergency [(61)].

Malignancies,chemotherapy & radiotherapy

Oropharyngeal candidiasis is one is the common fungal infection in immunocompromised individuals. Conditions like chemotherapy, malignancies and radiotherapy compromise the cell mediated immunity predisposing the individuals to fungal infections. Incidence of oral candidiasis has been reported among cancer patients head and neck malignancy, hematopoietic malignancy, and solid tumors on chemotherapy and or radiotherapy. A higher incidence of oral colonisation with non-candida albicans has been reported in patients with advanced stage of cancer. Although Candida albicans and non-Candida albicans are closely related, they differ in the antifungal susceptibility patterns. The colonised Candida can invade the underlying mucosa and enter the blood stream leading onto disseminated disease with considerable mortality and morbidity if not treated properly.

The various other risk factors are use of antibacterials and steroids, comorbid illnesses like diabetes, poor oral hygiene, and tobacco usage. Candida infection in patients with malignant diseases can lead to invasive infection and candidemia. The change in the etiology of oral candidiasis from Candida albicans, the commonly encountered species, to non-Candida albicans like Candida glabrata and Candida krusei, the more inherently medication resistant species, is particularly challenging for choosing the antifungal medication. Fluconazole is the first line of medication used to treat fungal infections in head and neck cancer. Increase in resistance to fluconazole is being reported among cancer patients. The common cancer type encountered is

carcinoma of oral cavity followed by malignancy of gastrointestinal tract. Dryness of mouth and pain in the oral cavity are the most frequently encountered symptoms. Chemotherapy or radiotherapy may cause dryness of mouth and can cause mucosal disruption facilitating infection by Candida. The association between the presence of a symptom and the isolation of Candida is not causal all the time. However, from a clinical viewpoint, associating certain clinical signs and symptoms with the microbiological findings will be helpful to ascertain the affliction of the sign/symptom and a necessity to identify and treat the cause. Such associations might be useful clinically. Direct gram staining of the specimen along with the clinical signs and symptoms for oral candidiasis can be a valuable tool in differentiating colonization from infection. In the present study, 95% of the Candida albicans showed germ tube test positivity. Although germ tube test, a simple rapid test, offers 95% consistency for identifying Candida albicans, it must be used in concurrence with other phenotypic tests for species identification. Oral candidial infection is seen highest among patients with carcinoma of oral cavity (68) (89%), followed by carcinoma of gastrointestinal tract (68%).

Candida albicans have demonstrated a higher percentage of resistance compared to Candida albicans for the empirically used medications like fluconazole and Itraconazole. High incidence of oral colonization and infection with such inherently medication resistant isolates becomes more challenging for choosing the prophylactic medication. Such medication resistant isolates can invade underlying mucosa and enter the blood stream causing invasive infections. Prevalence of fungal infections has increased several times among individuals with lowered immune status such as cancer patients on chemotherapy and radiotherapy. Cytotoxic therapy causes dryness of oral mucosa facilitating infections by various pathogens. Studies have reported that development of candidiasis is a two-step process consisting of colonization and subsequent invasion of epithelial layer. Once colonization establish, impaired cellular immunity permits invasion of epithelial layer. Neutropenia, irradiation, and chemotherapy will lead to mucosal disruption facilitating deeper invasion by Candida. The emergence of antifungal resistance within Candida species particularly in cancer patients is of serious concern because such medication resistant isolates may invade the deeper tissues leading to disseminated infection. The high prevalence of Candida in the oral cavity of cancer patients treated by chemotherapy/radiotherapy necessitates the need for routine periodic surveillance of fungal infections to determine the antifungal resistance pattern [(62)].

Congenital Conditions

Congenital candidiasis presents with a variety of clinical features, ranging from a diffuse erythematous skin eruption, with or without vesicles and pustules, to systemic disease in which the lungs are usually affected.Congenital candidiasis should be considered in the differential diagnosis of neonatal generalized maculopapular or pustular skin eruptions, along with some other disorders such as Listeria monocytogenes infection, impetigo, chickenpox, herpes virus infection, syphilis and epidermolysis bullosa. Diagnosis may be confirmed by microscopic examination and culture of the skin lesions. Infants with systemic fungal infections most commonly present with respiratory distress and an elevated white blood cell count with a left shift, reaching the level of a leukemoid reaction, particularly within the first 3 days of life. Persistent hyperglycemia and glycosuria are also seen. Full term infants with congenital candidiasis and skin involvement alone, the clinical course is often benign, and only topical therapy or no therapy at all is required. In infants with respiratory distress and clinical and laboratory signs of sepsis however, systemic antifungal therapy with amphotericin B is recommended. Very-low-birth-weight infants with congenital Candida infection are more likely to present with severe infections, such as pneumonia and widespread dermatitis with focal areas of superficial erosion and desquamation. Extremely-low-birth-weight infants with congenital cutaneous candidiasis are at greater risk of developing invasive fungal infection (66%) than low-birth-weight or term infants. vesiculopustular eruptions are common in newborns. Congenital candidiasis may present as this type of skin lesion. The rarity of this condition can lead to failure to recognize it promptly and so to unnecessary treatment and anxiety [(63)].

3.6. Laboratory diagnosis of oral candidiasis

The diagnosis of oral candidiasis is fundamentally clinical. A microbiological diagnosis is performed when the clinical diagnosis requires confirmation; for establishing a differential diagnosis with other diseases; in cases characterized by resistance to antifungal medications; and in hyperplastic candidiasis, where biopsies are made. The methods most frequently used for the diagnosis of primary candidiasis are smears, stains (10% KOH) and cultures (Sabouraud dextrose agar). In determining the species of Candida, the most widely used techniques are

CHROMagar, since they offer good sensitivity and specificity and are accessible and simple to use – allowing the presumptive identification of most Candida species.

a) Specimen collection

The specimen should always be collected from an active lesion and old 'burned out' lesions that do not frequently contain viable organisms. Accumulate the specimen under aseptic conditions. Collect sufficient specimen. Use sterile collection devices and containers Label the specimen appropriately; all clinical specimens should be considered as potential biohazards and should be handled with care using universal precautions. The specimen should be kept in moist environment and stored in a refrigerator at 4ºC. Due to various varieties of clinical forms of oral candidiasis, a number of different types of specimens may be submitted to the laboratory [(64)].

b) Smear

1. Using a pencil, label the frosted end of a microscope slide with the patient's name.
2. Remove cells from the oral mucosa with a Cytobrush or tongue blade and spread them evenly on the microscope slide.
3. Repeat procedure on a second microscope slide.
4. Spray the slides lightly and fixed immediately in ether/alcohol 1:1 or with spray fix.
5. Dry preparations may be examined by Gram stain method and periodic acid Schiff (PAS) method.

c) Swabs

1. Specimen type- Mouth swab, oral swab, tongue swab.
2. Container Sterile, individually wrapped rayon tipped swab with a semisolid transport medium.
3. Method of collection -Swab should be taken from the suspected area of infection (use of a tongue depressor or spatula may help) Denture fitting surfaces should be swabbed if present. Gently rotate the swab over the affected area. Place the swab into the transport medium immediately after swabbing.

4. Transportation- Place the labelled swab inside a sealed plastic bag attached to a correctly filled in microbiology request form, transport the specimen to the Microbiology Department.
5. Storage- Specimens that cannot be transported or processed immediately should be refrigerated (2-8°C) [64].

d) Biopsy

Oral Biopsy is a surgical procedure to obtain tissue from the patient's oral cavity, for microscopic examination, usually to perform a diagnosis.

Types of Oral Biopsy

There are six main types of Oral Biopsy. These are:

- **Cytology:** This type of Oral Biopsy can be used to diagnose lesions in the oral cavity due to post-radiation changes, herpes, and fungal infections. Cytology allows examination of individual cells but cannot necessarily result in an accurate and definitive diagnosis. It is recommended that this type of Oral Biopsy be performed along with an Excisional or Incisional Biopsy.
- **Aspiration Biopsy:** In this type of Oral Biopsy, the oral surgeon uses a needle and syringe to remove a sample of cells or contents of a lesion. If the oral surgeon is not able to withdraw fluid or air it probably means that the lesion is solid.
- **Incisional Biopsy:** This type of Oral Biopsy is performed only to sample a representative portion of the oral lesion. If the lesion is large or has many differing characteristics, it may require sampling of more than one area.
- **Punch Biopsy:** This is done with a punch tool for both incisional and excisional purposes. This type of Oral Biopsy is best suited for the diagnosis of oral manifestations of mucocutaneous and ulcerative conditions of the oral cavity, such as lichen planus.

- **Brush Biopsy:** In this type of Oral Biopsy, firm pressure with a circular brush is applied, and rotated give to ten times, causing light abrasion. The cellular material picked up by the brush is transferred to a glass slide, preserved and dried.
- **Excisional Biopsy:** This type of Oral Biopsy is performed for small oral lesions, usually less than 1 cm. On clinical exam, the lesion appears benign. This type of Oral Biopsy results in complete removal of the lesion.

Procedure of Oral Biopsy

Prior to performing an Oral Biopsy, the oral and maxillofacial surgeon should be fully aware of the patient's medical status. This would involve a thorough review of the patient's medical and dental history, including previous surgeries, medications, allergies, and any social behaviors, such as smoking, alcohol use, and illicit medication use. The oral surgeon should also perform a thorough clinical and radiographic exam.

An Oral Biopsy is usually performed under either a local anesthetic, applied locally with infiltration near the lesion, or then, regionally, with a nerve block technique. However, the anesthetic should not be applied directly into the lesion, or superficially in and around the lesion, as this may result in a false diagnosis by the oral pathologist.

Once the local anesthetic is applied, the oral surgeon should firmly retract the soft tissues surrounding the lesion, and should make an elliptical incision around the lesion, with a minimum of a 1 mm margin.

Once the lesion has been freed from the remaining soft tissues, it should be immediately placed into a sterile pathology specimen jar, containing 10% buffered formalin for fixation. The container must be labelled with accurate patient information, such as patient name, birth date, date, and site of biopsy [(64)].

e) **Imprint culture technique**

Sterile square shape 2.2 × 2.5 cm plastic foam pads are dipped in peptone water and then placed on the restricted area under study for 30-60 seconds only. Afterwards the pad is placed directly

on Pagano-Levin or Sabouraud's agar, left in situ for the first 8 hours of 48 hours incubation at 37ºC.

Then, the candidal density at each site is determined by a Gallenkamp colony counter and expressed as colony forming units per mm2 (CFU mm-2).Thus, it yields yeasts per unit mucosal surface.

It is useful for quantitative assessment of yeast growth in different areas of the oral mucosa and is thus useful in localizing the site of infection and estimating the candidal load on a specific area.

It is a sensitive & reliable method for oral sampling. Disadvantages of imprint culture is that a number of areas may have to be sampled, the appropriate sites for sampling is subjective, there can be accidental risk of inhalation of the foam pad by the patient and lastly, the need to store sterile foam pads or culture media in the clinic [65].

f) Impression culture technique

With the help of alginate maxillary and mandibular impressions taken, transporting them to the laboratory and casting in 6% fortified agar. Saliva samples are collected under standard conditions, immediately placed on ice, and cultured within 1 h. Subjects not stimulated to salivate is ask to expectorate into sterilized, wide-mouthed centrifuge tubes all saliva during a 15-min period. Such unstimulated whole saliva is made up of secretions from the parotid, submandibular, and minor salivary glands. The 15- min period was used to estimate the flow rate of unstimulated saliva and to provide adequate volumes.

A 0.5-ml amount of un-centrifuged saliva are spread on Sabouraud glucose agar plates containing 50 mg of chloramphenicol per ml; plates were incubated at 37°C for 48 h, and the number of CFU per milliliter of saliva are counted. Plates without fungal growth at 48 h were incubated for up to 2 weeks before being discarded as negative. The medium and culture conditions employed selected for yeast growth, and usually all the colonies were similar in appearance; therefore, one or a few colonies are sub-cultured and determined to be either C.

albicans or not C. albicans. Yeasts were identified as C. albicans on the basis of positive germ tube formation, growth on cornmeal agar, and carbohydrate assimilation tests [66].

g) Salivary Culture Technique

This technique is a sensitive & as accurate as an imprint culture .But the method involves considerable chair time,depending on the salivary flow rate of the patients .This simple technique involves requesting the patient to expectorate 2 ml of mixed unstimulated saliva into a sterile, universal container which is then vibrated for 30 second on a bench vibrator for optimal disaggregation.

This involves requesting the patient to expectorate about 2 ml of mixed unstimulated saliva into a sterile, universal container. The number of Candida expressed as CFU per milliliter of saliva is estimated by counting the resultant growth on Sabouraud's agar.

the quantitative culture of saliva is a useful adjunct in diagnosis of oral candidasis. They demonstrated that carriers and patients with oral Candida can be distinguished reliably (with95% confidence limits) on the basis of quantitative culture. Patients with clinical candidasis harbor greater than 400 CFU of Candida per milliliter of saliva [65].

h) Oral rinse technique:

In this method asked patient to rinse the mouth for 60 seconds, with 10 ml of phosphate-buffered saline supplied in a universal container. The patient then returns oral rinse to the universal container, which is sent to the laboratory. In the laboratory the oral rinse is concentrated by spinning for 10 minutes & resuspending the deposits in 1 ml of sterile phosphate-buffered saline. The concentrated oral rinse is transferred on appropriate media to assess CFU/ml of rinse sample using a spiral plater or Gallenkamp colony counte.

The concentrated oral rinse culture technique has a number of advantages over the imprint technique. It is simple to perform as it does not involve the clinician in judgement of the

sampling site. In addition to Candida species, a single rinse sample can be used for quantitation of other organisms such as coliforms [64].

Commercial identification kits

Identification method beginning with conventional approaches and followed by rapid screening tests, chromogenic media, and comprehensive manual and automated commercial methods. Immunologic methods like latex agglutination tests are also available for several remarkable yeast species.

Conventional Wickerham method

About 60 years ago, Wickerham described a broth method for assimilation and fermentation testing of yeasts. Assimilation tests determine the ability to use substrates as the sole source of carbon (e.g., sucrose) or nitrogen (e.g., KNO3). This method is a powerful tool and was used for definitive characterization and taxonomy of yeasts. Most Wickerham media are not commercially available and must be homemade. This is a long arduous process that only rare laboratories still employ. Basal media and various substrates are prepared, sterilized, and dispensed prior to inoculation. Since many types of yeast can 'carry-over' nutrients from the isolation medium, one must run negative controls for each test type and organism. For up to 4 weeks, assimilation tests are read for turbidity and fermentation tests are read for gas production. This 'gold standard' is inappropriate for use in a routine laboratory and has been replaced by the more practical methods available today.

Conventional auxanographic method

A more practical approach for comprehensive phenotypic identification was the dye pour-plate auxanogram. This dye pour-plate auxanogram improved the modified Wickerham medium with agar and pH indicator (bromocresol purple) in a tube slant with carbohydrate. With dye pour-plate auxanogram, one could test multiple substrates on an agar plate rather than multiple tubes. Addition of agar allowed for more rapid incubation. Suspending the organism within the medium allowed easier detection of weak reactions when compared to surface swab inoculation of dye pour-plate auxanogram plates. Bromocresol purple allowed for easier interpretation compared to turbidity measurements. Further modification employed a higher yeast nitrogen base concentration and eliminated the pH indicator that could inhibit certain yeasts or be

misinterpreted when reversions occurred. Another of the early commercial approaches included the Uni-Yeast-Tek introduced in 1975. The 1970s hosted the advent of these commercial yeast identification systems followed by many others in both manual and automated arenas. Several of these systems are discussed below but first we turn to rapid screening tests that were being developed for the most common opportunistic pathogens [67].

Rapid screening tests

In parallel to the evolution of rapid comprehensive commercial methods, there was also emphasis on development of key rapid tests that would enable presumptive identification of the most important etiologic agents i.e., C. glabrata, Candida albicans, and Cryptococcus neoformans of yeast infections. In addition, focus of these rapid tests has been to screen for species e.g., C. glabrata commonly associated with resistance to antifungal compounds.

10 mm × 10 mm of modified Nickerson medium and a plastic pouch used for incubation in the Microstix-candida system consists of a plastic strip to which is affixed a dry culture area. The O Yeast-I dent system is based on the use of chromogenic substances to measure enzyme activities. Ricult-N dip slide technique is similar to, but of higher sensitivity than Microstix-candida system [64].

Rapid Commercial Systems For Identification Of Clinically Important Yeasts [65]

Integral diagnostic systems

– API 20 C

– Auxodisk

– Iatron Candida check

– Micro-drop – Minitek(BBL)

– Mycotube(Roche)

– Uni-Yeast-Tek

– Randolph Multitest Mycology Plate

– Yeast Ident

Instrumental systems

– Abbott MS-2

– Autobac 1

– Auto Microbic System

i) Paper Points

For 10 sec an absorbable sterile point is inserted to the depth of the pocket and kept there and then transferred the point to a 2 ml vial containing Moller's VMGA III transport medium and which also facilitates survival of facultative and anaerobic bacteria.

3.7. Histological identification

Demonstration of fungi in biopsy specimens may require several serial sections to be cut. Fungi can be easily demonstrated and studied in tissue sections with special stains. The routinely used Hematoxylin and Eosin stain poorly stains Candida species. The specific fungal stains such as Periodic Acid-Schiff stain, Grocott-Gomori's methenamine silver and Gridley stains are widely used for demonstrating fungi in the tissues, which are colored intensely with these stains [(64)].

Very useful & popular primary culture media is Peptone – glucose (dextrose) or peptone – maltose agar .It was first coined in 1896 by Sabouraud & hence known as " Sabouraud's agar ",which shows Candida colonies as cream coloured smooth or rough, shiny or dull,convex in appearance.Candida albicans can also be formed on blood agar,where they appear as moist, opaque,whitish colonies characteristic of yeast.Candida albicans on a Dalmau plate appears as clusters of round blastoconidia are present at some septae,with thickwalled terminal chlamydospores (characteristic of C. albicans) are seen .Pagno – Levin Media allows various yeast species to be distinguished through visual differences in coloration eliciting degrees of pink ,blue and orange colouration..The medium is superior in detecting multiple yeast species in a single sample. Chrom Agar is another media detecting multiple yeast species in a single sample

CHROM agar can differentiate C.albicans as green colonies, C. krusei as pink colonies and C.tropicalis as blue colonies [66].

Although swabs and smears are essential for a micobiological diagnosis of a number of types of oral candidosis when candidal leukoplakia (chronic hyperplastic candidosis) is suspected, a biopsy specimen should be taken. Because Candida species stain poorly by hematoxylin and eosin, staining with periodic acid Schiff or Gridley's or Gomori's methenamine silver stains are used. In both Gridley's and the periodic acid Schiff procedure, the fungi appear a pinkish-red. The GMS technique stains yeast cell walls brownblack because of deposition of reduced silver. The presence of blastospores and characteristic pseudohyphae or hypae in the superficial epithelial tissues identifies the fungus as a species of Candida. However, as the speciation of the organism cannot be performed by this means alone, cultural studies should also be used.

Blastospores similar to those of Candida species may be seen in histoplasmosis or cryptococcosis, both of which are becoming increasingly important and may manifest orally with increasing frequency in Acquired immunodeficiency syndrome patients. Therefore, if only blastospores of Candida are seen in tissue sections of suspect patients, serial sections should be carefully searched for pseudohyphae or hyphae of Candida species [68].

3.8. Phenotypic methods

Serotyping Serotyping is limited to the two serotypes A and B, a fact that makes it inadequate as an epidemiologic tool. It has recently been shown that there can be wide discrepancies in the results obtained with different methods of serotyping,

Resistogram typingResistograms do not correlate with pathogenic potential and even though improvements have been made in the method growth end-points often present problems because of inoculum size, interpretation and reproducibility.

Yeast ‘Killer Toxin’ typing These authors initially used nine killer strains, developing a triplet code to distinguish between 100 strains of C. albicans and found 25 killer- sensitive types. This method was expanded by using 30 killer strains and three antifungal agents, which appeared to discriminate between sufficient numbers ofstrains of C. albicans.

Morphotyping This method has been used in a study of the morphotypes of 446 strains of C. albicans isolated from various clinical specimens.

Biotyping Williamson (1987) has proposed a simpler method. This system comprised three tests, the APIZYM system, the API 20C system and a plate test for resistance to boric acid. This system was found to distinguish a possible 234 biotypes, of which 33 were found among the 1430 isolates of C. albicans taken from oral, genital and skin sites.Protein typing Non-lethal mutations of proteins during the yeast cell cycle yield proteins of differing physical properties between strains, which may be distinguishable by one or two dimensional gel electrophoresis. These methods have been used to separate C. albicans at the subspecies level [(64)].

3.9. Genetic methods

The earliest molecular methods used for fingerprinting C. albicans strains were karyotyping, restriction endonuclease analysis REA and restriction fragment length polymorphism RFLP. In arbitrarily primed polymerase chain reaction analysis (synonym: randomly amplified polymorphic deoxyribonucleic acid RAPD analysis, the genomic deoxyribonucleic acid is used as a template and amplified at a low annealing temperature with use of a single short primer (9 to 10 bases) of an arbitrary sequence[(64)].

DNA fingerprinting with C3a prob

One of the most common methodologies employed in genotyping through deoxyribonucleic acid fingerprinting is Restriction Length Polymorphism followed by the Southern blot technique and specific probe hybridization. The most successful hybridization probes for fungi are fragments containing repetitive genomic sequences, such as Ca3. Ca3 is a moderately repetitive 11-kb C. albicans gene fragment which has been used as an effective deoxyribonucleic acid fingerprinting probe in several epidemiological studies.

When probed with the entire Ca3 fragment, EcoRIdigested deoxyribonucleic acid of C. albicans strain 3153A exhibit patterns composed of 10 to 20 bands of relatively high intensity and 4 to 6 bands of low intensity. While the entire pattern of hybridization has been used to assess the relatedness of isolates, the subset of hypervariable bands have been used to monitor the microevolution of strains at sites of infection or carriage. The software Dendron is employed to generate dendrograms, through computational analysis of the patterns obtained by Southern

blotting hybridization. The software combines the results of image processing, gel image analysis, computation of similarity coefficients, genesis of dendrograms, and is also able to storage the data for future retrospective analyses. Because Dendron retains the digitized Ca3 Southern blot hybridization pattern of every C. albicans strain analysed, and retrospectively compares all newly analysed strains with all previously analysed strains, a data bases for epidemiological studies have been developed. These data have been considered in the analysis of geographical distribution, transmission, and strain specialization.

The fingerprinting of C. albicans employing Southern blot hybridization with the midrepeat sequence Ca3 has proven to be reproducible and highly amenable to computer-assisted analysis. Pujol et al used Ca3 fingerprinting to analyse the genetic relatedness of a small collection of C. albicans isolates recovered in the United States of America, and clustered the samples into three groups named I, II and III. Subsequently, Blignaut et al analysed the genetic relatedness of C. albicans collected from the oral cavity of human immunodeficiency virus positive and healthy South African individuals. This work revealed a South African specific group, named SA. This C. albicans group is present in 53% of isolated collected from black South Africans and it is also present in 33% of the isolated collected from white South Africans. These results clear demonstrated an interesting pattern of racial differences in host colonization. Furthermore, Pujol et al identified the presence of a European-specific group (group E), and this group represented 26% of European C. albicans isolates. It is also interesting to note that when samples obtained in USA Southwest and South America were analysed from genetic relatedness, no isolates clustered into group II. The importance of this series of articles was the establishment of clades with specific characteristics that can be compared with future epidemiological assays.

Another interesting work was developed by Edelmann et al using deoxyribonucleic acid fingerprinting with Ca3 probe. They verified the genetic relatedness of C. albicans cultures obtained from human and animal sources. The phylogenetic analysis did not reveal the existence of species-specific lineages, which suggested that animals could be possible resources for Candida infection. However, although no species specificity has been demonstrated, different C. albicans clades may differ in the frequency in which they colonize various species. Deoxyribonucleic acid fingerprinting with the complex probe Ca3 thus represents an interesting

method for Candida genotyping which has been contributing to the understanding of the differences between strains traits.

Multilocus Sequence Typing

The technique is used for typing multiple loci in the genomic deoxyribonucleic acid and became the method of choice for Candida typing. MLST involves deoxyribonucleic acid amplification by Polymerase Chain Reaction followed by deoxyribonucleic acid sequence. It measures the deoxyribonucleic acid sequence variation in a set of housekeeping genes and characterizes strains by their unique allelic profiles. The characterization is based on the analysis of nucleotide polymorphisms of the sequences of approximately 450- to 500- bp internal fragments of housekeeping genes. For each housekeeping locus, different sequences present within the species are considered as distinct alleles. The large number of alleles at each housekeeping gene analyzed permits the construction of different allelic profiles. These data can be used to construct a dendogram using the matrix of pairwise differences between their allelic profiles that can be assumed to be derived from a common ancestor.

Bougnoux et al and Tavanti et al were the first researches that described a set of gene fragments for MLST of C. albicans. Based on these previous efforts, Bougnoux et al developed an optimized protocol for MLST of C. albicans. The authors proposed the following set of gene fragments as international standard protocol for MSLT in the fungi: AAT1a, MPIb, SYA1, VPS13, ACC1, ADPI, and ZWF1b. This set yielded unique diploid sequence types for each isolate tested. According to Chowdahry et al, MLST can be applied to define genetic relatedness of sequential C. albicans isolates, achieving equal or even better results than when the analysis is performed by Ca3 Southern hybridization. Similarly, Robles et al demonstrated that MLST is at least comparable with random amplified polymorphic deoxyribonucleic acid, multilocus enzyme electrophoresis and Southern blotting in discriminating C. albicans strains.

The correspondence between the clade systems developed by MLST and Ca3 hybridization is remarkable, because epidemiological studies performed with both methodologies can be compared. Regarding the anatomical sites, the proportion of isolates from blood and other sterile sites that belonged to clade I was lower than in the other major clades. Considering clade I isolates, Four out of five isolates belonged to cluster 1 when eBURST algorithm analysis was performed. Similarly, clade II isolates were related to cluster 2, clade SA isolates corresponded

to clade 3, and clade E isolates to clade 4. Clade III isolates, as determined by Ca3 hybridization, corresponded to clusters 9 or 10 when analyzed by eBurst algorithm. In a comparable study, Odds et al evaluated 1391 isolates and demonstrated that 97% of them could be grouped in one of the 17 groups or clades based on their MLST profile. Groups 1 to 4 comprised the majority of the isolates and these groups corresponded to clades I, II, III and SA respectively, as determined by Ca3 hybridization. Similarly, Clade 11 corresponded to clade E. This study also established that the proportions of A, B, and C genotypes, defined by the presence or absence of an intron in the ribosomal DNA region, differed significantly among clades. In this work, 93% of strains in clades 1 and 2 were type A. Furthermore, North American isolates were predominantly assigned as clades 1 and 3 and 40% of African isolates were grouped into clade 4.

The studies mentioned above demonstrated that results obtained by MLST can be compared with those obtained by Ca3 hybridization, although the correlation could not be totally demonstrated for all clades. This is an interesting point, due to the possibility of using the results obtained in epidemiological studies. Considering the practicability of the MLST experimental performance and analysis, it is considered as the method of choice for Candida genotyping, which has been largely contributing to the understanding of the evolutionary origins of Candida albicans different strains.

Microsatellites analysis

Microsatellites are short tandem repeated sequences interspersed randomly through the nuclear and organelles genome, and basically the repeating unit consists of fragments shorter than 10 bases pair. Between the individuals the repeating units number found in the microsatellite varies, which contributes with allele polymorphisms. Besides, the microsatellites are quite unstable and high polymorphic due to mispairing slippage during replication, which lead to their expansion or contraction, and also point mutations inside or outside the repeated region in the genomic structures. Interesting they present mendelian codominant inheritance and polymerase chain reaction typing simplicity which make them useful to identify hereditary relationships and for genotyping microorganisms. Microsatellite Deoxyribonucleic acid typing can provide important correlations between genotypes and location of infection, degree of virulence, or medication susceptibility. Such correlations are crucial for proper, large-scale epidemiological analysis.

C. albicans genotyping, with special emphasis to Ca3 hybridization, MLST and Microsatellites. The arrangement of C. albicans yeasts in clades or groups provided important information about this organism regarding the geographic maintenance of strains and in the mode of reproduction, which is probably clonal with certain degree of DNA rearrangement that contributes to yeast microevolution. Virulence factors and antifungal resistance can be associated to established clades, but a broad field of investigation must be explored; several physiological traits should be reevaluated on this basis [(69)].

3.10. Serological tests

Serological tests for invasive candidiasis

- Slide agglutination
- b- (1, 3)D-glucan
- Coelectosynersis
- Immunoprecipitation
- A and B immunofluorescence
- Nonspecific Candida

Antigens Immunodiffusion

- Phytohemagglutination
- Latex agglutination
- Detection of antibodies
- Immunobloting
- Cell Wall Components
- Cell Wall Mannoprotein

• Candida Enolase Antigen testing

Swab. A swab of a lesional site is a relatively simple method of detecting growth and semiquantitative estimation of Candida can be obtained. The sampling approach involves gently rubbing a sterile cotton swab over the lesional tissue and then subsequently inoculating a primary isolation medium such as Sabouraud's dextrose agar.

Concentrated Oral Rinse. The oral rinse technique involves the patient holding 10 mL of sterile phosphatebuffered saline 0.01 M, pH 7.2 in the mouth for 1 minute. The solution is then concentrated 10-fold by centrifugation and a known volume, usually 50 µL, inoculated on an agar medium using a spiral plating system. After 24–48 hrs incubation at 37◦C, growth is assessed by enumeration of colonies and expressed as candidal colony forming units per mL−1 of rinse.

Imprint Culture. The imprint method utilises a sterile foam pad of known size (typically 2.5 cm2), previously dipped in an appropriate liquid medium, such as Sabouraud's broth, immediately before use. The pad is then placed on the target site (mucosa or intraoral prosthesis) for 30 seconds and then transferred to an agar for culture.

Culture Media. The most frequently used primary isolation medium for Candida is Sabouraud dextrose agar and allow candida to grow, and find out that due to low pH growth of many species of oral bacteria delay. Incorporation of antibiotics into Sabouraud dextrose agar will further increase its selectivity. Incubated aerobically with Sabouraud dextrose agar at 37◦C for 24–48 hrs. Candida develops as cream, smooth, pasty convex colonies on Sabouraud dextrose agar and differentiation between species is rarely possible. It is estimated that more than one Candida species occurs in approximately 10% of oral samples and in recent years the ability to detect nonalbicans species has become increasingly important.

In recent years, other differential media have been developed that allow identification of certain Candida species based on colony appearance and colour following primary culture. The benefit of such media is that the presence of multiple Candida species in a single infection can be determined which can be important in selecting subsequent treatment options. Examples of these include PaganoLevin agar or commercially available chromogenic agars, namely, Albicans ID, Fluroplate, or CHROMagar Candida, Candichrom albicans.

Identification of Candida Species. Identification of yeasts based on primary culture media should be confirmed through a variety of supplemental tests traditionally based on morphological and physiological characteristics of the isolates.

Morphological Criteria. The germ-tube test is the standard laboratory techniqe for identifying C. albicans. The test involves the induction of hyphal outgrowths germ tubes and when subcultured in horse serum at 37◦C for 2– 4 hours. About 95% of C. albicans isolates produce germ tubes, property also shared by C. stellatoidea and C. dubliniensis. C. albicans and C. dubliniensis can also be identified from other species based on their ability to produce morphological features known as chlamydospores. Chlamydospores are spherical, refractile structures generated at the termini of hyphae following culture of isolates on a nutritionally poor medium like cornmeal agar. Isolates are inoculated in a cross hatch pattern on the agar and overlaid with a sterile coverslip. Agars are incubated for 24–48 hours at 37◦C and then examined microscopically for chlamydospore presence.

Physiological Criteria or Biochemical Identification. Candida species is mainly based on carbohydrate utilization. Traditional testing could have involved culture of test isolates on a basal agar lacking a carbon source. Carbohydrate solutions would then be placed within wells of the seeded agar or upon filter paper discs located on the agar surface. Growth in the vicinity of the carbon source would indicate utilization. Commercial systems are based on the same principle but test carbohydrates are housed in plastic wells located on a test strip. The Growth in each well is read by changes in turbidity or color changes in certain kit systems. Numerical codes obtained from the test results are used for comparasion.

Serology. Serological tests are commonly used to ascertain the clinical significance of Candida species isolates. Rising titers of lgG antibodies to C. albicans may reflect invasive candidiasis in immunocompetent individuals. The detection of IgM and IgA antibodies is important to identify an acute infection. Immunosuppressed individuals often show variability in antibody production and in such a case the use of an antigen detection test is recommended. And tests like enzyme linked immunosorbent assay and radio immuno assay for detection of candidial antigen, either cell-wall mannan or cytoplasmic constituents are now available in developed countries. Serological diagnosis is often delayed and the tests still lack specificity and sensitivity. Furthermore, antibody production in immunocompromised patients is variable, making diagnosis

complicated. And the fact that fungal antigens and metabolites are often cleared rapidly from the circulation and the presence of antibodies does not always imply a Candida infection, especially in person with serious underlying disease or who are taking immunosuppressive medications. Serologic tests are normally not a diagnostic tool for oral candidiasis. However, such tests may be a prognostic instrument in patients with severe oral candidiasis who respond poorly to antimycotic therapy.

Molecular-Based Identification Methods by analysis of genetic variability is a more stable approach than using methods based on phenotypic criteria. For the identification of Candida based on genetic variation are analyses of electrophoretic karyotype differences and restriction fragment length polymorphisms using gel electrophoresis or Deoxyribonucleic – Deoxyribonucleic hybridization. Species polymerase chain reaction approaches have also been used for Candida species identification. Several target genes have been reported for Candida species discrimination, although those most frequently amplified are the sequences of the ribosomal RNA operon. Identification can also be obtained based on polymerase chain reaction product sizes obtained following gel electrophoresis resolution, or polymerase chain reaction product sequence variation determined either by direct sequencing or through the use of restriction fragment analysis following cutting of polymerase chain reaction sequences with restriction endonucleases. Fluorescence in situ hybridization with peptide nucleic acid method is a new detection technique which targets highly conserved species-specific sequences in the abundant r- Ribonucleic acid of living C. albicans. Individual cells can be detected directly without the need for amplification. The technique achieves a sensitivity of 98.7–100%, with a specificity of 100%, allowing for the discrimination of C. albicans from the phenotypically similar C.dubliniensis. Molecular-based technology can also be used to identify strains of Candida species although the use of techniques such as Pulsed Field Gel Electrophoresis, Random Amplified Polymorphic Deoxyribonucleic acid analysis, and repeat sequence amplification polymerase chain reaction are largely reserved for epidemiological investigations in research of oral candidiasis.

In recent years a greater significance has been given for reliable identification of Candida species from human clinical samples. Candida is the resident microflora, appropriate isolation methods

and required to ascertain the presence in the mouth along with their number. Also important to identify the infecting strains of Candida because isolates of Candida species differ widely, both in their ability to cause infection and also in their susceptibility to antifungal agents. Various phenotypic techniques are available for identifying isolated Candida including using morphological culture tests, differential agar media, and biochemical assimilation tests. These methods are supplemented with recent molecular techniques mainly reserved for epidemiological investigations [(70)].

3.11. MANAGEMENT

Management of oral candidiasis is based on three foundations: Early and accurate diagnosis of the type of oral candidiasis, correction of the predisposing factors or underlying diseases, and the use of the most appropriate antifungal medications. Promotion of good oral hygiene and periodic oral examination, controlling predisposing or facilitating factors, are fundamental to prevent infection facilitate treatment if they occur. The choice of antifungal medication should take into account the patient immune status, the specific characteristics of oral candidiasis clinical presentation, aetiology, susceptibility to antifungal medications, organic location, dissemination and the pharmacological characteristics of the available antifungal medications administration, metabolism, elimination, interactions with other medications and toxicity. Three large families group the most commonly used antifungal medications: polyenes amphotericin B and nystatin, echinocandins anidulafungin, caspofungin and micafungin and azoles. Azoles constitute the most extensive group being divided into imidazoles clotrimazole, miconazole, ketoconazole, etc. and isavuconazole, itraconazole, triazoles fluconazole, posaconazole and voriconazole. Other medications with different antifungal actions and possible systemic use against superficial mycoses, such as and terbinafine, flucytosine, griseofulvin are not used generally in oral candidiasis. The other therapeutic alternatives under development involve the use of new antifungal medications, terpens, probiotics, peptides with antifungal activity, sera with polyclonal or monoclonal antibodies or cocktails of cytokines [(71)].

Mechanism of action

The mechanisms of antifungal action consist in the alteration of the membrane or the fungal cell wall by inhibition of molecules essential for these, such as ergosterol azoles or 1,3-ß-D-glucan echinocandins, or by binding to ergosterol polyenes, causing permeability of the cell membrane and formation. The actions of polyenes and echinocandins are generally fungicidal. Conversely, azoles are fungistatic for Candida at therapeutic dosespastilles, gels, mucoadhesive tablets, toothpastes, etc. for facilitating their therapeutic action that are very effective in curing most oral candidiasis in a few weeks. Although systemic azoles and echinocandins, with better tastes and less gastrointestinal adverse reactions, have provided new clinical options, topical therapy using nystatin polyene and miconazole azole are still the main recommended treatments for oral candidiasis due to its high efficacy, low cost, and less side effects, especially in low-income countries. The accumulated experience is important and their usefulness is clearly defined [(72)].

In 2016, the Infectious Diseases Society of America updated its clinical practice guidelines for the management of candidiasis, including oral candidiasis. These recommendations include the treatment of antifungal treatment of oral candidiasis can be topically or systemically, usually with oral formulations.

a) TROPICAL TREATMENT

Nystatin,is obtained from Streptomyces noursei, binds to the ergosterol of the fungal plasma membrane and forms pores that make it more permeable, causing a loss of intracellular potassium with a fungicidal effect. Nystatin also causes secondary cell damage by autooxidation. The anti-Candida spectrum of nystatin is quite broad. The antifungal cut-off point for polyenes in the in vitro susceptibility tests is a minimum inhibitory concentration of 1 µg/ml. Some of clinical isolates resistant or less susceptible have been described in Candida lusitaniae, Candida rugosa, Candida haemulonii, Candida lipolytica, C. guilliermondii, C. krusei and C. glabrata. Nystatin is not absorbed orally. Its parenteral use is toxic, but clinical trials have been conducted with a liposomal formulation that allows its intravenous administration. Its side effects include local irritation, and when administered orally vomiting, nausea, and diarrhoea. Nystatin is probably vary safe during breastfeeding and pregnancy. Treatment is effective only if nystatin is administered over a sufficient period. However, the unpleasant taste and this prolonged treatment pattern compromise compliance by the patient.

Nystatin suspension and pastilles in combination for two weeks achieved a higher clinical and mycological cure rates and using the nystatin pastilles alone might have a higher mycological cure rate,and when compared with using nystatin suspensions alone. Nystatin pastilles at a dose of 400,000 IU resulted in a remarkably higher mycological cure rate than that administrated at a dose of 200,000 IU. Furthermore, treatment with nystatin pastilles for four weeks gives better clinical efficacy than treatment for 2 weeks. Nystatin suspension was not a good option for infants, children, and HIV/ AIDS patients with oral candidiasis, probably because of its short-term action on the oral mucosa. Moreover, exposure to nystatin at a concentration 0.25 to 1 times the MIC value for 30 minutes resulted in a beneficial post-antifungal effect, the delay in fungal regrowth that persists after a brief exposure to an antifungal agent. Encapsulation of nystatin in nanoparticles or the inclusion in toothpastes or tissue conditioners exhibit properties that enable its in vitro functionality and can also provide the basis for new successful approaches for the treatment of oral candidiasis.

Amphotericin B is another polyene that has been used for many years in the treatment of oral candidiasis, but at present, it is practically not possible to find topical preparations of this antifungal medication in many countries, including Spain. IDSA recommends amphotericin B deoxycholate oral suspension as alternative for fluconazole-refractory oral candidiasis. In these severe situations, amphotericin B deoxycholate, the conventional intravenous formulation, and the less nephrotoxic formulations of amphotericin B liposomal and amphotericin B lipid complex may be used.

Miconazole and rest of other azoles imidazoles or triazoles block the fungal cytochrome P450-dependent lanosterol 14-α-demethylase. There action is fungistatic, causing a main alteration of the cellular permeability and the inhibition of the growth of the fungal cell. Azoles group develop their effect more slowly than polyenes, but have less toxicity because their action against fungal membranes is more selective than that of polyenes. Miconazole has a good in vitro antifungal activity against Candida but this activity is lower against some isolates of C. glabrata and C. krusei. Miconazole can be administered topically, orally or intravenously, but these latter two ways are very infrequent. It is administered as miconazole chewing gum, miconazole oral gel, miconazole buccal tablets and miconazole lacquer. In some countries, there is an alternative presentation of miconazole as one-daily 50-mg mucoadhesive buccal tablet. A daily application

is an advantage over applying nystatin four or five times a day to maintain patient compliance. Miconazole mucoadhesive tablets exhibited higher salivary concentrations & better tolerance for the patient miconazole was more effective than nystatin for pseudomembranous oral candidiasis. Single daily dose regimens of miconazole buccal tablets are useful for poorly compliant patients. However, in human immunodeficiency virus-infected patients, there was no significant difference in the efficacy between miconazole and other antifungal medications. There was no significant difference between miconazole and other antifungals in terms of the relapse rate. Despite its good properties, topical use of miconazole can cause itching or burning sensation due to local irritation less than 5% of patients. In addition, miconazole has the drawback of possible interaction with other medications, such as warfarin because inhibits the enzyme cytochrome P450. Other imidazoles for topical use are bifonazole, clotrimazole, eberconazole, fenticonazole, flutrimazole, oxiconazole, sertaconazole, sulconazole, terconazole, and thioconazole, with a broad-spectrum including Staphylococcus epidermidis and other Gram-positive bacteria [73].

b) **Systemic treatment**

Triazoles, such as isavuconazole, fluconazole, itraconazole, posaconazole, voriconazole and have a broader spectrum and they are used for the systemic treatment of many severe mycoses. There are presently oral and intravenous formulations but some of them are not available in many countries. The main disadvantages are that inhibit various isoforms of cytochrome P450, which causes an inhibition of the metabolism of other medications metabolized by this route, thus increasing their plasma concentrations. This usually occurs with immunosuppressant medications cyclosporine, tacrolimus, sirolimus, etc. oral anticoagulants warfarin, some statins, H1 antihistamines, benzodiazepines, human immunodeficiency virus infection, protease inhibitors, and calcium channel blockers. In addition, other medications, such as rifampicin, phenytoin, carbamazepine, H2 antihistamines, proton pump inhibitors and some antacids, when administered together with azoles, can reduce plasma concentrations of these. They are category C medications and you have to avoid them in pregnancy if there are other alternatives.

Oral fluconazole 100-200 mg daily for 7-14 days is recommended for treating moderate to severe oral candidiasis. Chronic suppressive therapy is generally unnecessary for oral candidiasis. If required for patients who have recurrent infection, fluconazole, 100 mg 3 times weekly

recommended. Fluconazole has a very good antifungal activity against most of the species of Candida. For the clinical isolates of albicans, parapsilosis and tropicalis, the in vitro susceptibility cut-off point is 2 μg/ml. In contrast, for isolates of glabrata, Minimum Inhibitory Concentration of fluconazole less than 32 μg/ml indicates a dose-dependent susceptibility, whereas all isolates of C. krusei are considered intrinsically resistant to fluconazole, independently of the Minimum Inhibitory Concentration. Infections caused by isolates of Candida glabrata with dose-dependent susceptibility to fluconazole can often be treated satisfactorily using doses of 800 mg/day or more. Fluconazole resistances have been described in some isolates of C. albicans and C. dubliniensis from human immunodeficiency virus -infected patients with repeated episodes of oropharyngeal candidiasis treated with fluconazole. The maximum activity of fluconazole against Candida is reached from a value of AUC24h/ Minimum Inhibitory Concentration of 25-100. Fluconazole is characterized by its excellent bioavailability and low toxicity. The incidence of adverse effects with fluconazole is low, among which the most frequent are headaches, rash, nausea, vomiting, abdominal pain and diarrhoea very serious side effects are very rare.

In fluconazole-refractory disease, itraconazole oral solution 200 mg once daily for up to four weeks is recommended. Itraconazole is a 1st generation lipophilic triazole of limited used because of its irregular oral absorption and its pharmacological interactions. It is active against so many clinical isolates of C. glabrata and C. krusei resistant to fluconazole. In the case of clinical isolates of Candida, the in vitro study susceptibility cut-off point is 0.125 μg/ml. The maximum activity against Candida is reached from an AUC24h/MIC index of 25- 100. The oral formulation can also cause gastric discomfort. There is a transient elevation of transaminases in about 2% of patients. Neuropathy, hallucinations, cerebellar alterations and hypertriglyceridemia have also been observed. It is contraindicated in patients with heart failure because of its negative inotropic effect. Oude Lashof AM et al. compared in one randomized study, the efficacy of fluconazole (100 mg/day for 10 days) and itraconazole (200 mg/day for 15 days) in cancer patients with oropharyngeal candidiasis. The fluconazole group got a clinical and mycological improvement of 66% compared to 54% for the group treated with itraconazole. Fluconazole had a significantly better clinical and mycological cure rate compared with itraconazole. Posaconazole suspension (400mg twice daily for three days followed by 400mg daily for up to four weeks) is an alternative for treating fluconazole-refractory oral candidiasis.

Posaconazole has a structure similar to that of itraconazole and shows one of the widest antifungal spectrum of all triazoles. The in vitro susceptibility cut-off point is 0.06 μg/ml and the maximum activity of posaconazole against Candida is reached from an AUC24h/MIC index of 25-100 of posaconazole. Its binding to plasma proteins is very high. The most frequent adverse effects are gastrointestinal discomfort, rash, headache and alterations in the electrocardiogram prolongation of the QT segment. Voriconazole is a second-generation triazole developed from fluconazole with also a broad antifungal spectrum that can be used as alternative for the treatment of fluconazole-refractory oral candidiasis. The in vitro study susceptibility cut-off point is 0.125 μg/ml for the clinical isolates of C. albicans, C. parapsilosis and C. tropicalis; however, it turn out to be established at 0.5 μg/ml for C. krusei. The maximum activity against Candida is reached from an AUC24h/MIC index of 25-100. It is widely distributed throughout the tissues and organs. Its fixation to plasma proteins is 60% and the oral formulation can cause gastric discomfort. Transient visual disturbances have appeared in one third of patients, abnormalities in liver enzymes that sometimes lead to suspension of treatment, rash, hallucinations, headache, confusion, hypotension and haematological alterations. Exceptional cases of clinical hepatitis, cholestasis and fulminant hepatic failure have been reported. lated to fluconazole and voriconazole, with one of the widest antifungal spectrum of all triazoles that presents a very high oral absorption not interfered by the presence of food, gastric pH modifications, or mucositis. Its distribution volume is high, in spite of it is highly bound to plasma proteins. The pharmacological interaction with other medications seems to be lower compared to other azoles, facilitating the management of this interaction and which is probably the most important advantage of this antifungal medication.

Some family of semisynthetic lipopeptides with a most selective target and the biosynthesis of 1,3-ß-D-glucan of the fungal wall by blocking the activity of the enzyme beta-glucan synthetase, with fungicidal effect against Candida and few toxic effects for human eukaryotic cells. Used exclusively intravenous. The cut-off points of echinocandins are set at a Minimum Inhibitory Concentration of 0.125 μg/ml for C. glabrata except for micafungin, for which it is 0.06 μg/ml, of 0.25 μg/ml for C. albicans, C. krusei and C. tropicalis, and 2 μg/ml for C. guilliermondii and C. parapsilosis. The pharmacodynamics indicator that is related to therapeutic success in the treatment of candidiasis is AUC24h over Minimum Inhibitory Concentration. The maximum activity against Candida would be achieved with serum concentrations of the free medication

four times higher than the Minimum Inhibitory Concentration. Among the advantages of echinocandins for the treatment of severe and recalcitrant oral candidiasis are their anti-biofilm activities and their prolonged post-antifungal effect. They can be first choice medications for the treatment of severe candidiasis in patients with immunodeficiency, the seriously ill and those with a high probability of medication interactions. They are category C medications in pregnancy and should be avoided if there is another therapeutic alternative, as during breastfeeding.

There are further more pharmacological preparations that have antifungal activity and can be used in combination with antifungal medications. Formulations are chlorhexidinesolutions of gentian violet, potassium permanganate, povidone iodine, propylene glycol, selenium sulphide, boric acid, methylene blue or sodium hyposulfite, and caffeic acid derivatives. Several investigational agents are presently under development against old targets, such as ergosterol tetrazoles VT-1129, VT-1161, and VT-1598 or 1,3-β-D-glucan extended half-life echinocandin CD101 or ibrexafungerp -SCY078- that may offer advantages over currently available medications. Ibrexafungerp has the advantages of its oral and intravenous administration and of being active against fluconazole- and echinocandin-resistant oral isolates of Candida. Several agents with novel mechanisms of action are also under development such as inositol acyltransferase, thdihydroorotate dehydrogenase inhibitor F901318, & VL-2397. These new antifungal medication may be less susceptible to mechanisms of antifungal resistance. Each of these investigational agents has the potential to improve patient outcomes in the treatment of oral candidiasis.

Treatment of oral candidiasis has several tools available to be victorious. Topical treatments with nystatin or miconazole are effective in most infections and systemic treatment with oral fluconazole shows a similar efficacy. The treatment should be personalized considering the different clinical characteristics of the patient to avoid physiological interactions (pregnancy, lactation etc. Pharmacological elderly with multiple treatments like critical patients, patients with neoplastic pathologies and immunodeficiency etc. In some case treatment failures, recurrent or relapsing infections and we can count on new antifungal agents with old and new mechanisms ofaction. Among these medications, the new triazoles, and echinocandins offer promising alternatives for treatment [(72)].

3.12. Natural course of candidiasis

Oral candidiasis is one of the most common opportunistic infection affecting the human oral cavity. In addition to Candida species, local and systemic co-factors, such as a reduced salivary flow rate, vitamin deficiency, oral mucosal erosion and generalized immune suppression also play contributory roles. Female patients are more vulnerable to Oral candidiasis than males, and old age may be a predisposing factor for oral candidiasis, possibly due to complex systemic conditions, increased medication intake, decreased salivary flow and denture wearing. Hence, oral candidiasis may reflect immunological changes and micro-environmental variations. Patients are more prone to develop oral candidiasis with immunodeficiency, under treatment with systemic immunosuppressants like pemphigus, pemphigoid, lupus, or graft-versus-host disease, with dry mouth like Sjögren's syndrome, xerostomia or head and neck radiation and under frequent topical steroid treatment like lichen planus and recurrent oral ulcers. Notably, patients with anaemia showed a rather high prevalence rate of oral Candida infection, and the possibility of Candida infection may be due to impaired cellular immunity and epithelial abnormalities. Oral candidiasis with potentially malignant disorders of the oral mucosa, found that oral candidiasis can present in potentially malignant oral disorders such as oral lichen planus, discoid lupus erythaematosus, and oral leukoplakia. Accumulated evidence suggests that oral candidiasis is a predisposing factor that increases the likelihood of malignant transformation in potentially malignant disorders. Although the exact pathologic significance of Candida infection remains unknown, it is generally accepted that there is a positive association between oral Candida infection and epithelial dysplasia. Therefore, accurate diagnosis and timely use of antifungal therapy are essential to manage these lesions [74].

4. MATERIALS & METHODS

PLACE OF THE STUDY

The study participants comprised of dental outpatients visiting the **Department of Oral Medicine and Radiology, Babu Banarasi Das College of Dental Sciences**, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

STUDY PARTICIPANTS & SAMPLE SIZE

For the study purpose 30 (thirty) participants will be enrolled, and divided into two study groups i.e. Group A and Group B. **Group A** consisting of 15 subjects on whom **3.6mg miconazole chewing gum** will be used. **Group B** consisting of 15 subjects on whom **2% miconazole gel** will be used. The subjects of either gender, satisfying the eligibility criteria and those willing to participate in the study were selected for the study.

ELIGIBILITY CRITERIA

A.) INCLUSION CRITERIA:

- Patients who are well oriented to time, place and person.
- Individuals willing to be a part of the study, who sign the informed consent form, and who find it convenient to appear for follow-ups as required by the study.
- Patients aged between 18-60 years in both the gender.
- Patients clinically and histologically diagnosed with Oral Candidiasis.
- Individuals willing to be a part of the study, who sign the informed consent form, and who find it convenient to appear for follow-ups as required by the study.
- Patients who agree not to use any other medication such as anesthetics and analgesics in either topical form or systemic form during the study.

B.) EXCLUSION CRITERIA:

- Patients not willing to be a part of the study or failing to give their consent.
- Patients suffering from any systemic disease.
- Patients who have undergone any previous treatment for Oral Candidiasis.
- Patients failing to give their consent.
- Pregnant and lactating female patients
- Patients with known medication allergy to Miconazole.

SAMPLING METHOD

1. The study was to comprise of 30 individuals within the age group of 18-60 years, clinically diagnosed with Oral Candidiasis.
2. The subjects to be selected according to the inclusion and exclusion criterion.
3. A detailed case history of each participant to be recorded using a case history proforma.
4. Exfoloative cytology was also performed in all the patients done.
5. Following the establishment of the diagnosis, each patient to be informed about the protocol and given the appropriate instructions after obtaining a written consent.

RANDOMIZATION

All the patients (30) to be included in the study were to be randomly divided into two groups A and B (consisting of 15 patients each).

ALLOCATION

The principal investigator was to carry out the initial as well as periodic evaluations of all study participants.

ARMAMENTARIUM

(Materials and Equipments used in the study with specifications and company)

MATERIALS

1. Gum base

Guar gum powder of endosperm by LOBA CHEMIE PVT.LTD.

(Manufactured by: Plot no D-22, Tarapur MIDC, Boisar, Palghar, Maharashtra, India.

Preparation of miconazole chewing gum

The raw material procured from the above mentioned company was formulated into a gel form at the multidisciplinary research laboratory CSIR-CDRI (Council of Scientific and Industrial Research- Central Drug Research Institute), Lucknow.

Concerned Departments:

1. Department of Pharmaceutics and Pharmacokinetics
2. Department of Microbiology

Equipments used:

Beaker, Conical flask, Measuring cylinder, Glass rod, Spatula, Filter paper, Funnel, Aluminium foil, Digital weighing balance, Magnetic stirrer and Propeller mixer.

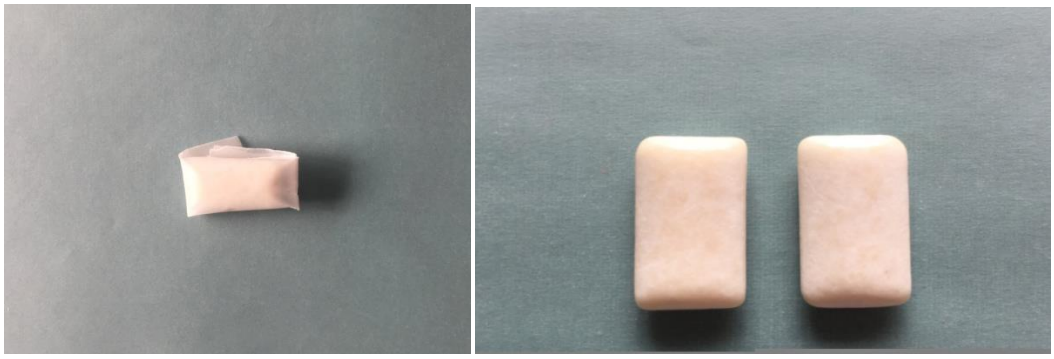
Chemicals used:

Gum base 29%, powder sorbitol 43%, 70% sorbitol solution 21%, glycerine 5%, peppermint flavor 1%, lecithin oil0.5 %, aspartame 0.33%.

CSIR-CDRI (Council of Scientific and Industrial Research- Central Drug Research Institute)

The preparation method applied is as follows [75].

- The gum base would be soften or melted at 50 to 70^{0}C and placed in a kettle mixer with blades.
- Powdered sweeteners, syrup, active ingredient and other ingredients would be added following an accurate time and schedule.
- During mixing procedure the flavoring agents would be added.
- The chewing gum mixture would then be cooled and rolled onto plates.
- Scored into strips and cut into pieces to produce sticks and tablets.



Miconazole chewing gum covered with butter paper

2. 2% gel (dose: 20 mg of miconazole)

2% miconazole gel was commercially purchased and applied by the study participants, falling in Group B.

Brand: Daktarin-gel 20g (Mconazole 2%w/w)

Manufactured By: Janssen pharmaceuticals

FOR CLINICAL EXAMINATION

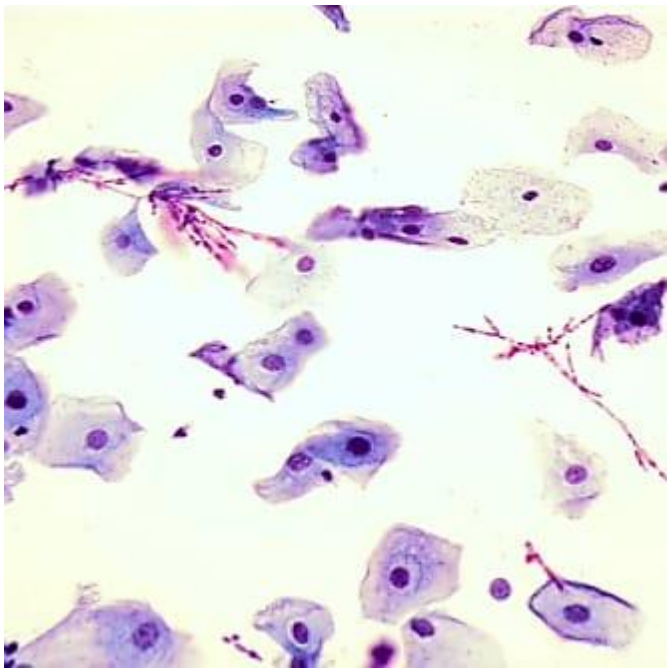
1. Dental chair
2. Sterile gloves
3. Disposable mouth masks
4. Sterilized intraoral mouth mirrors
5. Sterilized tweezers
6. Sterilized kidney trays
7. Sterilized cotton
8. Exfoliative cytology kit

METHODOLOGY

A total of 24 patients were enrolled in the study who gave consent to particpate and met the eligibility criteria. A detailed history and clinical findings & exfoliative cytology (Brush) were recorded in individual proformas designed especially for the study on the baseline visit.

The study participants were divided into 2 groups.

- **Group A:** Consisting of 12 participants
- **Group B:** Consisting of 12 participants



Histoilgy report- candidal hyphae

BASELINE VISIT PATIENT ASSESSMENT

All the relevant readings for all clinical parameters for each patient from baseline to subsequent visits were recorded and entered in the proforma.

1. Burning sensation score for Oral Lichen Planus was considered using the Visual Analogue Scale (VAS) ranging from: 0 (no burning sensation), 5 (burning sensation on eating hot and spicy food) to 10 (worst burning sensation occuring spontaneously).

Numerical rating scale									
1	2	3	4	5	6	7	8	9	10
Minimum									Maximum

2. Personal history like oral hygiene, tobacco chewing habit, smoking, alcohol consumption.

3. Different peri/intraoral sites were evaluated including lips, labial mucosa (upper and lower), buccal mucosa (right and left), vestibule (maxillary/ mandibular right and left), lateral borders of the tongue (right and left), dorsum of the tongue (right and left), ventral surface of the tongue, floor of the mouth (right and left), maxillary gingiva (right and left), soft palate hard palate, retromolar area, alveolar ridge/mucosa, and faucial pillars. And evaluate patch, plaque, scrapability and other associated features [(8)].

ACTIVE PHASE

The subjects were blinded i.e. the subjects were not aware of the nature of the drug they were receiving.

Patient regime:

- Group A will constitute of 12 patients who will be given Miconazole chewing gum, group B will constitute of 12 patients who will be given Miconazole gel.
- The patients will be instructed to apply the gel on the lesion twice a day for 30 days and refrain from eating, drinking and rinsing for at least 30 min after the topical application.
- Patients were asked to report immediately in case they encounter any adverse effects and they were managed on a case to case basis.
- In the active phase, the patients will be assessed for the effectiveness of topical applications in resolving the lesion and reducing burning sensation on the **7^{th}, 14^{th}, 21^{st}, 28^{th} day.**

FOLLOW UP PHASE

- The follow-up phase comprised of 3 months in total.

- Patient were followed up after at interval of 1 month each and noted for any recurrent lesions and associated resolution of such lesions.

STATISTICAL ANALYSIS

Group 1: Control group– consisted of 12 subjects who received miconazole chewing gum.

Group 2: Study group –consisted of 12 subjects who received miconazole gel.

Patients in both the groups were instructed who to use miconazole chewing gum 3.6mgfor 20-25 minutes, 3-4 times a day and miconazole gel 2% for 15-20 minutes 3 times a day.

Patients were asked to report immediately in case they encounter any adverse effects and they were managed on a case to case basis.

The following clinical parameters were assessed during follow up:

- Plaque/patch: present/absent
- Scrapability: present/absent/partially present
- Burning sensation
- Side effects

The patients were followed up after 7 and 15 days. Readings for all clinical parameters for each patient from baseline to subsequent visits were recorded. The patients were enquired for side effects, if any.

The data was entered into the computer using Microsoft excel and was analyzed using SPSS software for windows (version 22.0).

The data was tabulated and subjected to the following statistical analysis i.e the Cramer's V and chi square test. A p-value of 0.05 or less was considered as statistically significant.

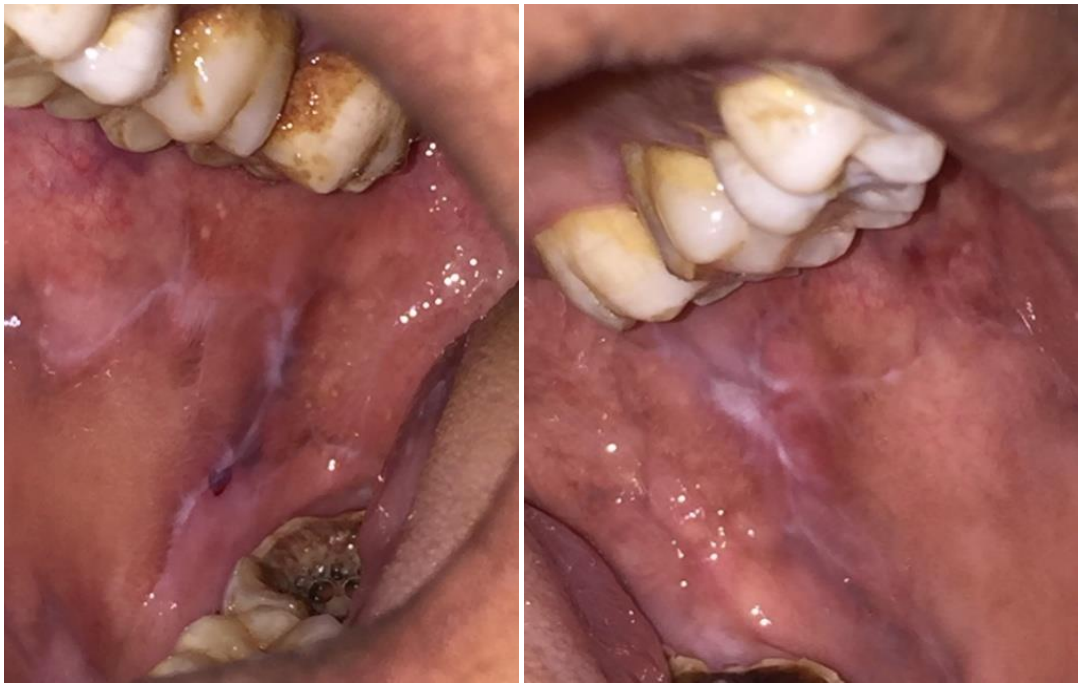


Photo 1 Group A: 1st visit base line

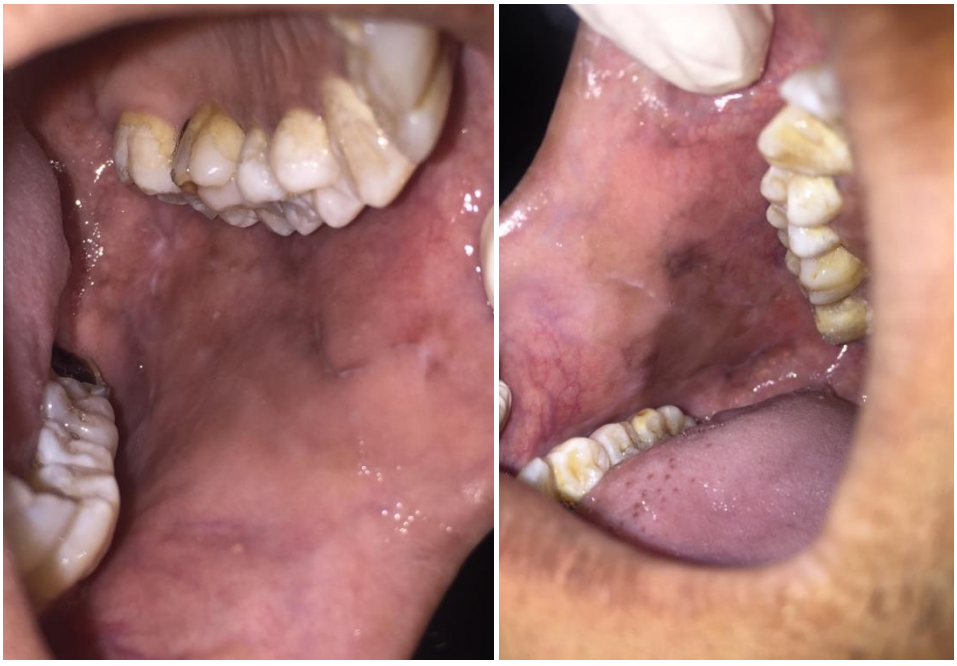


Photo 2 Patient: Group A Active phase 4th week

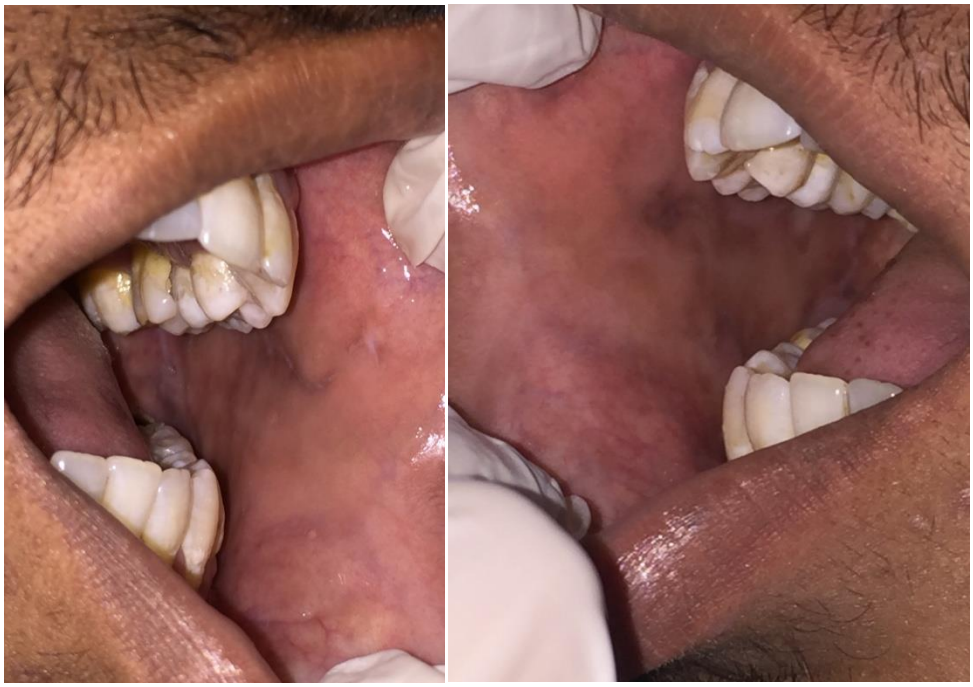


Photo 3 Patient: Group A 3rd month follow up

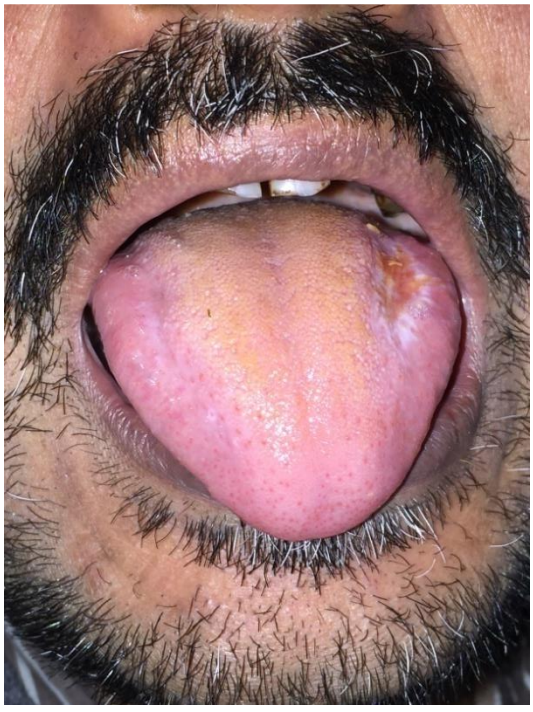


Photo 4 Group B: 1st visit base line

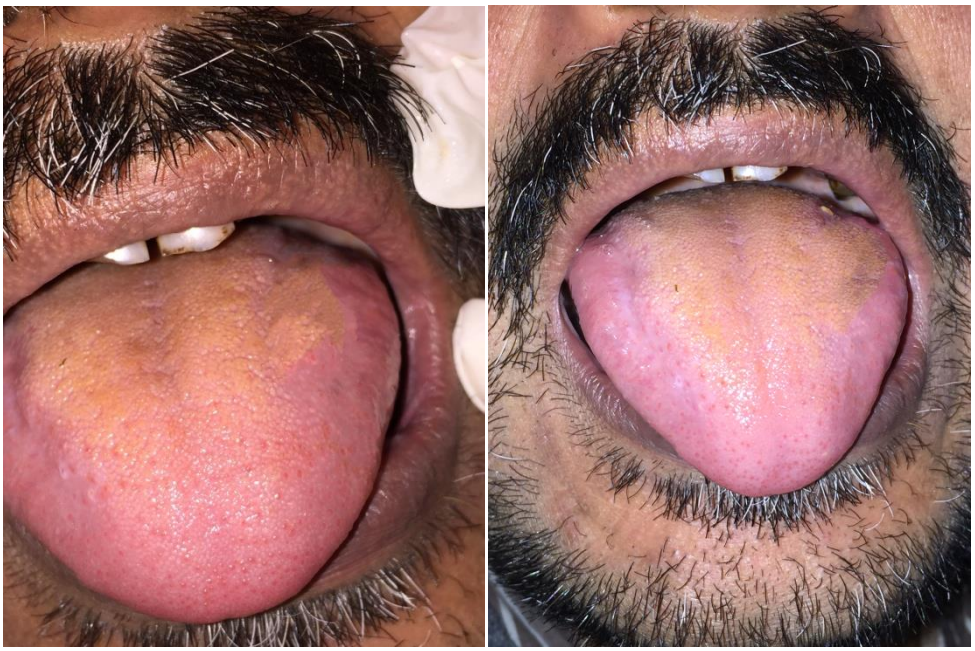


Photo 5 Patient: Group B Active phase 4th week Patient: Group B 3rd month follow up

5. Result

Statistical analysis

Microsoft excel was used for data tabulation and it is showed in number of patients (n) and percentage (%). Statistical analysis for all the data was performed using statistical tests contained in SPSS software version 23.0 (IBM Corporation). Differences between the groups were analyzed using independent t-test and dependent t-test, with the level of significance set at $p < 0.05$.

Results

The study group consisted of 24 patients with oral candidiasis harboring predominantly *Candida* species. Half of the patients chewed one piece of chewing gum (dose: 3.6 mg of miconazole) three times daily; the other half was given a 2% gel (dose: 20 mg of miconazole) to apply in the affected area three times daily.

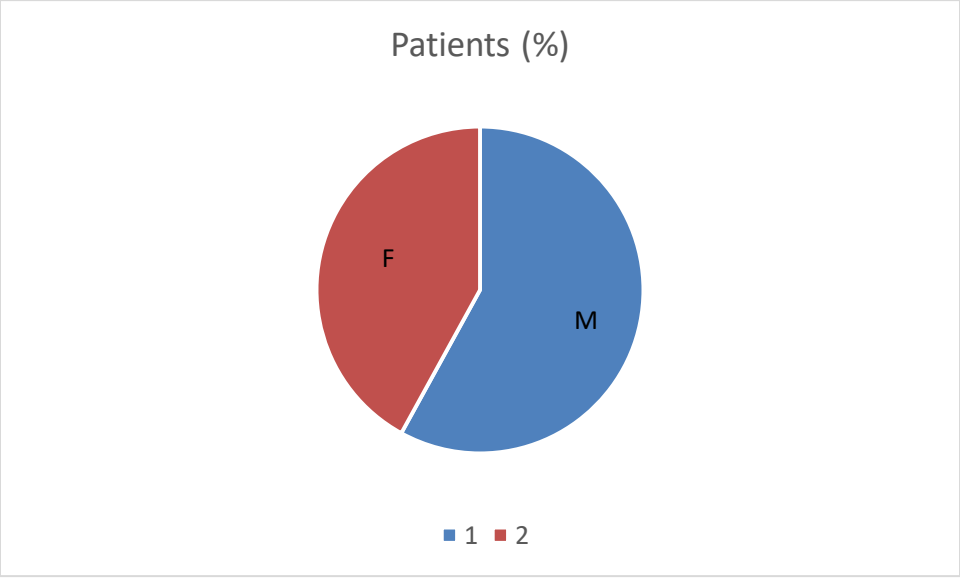


Figure 1 - Distribution of male and female patients included in the study

Distribution of male and female patients included in the study group is shown in **figure 1** and distribution of gender according to the type of lesion involved in the two study groups is shown in **figure 2.** Acute Pseudomembranous Candidasis was seen in 50% males while 42% in female. In male, Acute Erythematous Candidiasis is seen in 4% while in female none of the patient presented the same . Chronic Atrophic Candidiasis is 4% in male while none in female.

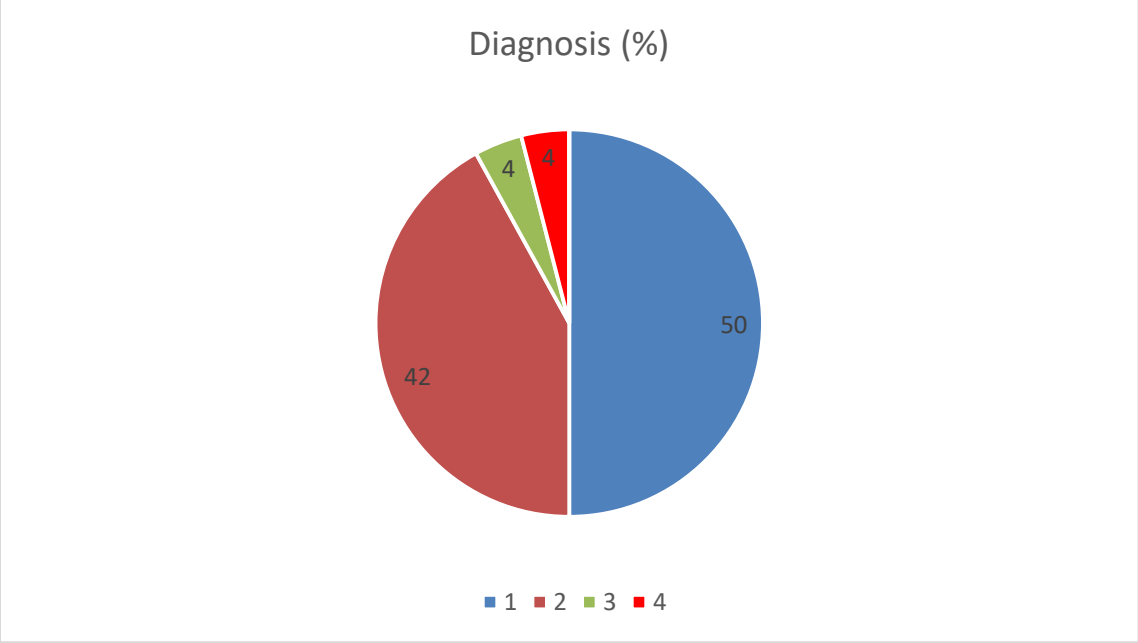


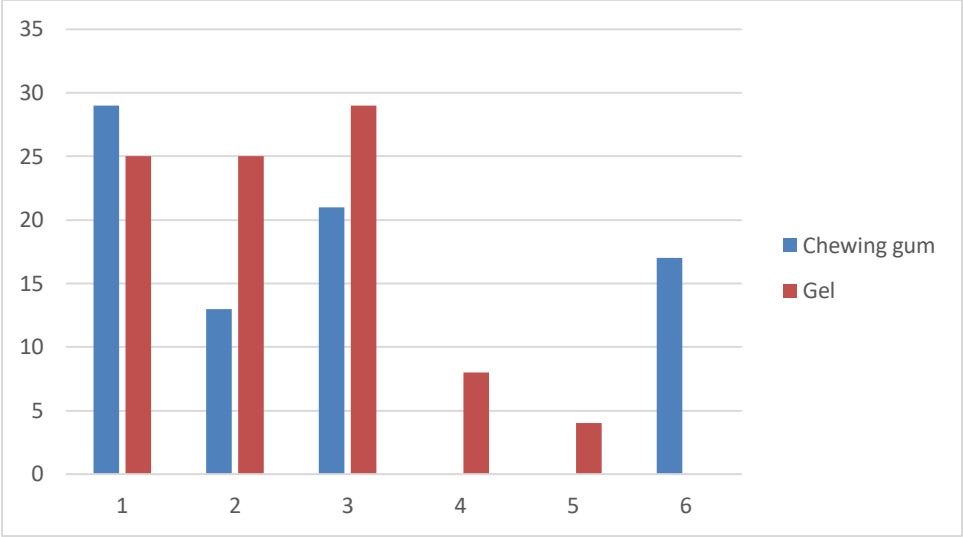
Figure 2 - Distribution of gender according to the type of lesion. 1: MALE: Acute Pseudomembranous candidasis, 2: FEMALE: Acute Pseudomembranous candidasis, 3: MALE: Acute Erythematous candidiasis, 4: MALE: Chronic atrophic candidiasis.

Table 1 showing number of patients (%) with type of lesions at multiple sites treated by different delivery system. Out of the 24 patient only 1 patient was diagnosed to have erythematous

candidiasis and 1 was diagnosed with chronic atrophic candidiasis, rest others were diagnosed to have pseudomembranous form of candidiasis in the various sites of the oral cavity which were treated by the two drug delivery system (chewing gum and gel).

	Type of lesion					
	Acute Pseudomembranous Candidiasis		Acute Erythematous Candidiasis		Chronic atrophic candidiasis	
Sites	Chewing gum	Gel	Chewing gum	Gel	Chewing gum	Gel
Dorsum of tongue	29	25		4		
Buccal mucosa (left)	13	25		4		
Buccal mucosa (right)	21	29		4		
Hard palate		8		4		4
Lower buccal vestibules		4				
Multiple sites	17					

Table 3 - No of patients (%) with type of lesions at multiple sites treated by different delivery system.



Graph 1- Graphical representation of type of lesions at multiple sites.1: Dorsum of tongue, 2: Buccal mucosa (left), 3: Buccal mucosa (right), 4: Hard Palate, 5: Lower buccal vestibule, 6: Multiple sites.

Lesions involved to have affected on right and left buccal mucosa, dorsum of the tongue, palatal and gingival areas, that has been represented in bar diagram in **figure 3**. Some cases were having involvement of multiple sites.

	Start of Treatment				(ACTIVE PHASE 2ND VISIT)				(ACTIVE PHASE 4TH VISIT)				(FOLLOW UP PHASE 1ST MONTH)				(FOLLOW UP PHASE 3RD MONTH)			
	(n)		%		(n)		%		(n)		%		(n)		%		(n)		%	
	CG	G	CG	G	CG	G	CG	G	CG	G	CG	G	CG	G	CG	G	CG	G	CG	G
BURNING SENSATION *VAS SCALE (Scale of above "0")	9	8	75	67	9	8	75	67	4	6	33	50	3	3	25	25	0	1	0	8
PATCH/ PLAQUE PRESENT	12	12	100	100	12	12	100	100	5	7	42	58	1	1	8	8	1	2	8	17
SCRAPABILITY PRESENT	12	12	100	100	10	12	83	100	3	2	25	17	0	0	0	0	1	2	8	17

Table 4 - No of patients (%) with clinical evaluation after treated by different delivery system

Treatment with chewing gum and gel - $p < 0.001$ and $p < 0.05$ respectively

Patch/plaque and scrapability - $p < 0.001$.

Table 2 number of patients treated by different drug delivery system. Burning sensation (75%), presence of patch or plaque (100%) and scrapability (100%) from the lesion were noted at the start of the treatment which were treated by chewing gum and burning sensation (67%), presence of patch or plaque (100%) and scrapability (100%) was noted from the gel group.

At the end of 3 month follow up period significant reduction in the burning sensation (0%), presence of patch or plaque (8%) and scrapability (8%) was noted in the group treated by chewing gum and burning sensation (8%), presence of patch or plaque (17%) and scrapability (17%) was noted from the group of patient who were treated by gel.

However, recurrence was noted in 1 patient from the chewing gum group and 2 patients from the gel group at the end of the 3 months follow up.

There was significant reduction in the candida infection after 4 weeks of active treatment and 3 months of follow up of treatment from the affected area in the two groups.

There is significant difference was evident for burning sensation scale before treatment and after treatment with chewing gum and gel with $p<0.001$ and $p<0.05$ respectively. Significant difference was also evident for patch/plaque and scrapable availability before treatment and after treatment with chewing gum and gel with $p<0.001$ at the end of the follow up of the treatment.

However, no significant difference was evident between chewing gum and gel for burning sensation scale, patch/plaque and scrapable availability parameters at the end of the study period. It seems that both delivery system of Miconazole was effective in treating chronic oral candidiasis.

6. Discussion

The opportunistic infections whether bacterial, viral or fungal are highly prevalent these days owing to altered life style of majority of individuals. Among the opportunistic variant fungal infection are most prevalent. Altered life style include compromised immunity, tobacco and tissue abusive habit, diabetes status, poor oral hygiene to name a few.

Depending on its virulence, location and type of candidiasis treatment protocol has to be decided. First the use of conservative measures is supported before starting drug treatment, promoting good oral hygiene along with removing the dentures at night; thereby it will benefit the removal of the biofilm layer generated in the prosthetic surface.

Miconazole is used widely as a topical medication. We found it in the form of gel, applying it directly on the affected area, at doses of 200-500 mg per day, divided into 4 times. Despite its good properties it has the limitation of possible interaction with mediction such as warferin , such as warfarin. This is because the antifungal inhibit the enzyme cytochrome P-450, which affects the clearance of certain medications. In addition, this medication is absorbed by the intestine; therefore care must be taken when administrated [(2)].

In 2011 Nairy, H.M. conducted a pseudo-randomised clinical trial of *in* situ gels of fluconazole for the treatment of oropharngeal candidiasis in which he found all patients had mycological documented oropharyngeal candidiasis and were treated with fluconazole (0.5%w/v) *in situ* gels for 14 days. Severity of disease was scored clinically before treatment and at different predetermined time intervals along with semi quantitative culture of oral swabs. The clinical response rate showed 97% cure after 14 days in the treated group with *in situ* gel. In comparison, the control group treated with fluconazole tablets showed 85% improvement in symptoms of oral candidiasis. The patients suffering from HIV infection showed relapse in oral candidiasis at the end of 21 days. The patients having oral candidiasis due to partial or complete dentures showed complete recovery and were free from signs and symptoms of oral candidiasis. Gel formulation of fluconazole was well tolerated with no severe adverse reaction and offers a better alternative to tablet formulation in the treatment of oropharyngeal candidasis[(76)].Similarly my study also shows the better efficacy of topical use of miconazole.

Now a day's chewing gum is a novel drug delivery system which is going to advance more and more in researches and it seems to get more standardized in future industry because it can deliver either pharmaceuticals or nutrients which are known as medicated chewing gum (MCG). MCG is supposed to act as an extended release dosage form that provides a continuous release of medicine contained. In **2015 Aslani A et al** conducted a study on a medicated chewing gum as a novel drug delivery, like concurrently supporting both local and systemic delivery, protection against acids and enzymes, low first pass metabolism, elevating alertness and cognitive function and good stability.

Some studies found out about its advantages like increased rate of effectiveness rather than other oral delivery systems, reduced risk of overdosing while it's whole swallowed, good for rapid delivery, reduced risk of intolerance to gastric mucosa, requiring no water to drink, good stability against light, oxygen, and moisture., an nihilation of xerostomia and help swallowing in people with dry mouth, reduced pains and difficulties in swallowing.

Some of the disadvantages of medicated chewing gums are disappearing of drug in oral cavity following salivary dilution, different release profiles because of chewing style differences, allergic reaction to artificial sweeteners, short time of administration due to eating, speaking, and drinking, continuous stress on jaws may cause temporo-mandibular joint disorder, teeth decay through being coated by sugar [(77)].

Similar study was conducted to evaluate the effect of miconazole as a tablet and gel form in a trail having equal number of male and female patients in **2004 by J.-M. Cardot et al**. In the mentioned study total sample number was 18(50% male & 50% female) and the dose of miconazole tablets was 50 or 100 mg and gel 3 times a day, 375 mg /day [(78)]. While in my study the total number of patient is 24 (42% female, 58% male) and the dose of chewing gum (dose: 3.6 mg of miconazole) three times daily; the other half was given a 2% gel (dose: 20 mg of miconazole) to apply in the affected area three times daily. However no major difference was found between these two groups pertaining to effectiveness and safety.

One similar study was conducted in the year **2003 By JM Cardot**, in which he investigated the safety, adhesion and general comfort of new bioadhesive buccal tablets of miconazole. The trial was randomized, cross-over phase I conducted in 18 healthy volunteers, single administrations

of two dosages of the bioadhesive tablet (50 and 100 mg of miconazole) were compared to miconazole buccal gel (125 mg three times consecutively at four hour intervals) with a washout period of 7 days between the 3 treatments. Bioadhesive tablets were stuck in the cuspid fossa and stayed in the oral cavity until erosion or detachment. The weight of the 100 mg miconazole tablet was twice the 50 mg miconazole tablet, and found that the average adhesion duration for the tablets was 15 hours. Bioadhesion of the 50 mg tablet was 9 h or more in all subjects. For this tablet, termination of adhesion was mainly due to erosion. Overall the tablets, especially the 50 mg tablet, demonstrated better tolerance and acceptability than the gel, without any significant local side-effects. One subject noted a bad taste for the 50 mg tablet versus thirteen subjects for the gel.While in my study the total number of patients is 24, and we have used 3.6gm miconazole chewing gum and miconazole gel 2%, in which we found that no patient had copmplained about the bad taste and discomfort. They also further elaborated the adhesion of miconazole tablet in the fossa and cusp [(79).]

One more similar study was conducted in **2007 by Bensadoun J.R** et al, They compared the efficacy and safety of miconazole 50-mg mucoadhesive buccal tablets with miconazole 500-mg gel in the treatment of oropharyngeal candidiasis. In which the men and women aged >18 years who underwent radio therapy for head and neck cancer with clinical signs and symptoms of oropharyngeal candidiasis and had either a first episode or recurrence of the oropharyngeal candidiasis are eligible for the study. Oropharyngeal candidiasis had to be confirmed by direct mycologic examination and positive fungal culture with >100 colonies.And they found that the success rate was statistically not inferior ($P < .0001$) in the mucoadhesive buccal tablet population to the rate observed in the miconazole oral gel group (56% vs 49%, respectively; $P < .0001$). After adjustment for the extent of lesions and salivary secretions, a trend toward superiority was observed in favor of mucoadhesive buccal tablet ($P = .13$), particularly among patients with multiple lesions ($P = .013$). Results for secondary endpoints were comparable to those observed for the primary endpoint. Compliance with mucoadhesive buccal tablet was excellent, and >80% of patients completed treatment. Both treatments were safe [(80)].While in my study, comparison was done of the efficacy and safety of miconaole chewing gum dose: 3.6 mg of miconazole and miconazole gel 2% (dose: 20 mg of miconazole).And we too had subject whose age is more then 18, but in my study there is no case of oropharyngeal candidiasis included. Significant difference was evident for burning sensation scale before treatment and

after treatment with chewing gum and gel with $p<0.001$ and $p<0.05$ respectively. Significant difference was also evident for patch/plaque and scrapability before and after treatment with chewing gum and gel with $p<0.001$ at the end of the follow up of the treatment.

In **2010 Vazquez et al** compared the miconazole buccal tablet with clotrimazole troches for treatment of oropharyngeal candidiasis. The study was double-blind, double-dummy, multicenter trial and evaluated 578 randomized patients with HIV infection and oropharyngeal candidiasis. The study compared the efficacy and safety of miconazole buccal tablet once daily with clotrimazole 10 mg troches 5 times daily for 14 days. And they found that once-daily MBT was shown to be superior to clotrimazole troches 5 times daily in the treatment of oropharyngeal candidiasis in HIV-positive patients. Miconazole buccal tablet offers an effective, safe, and well-tolerated topical treatment option for **oropharyngeal** candidiasis administered as a convenient once-daily dose [(81)]. While in my study the comparison was between miconazole gel and chewing gum, and there was no patient of oropharyngeal candidiasis, with the smaller sample size. But the similarity was in the effectiveness, safety and tolerance of miconazole.

Similar study was conducted in the year **1993 by Rindutn JL et al**, where among 32 patients, both erythematous, plaque-like, and nodular lesions were found in the commissural area. Erythematous tongue lesions (6% in both groups) were seen to be associated with degrees of loss of filiform and fungiform papillae. Palatal erythematous lesions were found in, respectively, 25% and 19% of the patients in the two groups of treatment. Tongue and palatal lesions were found at the same time in 44% and 25%, respectively. Multifocal pseudomembranous lesions were seen in 6% and 13%. However in my study the acute pseudomembranous candidiasis in dorsum of tongue (Chewing gum- 29%,gel – 25%) , Buccal mucosa (Chewing gum- 13%,gel – 25%), hard palate (gel – 25%), Lower buccal vestibules (gel – 4%), Multiple sites (chewing gum-17%). Acute Erythematous Candidiasis in dorsum of tongue (gel – 4%), Buccal mucosa (gel – 4%), hard palate (gel – 4%). Chronic atrophic candidiasis in hard palate (gel - 4%) were observed. Symptoms and oral mucosal lesions were recorded at the beginning of treatment, 3 and 6 weeks after start of treatment, and 4 weeks after termination of treatment. Both antifungal treatments were successful in all patients. A treatment period of 3 wk was clinically insufficient for disappearance of lesions in 69% of patients treated with chewing gum and in 38% of patients treated with gel [(8)]. While in my study Burning sensation (75%), presence of patch or plaque

(100%) and scrapability (100%) from the lesion were noted at the start of the treatment with miconazole chewing gum and burning sensation (67%), presence of patch or plaque (100%) and scrapability (100%) was noted from the gel group. At the end of 3 months follow-up period significant reduction in the burning sensation (0%), presence of patch or plaque (8%) and scrapability (8%) was noted in the group treated by chewing gum in comparison to burning sensation (8%), presence of patch or plaque (17%) and scrapability (17%) in the group of patient who were treated by gel. The major differences between these two studies are that they included the systemic condition HIV also which is excluded in my study.

A study was done in **2004 by Roey JV et al** in which they compared the efficacy of topical therapy with a slow-release mucoadhesive buccal tablet containing miconazole nitrate versus systemic therapy with ketoconazole in HIV-positive patients with oropharyngeal candidiasis. They assessed the efficacy and safety of a 10-mg once-daily topical regimen of miconazole nitrate mucoadhesive buccal tablet versus a 400-mg once-daily systemic regimen of ketoconazole in HIV-positive patients with oropharyngeal candidiasis. A total of 357 patients were treated for 7 or 14 days depending on response after 7 days of treatment. Clinical response was the primary outcome variable and secondary outcomes included microscopy, time to cure, symptom scores, and safety outcomes. A per-protocol analysis of 332 patients demonstrated that miconazole nitrate was not statistically significantly inferior to ketoconazole treatment. At day 7, the clinical response rate was 135 of 156 (87%) for miconazole nitrate and 137 of 153 for ketoconazole (90%)confidence interval of the treatment difference. At the end of treatment, dysphagia was 1% in both the groups. Microscopic findings paralleled the clinical results. The mucoadhesive tablet was generally well tolerated. A higher incidence of gastrointestinal disorders and drug-related adverse events was seen during ketoconazole treatment [(82).]

In my study we treated the patient under active phase 4 weeks and followed them till 3 months with miconazole chewing gum and gel whereas in above mentioned study the patient reported on 7th and 14th day with miconazole nitrate buccal tablet and 400 mg ketoconazole. They compared the systemic with topical route. They also found that the miconazole tablets were more tolerated, as similar to my study where no adverse effect was seen.

Similar study was conducted in the year **1993 by Rindutn JL et al**, among 32 patients in which efficacy between the miconazole chewing gum 3.6gm and 2% miconazole gel was done. They found recurrence in one patient in the chewing gum group where recurrence was seen in symptoms and lesions (multifocal pseudomembranous candidaisis), while in my study 3 recurrences occured one in chewing gum group and two patients in gel group[8].

In 2003 J.-M. Cardot et al, conducted a trail in 18 healthy volunteers, where single administrations of two dosages of the bioadhesive tablet (50 and 100 mg of miconazole) were compared to miconazole buccal gel (125 mg three times consecutively at four hour intervals) with a washout period of 7 days, without any follow up. Thus no conclusion could be elaborated for recurrence. In **2004 J.-M. Cardot et al**, conducted another trail in which they included 18 subjects (50% male & 50% female) and the dose was miconazole tablets 50 or 100 mg and gel 3 times a day, 375 mg day for 1 week active phase and no further follow-up. **In 2004 Roey JV et al,** compared the efficacy and safety of a 10-mg once-daily topical regimen of miconazole nitrate mucoadhesive buccal tablet versus a 400-mg once-daily systemic regimen of ketoconazole in HIV-positive patients with oropharyngeal candidiasis for 14 days only and no further follow up. **In 2007 Bensadoun J.R** et al, compared the efficacy and safety of miconazole 50-mg mucoadhesive buccal tablets with miconazole 500-mg gel in the treatment of oropharyngeal candidiasis for 2 weeks active phase only and they too have not mentioned about the recurrence. **In 2010 Vazquez et al** compared the efficacy and safety of miconazole buccal tablet once daily with clotrimazole 10 mg troches 5 times daily for 14 days only and no follow up. In **2011 Nairy, H.M.** conducted a study on fluconazole gel for the treatment of oropharngeal candidiasis under the active phase for 21 days but there was no further follow up, and the patient suffering from HIV infection showed relapse in oral candidiasis at the end of 21 days.

In all the above mentioned studies the patients were assessed only in the active phase without giving emphasis to recurrence. However all the opportunistic infection has a high tendency to show recurrence pertaining to sing and symptom. Considering this important aspect of recurrence following active phase of 4 weeks we have even followed up the patients for 3 months. We found out that miconazole chwing gum give better effect then miconazole gel in context of recurrence. We got one recurrence in chewing gum and 2 recurrences gel group. We got 1 recurrence in symptom and sing in one subject of chewing group and two subjects in gel

group. Thus we can say that chewing gum works better because of supporting both local and systemic delivery, protection against acids and enzymes, low first pass metabolism, elevating alertness and cognitive function and good stability.

7. Summary and conclusion

In recent researches new formulation technologies have been developed in oral route of drug administration. Among this chewing gum is a modern drug delivery system. The most notable asset of medicated chewing gum is an extended release dosage form that provides a continuous release of medicine contained and can have both topical as well as systemic effect ones dissolves and is swallowed.

We to in our study compared the efficacy, biocompatibility, tolerance of standard miconazole gel with miconazole chewing gum. It was decided to incorporate and asses 30 (thirty) participants. These participants were equally divided into two study groups i.e. Group A and Group B. Group A consisting of 15 subjects on whom 3.6mg miconazole chewing gum will be used. Group B consisting of 15 subjects on whom 2% miconazole gel will be used. The subjects of either gender, satisfying the eligibility criteria and those willing to participate in the study were selected for the study. However a total of 24 patients were finally enrolled in the study who gave consent to particpate and met the eligibility criteria. A detailed history and clinical findings & exfoliative cytology (Brush) were recorded in individual proformas designed especially for the study on the baseline visit. In the active phase, the patients will be assessed for the effectiveness of topical applications in resolving the lesion and reducing burning sensation on the 7^{th}, 14^{th}, 21^{st}, 28^{th} day and will be followed up for 3 months. At the end of active phase no statical significant difference was found between two groups. However, recurrence was noted in one patient 1 patient from the chewing gum group and 2 patients from the gel group at the end of the 3 months follow up.

Thus, it can be concluded that miconazole chewing gum and gel offers an effective, safe, and well-tolerated topical treatment for oral candidiasis. Chewing gum is a very stable product due to its low moisture content and less reactive nature than that of other oral ingredients. Chewing gum act as both local and systemic delivery, protection against acids and enzymes, low first pass metabolism, elevating alertness and cognitive function, good stability and a lot more; we can conclude that chewing gum will be much more familiar to patients and market in the next few years. Patient accepted towards chewing gum is also good. In the 3 month follow up period recurrence was noted in 1 patient from the chewing gum group and 2 patients from the gel group

at the end of the 3 months follow up. But no significant difference was evident between chewing gum and gel for burning sensation scale, patch/plaque and scrapable availability parameters at the end of the study period. It seems that both delivery system of miconazole was effective in treating oral candidiasis.

However, similar studies with large sample size can be taken up to comment further on the efficacy of chewing gum as a drug delivery mode in reliving the symptom & signs in various mucosal lesions.

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ANNEXURE-1

CASE HISTORY PROFORMA

A COMPARATIVE EVALUATION OF THE EFFICACY BETWEEN TOPICAL APPLICATIONS OF PROPOLIS AND TACROLIMUS IN MANAGEMENT OF SYMPTOMATIC ORAL LICHEN PLANUS PATIENTS

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY
BabuBanarasi Das College of Dental Sciences, Lucknow (U.P.)

OPD NO: Case No:

Name: Age: Sex:

Marital status: Occupation:

Address:

Contact No:

CHIEF COMPLAINT:

HISTORY OF PRESENT ILLNESS:

Numerical rating scale

1	2	3	4	5	6	7	8	9	10
Minimum									**Maximum**

PAST MEDICAL HISTORY:

DRUG ALLERGY:

PAST DENTAL HISTORY:

INTRAORAL SOFT TISSUE EXAMINATION:

PROVISIONAL DIAGNOSIS:

TREATMENT PLAN:

PATIENT ASSESSMENT:

ACTIVE PHASE

1ST VISIT

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

2ND VISIT

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

3RD VISIT

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

4TH VISIT

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

FOLLOW-UP PHASE

1ST MONTH

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

2ND MONTH

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

3RD MONTH

BURNING SENSATION (VAS)	**SITE**	**ERYTHEMA**	**ULCERATION**

SIGNATURE OF STUDENT SIGNATURE OF GUIDE

ANNEXURE -2

CONSENT FORM

Title of the study…….

Study Number…….

Subject's Full Name……

Date of Birth/Age……

Address of the Subject……

Phone No. and email address……..

Qualification……

Occupation: Student/Self employed/Service/Housewife/Other

1. I confirm that I have read and understood the Participant Information Document dated ……. for the above study and have had the opportunity to ask questions

   OR

   I have been explained the nature of the study by the investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with the free will without any duress and that I am free to withdraw at any time, without given any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trail. However, I understand that my identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use any data or results that arise from this study provided such a use is only for scientific purpose(s).

5. I agree to participate in the above study for the future research

   Yes [ ] No [ ] Not Applicable [ ]

6. I have been explained about the study, and have fully understood them. I have also read and understand the participant/volunteer's information document given to me.

Signature/Thumb impression of the subject/Legally acceptable Representative……....

Signatory's Name…….....Date…….....

Signature of Investigator's Name……..……..…

Study Investigator's Name……...Date……..

Signature of the witness……..…

Name of witness……...Date……..

Received a signed copy of the duly filled consent form

Signature/Thump Impression of the subject/Legally acceptable representative……Date……

ANNEXURE -3

सहमति पत्र

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ANNEXURE- 4

ETHICAL APPROVAL FORM

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparison of the Efficacy and Tolerance Between Miconazole as Chewing Gum and as Gel Application in Patients With Oral Candidiasis."** submitted by **Dr Ismat Fakhra** Post graduate student from the **Department of Oral Medicine & Radiology** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **27th November 2018** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE-5

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 10 **BBDCODS/01/2019**

Title of the Project: Comparison of the Efficacy and Tolerance Between Miconazole as Chewing Gum and as Gel Application in Patients With Oral Candidiasis.

Principal Investigator: Dr. Ismat Fakhra **Department:** Oral Medicine & Radiology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Ismat Fakhra,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10th January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 22/01/19

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member Secretary
Institutional Ethic Committee
Babu Banarasi Das College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURE-6

MASTER CHART

1	21	F	Miconazole chewingum - 3.6 mg
2	38	M	Miconazole chewingum - 3.6 mg
3	40	F	Miconazole chewingum - 3.6 mg
4	35	M	Miconazole chewingum - 3.6 mg
5	45	F	Miconazole chewingum - 3.6 mg
6	62	M	Miconazole chewingum - 3.6 mg
7	19	M	Miconazole chewingum - 3.6 mg
8	40	M	Miconazole chewingum - 3.6 mg
9	23	F	Miconazole chewingum - 3.6 mg
10	38	M	Miconazole chewingum - 3.6 mg
11	36	M	Miconazole chewingum - 3.6 mg
12	45	M	Miconazole chewingum - 3.6 mg
13	80	M	Miconazole Gel - 2%
14	47	F	Miconazole Gel - 2%
15	48	F	Miconazole Gel - 2%
16	32	M	Miconazole Gel - 2%
17	45	M	Miconazole Gel - 2%
18	31	F	Miconazole Gel - 2%
19	74	M	Miconazole Gel - 2%
20	39	F	Miconazole Gel - 2%
21	37	F	Miconazole Gel - 2%
22	30	F	Miconazole Gel - 2%
23	45	M	Miconazole Gel - 2%
24	25	M	Miconazole Gel - 2%

BURNING SENSATION *VAS SCALE	PATCH/ PLAQUE	SCRAPABILITY PRESENT/ABSENT/
7	Plaque	Partially scrapable
8	Patch +Plaque	Partially scrapable
0	Plaque	Partially scrapable
8	Plaque	Partially scrapable
5	Plaque	present
0	Patch +Plaque	present
4	Patch +Plaque	Partially scrapable
5	Plaque	Partially scrapable
0	Plaque	Present
7	Patch +Plaque	present
7	patch+plaque	present
5	Plaque	present
8	patch+plaque	present
5	Plaque	present
0	patch+plaque	present
0	patch+plaque	Partially scrapable
0	Plaque	present
8	Patch +Plaque	present
8	patch+plaque	present
4	Plaque	present
8	patch+plaque	present
0	Plaque	present
4	Plaque	present
6	Plaque	present

Baseline visit

BURNING SENSATION *VAS SCALE	PATCH/ PLAQUE PRESENT/ABSENT	SCRAPABILITY PRESENT/ABSENT/ PARTIALLY
8	present	Partially scrapable
7	Present	present
0	present	Partially scrapable
5	present	Partially scrapable
3	present	Partially scrapable
0	present	present
2	present	absent
3	present	Partially scrapable
0	present	present
4	present	absent
5	present	present
4	present	present
5	present	present
3	present	present
0	present	present
0	present	Partially scrapable
0	present	present
5	present	present
6	present	present
3	present	Partially scrapable
5	present	present
0	present	present
2	present	present
3	present	Partially scrapable

2nd week active phase

BURNING SENSATION *VAS	PATCH/ PLAQUE PRESENT/ABSENT	SCRAPABILITY PRESENT/ABSENT/
3	absent	absent
4	Absent	Absent
0	absent	absent
0	absent	absent
0	absent	absent
0	present	partially scrapable
0	absent	absent
0	present	absent
0	absent	absent
0	present	absent
1	present	partially scrapable
3	present	partially scrapable
0	absent	absent
0	absent	absent
0	present	partially scrapable
0	present	absent
0	present	absent
3	present	absent
4	present	partially scrapable
2	present	absent
1	absent	absent
0	absent	absent
2	absent	absent
2	present	absent

4th week active phase

BURNING SENSATION *VAS	PATCH/ PLAQUE PRESENT/ABSENT	SCRAPABILITY PRESENT/ABSENT/
1	absent	absent
2	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	Buccal mucosa (right)	absent
1	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
1	absent	absent
2	present	absent
0	absent	absent
0	absent	absent
0	absent	absent
0	absent	absent
2	absent	absent

1st month follow up

PATCH/ PLAQUE PRESENT/ABSENT	SCRAPABILITY PRESENT/ABSENT/	RECURRENCE
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
plaque present	partially scrapable	**RECURRENCE**
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
present	partially scrapable	**RECURRENCE**
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
Absent	Absent	NO- Recurrence
present	present	**RECURRENCE**
Absent	Absent	NO- Recurrence

3rd month follow up

URKUND

Urkund Analysis Result

Analysed Document: Ismat thesis 2.docx (D110152894)
Submitted: 7/5/2021 11:41:00 AM
Submitted By: drsaurabh2002@bbdu.ac.in
Significance: 10 %

Sources included in the report:

ABHILASHA PLAG FILE.pdf (D61631816)
RAMESH FINAL 21.07.2019.docx (D54524033)
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