DETECTION OF CANDIDA SPECIES IN SALIVA OF TYPE 2 DIABETIC AND NON-DIABETIC INDIVIDUALS USING CHROM AGAR MEDIA AND PCR – A COMPARATIVE STUDY

DISSERTATION

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BRANCH - VI

ORAL PATHOLOGY AND MICROBIOLOGY

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CERTIFICATE

Certified that the dissertation entitled: **"DETECTION OF CANDIDA SPECIES IN SALIVA OF TYPE 2 DIABETIC AND NON - DIABETIC INDIVIDUALS USING CHROM AGAR MEDIA AND PCR - A COMPARATIVE STUDY"** is a bonafide record of the work done by **Dr. ABILASHA J.V.** under our guidance during her post graduate study during the period of 2017-2020 under THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI, in partial fulfilment for the degree of MASTER OF DENTAL SURGERY IN ORAL PATHOLOGY AND MICROBIOLOGY, BRANCH -VI. It has not been submitted (partial or full) for the award of any other degree or diploma.

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DECLARATION

I hereby declare that this dissertation titled **"DETECTION OF CANDIDA SPECIES IN SALIVA OF TYPE 2 DIABETIC AND NON-DIABETIC INDIVIDUALS USING CHROM AGAR MEDIA AND PCR – A COMPARATIVE STUDY"** is a bonafide record of work undertaken by me and that this thesis or a part of it has not been presented earlier for the award of any degree, diploma, fellowship or similar title of recognition.

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Dr. Abilasha J.V.

CONTENTS

LIST OF ABBREVIATIONS

Abc	ATP Binding Cassette
ADA	American Diabetes Association
AIDS	Agglutinin –Like Sequence
ALS	Acute Immuno Deficiency Syndrome
CHS	Chitin Synthase Gene
CSH	Cell Surface Hydrophobicity
DM	Diabetes Mellitus
DPP-IV	Dipeptidyl Peptidase- 4 inhibitor
DS	Denture Stomatitis
DSND	Denture Stomatitis Non Diabetics
ECE	Endothelin- Converting Enzyme
ECM	Extra Cellular Matrix
Epa	Epithelial Adhesives
FPG	Fasting Plasma Glucose
GAD	Glutamic Acid Decarboxylase
GLP-1	Glucagon Like Peptide- 1
GPI	Glycosyl Phosphatidylinositol
HbA1c	Hemoglobin A 1 c
Hinfl	Haemophilus Influenza
HLA-DR/DQ	Human Leukocyte Antigen- DR/DQ isotype
HLA	Human Leukocyte Antigen
HIV	Human Immunodeficiency Virus
IDDM	Insulin Dependent Diabetes Mellitus
ITS	Internal Transcribed Spacer

LDH	Lactate Dehydrogenase
MDR	Multidrug resistance
NAC	Non Albican Candida
NHANES	The National Health and Nutrition Examination Survey
NOD	Non- Obese Diabetic
OGTT	Oral Glucose Tolerance Test
PEM	Proton Exchange Membranes
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction- Restriction Fragment Length Polymorphism
PL	Phospholipase
RapID	Random Amplification of Polymorphic DNA
SAP	Secretary Aspartyl Proteinase
SDA	Sabouraud Dextrose Agar
TNF-ALPHA	Tumor Necrosis Factor- alpha
WHO	World Health Organisation

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ABSTRACT

Background

Oral candidiasis is an opportunistic infection of the oral cavity. It can be a mark of systemic disease, such as diabetes mellitus and is a common problem among the immune-compromised. It is caused by overgrowth or infection of the oral cavity by yeast-like fungus, candida. The important ones are C. albicans, C. tropicalis, C. glabrata, C. pseudotropicalis, C. guillierimondii, C. krusei, C. lusitaniae, C. parapsilosis, and C. stellatoidea. C. albicans represents more than 80% of isolates from clinical infection. Over the past decade, the incidence of non albicans candida species has dramatically increased. Recently, an increasing number of publications have described populations with increased incidences of C. glabrata and their resistance to commonly used antifungal agents.

Aims and objectives

This study aims to identify and compare different candida species in saliva of Type 2 diabetic patients and non-diabetic individuals. The objectives are to identify the candida species in saliva of type 2 diabetic patients and healthy individuals using chrom agar media and to confirm the identified candida species using PCR. This is followed by quantification of the colonies of different candida species in both diabetic and healthy individuals and its correlation.

Materials and methods

A total of 200 participants in the age group of 45-64 years were included in the study. 100 were uncontrolled type 2 diabetic patients and 100 normal healthy individuals. After collection the saliva sample was concentrated by centrifugation at 5000 rpm for five minutes. The pellet which remained at the bottom of the tube

was streaked onto chrom agar media plates. These plates were then incubated at 37°C for 3-4 days. Candida speciation is done based on the different colored colonies appearing on chrom agar culture media. Total numbers of positive cultures of each species were noted in the proforma. The mean value of number of colonies of all the candida species were calculated for the two groups. These cultured species were then sent for PCR using sabouraud dextrose agar slants for species confirmation.

Results

Comparison of candida species within and between the groups was done by applying the unpaired t-test. The results obtained showed that there is significantly increased prevalence of candida in diabetic group compared to the control group. All the species of candida, namely, candida albicans, candida glabrata, candida parapsilosis and candida krusei showed a significantly higher ($p<0.05$) occurrence in the diabetic group compared to the healthy group. The highest identified species was C. glabrata followed by C. albicans in both the groups. Also the diabetic group showed more samples with multiple species of candida compared to the healthy group.

Conclusion

Antifungal resistance is becoming an increasingly concerning problem hence understanding the mechanisms of resistance is the key to the discovery of new treatment therapies. To date, there is a lack of understanding surrounding how C. glabrata interacts within a host and the host's defense mechanisms while simultaneously retaining a commensal existence in areas of the body including the

mouth, intestines and vaginal mucosal surfaces. We can learn much from candida glabrata, and indeed it deserves more attention, since it has an existence in a class all of its own.

Keywords

Chrom agar, C.albicans, C. glabrata, Diabetes mellitus, Oral candidiasis

INTRODUCTION

One of the increasing fungal infections affecting the oral mucosa is oral candidiasis[1].It is an occasional oral cavity infection. It is common and underdiagnosed among elderly people, especially in those who wear dentures and can often be avoided with a good oral hygiene care. It can also be a mark of systematic disease, such as diabetes mellitus, and is common among immunocompromised individuals[2]. Many species of candida are seen in the oral cavity like *c*andida albicans, candida glabrata, candida pseudotropicalis, candida guillermondii, candida stellatoidea, candida krusei, *c*andida parapsilosis, and candida tropicalis.

There are different clinical types of oropharyngeal candidiasis including acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis and angular cheilitis. The most discrete lesion represents conversion from benign colonisation to pathological overgrowth[2]. Upon modifying the oral cavity environment, a switch from the benign commensal nature of candida to a pathogenic state may occur to one that facilitates candida development. The origins of these shifts are the so-called predisposing factors of candida infection (candidiasis), most commonly associated with a loss of host immune defences[3].

Diabetes mellitus (DM) is a progressive metabolic disease which develops mainly in adolescents and is often combined with host of other risk factors. Defect in the body's control cells of the insulin signaling system triggers type 2 diabetes, as these cells will not be able to use insulin circulating in the blood[1.] Multiple immune changes have been defined in diabetes with more weakened cellular immunity and improvements in monocytes, polymorphonuclear cells, and lymphocytes. Individuals of DM have higher quantities of plasma glucose similar to safe people[4].

It is recognized that candida albicans is the most popular commensal among all candida species in the oral cavity. Recently, non-albican candida species viral potential has improved in oral cavity. These species differ in their disease causing potential and response to antifungals that are commonly used. Thus it has become important to identify these species[4].

Chrom agar contains specific substrates that are chromogenic. Enzymes are generated by microorganisms. These interfere with each other, producing different coloured colonies on the chrom agar media. Each colour represent different species of candida. On chrom agar media, glabrata appears purple, whereas albicans appears light green. C.glabrata has a haploid genome which distinguishes it from the albicans and few other non albicans which has a diploid genome. Many recent publications have used Polymerase Chain Reaction (PCR) to recognize different species of candida[4].

Fluconazole is a potent and selective inhibitor of fungal enzymes involved in the synthesis of ergosterol, an important constituent of the plasma cell membrane. Nowadays, candida species showing resistance to antifungal agents has been reported by many researchers. Because of the increase in the level of non-albicans, it is important to find a reliable diagnostic tool for treating of candida-related infections[5].

The present study was done to evaluate the prevalence of different candida species present in the saliva by estimating the colony forming units (CFU/ml) counts in the saliva of uncontrolled diabetic patients. This study also aims to confirm the candida species previously cultured in chrom agar media using PCR technique. The results of this study can help in improving patient treatment outcomes and reducing health care costs.

AIMS & OBJECTIVES

AIM

To identify and compare different candida species in saliva of Type 2 diabetic patients.

OBJECTIVES

- To identify the candida species in saliva of type 2 diabetic patients using chrom agar media.
- To identify the candida species in saliva of normal healthy individuals using chrom agar media.
- To confirm the identified candida species using PCR.
- To quantify the colonies of different candida species in both diabetic and healthy individuals.
- To compare and correlate different candida species in saliva of Type 2 diabetic patients with that of control.

REVIEW OF LITERATURE

CANDIDIASIS

Candida is a fungus and was isolated from a tuberculous patient's sputum for the first time in 1844. They are non-photosynthetic, eukaryotic organisms, like other fungi, with a cell wall that lies outside the plasma membrane. Within the nuclear membrane, there is a nuclear pore complex. There are large quantities of sterols in the plasma membrane, usually ergosterol. The morphological features of the various candida species are identical apart from a few exceptions. Under both aerobic and anaerobic environments, they can metabolize glucose.[1]

Candida is harmless in the mucous membranes of healthy individuals. It is present in the natural flora" and are sometimes useful but induce candidiasis when there is an imbalance.[2]

Many diseases are associated with candidiasis. Candida albicans is the most common species which is said to cause oral infection. They may also be caused by other species like candida glabrata, candida parapsilosis, and candida tropicalis. Rarely infections are also caused by organisms such as candida krusei, candida lusitaniae, and candida guilliermondii[1].

The Pathogen

Candida species reproduce by budding like yeasts. Most species except C. glabrata, form pseudohyphae[2].

Candida species cause various diseases extending from localized inflammation of the mucous membrane to life-threatening disseminated disease. The host's immune response is the main determinant of the extent of the infection. Overgrowth of candida caused due to changes in the normal flora lead to infections

locally. Invasive diseases that linger in an organ system, such as infections of the urinary tract, usually occur due to local anatomical abnormalities[3].

C. albicans are the only common cause of infection with oral fungus or yeast. C. albicans can be serologically classified into A and B serotypes, distributed equally among healthy individuals, but in immunocompromised patients there is a significant shift to type B[4]. Species that are not C. albicans (especially C. krusei) are increasingly seen, particularly in people with impaired immunity[4].

Oral candidiasis

C. albicans, C. guillermondii, C. krussei, C. glabrata, C. parapsilosis, C. pseudotropicalis, C. stellatoidea, C. tropicalis are the different species of candida in the oral cavity[4]**.**

There are various types of oropharyngeal candidiasis, including acute pseudomembrane, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis, and angular cheilitis. Conversion from benign invasion to pathological overgrowth is the most distinct lesion.

Oral candidiasis- Proposed revised classification[5]

Primary oral candidiasis:

Acute candidiasis

- Pseudomembranous type
- Erythematous type

Chronic candidiasis

- Erythematous type
- Pseudomembranous type

- Hyperplastic type
- Nodular type
- Plaque-like
- Candida-associated lesions
  - Denture stomatitis
  - Median rhomboid glossitis
  - Angular cheilitis
- Keratinized primary lesions superinfected with candida
  - Lichen planus
  - Lupus erythematosus.
  - Leukoplakia

Secondary oral candidiasis:

- Oral manifestations due to systemic mucocutaneous diseases
- Candidiasis as a result of diseases like candidiasis endocrinopathy syndrome and thymic aplasia.

Pseudomembranous candidiasis

Large white pseudomembranes consisting of desquamated epithelial cells, fibrin, and fungal hyphae characterize pseudomembranous candidiasis (Thrush). White patches exist on the surface of the hard and soft palate, paradontal tissue, labial and buccal mucosa, lip, and oropharynx. Usually, scrapping with a swab exposes the erythematous mucosa underlying it. Diagnosis is typically simple as it is easy to see and is one of the most common forms of oropharyngeal candidiasis which accounts for almost a third of the disease. Diagnosis can be verified

microbiologically either by staining a smear from the affected area or by cultivating an oral rinse swab[1].

Predisposing factors include age extremes, diabetes mellitus, HIV / AIDS, leukaemia patients, inhalers of steroid aerosols, broad spectrum antibiotics, and psychotropic drugs, and terminally ill patients. Lichen planus, squamous cell carcinoma, lichenoid reaction, and leukoplakia are other conditions that may cause white patches in the mouth.

Acute atrophic candidiasis is usually associated in the mouth or on the tongue with a burning sensation. The tongue may be bright red, similar to a low B12 serum, low folate, and low ferritin. Diagnosis may be difficult but should be taken into account in the differential diagnosis of a sore tongue, especially in a vulnerable older patient with dentures who has undergone antibiotic therapy or who is on inhaled steroids. A swab from the mucosa of the tongue / buccal area may help to diagnose[4].

Chronic hyperplastic candidiasis

Chronic hyperplastic candidiasis occurs as speckled or homogeneous white lesions on the buccal mucosa or lateral border of the tongue. The lesions usually occur on the tongue's buccal mucosa or side edges. There is a correlation with smoking and it seems that full resolution relies on smoking cessation. This condition may lead to severe dysplasia or malignancy and is sometimes called candidal leukoplakia. It has been suggested that finding candida species is a complicating rather than causative factor in premalignant lesions like lichen planus, pemphigoid / pemphigus, and squamous cell carcinoma[2].

Chronic atrophic candidiasis

Also known as "denture stomatitis," chronic atrophic candidiasis is characterized by localized chronic erythema of tissues covered by dentures. In the palate and upper jaw, lesions usually occur, but can also affect the mandible tissue. Diagnosis includes removal of dentures and close inspection; for verification, swabs may be taken. Incidence levels of up to 65 percent are stated to be quite normal[2].

Median rhomboid glossitis

The chronic symmetrical area anterior to the circumvallate papillae of the tongue is the site for median rhomboid glossitis. It consists of filiform atrophic papillae. This area's biopsy usually yields candida in more than 85% of cases. Smoking and the use of inhaled steroids tend to be associated with it[2].

Angular cheilitis

Angular cheilitis is an erythematous fissuring lesion in one or both corners of the mouth and is usually associated with a candida infection. Staphylococci and streptococci are the other species involved. The reservoir is normally the anterior region of the nostrils in the case of staphylococci and has been reported to spread to the angles of the mouth.

Facial wrinkling in the corners of the mouth and along the nasolabial fold, especially in the elderly, leads to a chronically moist environment predisposing to this lesion. This wrinkling is worse in long-term denture wearers as there is bone resorption on which the dentures rest, leading to a decrease in the lower face height when the mouth is closed. Other factors involved in this condition's etiology are iron deficiency anaemia and lack of vitamin B_{12}[2].

Factors predisposing to oral candidiasis

- **Local factors influencing oral immunity or ecology includes:**
    - Hyposalivation
    - Smoking
    - Broad-spectrum antimicrobials
    - Corticosteroids
    - Dental appliances
    - Irradiation involving the mouth or the salivary glands

- **Systemic immune defects, such as those caused by;**
    - Extremes of age
    - Malnutrition
    - Cytotoxic chemotherapy
    - Immune t-cell defects, especially HIV infection, leukaemias, lymphomas and cancer.
    - Leukocyte defects, such as in diabetes and immunosuppressant drugs
    - Anaemia

Diabetes Mellitus

Diabetes Mellitus, commonly known as diabetes, is a metabolic problem with high glucose levels over a delayed period of time or insulin emission deformity, insulin activity, or both. Therefore, insulin deficiency causes constant hyperglycemia with the aggravation of the starch, fat and protein digestion system. It is the most common endocrine problem and by 2010, more than 200 million people worldwide were expected to have DM and 300 million by 2025. As the disease

progresses, serious diabetic disorders such as retinopathy, neuropathy, nephropathy, cardiac complications, and ulceration result in tissue or vascular injury. Therefore, diabetes covers a wide variety of heterogeneous diseases. High symptoms of glucose include increased micturition, increased appetite, and increased cravings. Diabetes may cause multiple off chance problems that have stayed untreated. Acute symptoms may include diabetic ketoacidosis, non ketotic hyperosmolar coma, or death. Serious complications of the long haul include coronary disease, stroke, constant kidney failure, foot ulcers, and eye damage[6].

Classification of diabetes

Classification is dependent on insulin production by the pancreas or the body's reaction to insulin production properly. There are three main types of diabetes mellitus.

Type 1 Diabetes Mellitus

In this type of diabetes, pancreas does not properly produce insulin, or there is no insulin in the pancreas. It is also known as early diabetes or insulin-dependent diabetes mellitus (IDDM). The etiology for type 1 diabetes is unclear. It is less common than type 2 diabetes. People with type 1 diabetes must take insulin injections for the rest of their lives[7].

Immune-mediated diabetes

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously covered by insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from cell-mediated autoimmune destruction of the pancreatic β-cells. Markers of β-cell immune destruction include autoantibodies of

islet cells, insulin autoantibodies, GAD (Glutamic acid decarboxylase) autoantibodies (GAD65), and tyrosine phosphatasesIA-2 and IA-2β autoantibodies. One and possibly more of these autoantibodies are present in 85–90% of individuals when the initial diagnosis of fasting hyperglycemia occurs.

The disease also has strong associations with HLA (human leukocyte antigen), associated with the genes of DQA and DQB, and is influenced by the genes of DRB. Such alleles of HLA-DR / DQ may be predisposing or defensive[8].

Idiopathic diabetes

There are no known etiologies in some forms of type 1 diabetes. Some of these patients have permanent insulinopenia and are susceptible to ketoacidosis, but have no autoimmune evidence. Although this category includes only a minority of patients with type 1 diabetes, most of those are Africans or Asians. Individuals with this type of diabetes have episodic ketoacidosis and varying degrees of insulin deficiency between episodes. This diabetes type is heavily inherited, lacks immunological evidence of β-cell autoimmunity, and is not associated with HLA. In affected patients, an absolute requirement for insulin replacement therapy may come and go[8].

Type 2 Diabetic Mellitus

Diabetes mellitus can be one of the human being's oldest known diseases. It was first written about 3000 years ago in Egyptian manuscript. There was a clear distinction between Type 1 and Type 2 DM in 1936. Hyperglycemia, insulin resistance, and insulin deficiency are the most common cause of type 2 DM. Type 2 DM results from the combination of risk factors such as genetics, climate and behavior[7].

This form of diabetes, which accounts for between 90 and 95 percent of people with diabetes, previously referred to as non-insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, includes people with insulin resistance and usually have relative (rather than absolute) insulin deficiencies. These individuals do not need insulin therapy to survive at least initially and often during their lifespan. This form of diabetes is probably caused by many different causes. While the specific etiologies are not known, there is no autoimmune destruction of β-cells and there are no other causes of diabetes in patients[8].

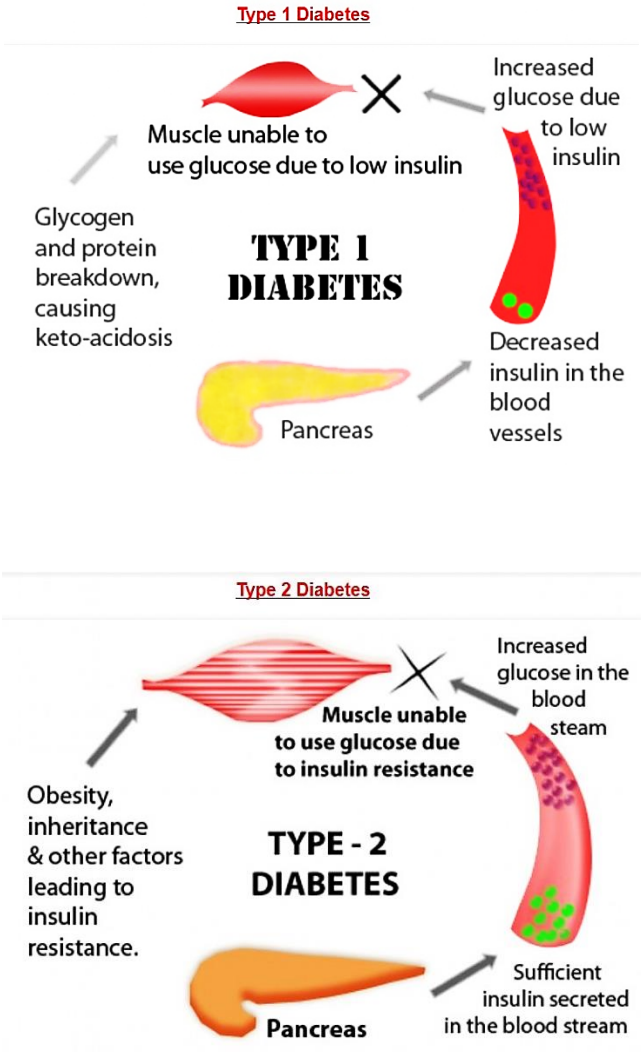


Figure1: Type I diabetes mellitus and Type 2 diabetes mellitus

Gestational diabetes

It is the third type of diabetes. Women are affected by this form during pregnancy. Some ladies have large amounts of glucose in their blood, and their bodies are unable to generate enough insulin to bring most of the glucose into their cells, resulting in dynamically increasing glucose levels. Pregnant women with gestational diabetes may develop type 1 or type 2 diabetes. Later many times in the second trimester of pregnancy (weeks 14-26) gestational diabetes occurs and disappears after pregnancy Gestational diabetes may build the danger of well-being issues in an unborn child. Consequently, it is important that it is detected early and properly handled. In the middle of pregnancy, gestational diabetes is diagnosed. Gestational diabetes can be controlled by exercise and diet. Approximately 10% and 20% of them should take some sort of solutions for regulating blood glucose. Undiscovered or uncontrolled gestational diabetes may increase the risk of interference associated with work.[6]

Epidemiology

366 million people were estimated to have DM in 2011; this would have risen to 552 million by 2030. With 80 percent of people with DM living in low and middle income countries, the number of people with Type 2 DM is growing in each region. In 2011, DM resulted in 4.6 million deaths. It is estimated that by the year 2030 there would be 439 million people with type 2 DM. Due to environmental and lifestyle risk factors, the occurrence of Type 2 DM varies greatly from one geographic region to another. The prevalence of DM in adults of which type 2 DM is becoming prominent is predicted to increase over the next two decades and much of the increase will occur in developing countries where most patients are between

45 and 64 years of age. It is estimated that the latter in developing nations would surpass or even exceed the former, resulting in a double burden as a result of the current transformation pattern from communicable to non-communicable diseases[7].

Pathophysiology

Type 2 DM is characterized by insulin insensitivity due to insulin resistance, decreased insulin production and subsequent pancreatic beta-cell failure. It results in a reduction in the transfer of glucose into the liver, muscle cells and fat cells. The involvement of impaired alpha-cell function in type 2 DM pathophysiology has recently been recognized[9].

Glucagon and hepatic glucose levels that increase during fasting are not decreased with a meal as a result of this dysfunction. Hyperglycemia results due to low insulin levels and increased insulin resistance. While GIP activity in those with type 2 DM is impaired, Glucagon like peptide-1 (GLP-1) insulinotropic effects are retained and therefore GLP-1 is a potentially beneficial therapeutic choice[9]. Nevertheless, like Gastric inhibitory polypeptide (GIP); DPP-IV in vivo easily inactivates GLP-1.

Two therapeutic approaches to this problem have been developed: GLP-1 analogs with increased half-lives, and DPP-IV inhibitors that prevent the breakdown of endogenous GLP-1 and GIP[9]. Both groups of agents have shown promise, with potential not only to normalize fasting and post-prandial glucose levels, but also to improve the mass of functioning beta-cells. Studies on the role of mitochondrial dysfunction in insulin resistance production and type 2 DM etiology are ongoing.

Adipose tissue is also very significant as an endocrine organ hypothesis (secretion of various adipocytokines, i.e. leptin, TNF-alpha, resistin and adiponectin involved in insulin resistance and probably dysfunction of the beta-cell) [9].

Many individuals with type 2 DM are obese with central visceral adiposity. The adipose tissue therefore plays a crucial role in type 2 DM pathogenesis. Although the predominant theory used to explain this link is the portal / visceral hypothesis that plays a key role in high concentrations of non-esterified fatty acids, the ectopic fat storage syndrome (deposition of triglycerides in muscle, liver and pancreatic cells) is two new emerging theories. This two theories form the basis for the study of the interplay in type 2 DM between insulin resistance and beta-cell dysfunction as well as between our obesogenic environment and DM risk in the next decade[9].

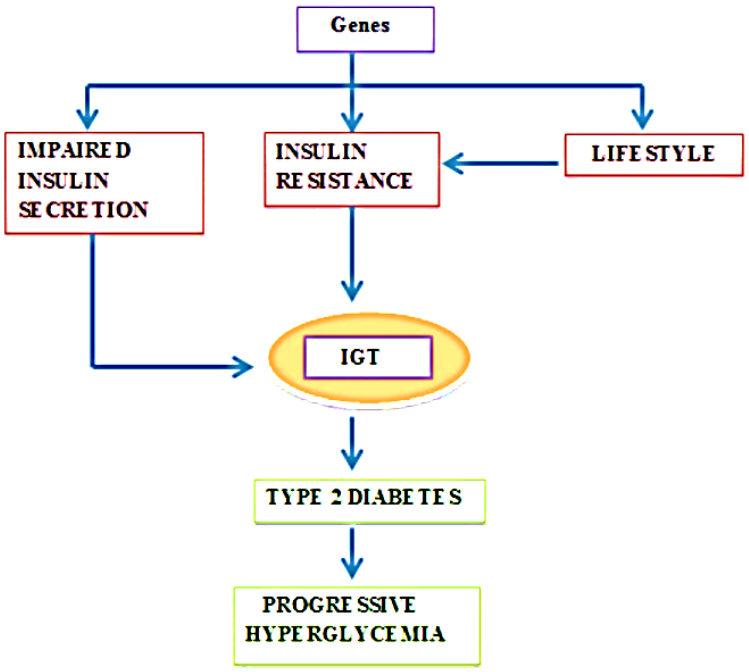


Figure 2: Pathogenesis of type 2 diabetes characterized by impaired insulin secretion and insulin resistance

Diagnostic criteria for diabetes mellitus

Diabetes mellitus is diagnosed either with plasma glucose approximation (Fasting Plasma Glucose (FPG) or Oral Glucose Tolerance Test (OGTT) or with HbA1c. Estimation of the cut off values for glucose and HbA1c is based on the association of FPG or HbA1c with retinopathy. Fasting plasma glucose of ≥ 126 mg/dL(7.0 mmol/L), plasma glucose after 2-h OGTT ≥ 200 mg/dL (11.1 mmol/L), HbA1c ≥ 6.5% (48 mmol/mol) or a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) along with symptoms of hyperglycemia is diagnostic of diabetes mellitus. In addition to tracking diabetes treatment, the International Committee of Experts proposed that HbA1c be diagnosed with diabetes in 2009 and supported by ADA, the Endocrine Society, the WHO[6] and many scientists and related organizations worldwide.

Sacks et al. in 2012 reviewed the advantages and disadvantages of the various tests used to diagnose diabetes. The benefits of using hemoglobin A1C(HbA1c) compared to FPG for diagnosing diabetes include greater flexibility and preanalytical stability, stronger association with microvascular complications, particularly retinopathy, and a marker for glycemic control and protein glycation that is the direct link between the disease and its diagnosis. Repeating the HbA1c test in asymptomatic patients within two weeks is recommended in order to reaffirm a seemingly single diagnostic result.

Many countries and different ethnic groups have endorsed a cut-off value of 6.5% for HbA1c (48 mmol / mol), yet ethnicity appears to affect the cut-off values for diagnosing diabetes. Cut-off values of 5.5% (37 mmol / mol) and 6.5% (48 mmol / mol) were reported in a Japanese study, 6.0% (42 mmol / mol) in a

National Health and Nutrition Survey (NHANES III), 6.2% (44 mmol / mol) in a Pima Indian study, 6.3% (45 mmol / mol) in an Egyptian study as reported by Davidson, and three cut-off values for Chinese. Two threshold levels are proposed by the Australians: about 5.5% for "rule-out" and about 7.0% "rule-in" diabetes[10]. Variations have been documented in the prevalence of diabetes and prediabetes due to ethnicity[11]. Some studies have reported fewer diabetes subjects with HbA1c than with FPG or OGTT. Other studies, however, reported more diagnosed subjects using HbA1c.

Features of candida species causing candidiasis in diabetic patients

Enzymatic activity

Reduction in the salivary flow rate results from longstanding hyperglycemia. It also damages the eyes, nerves, kidneys, blood vessels and heart[12]. Reduced salivary flow due to acute hyperglycemia causes buccal or oral alterations like: a) impaired production or factors of many antimicrobial actions; b) increased mucin and glucose concentration; c) absence of a gustin a metalloprotein, that contains zinc which is responsible for the taste papillae maturation; d) coated tongue; e) increased proliferation of pathogenic microorganisms f) increased cells exfoliation after contact, because of poor lubrication; g) oral candidiasis; h) bad taste; i) halitosis; and many others occur. In the presence of inadequate glycemic control, buccal alterations found in diabetic patients will have increased incidence and progression[13].

Numerous studies have reported a link between the pathogenesis of candida and enzymes that cause hydrolysis. Higher blood glucose concentration is the main cause of candidiasis in diabetics. Higher esterase and hemolytic activity may lead to increased enzyme activity in diabetics. It has been suggested that these species are

more pathogenic in diseases such as diabetes mellitus. Enzymes like Secreted aspartyl proteinases (SAP) which degrade various substrates making the host proteins present in the oral cavity were studied and they were found to help candida species[14]. These can lead to increased vascular permeability thus causing inflammatory reactions and clinical findings that can disturb the humoral host's defense. Similarly, phospholipase (PL) targets and digests the phospholipids of the membrane, initiates cell lysis and facilitates infectious fungi penetration. This enzyme facilitates the aggregation of plasma and inflammatory cells, producing several inflammatory mediators in vivo. By secreting a cytolytic peptide toxin called candidalysin, albicans hyphae cause epithelial damage as well as innate immunity[15]. Hypha-associated ECE1 gene encodes this enzyme. This is said to be the first toxin found in human fungal pathogen. Candida lysin triggers the infiltration of calcium ions into oral epithelial cells and the release of lactate dehydrogenase (LDH) as cell damage and membrane destabilization[16].

Biofilm Formation

Biofilms are colonies of microorganisms trapped in an extracellular matrix that imparts significant resistance to antifungal treatment and enhances the host's immune response. Biotic surfaces (e.g. mouth mucosae) or abiotic surfaces (e.g. catheter) can form such communities. Nonetheless, candidemia is the world's most dangerous invasive mycoses, with mortality rates close to 40%. Candidemia causes urinary tract infections, and pneumonia are frequently known[17]. The infections are linked in nearly all of these cases to the use of a medical device and the formation of biofilms on its surface. Venous catheter is the most widely colonized device by the fungus which is mainly used for the delivery of blood, nutrients and medication. The

catheter contamination can occur either from the patient or from the hands of health professionals, or from migration of pre-existing lesion into the catheter18. Less frequently candida species can infiltrate the intestinal mucosa and propagate through the bloodstream if the digestive tract is colonized as a commensal starting to acquire pathogenic behaviour. Thus, circulating yeast cells endogenously colonize the catheter and is more common in patients who are undergoing chemotherapy as it destroys the intestine's mucosa. In the other instances, catheters contaminated with endemic invasive candidiasis were the most important source of bloodstream infections[19]. Production of biofilm is explained in four steps namely

1: Adherence-initial adherence of yeast and planktonic cells (first 1–3 h);
2: Intermediate phase – Biofilm development (11–14 h);
3: The polymer matrix (PEM) maturation process which penetrates all cell layers completely and are adhered to the substrate in a three-dimensional framework (20–48 h);
4: The most rudimentary step in which the cell form is scattered[20].

A mature biofilm is therefore characterized as a dense network of cells showing yeasts, hyphal, or pseudohyphal forms and are involved in the proton exchange between the cells via membranes and water channels. This helps the spread of nutrients from the atmosphere to the lower layers by biomass and also it allows waste removal. In vivo templates were used to develop candida species. Biofilms seem to adopt the same pattern of in vitro development. The thickness decreases by the rapid maturation phase[21]. Candida species is responsible for the final configuration of the biofilm and is unpredictable. Growth factors are concerned on the substratum where it is built. High glucose levels are believed to act as the

energy source of carbohydrates which serve the candida organisms. Sessile cells secrete the polysaccharide layer, which is likely to be necessary to protect against environmental challenges for the creation of biofilms. Candida species isolated from DM patients have a greater pathogenic capacity for the production of biofilms[17, 20].

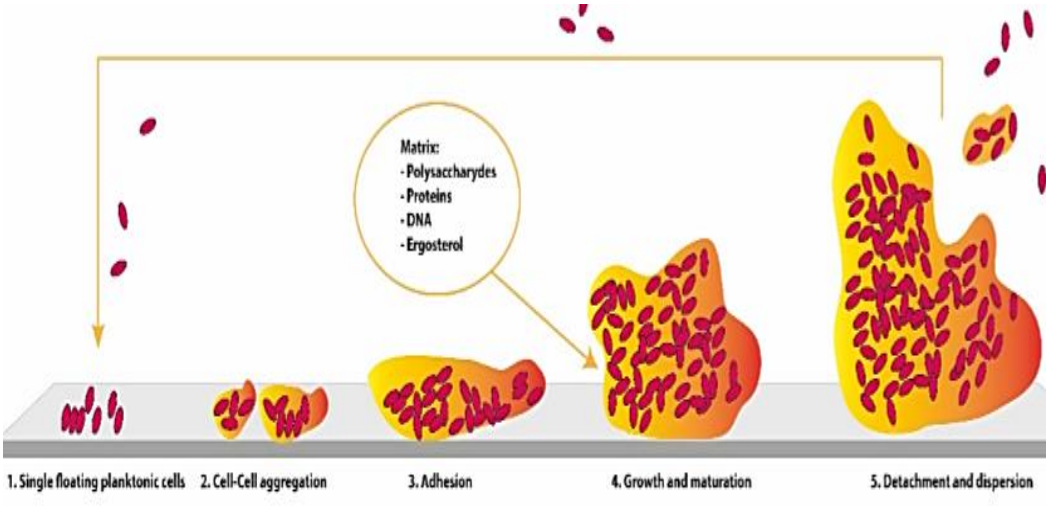


Figure 3: Development of a candida species biofilm in a surface

Hydrophobicity

The adhesion phenomenon in candida is mediated by glycosyl phosphatidyl inositol (GPI) Glycosyl Phosphatidyl Inositol (GPI)-like agglutinin-like (Als) proteins connected to β-1-6 glucans in the fungal cell wall. Als dependent cell adhesion is associated with increased cell surface hydrophobicity (CSH) [22]. These candida species increases their virulent property by promoting adhesion of the host tissue. C. albicans Als3p (hypha-associated) is a major epithelial adhesive that is highly upregulated during disruption of the gene ALS3 decreases adhesion in vitro and epithelial in vitro infection. Adhesion is also decreased by reducing the expression of the ALS2 gene. The epithelial adhesives in C. glabrata, (Epa) have a structure similar to Als proteins.

Along with adhesion ability, hydrophobicity, is a virulent trait that is gene-regulated and is correlated positively with metabolic activity as hydrophobic interactions tend to be crucial in promoting tissue invasion through the candida species' mycelial phase[24]. They may expand under anaerobic conditions, although fermentation under these conditions is the dominant path for ATP production. Data from Hoyer et al. (2007) found that 51.97% of diabetic patient isolates are strongly hydrophobic under anaerobic conditions relative with 21.90% under aerobic conditions. It is recognized that germ tubes can adhere to and supplement fibronectin through cell surface receptors, help filament yeasts to be attached to extracellular matrix components (ECM) and cause phagocytosis impairment, thereby increasing blood clearance resistance and candida species virulence[25, 26].

Pulative virulence factors of Candida albicans

•	**Virulence factor**	•	**Effect**
•	**Adherence**	•	**Promotes retention in the mouth**
•	Cell surface hydrophobicity	•	Non-specific adherence
•	Expression of cell surface adhesions	•	Specific adherence
•	**Evasion of host defences**	•	**Promotes retention in the mouth**
•	Phenotypic switching	•	Antigenic modification
•	Hyphal development	•	Reduces phagocytosis
•	Secreted aspartyl proteinase production	•	Secretory IgA destruction
•	Binding of complement	•	Antigenic masking
•	**Invasion and destruction of host tissue**	•	**Enhances pathogenicity**
•	Hyphal development	•	Promotes invasion of oral epithelium
•	Hydrolytic enzyme production	•	Host cell and extracellular matrix damage

C. glabrata

C. glabrata, is a normal microbiota in mouth, gastrointestinal and vaginal tract in humans, and cause no disease in most individuals. However disruptions in the normal environment lead to disease particularly in immunosuppressed hosts,

becomes a cause of disease [27, 28]. In view of the limited number of active antimicrobial therapies currently available and increase in antimicrobial resistance, Rodrigues et al. in 2009 have noted that C. glabrata's pathogenicity may be attributed to its ability to make biofilms and its high resistance to currently available conventional antifungal therapies. The increasing use of immunosuppressive drugs in modern medicine may also lead to an increased incidence of infections with C.glabrata[27].

Currently, virulence factors related to C. glabrata, relative to other species such as C. albicans are nonsensical. In analogy with C. albicans, C. glabrata can be called "less virulent"[29,30,31]. It was originally called Torulopsis glabrata[27] because of its inability to secrete proteases. Due to its human pathogenicity, the genus was only reclassified as Candida[29]. The two major functional differences between C. glabrata and C. albicans is its incapacity to form true hyphae and to secrete certain proteases[32].

Until date, there has been a lack of comprehension about how C. glabrata communicates with the host's defensive mechanisms while maintaining a commensal presence in areas of the body including the mouth, intestines and mucous surfaces of the vagina[27, 29]. Glabrata's non-pathogenic existence indicates that it has few virulence factors. The incidence of C. glabrata infections in various people like transplant recipients, cancer patients, and HIV victims, who have reduced T-cell functionality, may suggest that T cells play a role in protecting healthy people from C. glabrata infections[29]. Nevertheless, no reported indications of increased prevalence of C. glabrata infections in persons with impaired B-cells imply that antibodies may not be a tool used to defend the host from C. glabrata[29].

Pathogenic strains of bacteria are always clusters of chromosomal genes that encode for virulence traits; while comparing virulence traits and, more precisely, virulence genes in bacterial cells. These are absent in bacterial species that are very similar but not pathogenic. C. glabrata is hypothesized to have developed in a similar fashion, gaining new virulence traits and associated genes that are not present in closely related S. cerevisiae or C. albicans.

Virulence of candida glabrata

Compared with C. albicans, C. glabrata demonstrated relatively lower virulence and pathogenicity in animal models (Fidel et al., 1999). So far, only two animal model of mucosal C. glabrata infections have been reported. One is C. glabrata vaginitis in non-obese diabetic (NOD) mice (reviewed in Fidel et al., 1996), and the other is C. glabrata intestinal colonization in CF1 mice (Stiefel et al., 2004).

Culture Media

Sabroud Dextrose Agar (SDA) is the most frequently used primary isolation medium for candida. They permit the growth of candida simultaneously suppressing the growth of many other bacterial species because of its low pH[32]. Selectivity is further increased by adding antibiotics into SDA. Aerobically SDA is incubated at 37°C for 24–48 hrs. Candida appear as smooth, creamy, pasty convex colonies on SDA. Differentiation between species is rarely possible. It has been estimated that approximately 10% of oral samples have more than one candida species and recently the ability to detect non albicans species has become increasingly important because of numerous factors[32].

In recent years, various other differential medias have been developed. These medias allows the identification of different species of candida based on colony morphology and colour following primary culture. The presence of multiple candida species causing an infection can be determined by selecting subsequent treatment options. This is the advantage of such medias. Examples: Pagano-Levin agar or commercially available chromogenic agars, namely, Fluroplate, or Candi chrom albicans, chrom agar candida, albicans ID[31].

Distinguishing candida species based on reduction of triphenyl tetrazolium chloride is done with the help of Pagano-Levin agar. C. albicans produces pale coloured colonies compared to other species exhibiting pink coloration. The sensitivity of Pagano-Levin agar is similar to SDA but can detect more than one species in the sample[31], using chrom agar candida albicans, candida tropicalis, and candida krusei can be identified based on the colour of the colonies produced[34]. Studies have reported 95% specificity for candida albicans in chrom agar[35] and 98.6% for in Fluroplate agars[34]. On chrom agar candida dubliniensis appear as darker green colonies compared with those of C. albicans which produces light green colonies[36]. Discriminating between these two species using chrom agar decline upon subculture and storage of isolates. Recently it has been suggested that candida dubiliniensis fails to grow on agar media at the elevated incubation temperature of 45°C.

Chromagar

Chrom agar is a tool for yeast isolation. Chrom agar candida's structure has been designed to allow primary yeast separation from clinical product. Some of the isolated yeasts was C. albicans and their green color on chrom agar candida are

easily recognizable as such[32]. In terms of its ability to inhibit bacterial growth, chrom agar candida was slightly superior to the Sabouraud agar used for contrast. Chrom agar candida seems to be an effective tool for mycological medicinal use. It can act as a primary isolation and separation medium for medical samples likely to contain yeasts, as well as an adjunctive differential medium for distinguishing isolated yeasts on other media[32].

A PCR-based assay was used in a single reaction to amplify a portion of a chitin synthase gene (CHS) from these four species of candida. The reasoning for picking the gene for CHS1 was as follows. The CHS1 gene is chosen for amplification because there is no homologous mammalian allele, an important point in preventing false-positive results and because there are significant amounts of mammalian DNA in medical samples. The PCR assay should identify and distinguish between these four candida isolates in less time than is normally required for culture-based approaches since the early initiation of antifungal therapy is a crucial factor in reducing morbidity and mortality[38].

In a study by Jeanne A Jordan in 1994 a single pair of primers was used in a PCR assay to amplify and identify the DNAs from four medically important candida species: C. albicans, C. parapsilosis, C. tropicalis, and C. (Torulopsis) glabrata. The first successful amplification of a chitin synthase-specific fragment from four different candida species which are responsible for more than 91% of all cases of neonatal candidemia have been reported. The sequence of the first pair was based on that of the C. chitin synthase gene for albicans, CHS1. Each of the four enhanced products is a different size single unit. In a retrospective study of 27 paired blood samples from 16 patients with culture-proven candidemia, PCR analysis was

effective in detecting species level 26 of the 27 candida isolates and correctly identifying them. This PCR-based technology's speed and accuracy allow it a very powerful tool for candidemia identification and diagnosis. Implementation of this blood sample review test would contribute to more effective diagnosis of neonatal candidemia, thereby reducing morbidity and mortality[38].

Verghese et al in the year 2001 did a study to study the resistance of candida species to fluconazole using chrom agar media. The candida strains which were routinely isolated from clinical specimens like blood, urine, sputum, pus, fluid and homograft isolates were included in the study. 142 candida isolates were tested by using chrom agar candida incorporated with fluconazole. 16 strains were found to be resistant to fluconazole and 126 strains sensitive to fluconazole. Nine were C.tropicalis, three C.krusei, two C .guillermondii, one Geotrichum candidum and one was an unidentified strain of Candida[37].

In 2006 Hospenthal et al evaluated the usefulness of chrom agar candida in identifying C. albicans, C. glabrata, C. parapsilosis, C. guilliermondii, C. inconspicua, , C. lusitaniae, C. norvegensis, and C. rugosa and several other candida species and have found that most non albicans candida produced colonies that were shades of pink, lavender, or ivory. Several isolates of C. firmetaria and C. inconspicua developed colonies that were difficult to differentiate from C. krusei. Except in a light blue-green color, most C. rugosa isolates produced special colonies with morphology such as C. krusei. C. glabrata isolates produced small dark violet colonies that could be differentiated from the pink and lavender colors produced by other species. All isolates of C. dubliniensis produced green colonies similar to those produced by C. albicans[38].

Carvalho et al in 2007, described the identification of 8 clinically relevant yeasts using multiplex PCR, namely C. albicans, C. guilliermondii, C. parapsilosis, C. lusitaniae, C. tropicalis, C. krusei, C. glabrata, and C. dubliniensis. Results from the identification of 231 clinical isolates are presented pointing to the high specificity of this procedure. Furthermore, several candida isolates were identified directly from clinical specimens which also attests to the method's direct laboratory application. The results from the multiplex reactions with other microorganisms that usually coinfect patients also confirmed its high specificity in the identification of candida species. Moreover, this method is simple and presents a sensitivity of approximately 2 cells per ml within 5 hours. Furthermore, it allows discrimination of individual candida species within polyfungal samples. This novel method may therefore provide a clinical diagnostic procedure with direct applicability[39].

In a review by Trofa et al in 2008, they have said that candida parapsilosis has an extensive distribution in nature. There is currently no consensus on the treatment of invasive C. parapsilosis diseases, although the therapeutic approach typically includes the extraction of any removable foreign bodies and the administration of a systemic antifungal. Fluconazole is the most frequently administered alternative to amphotericin B. In vitro resistance to fluconazole has been documented among non C. albicans candida species, particularly C. glabrata and C. krusei. Additionally, there are conflicting opinions as to whether or not fluconazole use has resulted in a shift toward non albicans species causing candidemia. While some favour the argument that it has, it should be noted that others conclude that azole usage has not influenced the prevalence of certain candida species causing infections[40].

Nadeem et al did a study in 2010; for identifying yeast species isolated from specimens that has become important increasingly. An ever-increasing variety of known pathogens, an ever-increasing number of immunosuppressed patients and the development of antifungal susceptibility add to this need. The efficacy of chrom agar was tested using conventional methods like morphology using API 20 C AUX, on corn meal tween 80 agar and biochemical methods. The results confirmed that chrom agar can be used to identify and accurately differentiate three candida species namely, candida albicans, candida tropicalis, and candida krusei. For candida albicans the specificity and sensitivity of chrom agar media is 99%, for C. tropicalis it is 98%, and for C. krusei it is 100%. Their results promote the use of chrom agar media to quickly identify candida organisms directly from clinical collections in resource-limited environments, which could be very useful in designing effective therapeutic approach and patient care[41].

Sanita et al in 2011 compared the prevalence of candida species. Samples were collected from 90 healthy participants, 40 diabetic denture stomatitis and 80 non diabetic denture stomatitis, inorder to classify candida organisms. For analysis they have used Fisher exact test and have found that the predominant species isolated was candida albicans (81.9%), with C. tropicalis and C. glabrata demonstrating 15.71% and 15.24%, respectively. C. albicans and C. tropicalis were found to be significantly higher in the Denture Stomatitis groups than in the control group. C. tropicalis prevalence was increased significantly with the highest degree of inflammation. Candida species prevalence in diabetic and non-diabetic patients with denture stomatitis was similar. However candida tropicalis play a role in the advancement of diabetic stomatitis[42].

In a review by Tam et al in 2015, they have said that of all the species of candida, C. glabrata seems to be the least vulnerable to azoles. In turn, the growing use of azole antifungals to combat invasive candidiasis has resulted in numerous resistant strains being identified.

The antifungal resistance in C. albicans are largely agreed to be due to: (i) cell wall changes or plasma membrane changes leading to reduced drug uptake; (ii) P-450 lanosterol demethylase enzymatic changes, encoded by ERG11, which results in loss of drug affinity or overexpression of ERG11; and (iii) Use of an energy-dependent product efflux pathway regulated by ATP binding cassette membrane transport proteins (ABC) transporter superfamily or the major facilitator (MDR) superfamily. The latter two are the most frequently discussed of these three processes and were described as resistance mechanisms used by C. glabrata.[43]

Mohhammed et al did a study in 2016 to identify and equate the colonization rates of organisms in diabetic and non-diabetic individuals in the oral cavity. The candida species were identified using the restriction enzymes HinfI and MspI with PCR-restriction fragment length polymorphism (RFLP) and were differentiated by chrom agar culture medium. Among diabetic patients, the prevalence of candida organisms was greater than in non-diabetics. Candida albicans were more prevalent in diabetics (%36.2) followed by candida krusei (%10.4), candida glabrata (%5.1), and candida tropcalis. (%3.4). Also candida albicans was the most frequent species (%27) in the non-diabetic individuals. It was found in this analysis that the use of chrom agar and PCR-RFLP methods concurrently leads to more precise isolate detection.

The aim of the 2016 research by Zomorodian et al was to establish the frequency of oral candida colonization in patients with diabetes and its interaction with variables such as candida species, serum glucose levels and independent heat sensitivity to antifungal disease. The study included 113 type 2 diabetes, 24 type 1 diabetes, and 105 healthy controls. The samples were taken from people with diabetes mellitus and healthy individuals by swabbing oral mucosa and these were then inoculated on chromagar media. The yeast isolates were identified by PCR-RFLP and RapID methods. These have also been exposed to antibiotic drug resistance research. For analysing glycosylated hemoglobin (HbA1c), blood samples were taken. Although the rate and density of candida carriage in diabetics were statistically higher than healthy individuals, there was no direct association between high candida-burden and glycosylated hemoglobin. Candida albicans was the species found more in both diabetics and controls[45].

In 2016 Rajakumari et al did a study to assess the incidence of candida in the diabetic and non-diabetic buccal cavity and to evaluate the collected isolates antifungal susceptibility profile. Their results revealed that, compared to non-diabetic healthy individuals, the incidence of candida species in diabetics was greater. From their findings the most predominantly isolated species in both the groups was C. albicans. Among the non-albicans C. tropicalis was predominant in both the groups. Among denture wearers C. glabrata was predominant. In vitro antifungal susceptibility testing was done and found that commonly used antifungal agents showed increased effectiveness against itraconazole, ketoconazole and fluconazole[46].

In 2017, Sharma et al conducted a study to classify and evaluate different candida organisms in oral cavity of Type II diabetic patients. Student's t-test was

done for species comparison between two groups. Candida albicans, candida dubliniensis, candida krusei, candida glabrata, candida parapsilosis showed a significantly higher occurrence in the diabetic group compared to the control group. In both the groups, the highest identified species was C. parapsilosis, followed by C. albicans[47].

In 2018, Hassan et al conducted a study to classify and evaluate species variability, colony forming species units and antifungal resistance from oral cavity of individuals in well controlled, moderately controlled and poorly controlled diabetes. For poorly controlled diabetes, there was a significant difference for candida incidence relative to adequately managed diabetes, well-controlled diabetes, and average patients. It was found that in well controlled diabetes a higher number of colony count were seen than the other three groups. Comparatively small number of non-albicans was seen in healthy individuals. C. albicans demonstrated improved fluconazole resistance in patients with diabetes mellitus compared to the control group[48].

MATERIALS & METHODS

Study Setting:

The present study was carried out in the Department of Oral Pathology, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Kanyakumari District, Tamil Nadu, after obtaining clearance from the Institutional Research Committee (IRC) and Institutional Human Ethics committee (IHEC).

Study design: Analytical Cross-sectional study

Study period: One year

Number of groups to be studied: Two groups

Detailed description of the groups:

Group I : Healthy individuals (100 Individuals)

Group II : Type 2 Diabetic patients (100 Individuals)

SAMPLING:

Sample size of each group

Group I- 100

Group II- 100

Total sample size of the study: 200

Scientific basis of sample size used in the study: $n = \frac{z_{\alpha}^2 pq}{d^2}$

p = Percentage of any one variable in study

q → 100 – q

d → 20% of q

Level of confidence=95%

Level of power= 80%

With reference to the study conducted by Zomorodian et al (2016)

The obtained p = 65.2

q =34.8

d = 13.04

Sample size = 92

Here 100 is the sample size

Inclusion criteria:

Study group: Any participant having type 2 DM without any other oral lesions satisfying the following criteria were included in the study.

Fasting blood sugar (FBS) >126 mg/dl

Random blood sugar (RBS) ≥200 mg/dl or

Control group: includes healthy individuals without diabetes mellitus or any other systemic illness.

Age group: 45-64 years

Exclusion criteria: Patients with any other oral lesions or systemic illness other than type 2 diabetic mellitus or patients who have been diagnosed with diabetes since 6 years or those undergoing antifungal drugs are excluded from the study.

Preparation of culture medium

- Chrom agar media was prepared by adding 44.2 g of the agar in 1000 ml of distilled water.
- It was gently agitated and then heated to boil until the powder was completely dissolved.
- The solution was then poured on to petri plates under laminar air flow.

- Once the media sets the plates were then preserved inside the refrigerator to avoid contamination.

Sample collection

- In a well-ventilated room the participants were made to sit and their demographic details were recorded in the data sheet. Random Blood Sugar (RBS) or Fasting Blood Sugar (FBS) was recorded.
- They were asked to rinse their mouth for 10 minutes before sample collection to avoid food debris.
- From each participant 2-3 ml of unstimulated sample was collected by allowing the participant to drool into sterile and clean sample container.
- The lids were closed immediately after collection and within 30 minutes the samples were transferred to laboratory.
- These samples were stored at 4°C, until centrifugation and inoculation.

Equipment and armamentarium:

For candida culture

- Refrigerator (Whirlpool 19 premier India)
- Digital weighing machine. (Infra.Digi.Model no.IN 101 L, India)
- Light microscope (Adelta, India)
- Beakers (Borosil, India)
- Measuring jars(Borosil, India)
- Incubator (Kemi, India)
- Laminar air flow (Labline)
- Sample container (Labtech Medico)

- Petri plates (Aqua bio science)
- Micropipettes (Accupipetes, India)
- Bunsen burner
- Inoculation loop (Hi-media)

Reagents for preparation of culture medium

- Deionized distilled water (NICE, India)
- Himedia chrom agar

PCR set up:

18s r RNA Primer used for amplification (Covering the region of ITS1 and ITS2 region)

Takara Master mix: 15ul

Distilled H2O : 5 ul

Forward Primer : 1 ul (10picomolar)

Reverse Primer : 1 ul (10picomolar)

Template DNA : 3 ul

Total Reaction : 25 ul

Reagents for PCR

- CTAB (Sigma H6269)
- Chloroform (Sigma C2432)
- Isoamyl alchohol (Sigma I9392)
- Isopropyl alcohol (VWR PX-183514)
- Rnase (100mg/ml Dnase free) (Qiagen 19101)
- TE (10 mM tris, 1mM EDTA) (Ambion 9858)

- Proteinase K (Qiagen 19131)
- NaCl (Sigma S3014)
- Mercaptoethanol (Sigma M3148)
- Ammonium acetate (Sigma A1542)

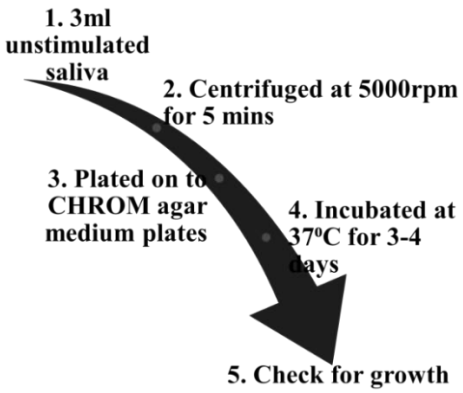
Procedure in detail:

- Written consent was obtained from each participants.
- After collection the sample was centrifugated at 5000 rpm for five minutes
- The suspension which was at the bottom of the tube was taken in a inoculation loop and inoculated onto previously prepared chrom agar media plates under laminar air flow
- The plates were then incubated for 3 to 5 days at 37°C.
- These were checked every day at 24 hours, 48 hours, and 72 hours for colonial growth.
- The speciation of candida was done depending upon the various colored colonies appearing on the culture media.

Candida species	**Colour obtained on chrom agar**
C. albicans	Light green colured colonies
C. parapsilosis	Cream colored colonies
C. glabrata	Pale edges on dark pink (purple) coloured colonies
C. krusei	Fuzzy, rough, large, pink colonies
C. tropicalis	Dark blue with halo colonies
C. dubliniensis	Dark green colonies

- The number of positive cultures of each species were noted.
- The mean values were calculated for all the species in both the groups, namely, Type 2 diabetic group and the healthy control group.
- These cultured species were sent for PCR using Sabouraud Dextrose Agar slants.
- Polymerase chain reaction allows the identification of clinically relevant yeasts of the candida genus
- Briefly, genomic DNA was isolated and purified; amplification was established utilizing glass bead preparation and Candida organisms.

Procedure in brief:



Evaluation:

The number of colonies of each species obtained from each sample is recorded. All the data were entered in a prepared data entry sheet. Data were entered in Microsoft excel application.

Statistical analysis:

The results obtained were analyzed by statistical package for SPSS20.0 version. Statistical tests to be used for data analysis: Mean, Standard Deviation, Student t-test.

RESULTS & OBSERVATIONS

The present study was carried out in the Department of Oral pathology and Microbiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam. Identifying and comparing the different candida species in the saliva of Type II diabetic individuals and normal healthy individuals was the aim of this study. This study took place for a period of twelve months. Unpaired t-test was done to compare candida species between the two groups. All candida species, namely candida albicans, candida glabrata, candida krusei, candida parapsilosis, showed a significantly higher incidence ($p<0.05$) in the diabetic group than in the healthy group. C. glabrata was the highest identified species, which is followed by C. albicans in both the groups.

This study included 2 groups, Group I and II. Group I consisted of 100 normal healthy individuals and group II consisted of 100 Type 2 diabetic patients. Out of the 100 patients in group I, 53 were males and 47 were females. In group II, 45 were males and 55 were females. All 200 participants were within the age group of 45 to 64. Comparison was done between the number of colony forming units in both the groups and the species differentiation within and between the groups.

Saliva sample was collected from each patient, centrifuged and cultured in chrom agar media plates. Following the incubation procedure the number of fungal colonies was counted in each plate and tabulated depending upon the colour. Candida speciation was done based on the different coloured colonies appeared in the culture media plates.

Species	Colonies on chrom agar
C. albicans	Light green coloured colonies
C. glabrata	Pale edges on dark pink (purple) coloured colonies
C. parapsilosis	Cream coloured colonies
C. krusei	Fuzzy, rough, large, pink coloured colonies
C. tropicalis	Dark blue with halo coloured colonies
C. dubliniensis	Dark green coloured colonies

Comparing the total number of positive candida cultures between the groups 74% positive cultures were seen in group I and 92% were seen in group II. The obtained results showed that there was significant difference in the frequency of candida in uncontrolled diabetes when compared to normal healthy individuals (p=0.03).

Comparison of pure growth cultures of each candida species was done between the groups. Pure C. albicans growth was seen in only 15% of diabetic and 12% of healthy individuals whereas pure C. parapsilosis growth was seen in 6% of diabetic and 3% of healthy individuals. The results obtained were statistically insignificant (p=0.34 and p=0.74 respectively). Similarly pure C. glabrata growth was seen in 23% of diabetic and 18% of healthy individuals. This was statistically significant (p=0.05). Pure C. krusei growth was seen in 3% of diabetic patients and not seen in any of the healthy individuals (p=0.05).

On comparing the mixed growth of candida culture between the groups significant difference was seen (p=0.03). Group I showed 40% mixed colony

cultures whereas Group II showed 27%. Comparison of mixed growth culture of each candida species between diabetic and control group was done and it was found that mixed colonies of, C. glabrata + C. parapsilosis, C. albicans + C. parapsilosis, C. albicans + C. Krusei and and C. albicans + C. glabrata + C. parapsilosis were found to be significantly higher in diabetic group compared to the control group. However mixed culture of C. albicans + C. glabrata was more prevalent in both the groups. (Control=12% and Diabetic=13%).

On comparing the mean colony forming units of different candida species within the control group, a mean value of 19.77 ±6.857, 39.14 ±8.81, 10.37± 5.14 and 0.81± 0.907 were obtained for C. albicans, C. glabrata, C. parapsilosis and C. krusei respectively. Multiple comparisons of mean values of each species within the control group showed C. glabrata to be more prevalent followed by C. albicans and the values were found to be statistically significant ($p<0.05$).

Similarly on comparing the mean colony forming units of different candida species within the diabetic group, a mean value of 30.09 ±7.88, 41.12±8.28, 14.64 ±5.741 and 4.58± 3.602 were obtained for C. albicans, C. glabrata, C. parapsilosis and C. krusei respectively. Multiple comparisons of mean values of each species within the diabetic group showed C. glabrata to be more prevalent followed by C. albicans and the values were found to be statistically significant ($p<0.05$).

On multiple comparison of mean of each candida species between the control and the diabetic group, C. glabrata was found to be more prevalent in both the groups and the values were statistically significant when compared with the other species between the groups.

The identification of the candida species using chrom agar media was confirmed using polymerase chain reaction (PCR). Based on sequence homology and phylogenetic analysis, the purple coloured fungal culture was found to contain candida glabrata ribosomal RNA (CAGL0L13398r). The light green coloured fungal culture was found to contain candida albicans small subunit ribosomal RNA gene. The creamy white fungal culture was found to contain candida parapsilosis small subunit ribosomal RNA gene. The fuzzy, round, large pink fungal culture was found to contain candida tropicalis small subunit ribosomal RNA gene.

TABLES

Table-1: Demographic data's of the study groups

Description of group		**Group I (Healthy individuals)**	**Group II (Type 2 diabetic patients)**
Sample size (n)		100	100
Age range Mean age		45 to 64 years 55years	45 to 64 years 55years
FBS		<125mg/dl	126 to 200mg/dl
RBS		<200mg/dl	≥200mg/dl
Parameters		Number of candida colonies Number of different species	
Sex Distribution	Male	53%	45%
	Female	47%	55%
Sample	Unstimulated saliva		

Table-2: Comparison of total number of positive candida cultures between the groups

Groups	**Number**	**Percentage**	**p value**
Diabetic	92	92%	0.03
Control	74	74%	

(*p<0.05 significant compared between the groups)

Table-3: Comparison of pure growth culture of each candida species between the groups

Group	C.albicans	C.glabrata	C.parapsilosis	C.krusei
Diabetic Group	15	23	6	3
Control Group	12	18	3	0
p value	0.34	0.05	0.74	0.05

(*p<0.05 significant compared diabetic with control group)

Table-4: Comparison of mixed growth candida culture between the groups

Groups	>1 species	
	Number	Percentage (%)
Diabetic	40	40.00
Control	27	27.00
p value	0.03	

(*p<0.05 significant compared diabetic with control group)

Table-5: Comparison of mixed growth culture of each candida species between diabetic and control groups

Groups	C.albicans + C. glabrata	C. albicans + C. parapsilosis	C. albicans + C. Krusei	C. glabrata + C. parapsilosis	C. albicans, C. glabrata + C. parapsilosis
Diabetic	13	0	3	12	12
Control	12	3	0	8	4
p value	0.65	0.05	0.05	0.05	0.04

(*p<0.05 significant compared diabetic with control group)

Table-6: Comparison of mean colony forming units of different candida species between diabetic and control groups

Groups	C. albicans	C. glabrata	C. parapsilosis	C. krusei
Diabetic Group	30.09 ±7.88	41.12± 8.28	14.64 ±5.741	4.58± 3.602
Control Group	19.77±6.857*	39.14 ±8.81*	10.37± 5.14*	0.81± 0.907*
P value	0.03	0.04	0.04	0.03

(*p<0.05 significant compared diabetic with control group)

Table-7: Multiple comparisons of mean values of each candida species within control group

Organism	Mean ± SD
C. albicans	19.77±6.85
C. glabrata	39.14±8.81*
C. parapsilosis	10.37±5.14*,#
C. krusei	0.81±0.90*,#,$

(*p<0.05 significant compared *C.* albicans with others,
#p<0.05 significant compared C. glabrata with others,
$p<0.05 significant compared C. parapsilosis with others)

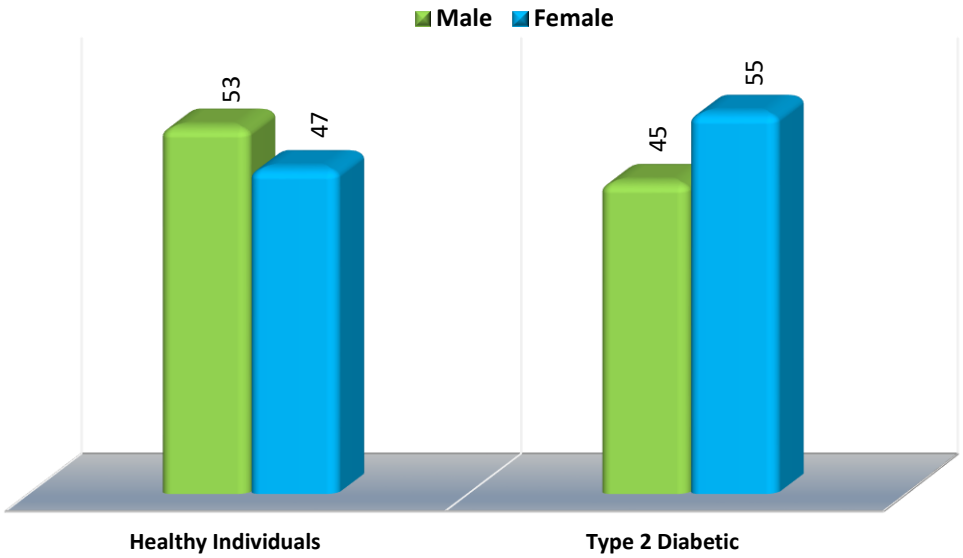
Table-8: Multiple comparisons of mean values of each candida species within diabetic group

Organism	MEAN±SD
C. albicans	30.09±7.88
C. glabrata	41.12±8.28*
C. parapsilosis	14.64±5.74*,#
C. krusei	4.58±3.60*,#,$

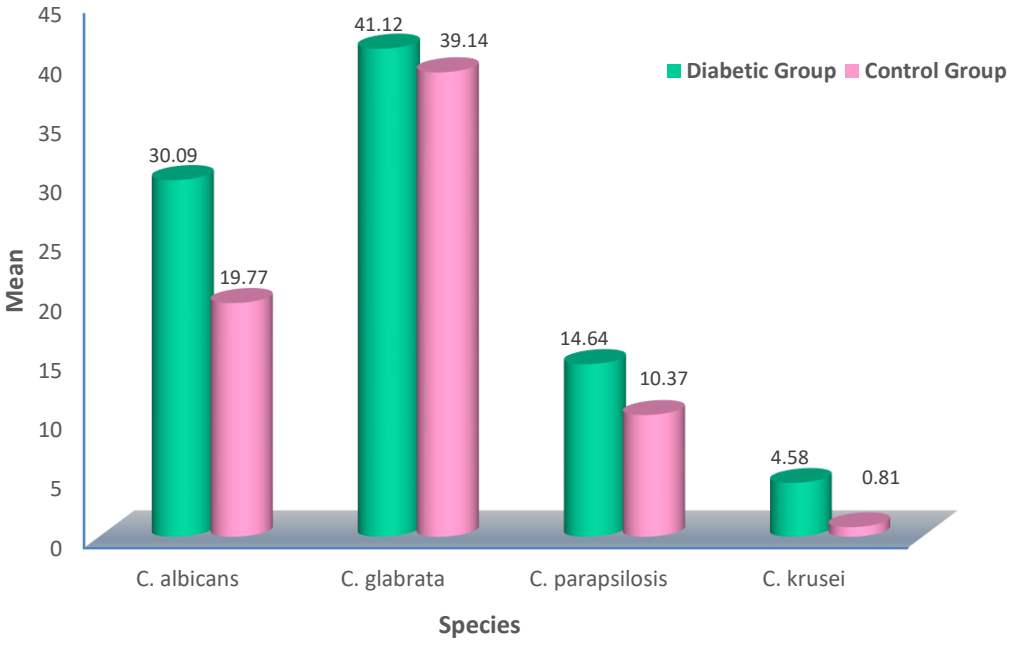
(*p<0.05 significant compared C. albicans with others,
#p<0.05 significant compared C. glabrata with others,
$p<0.05 significant compared C. parapsilosis with others)

GRAPHS

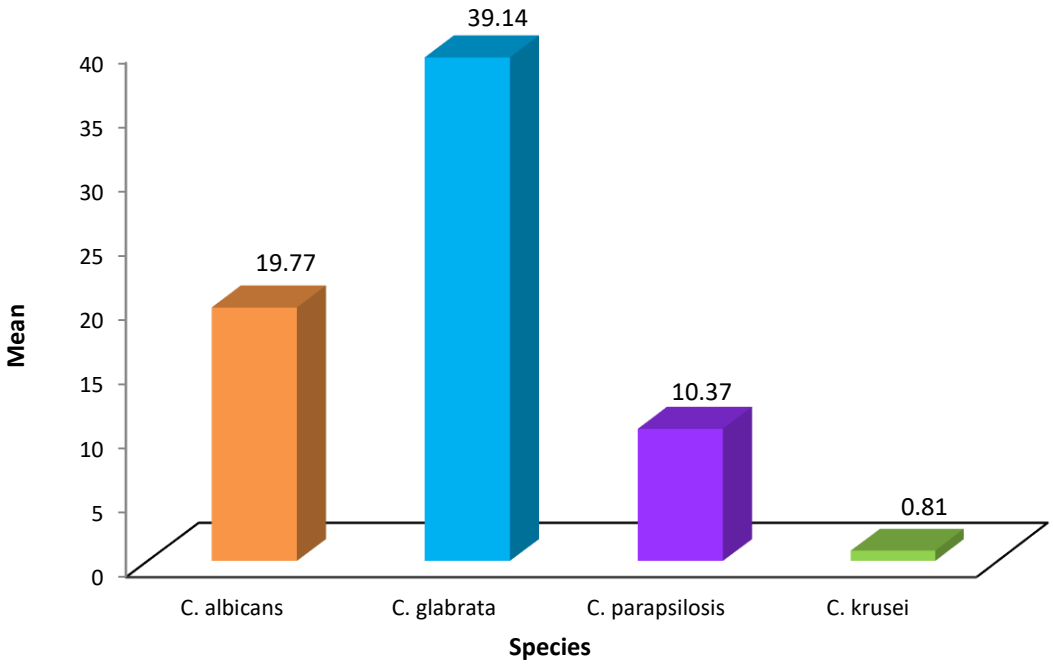
Graphs-1: Distribution of gender among the groups



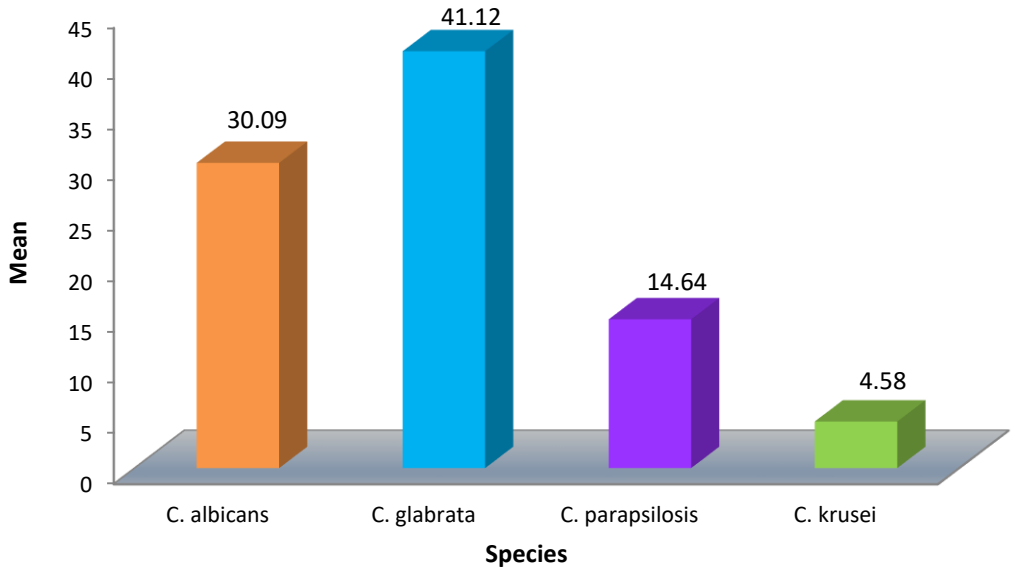
Graph-2: Comparison of mean colony forming units of different Candida species between diabetic and control groups



Graph-3: Multiple comparisons of mean values of each candida species within control group



Graph-4: Multiple comparisons of mean values of each candida species within diabetic group



DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, with several etiologies. The normal metabolism of food, fat and carbohydrate is influenced and impaired by it. It may be attributed to insulin secretion dysfunction, its operation, or both. In the world, particularly among the immune-suppressed, candida infections are a huge problem. The co-occurrence of diabetes mellitus with candidiasis has long been established, but this connection is still being challenged[1]. Oral candidiasis is caused by overgrowth or infection of the oral cavity by yeast-like fungus candida. This is a characteristic of certain autoimmune diseases such as diabetes mellitus and is more common in people who are immunocompromised and hospitalized[2].

C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, and C. stellatoidea are some of the common candida species seen[1]. Nevertheless, it has been said that candida albicans are the main cause of candidiasis isolated from many patients but, there has been a change in this pattern in recent years. The non-albicans mainly like C. glabrata and C. parapsilosis are coming into play. They differ widely from the albicans because of their pathogenesis and vulnerability. They also show increased resistance to fluconozole [2].

In this study, compared to the control group a higher prevalence of candida species was seen in the diabetic patients. This was in accordance with the studies conducted by various authors.

Mohammadi F et al. in 2016 have found higher incidence of candida in diabetic patients compared to non-diabetics. Their finding was in accordance with our study. The most popular candida species in their analysis was candida albicans (36.2 %) proceeded by C. krusei, C. glabrata and C. tropicalis in both diabetic and

non-diabetic patients. But in our study the most commonly isolated species was candida glabrata followed by candida albicans[44].

Aitken-Saavedra et al. in 2018 revealed that in their study they have isolated more than 60% of c. albicans in diabetic patients and 20.7% of C. glabrata (20.7%) [58].

The study presented by Lydia Rajakumari and Saravana Kumari in 2016 showed that in diabetic patients higher candidal colonization was due to lower glycemic control in diabetic patients. The most common species identified in their study was C. albicans, in denture wearers, C. glabrata was found to be more predominant[59].

Bhuyan et al in 2018 had reported that among poorly controlled diabetics, there was a higher number of candida colony counts than well-controlled, reasonably regulated and non-diabetic groups. The most common species in their sample is candida albicans. Researchers thought that this rapid development of yeast could be attributed to their increased levels of salivary glucose and altered oral microenvironment that facilitates candida growth in patients with diabetes. Such patients may find the history of high candida colonization for a longer period of time as a potential risk factor for candidiasis[48].

Zomorodian et al. in 2016 in their study had have not found any significant difference in growth of candida between diabetic healthy individuals[45]. In our study the most prevalent species in both the groups was found to be candida glabrata followed by candida albicans and then candida parapsilosis.

Li et al in 2007 had stated C. glabrata as an emerging pathogenic agent in the oral mucosa. C. glabrata associated oropharyngeal candidiasis infections in HIV and

cancer patients are more severe and more difficult to treat than infections due to C. albicans alone. Sometimes they act as a co-infectant with C. albicans, or as the only detectable species from oral lesions. Such fungi are highly resistant to antifungal agents and are particularly capable of producing biofilms that are highly refractory to antimicrobial treatment, making them very difficult to treat with conventional antifungal drugs[28]. In our study also there was an increased occurrence of candida glabrata in both diabetic and control groups.

A case of untreated diabetes mellitus and an ulcer on the perineum resulting in necrotizing soft tissue infection with candidemia by C. glabrata was reported by Shindo et al. in 2009[52]. Similarly in 2008 Bagg et al. have reported C. glabrata as an emerging pathogen in their study on patients with denture stomatitis[53].

C. glabrata, like most types of candida, is part of the normal mouth microbiome, of humans and does not cause disease in most people. Nevertheless, disruptions in the normal environment seem to trigger C. glabrata to become a cause of disease, especially in immunosuppressed hosts. [17]. Given the rise of antimicrobial resistance and the limited number of efficient antifungal drug treatments currently available, Rodrigues et al. in 2014 had noted the pathogenicity of glabrata may be attributed to its biofilm capacity and its relatively high resistance to traditional antifungal therapies. The use of immunosuppressive treatments in modern medicine can also contribute to increased glabrata infection occurrence[17].

Fidel et al. in 1999 have stated that, although both organisms have comparable cell surface hydrophobicity properties, C. glabrata is not prone to environmental conditions as C. albicans [49]. Nonetheless, a more recent study by Bialkova and Subik in 2006 had found that C. glabrata has a significantly higher

relative hydrophobicity of the cell surface than other species of candida[50]. In fact; C. glabrata has a four-fold hydrophobicity property of the cell surface relative to C. albicans [28]. Fidel et al has also indicated that albicans are better compared to other candida organisms in a variety of environments; C. glabrata is slightly more stable in in-vitro test conditions[49]. It should also be remembered that far more work has been done on albicans in relation to C. glabrata.

The emergence of drug resistance in candida isolates associated with epidemiological differences in natural flora of candida has important morbidity and mortality implications[56, 57]. The extensive use of medications, especially azoles, has promoted the selection of resistant species by shifting colonization to more naturally resistant candida species, such as C. glabrata, C. dubliniensis, and C. krusei. Apparently, the worldwide spread of candida organisms is a characteristic of the area's epidemiology[55].

Another study by Usha Sharma et al. in 2017 had reported C. parapsilosis as the highest identified species in both diabetic and non-diabetic group, followed by C. albicans which was explained to be due to change in pattern of candida species found in human oral flora. In this study candida parapsilosis ranks third[4].

Multiple isolation of candida species, including C. glabrata, C. albicans, C. parapsilosis, C. tropicalis and C. krusei were identified more in diabetic group compared to the control group which is similar to the studies conducted by Sanita et al in 2011[42] and Kamran et al in 2009[54]. This shows that there is increased prevalence of various non albicans species compared to albicans in diabetic group.

It is necessary to find a good diagnostic method for treating candidiasis as there is increase in non-albicans candida species and azole-resistant isolates. For the isolation and identification of candida cells, the chrom agar media is a chromogenic culture device. This will also encourage health professionals to make appropriate antifungal decisions more easily, thereby decreasing the morbidity and mortality of the patient. Speciation of candida was done based on the color exhibited by the colonies on chrom agar [54].

Nadeem et al. in 2010[41] have reported that chrom agar candida have correctly identified 99% of C. albicans, 98% C. tropicalis, 100% C. krusei and 94% of C. glabrata in their study. They have confirmed their finding by API 20C Aux yeast identification panel (Biomuriex, France), based on assimilation of carbohydrates.

In the present study, the results obtained from polymerase chain reaction and the colony features candida on chrom agar media demonstrated perfect agreement which is similar to the study conducted by Mohammedi et al in 2016[44].

From all the above findings we can conclude that it is necessary to do speciation in candidiasis as these emerging non-albicans are resistant to commonly used antifungal drugs.

SUMMARY & CONCLUSION

The aim of this study was identifying and comparing different candida species in the saliva of Type 2 diabetic patients and non-diabetic individuals. A total of 100 saliva samples were collected from each group, centrifuged and inoculated in to chrom agar media plates. Chrom agar plates were examined for up to 5 days to check for growth. Based on the different coloured colonies the candida speciation is done.

For both Type 2 diabetic group and the healthy control group the mean value of colonies of each candida species was calculated. Purple, green, creamy white and fuzzy, rough, large, pink coloured colonies were observed in the media plates. These cultured species were then sent for polymerase chain reaction (PCR) using sabouraud dextrose agar slants. Using polymerase chain reaction it was confirmed that the Purple coloured colonies were C. glabrata, green were C. albicans, creamy white were C. parapsilosis and fuzzy, rough, large, pink coloured colonies were C. krusei.

It was observed that there is increased prevalence of candida growth in diabetic patients compared to healthy individuals. Also found that the prevalence of C. glabrata was found to be more followed by C. albicans and C. parapsilosis in both the groups. The values obtained were statistically significant for all the species within the groups. Similarly multiple species growth was significantly more in diabetic group compared to control group.

It has long been known that candidiasis is an opportunistic infection. Oral candidiasis is more common in systemic diseases like diabetes mellitus and immune-compromised individuals. C. albicans was said to be the most common cause of this. However the trend has been changed in the recent years. C. glabrata and C. parapsilosis have now currently emerging as human pathogens. A greater proportion of these non-albican strains and their high sensitivity to the commonly prescribed diabetes antifungal agents suggest the need for specific diagnosis and therapeutic treatment approaches This highlights the importance of regular candida speciation and correct selection after susceptibility testing of prophylactic antifungal therapy, especially during periodic visits. This could prevent this opportunistic yeast from being disseminated systemically in such patients. In addition, antifungal resistance is becoming increasingly problematic, so understanding the resistance mechanisms is the key to discovering new therapies for treatment. To date, there is a lack of understanding of how C. glabrata communicates inside a host and the defensive systems of the host when retaining a commensal nature in areas of the body including the mucous surfaces of the mouth, intestines and vagina. We can learn a lot from candida glabrata and it deserves more attention because it has its own existence in a class.

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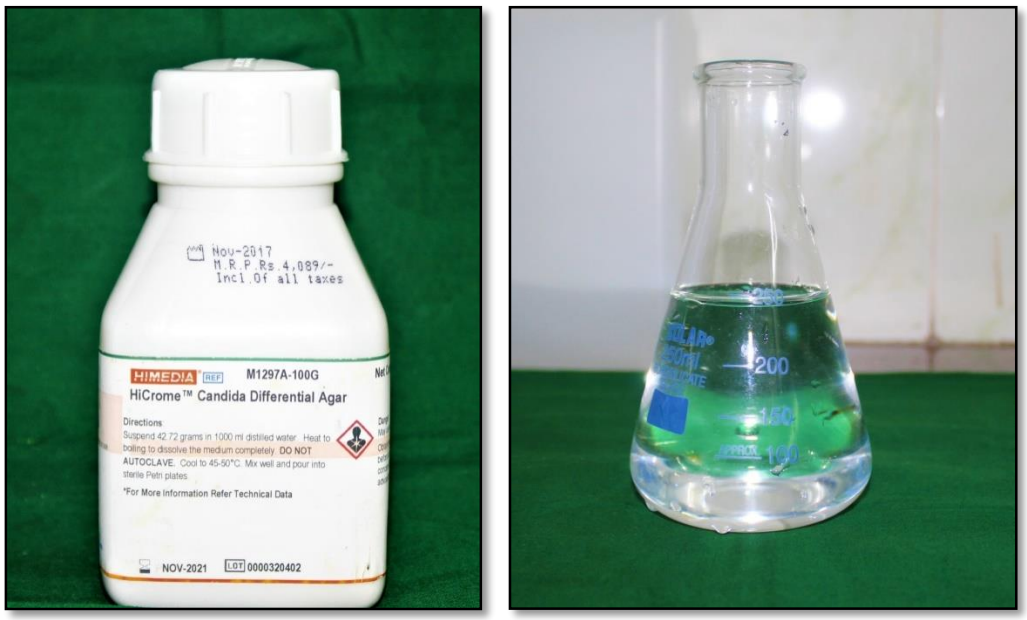
COLOR PLATES

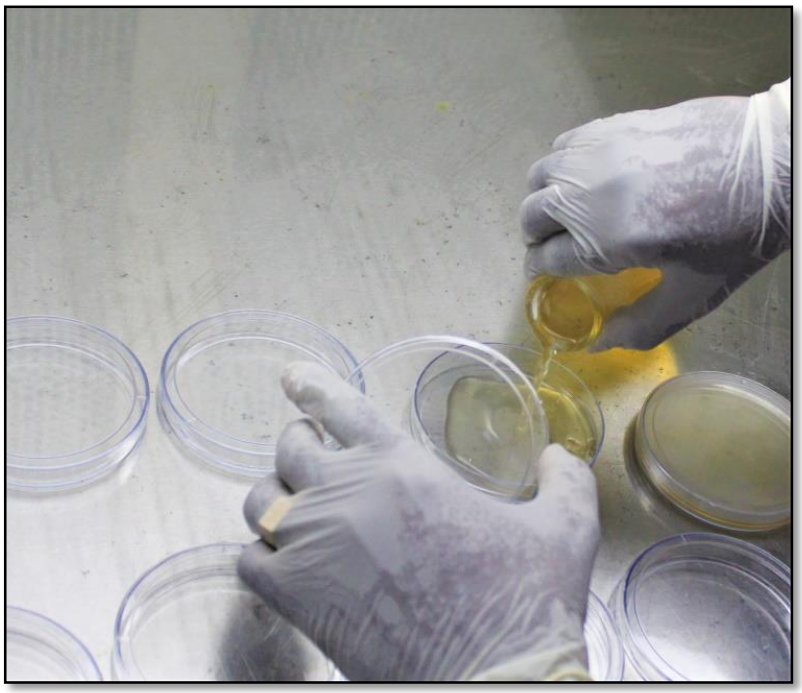
CP-1: Collection of sample and storage

CP-2: Centrifugation of samples

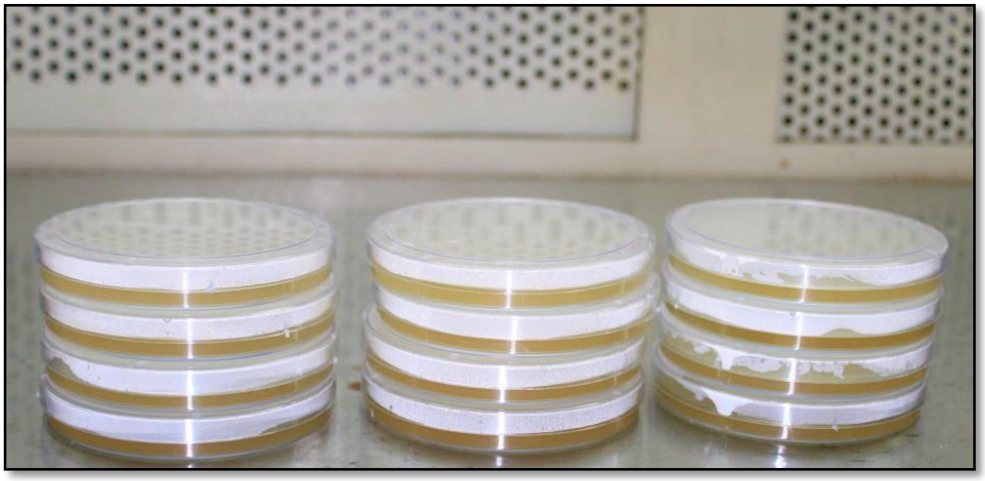


CP-3: Chrom agar media and distilled water

CP-4: Media preparation



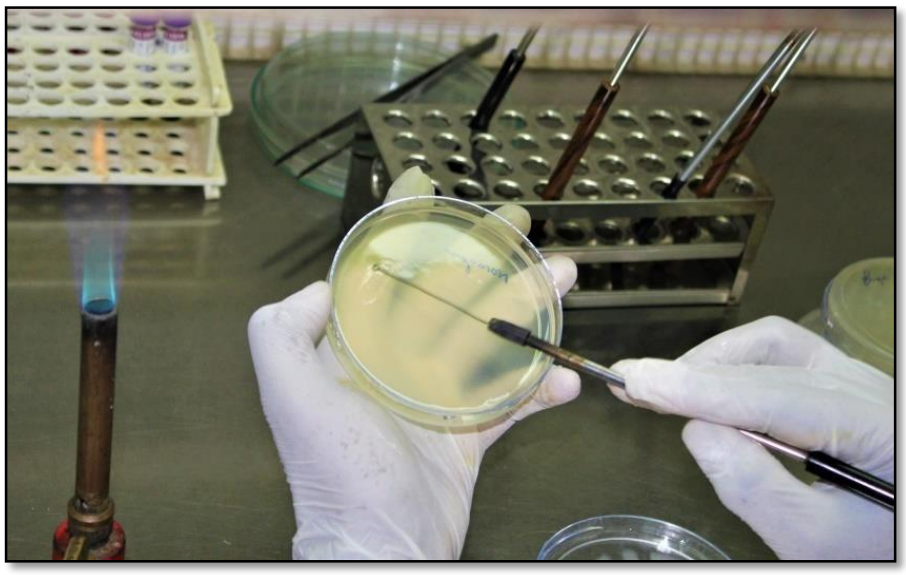
CP-5: Pouring of media in to plates



CP-6: Petri plates with chrom agar media

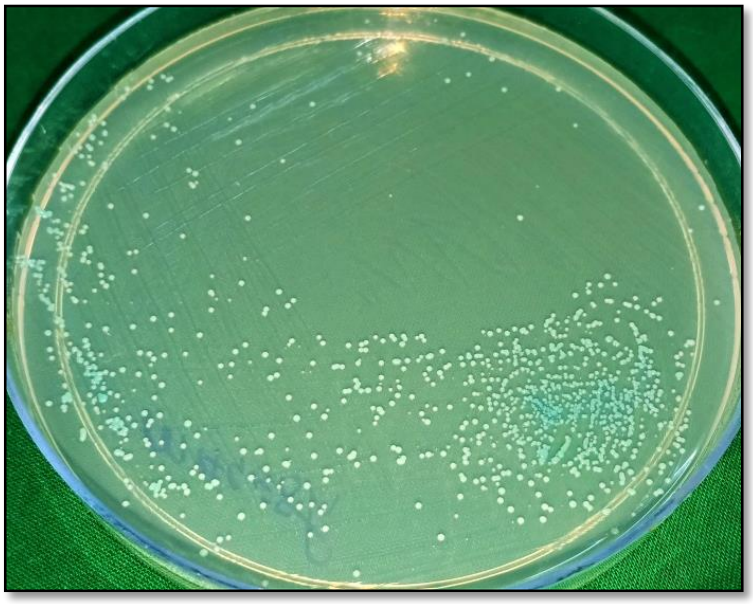
CP-7: Workstation for sample inoculation

CP-8: Loops used for inoculation



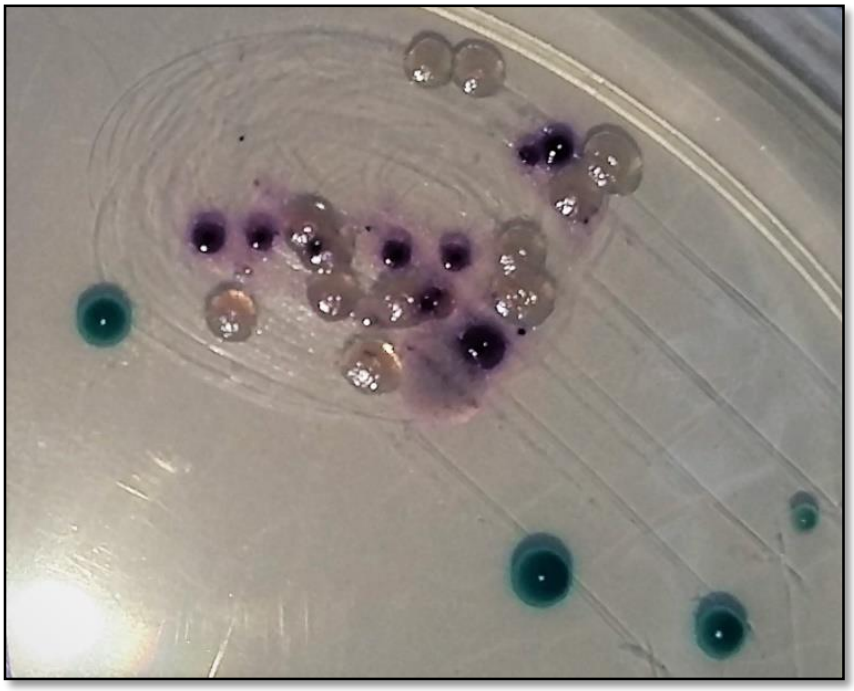
CP-9: Inoculation of salivary sample in to media under laminar airflow

CP-10: Incubation of petri plates

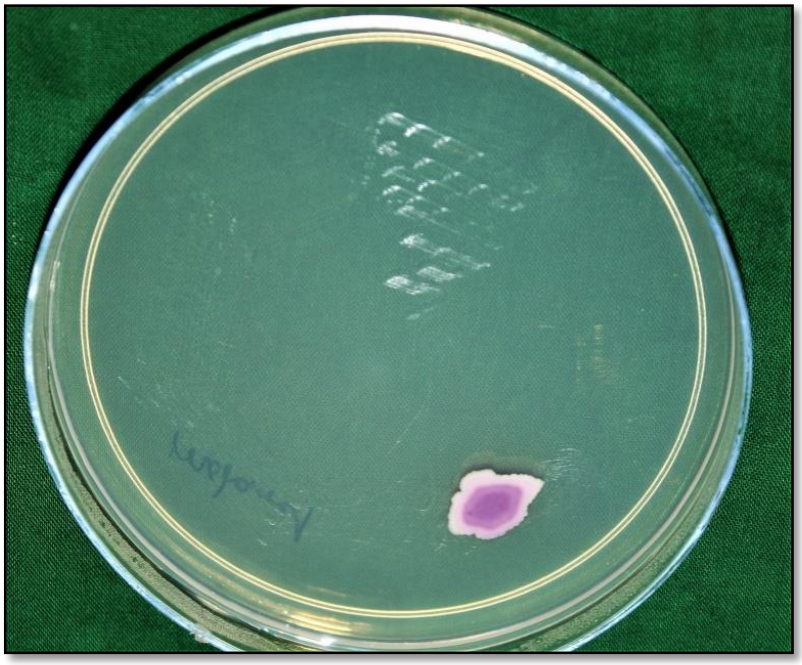


CP-11: Media showing candida albicans

CP-12: Media showing candida glabrata

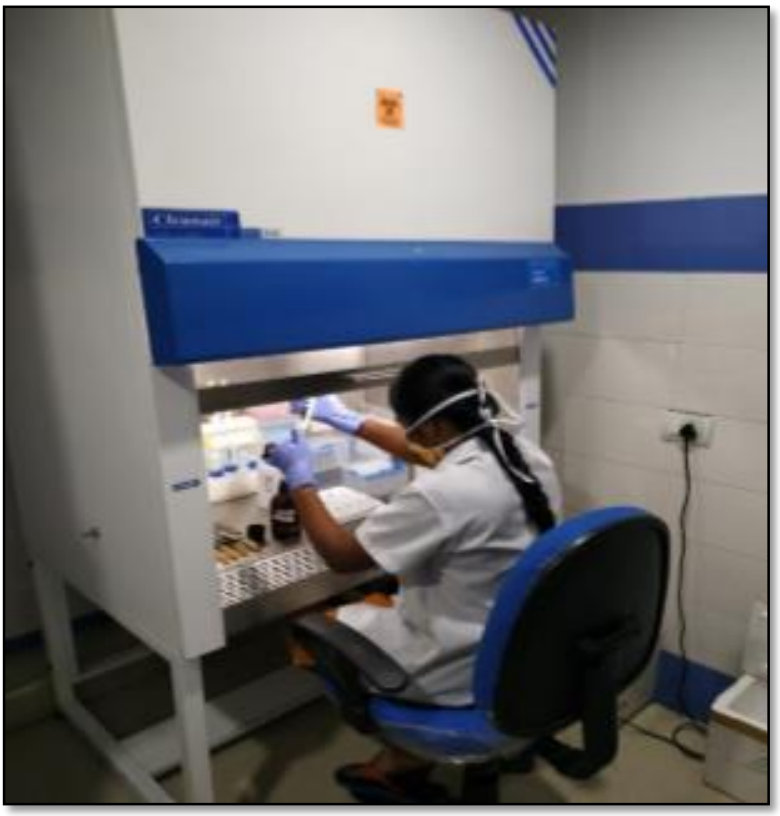


CP-13: Media showing colonies of C. albicans, C. glabrata and C. Parapsilosis



CP-14: Media showing colonies of C. Kruse

CP-15: Reagents used in PCR technique



CP-16: DNA extraction process

CP-17: Gel electrophoresis

CP-18: Genetic analyzer

ANNEXURE

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES

KULASEKHARAM, KANYAKUMARI DIST., TAMIL NADU, INDIA.

INSTITUTIONAL RESEARCH COMMITTEE

Certificate

This is to certify that the research project protocol, ***Ref no. 08/08/2018*** titled, ***"Detection of Candida species in saliva of Type 2 diabetic and non-diabetic individuals using Chromagar media and PCR – a comparative study"*** submitted by ***Dr. Abilasha J.V., II Year MDS, Department of Oral Pathology and Microbiology*** has been approved by the Institutional Research Committee at its meeting held on ***3rd September 2018.***

Convener
Dr. T. Sreelal

Secretary
Dr. Vineet R.V

INSTITUTIONAL HUMAN ETHICS COMMITTEE

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES,
KULASEKHARAM, TAMILNADU

Communication of Decision of the Institutional Human Ethics Committee(IHEC)

SMIMS/IHEC No: 1 / Protocol no: 17 / 2018

Protocol title: DETECTION OF CANDIDA SPECIES IN SALIVA OF TYPE 2 DIABETIC AND NON DIABETIC INDIVIDUALS USING CHROM AGAR MEDIA AND PCR- A COMPARATIVE STUDY
Principal Investigator: Dr.J.V.Abilasha
Name& Address of Institution: Department of Oral Pathology and Microbiology Sree Mookambika Institute of Dental Sciences ☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y): 06-12-2018 Date of previous review , if revised application:
Decision of the IHEC: ☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:
Recommended for a period of :One Year

Please note*

- Inform IHEC immediately in case of any Adverse events and Serious adverse events.
- Inform IHEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above.
- Annual report to be submitted to IHEC.
- Members of IHEC have right to monitor the trial with prior intimation.

Reneega

Signature of Member Secretary (IHEC)

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES * KULASEKHARAM *
IHEC
Date 21/12/18

SREE MOOKAMBIKAINSTITUTEOFDENTALSCIENCES

KULASEKHARAM, KANYAKUMARI DISTRICT – 629161

DEPARTMENTOF ORAL PATHOLOGY AND MICROBIOLOGY

BRIEF CASE RECORD PROFORMA

Sample No: Group:

OP .No :

Patient Name:

Age:

Sex:

Blood sugar values:

FBS:

RBS:

Diabetic Since:

SREE MOOKAMBIKAINSTITUTEOFDENTALSCIENCES

KULASEKHARAM, KANYAKUMARI DISTRICT – 629161

DEPARTMENTOF ORAL PATHOLOGY AND MICROBIOLOGY

DATA FORM

Title of Research study: "Detection of candida species in saliva of type 2 diabetic and non-diabetic individuals using Chrom Agar media and PCR- A comparative study"

SAMPLE SIZE: 200

Group I: Normal healthy individual (n=100)

Group II: Type 2 diabetic patients (n=100)

Group: Sample No:

Candidal species	No. Of colonies
Candida albicans	
Candida glabrata	
Candida parapsilosis	
Candida krusei	

DATA ANALYSIS SHEET

CONTROL GROUP (GROUP I)

Sample No	Demographic details				No of colony forming units(CFU)			
	Age	Sex	FBS	RBS	C.albicans	C.glabrata	C.parapsilosis	C.krusei
1.	45	M	72	142	0	98	2	0
2.	64	M	68	130	0	0	0	0
3.	43	F	90	128	0	100	0	0
4.	56	M	76	110	67	33	0	0
5.	49	F	85	135	50	0	50	0
6.	61	M	83	124	0	90	10	0
7.	46	F	96	134	0	100	0	0
8.	60	F	64	128	0	0	0	0
9.	47	M	75	145	0	0	0	0
10.	52	M	79	160	0	100	0	0
11.	53	F	86	132	38	62	0	0
12.	64	F	83	169	0	0	0	0
13.	59	M	98	146	62	38	0	0
14.	48	M	73	175	0	0	0	0
15.	44	M	94	154	0	100	0	0
16.	54	F	83	146	7	0	67	27
17.	62	F	89	128	0	50	50	0
18.	55	M	87	139	100	0	0	0
19.	61	F	100	167	0	0	0	0
20.	45	M	81	157	2	0	0	0
21.	56	M	93	136	100	0	0	0

22.	45	F	88	169	0	100	0	0
23.	64	M	71	145	0	100	0	0
24.	43	F	80	178	0	100	0	0
25.	56	M	83	163	100	0	0	0
26.	49	M	74	116	0	0	100	0
27.	61	F	79	104	0	100	0	0
28.	46	F	94	142	33	67	0	0
29.	60	M	84	130	0	0	0	0
30.	47	M	69	160	100	0	0	0
31.	48	M	84	119	0	0	0	0
32.	44	F	104	128	0	0	100	0
33.	54	M	75	135	0	0	0	0
34.	62	M	87	146	0	0	0	0
35.	55	F	79	139	0	100	0	0
36.	61	M	83	112	0	98	2	0
37.	45	F	96	109	0	0	0	0
38.	56	M	64	136	0	100	0	0
39.	45	M	75	143	67	33	0	0
40.	64	F	76	129	50	0	50	0
41.	43	F	86	146	0	90	10	0
42.	45	F	83	125	0	100	0	0
43.	56	M	98	120	0	0	0	0
44.	45	F	72	115	0	0	0	0
45.	64	M	94	124	0	100	0	0
46.	43	F	83	153	38	62	0	0
47.	56	M	89	119	0	0	0	0
48.	49	F	87	168	62	38	0	0
49.	61	M	95	134	0	0	0	0

50.	46	M	69	146	0	100	0	0
51.	60	M	76	127	7	0	67	27
52.	49	M	77	173	0	50	50	0
53.	61	F	79	159	100	0	0	0
54.	46	F	73	136	0	0	0	0
55.	60	M	83	135	2	0	0	0
56.	47	M	69	130	100	0	0	0
57.	52	F	78	105	0	100	0	0
58.	53	M	96	114	0	100	0	0
59.	64	F	78	143	0	100	0	0
60.	59	M	85	109	100	0	0	0
61.	49	F	83	158	0	0	100	0
62.	57	F	79	124	0	100	0	0
63.	55	M	81	156	33	67	0	0
64.	62	M	93	137	0	0	0	0
65.	60	F	87	157	100	0	0	0
66.	54	M	71	147	0	0	0	0
67.	47	F	80	126	0	0	100	0
68.	46	F	83	159	0	0	0	0
69.	61	M	74	135	0	0	0	0
70.	60	M	79	168	0	100	0	0
71.	49	F	94	153	0	98	2	0
72.	61	M	89	106	0	0	0	0
73.	46	F	69	114	0	100	0	0
74.	60	M	84	132	67	33	0	0
75.	47	F	101	120	50	0	50	0
76.	52	F	75	150	0	90	10	0
77.	53	M	87	109	0	100	0	0

78.	64	F	79	118	0	0	0	0
79.	59	F	89	125	0	0	0	0
80.	48	M	96	136	0	100	0	0
81.	44	M	64	129	38	62	0	0
82.	54	F	75	102	0	0	0	0
83.	62	M	86	119	62	38	0	0
84.	55	F	78	126	0	0	0	0
85.	61	F	90	133	0	100	0	0
86.	45	M	68	119	7	0	67	27
87.	56	M	73	136	0	50	50	0
88.	45	F	70	155	100	0	0	0
89.	64	M	86	100	0	0	0	0
90.	43	F	84	172	2	0	0	0
91.	45	M	84	160	100	0	0	0
92.	56	F	79	128	0	100	0	0
93.	46	F	75	130	0	100	0	0
94.	58	M	87	165	0	100	0	0
95.	59	M	87	104	100	0	0	0
96.	64	M	89	114	0	0	100	0
97.	52	M	75	168	0	100	0	0
98.	46	F	78	125	33	67	0	0
99.	54	F	87	143	0	0	0	0
100.	65	M	75	132	100	0	0	0

DIABETIC GROUP (GROUP II)

Sample No	Demographic details				No of colony forming units(CFU)			
	Age	Sex	FBS	RBS	C.albicans	C.glabrata	C.parapsilosis	C.krusei
1.	60	M	297	440	0	9	18	0
2.	54	F	283	369	0	100	0	0
3.	47	F	222	272	67	28	5	0
4.	46	M	102	208	0	0	100	0
5.	61	F	192	332	43	14	43	0
6.	60	M	153	339	15	50	35	0
7.	49	F	362	482	100	0	0	0
8.	61	F	180	288	14	86	0	0
9.	46	M	239	370	100	0	0	0
10.	60	F	169	189	100	0	0	0
11.	47	M	178	268	100	0	0	0
12.	52	M	331	425	0	100	0	0
13.	53	F	240	384	0	100	0	0
14.	64	M	155	293	20	80	0	0
15.	59	F	134	253	0	0	100	0
16.	48	M	135	329	0	44	56	0
17.	44	F	242	363	0	20	80	0
18.	54	F	133	256	0	0	0	100
19.	62	M	147	230	50	50	0	0
20.	55	F	156	229	0	99	1	0
21.	61	M	269	499	89	11	0	0
22.	45	F	234	417	100	0	0	0
23.	56	F	209	340	0	0	0	0

24.	45	M	158	207	0	100	0	0
25.	64	F	229	374	0	50	50	0
26.	43	F	140	259	25	63	0	13
27.	45	M	167	314	0	100	0	0
28.	56	M	204	304	0	0	0	0
29.	46	M	120	237	100	0	0	
30.	58	F	370	473	8	0	0	38
31.	54	F	139	231	0	100	0	0
32.	52	M	222	390	0	100	0	0
33.	64	M	303	391	0	100	0	0
34.	61	F	130	261	0	0	0	0
35.	49	M	254	260	72	9	18	0
36.	56	F	169	317	0	100	0	0
37.	58	M	227	305	67	28	5	0
38.	51	F	158	297	0	0	100	0
39.	45	F	213	376	43	14	43	0
40.	57	M	150	281	15	50	35	0
41.	62	M	271	374	100	0	0	0
42.	61	F	131	243	14	86	0	0
43.	58	F	128	283	100	0	0	0
44.	54	F	149	276	100	0	0	0
45.	46	F	168	302	100	0	0	0
46.	49	M	174	263	0	100	0	0
47.	50	M	163	237	0	100	0	0
48.	63	F	142	287	20	80	0	0
49.	51	M	126	270	0	0	100	0
50.	60	F	130	261	0	44	56	0
51.	56	M	254	220	0	20	80	0

52.	45	F	169	307	0	0	0	100
53.	64	F	227	355	50	50	0	0
54.	43	M	157	277	0	99	1	0
55.	45	M	213	336	89	11	0	0
56.	56	F	150	291	100	0	0	0
57.	46	F	271	344	0	0	0	0
58.	58	F	131	273	0	100	0	0
59.	46	F	129	223	0	50	50	0
60.	61	M	149	246	25	63	0	13
61.	60	F	168	352	0	100	0	0
62.	49	M	174	243	0	0	0	0
63.	61	F	163	267	100	0	0	0
64.	46	F	142	257	8	0	0	38
65.	60	F	176	280	0	100	0	0
66.	47	M	128	293	0	100	0	0
67.	52	F	163	247	0	100	0	0
68.	53	F	242	353	0	0	0	0
69.	64	M	153	286	72	9	18	0
70.	59	M	147	250	0	100	0	0
71.	48	M	137	269	67	28	5	0
72.	44	F	269	499	0	0	100	0
73.	54	M	234	417	43	14	43	0
74.	62	F	209	340	15	50	35	0
75.	55	M	158	207	100	0	0	0
76.	61	F	229	374	14	86	0	0
77.	45	F	147	259	100	0	0	0
78.	56	M	167	314	100	0	0	0
79.	45	M	204	314	100	0	0	0

80.	64	F	163	227	0	100	0	0
81.	43	F	370	443	0	100	0	0
82.	54	M	139	251	20	80	0	0
83.	62	F	222	360	0	0	100	0
84.	55	M	303	381	0	44	56	0
85.	61	F	297	430	0	20	80	0
86.	45	M	283	379	0	0	0	100
87.	56	M	222	242	50	50	0	0
88.	45	F	154	288	0	99	1	0
89.	64	F	192	392	89	11	0	0
90.	43	M	153	319	100	0	0	0
91.	45	F	362	402	0	0	0	0
92.	56	F	180	238	0	100	0	0
93.	45	M	239	350	0	50	50	0
94.	64	M	169	179	25	63	0	13
95.	43	M	178	238	0	100	0	0
96.	56	F	331	495	0	0	0	0
97.	48	F	240	314	100	0	0	0
98.	55	M	155	233	8	0	0	38
99.	60	F	136	253	0	100	0	0
100.	57	M	135	359	0	100	0	0

CONSENT FORM

PART 1 OF 2
INFORMATION FOR PARTICIPANTS OF THE STUDY

1. **Title of the study:** Detection of candida species in Type 2 diabetic patients using chrom agar and PCR.

2. **Background information:** Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of candida species. It can also be a mark of systemic disease, such as diabetes mellitus and is a common problem among the immune compromised. A great number of reports suggest that C. albicans is the most common species that harbor in the oral mucosa of these sensitive patients in high levels. The other candida species include candida tropicalis, candida glabrata, candida pseudotropicalis, candida guillierimondii, candida krusei, candida lusitaniae, candida parapsilosis, and candida stellatoidea, .Recently, there has been a greater surge in the infectious potential of non-albicans candida species in the oral cavity. Identification of these species has become important because they differ both in their potential to cause the disease as well as in their response to the antifungal agents.

3. **Aims and Objectives**

Aim of the study is to identify and compare different candida species in the oral cavity of type 2 diabetic patients.

4. **Scientific justification of the study:**

This research project is aimed to determine the distribution of candida species in the oral cavity using chrom agar and to confirm the type of candida species using PCR in diabetic and non-diabetic individuals. This is because the candidal load of oral mucosa in type 2 diabetic mellitus patients were found to be significantly higher than those of the control group. The decreased immunity and change in oral habitat in diabetic patients creates a diversification in various species of candida. These vary in their susceptibility and pathogenesis. A definite identification of these diverse species in the oral cavity of such patients and their susceptibility mandates proper management to avoid recurrence and drug resistance. Our results can help with improving patient treatment outcomes and reducing healthcare costs.

5. **Procedure for the study:**

- Participants will be asked to rinse their mouth thoroughly 10 minutes before collection to avoid the collection of food debris.

- 2-3 ml of unstimulated saliva will be collected by allowing the participant to drool or gently expectorate into clean, sterile sample containers.
- Immediately after collection, the lid was closed and transferred to laboratory within 30 minutes of collection. These samples will be stored at 4°C, until analysis.
- The solution is concentrated by centrifugation at 5000 rpm for five minutes after this procedure; the pellet which remained at the bottom of the tube is plated onto chrom agar medium plates (HiMedia, India) and incubated at 37°C for at least 3-4 days.
- Chrom agar plates are visualized daily at 24 hours, 72 hours, and followed up to seven days to check for colonial growth. Candida speciation is done based on the different colored creamy colonies appeared on chrom agar culture media.
- Then the plates are transferred to Doctors Diagnostic centre, Trichy for further confirmation using PCR.
- Polymerase chain reaction-(PCR) provides a unique species-specific pattern for the most prevalent candida species
- No Personal Information shared.

6. **Expected risks for the participants:** Nil

7. **Expected benefits of research for the participants:**
   - There is no direct benefit to you. The collected saliva sample will be used only for research purpose without any loss of details regarding patient and sample collected.
   - The study will help the health care practitioners to know about the most prevalent species of candida in Type 2 diabetic patients.

8. **Maintenance of confidentiality:**
   a. You have the right to confidentiality regarding the privacy of your medical information

   (Personal details, results of physical examinations, investigations, and your medical history)

   b. By signing this document, you will be allowing the research team investigators, other study Personnel, sponsors, institutional ethics committee and any person or agency required by law to view your data, if required.
   c. The results of study performed as part of this research may be included in your medical record.
   d. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

9. **Why have I been chosen to be in this study?**
   a. Chosen because of grouping under the inclusion and exclusion criteria
   b. Need of good sampling size
   c. No invasive procedure that harm your health and it helps in diagnosis and helpful for the society

10. **How many people will be in the study?** 200

11. **Agreement of compensation to the participants (In case of a study related injury):**

    No related injury anticipated. Patient will be taken care in case of complication and medical treatment will be provided.

12. **Can I withdraw from the study at any time during the study period?**
    - The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.
    - However, it is advisable that you talk to the research team prior to stopping information.

13. **If there is any new findings/information, would I be informed?**

    Yes

14. **Expected duration of the participant's participation in the study?**

    One day

15. **Whom do I contact for further information?**

For any study related queries, you are free to contact:

Dr. XXXXXXXX.,
Post graduate Student,
Department of Oral Pathology and Microbiology,

Place:

Date: **Signature of Principal Investigator**

Signature of the participant

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled: "Detection of candida species in saliva of type 2 diabetic and non-diabetic individuals using chrom agar media and PCR – A comparative study".

Serial no / Reference no:

Name of the participant:

Address of the participant:

Contact number of the participant:

Signature / thumb impression of the Parent / Legal guardian

Witnesses:

1.

2.

Date:

Place:

ஒப்புதல் வாக்குமூலம்
முதல் பாகம்
பங்கேற்பாளர்களுக்கு ஆய்வினைக் குறித்த தகவல்

1. ஆராய்ச்சியின் தலைப்பு

கேன்டிடா பூஞ்சையின் அளவை நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளிடம் கண்டறிதல்.

2. குறிக்கோள்

கேன்டிடா பூஞ்சையின் அளவை நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளிடம் கண்டறிந்து வகைப்படுத்துதல்.

3. பின்புல தகவல்கள்

கேன்டிடா பூஞ்சைத் தொற்றானது வாயில் ஏற்படும் முக்கிய நோய்களில் ஒன்று. இது உடலில் ஏற்படும் மற்ற நோய்களான நீரிழிவு உள்ளிட்ட நோய்களிலும் ஏற்படும். இந்த வகை தொற்றானது நோய் எதிர்ப்பு சக்தி குறைவாக உள்ளவர்களிடம் ஏற்படுகிறது. கேன்டிடா அல்பிகன்ஸ் வகையே பெரும்பாலான நோயாளிகளில் கண்டறியப்பட்டுள்ளது. மற்ற வகை கேன்டிடா பூஞ்சைகள் பின்வருமாறு கேன்டிடா ட்ராபிகாலிஸ், கேன்டிடா க்லாப்ராடா, கேன்டிடா க்ருசெய், கேன்டிடா பராப்ஸ்லோசிஸ். கேன்டிடா பூஞ்சையின் அளவை கண்டறிவது அதன் நோயை கண்டுபிடிப்பது மட்டுமல்லாமல் அதற்கான சிகிச்சை முறைக்கும் வழி வகுக்கிறது.

4. ஆராய்ச்சியை அறிவியல் ரீதியாக உறுதிப்படுத்துதல்

கேன்டிடா பூஞ்சையின் அளவு PCR என்ற முறையில் கண்டறியப்படுகிறது. இந்த பூஞ்சையின் அளவு பொதுவாக நீரிழிவு நோயாளிகளிடம் அதிகமாக காணப்படும். நீரிழிவு நோயாளிகளில் நோய் எதிர்ப்பு சக்தி குறைவதாலும் நுண்ணுயிரிகளின் அளவு மாறுவதாலும் இந்த வகை பூஞ்சை தொற்று ஏற்படுகிறது. இந்த ஆய்வின் மூலம் கேன்டிடா பூஞ்சையின் அளவைக் கண்டறிவது நோயாளிகளுக்கு சிகிச்சையின் தரத்தை கூட்டுவதற்கு பயன்படும்.

5. செய்முறை

- ✓ ஆய்வினைக் குறித்த ஒப்புதல் வாக்குமூலம் பெற்ற பிறகு நோயாளிகள் வாயை நன்றாக கொப்பளிக்க வேண்டும்.
- ✓ அதன் பின்பு தூண்டப்படாத உமிழ் நீர் சேகரிக்கப்பட்டு ஆய்வகத்திற்கு அனுப்பப்படும்.
- ✓ ஆய்வகத்தில் கேன்டிடா பூஞ்சையின் அளவு PCR என்ற முறையில் கண்டறியப்படுகிறது.

6. எதிர்பார்க்கும் பக்கவிளைவுகள் : ஏதுமில்லை

7. ஆராய்ச்சியில் பங்கு பெறுபவர்களுக்கு கிடைக்கும் நன்மைகள்

உங்களுக்கு நேரடி நன்மைகள் கிடைக்கப்பெறவில்லை என்றாலும் உங்களிடம் இருந்து எடுக்கப்படும் உமிழ் நீரில் கேன்டிடா பூஞ்சையின் அளவைக் கண்டறிவது நோயாளிகளுக்கு சிகிச்சையின் தரத்தை கூட்டுவதற்கு பயன்படும்.

8. தங்களைப் பற்றிய விவரங்கள் அனைத்தும் பாதுகாக்கப்படுமா?

இந்த ஆய்வுரையின் குறிப்புகள் அனைத்தும் நம்பகமாக வைக்கப்பட்டிருக்கும். உங்களை குறித்து உண்மையான அடையாளங்கள் யாவும் வெளியிடப்பட மாட்டாது.

9. எதற்காக நான் இந்த ஆராய்ச்சிக்கு தேர்ந்தெடுக்கப்பட்டேன்?

உங்களுக்கு நீரிழிவின் இரண்டாம் வகை இருப்பதால் தேர்ந்தெடுக்கப்பட்டுள்ளீர்கள்.

10. **இந்த ஆராய்ச்சியில் எத்தனைநபர்கள் பங்குபெறுகிறார்கள்?**

குறைந்தபட்சம் 200 நபர்கள் பங்குபெறுகிறார்கள்

11. இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் அது எவ்வாறு ஈடு செய்யப்படும் ?

இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் அதற்கான மருத்துவ செலவை முதன்மை ஆய்வாளர் ஏற்றுக்கொள்வார் .

12. இந்த ஆராய்ச்சியில் ஏதேனும் தீங்கு ஏற்ப்பட்டால் இடையில் விலகிக்கொள்ளலாமா?

தங்களுக்கு இந்த ஆராய்ச்சியில் பங்கு பெறவும் விலகிக்கொள்ளவும் முழு சுதந்திரம் உண்டு .

13. இந்த ஆராய்ச்சியில் ஏதேனும் புதிதாக கண்டுபிடிக்கப்பட்டால் எனக்கு அறிவிக்கப்படுமா?

ஆம் ஆராய்ச்சியில் புதிதாக ஏதேனும் கண்டுபிடிக்கப்பட்டால் கண்டிப்பாக உங்களுக்கு அறிவிக்கப்படும்.

14. எத்தனை நாட்கள் இந்த ஆராய்ச்சியில் பங்கு பெற வர வேண்டும்?

ஒரு நாள்

15. மேற்கொண்டு நான் தொடர்பு கொள்ள என்ன செய்ய வேண்டும் ?

<u>ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டிய முகவரி:</u>
XXXXXXXXXXXXXX
XXXXXXXXXXXX
XXXXXXXX

இடம் முதன்மை ஆராய்ச்சியாளரின்

கையொப்பம்

நாள்

ஒப்புதல் படிவம்

இரண்டாம் பாகம்
பங்கேற்பாளரின் ஒப்புதல்

இந்த ஆய்வின் தகவல்கள் அனைத்தும் எனக்கு வாய் மொழியாகவும் எழுத்து மொழியாகவும் தெரிவிக்கப்பட்டது. இந்த ஆய்வில் நானாக முன்வந்து பங்கு பெறுகிறேன். இதன்மூலம் எனக்கு நேரடி நன்மைகள் கிடைக்கபெறவில்லை எனினும் இந்த ஆய்வின் முடிவுகள் மருத்துவத்துறையின் முன்னேற்றத்திற்கு பயன்படும் என்பதை அறிந்து இதற்க்கு சம்மதிக்கிறேன். இந்த ஆய்வைக் குறித்த விரிவான தகவல்கள் அடங்கிய படிவம் எனக்கு தரப்பட்டுள்ளது.

நான் **"கேன்டிடா பூஞ்சையின் அளவை நீரிழிவு வகை இரண்டு உள்ள நோயாளிகளிடம் கண்டறிதல்**'' என்ற இந்த ஆய்வில் பங்கேற்க முழு மனதுடன் சம்மதிக்கிறேன் .

குறிப்பு எண்

பங்கேற்பவரின் பெயர் :

பங்கேற்பவரின் முகவரி :

தொலைபேசி எண் :

பாதுகாவலர் அல்லது
பங்கேற்பவரின் கையொப்பம்

சாட்சி :

1.

2.

நாள் :

இடம் :

സമ്മത പത്രം - ഭാഗം - 1
പഠനവുമായി സഹകരിക്കുന്ന വ്യക്തികളുടെ അറിവിലേയ്ക്ക്

1. പഠനത്തിന്റെ പേര്:

ടൈപ്പ് 2 പ്രമേഹരോഗികളിലെ കാൻഡിഡ രോഗാണുക്കളിലെ വിവിധ ഇനങ്ങൾ പ്രത്യേകമായി തിരിച്ചറിയൽ

2. അടിസ്ഥാന വിജ്ഞാനം ?

ഓയിലെ വളരെ സാധാരണമായ ഒരു രോഗാണുബാധയാണ് ഓറൽ കാൻഡിഡിയാസിസ്. കാൻഡിഡ ഇനത്തിൽപ്പെട്ട രോഗാണുക്കൾ അനുകൂലസാഹചര്യം മുതലെടുത്ത് അമിതമായി വളരുമ്പോഴാണ് ഈ രോഗം ഉ—ാകുന്നത്. പ്രമേഹരോഗികളിലും ശരീരത്തിന്റെ പ്രതിരോധശക്തി കുറഞ്ഞ സ¡ർഭങ്ങളിലും ഈ രോഗം കൂടുതലായി ക—ുവരുന്നു. ഇന്നത്തെ രോഗികളിൽ വായിലെ നേർത്ത ചർമ്മത്തിൽ ധാരാളമായി ക—ുവരുന്ന രോഗാണു കാൻഡിഡാ ഗ്ലാബ്രാറ്റാ കാൻഡിഡ സ്യൂഡോടോപിക്കാലിസ് കാൻഡിഡാ ഗ്രില്ലറിക്കേ—ി, കാൻഡിഡാ ക്യൂസി, കാൻഡിഡാ ലൂസിറ്റാനിയേ, കാൻഡിഡാ പാരാപ്സിലോസിസ്, കാൻഡിഡാ സ്റ്റെല്ലറ്റോയിഡിയ തുടങ്ങിയവയാണ് മര്റ് കാൻഡിഡ ഇനങ്ങൾ. അടുത്തകാലത്തായി കാൻഡിഡ ഇനങ്ങൾ അടുത്ത കാലത്തായി കാൻഡിഡാ ആൽബിക്കൻസ് അല്ലാത്ത കാൻഡിഡ ഇനങ്ങൾ മൂലമുള്ള രോഗമാണ് കൂടതലായി കാണപ്പെടുന്നു. രോഗമു—ാകാനുള്ള കഴിവും ആന്റിഫംഗൽ ഔഷദങ്ങളോടുള്ള ഇവയുടെ പ്രതികരണവും വ്യത്യസ്തമായതിനാൽ ഈ ഇനങ്ങളെ തിരിച്ചറിയുന്നത് വളരെയധികം പ്രാധാന്യം അർഹിക്കുന്നു.

3. ഉദ്ദേശവും ലക്ഷ്യവും

ടൈപ്പ് 2 പ്രമേഹ രോഗികളുടെ വായിലുള്ള വിവിധതരം കാൻഡിഡ ഇനങ്ങളെ തിരിച്ചറിയലും താരതമ്യം ചെയ്യലും.

4. പഠനത്തെക്കുറിച്ചുള്ള ശാസ്ത്രീയ ന്യായീകരണം

പ്രമേഹ രോഗികളിലും, പ്രമേഹരോഗമില്ലാത്തവരിലും ക്രോംഅഗാർ മീഡിയം ഉപയോഗിച്ച് വിവിധ തരം കാൻഡിഡ ഇനങ്ങളെ തിരിച്ചറിയുകയും, പി.സി.ആർ. സങ്കേതം ഉപയോഗിച്ച് വിവിധ കാൻഡിഡ ഇനങ്ങളെ തരം തിരിക്കുകയും ചെയ്യുകയാണ്. ഈ പഠനത്തിന്റെ ലക്ഷ്യം. ടൈപ്പ് 2 പ്രമേഹരോഗികളിൽ വായിലെ കാൻഡിഡ രോഗാണുകളുടെ സാ¡തയുംവിവിധ തരം കാൻഡിഡ രോഗാണുക്കൾ വളരുന്നതിലേയ്ക്ക് നയിക്കുന്നു. ഇവ രോഗമു—ാക്കാനുള്ള കഴിവിലും ഇത്തരം വ്യത്യസ്ത രോഗാണുക്കളുടെ പൂർണ്ണമായ തിരിച്ചറിയൽ അവ ഉ—ാക്കുന്ന രോഗത്തിന്റെ രോഗനിർണ്ണയത്തിനും ഔഷധങ്ങളുടെ ശരിയായ പ്രയോജനത്തിനും ആവശ്യമാണ്.

5. പഠനത്തിന്റെ രീതി

- ഭക്ഷണ പദാർത്ഥങ്ങളുടെ അവശിഷ്ടങ്ങൾ നീക്കുവാനായി പഠനത്തിൽ പങ്കെടുക്കുന്നവർ 10 മിനിറ്റ് മുമ്പ് വായ കഴുകി വൃത്തിയാക്കേ—താണ്.
- രോഗാണു വിമുക്തമാക്കിയ കുഷിയിലേക്ക് 2-3 മില്ലി ലിറ്റർ ഉമിനീർ ശേഖരിക്കുക.
- ഉടൻ തന്നെ കുഷി അടച്ച് 30 മിനിട്ടുകൾക്കകം ലബോറട്ടറിയിൽ എത്തിക്കുക.
- ഈ സാംപിളുകൾ 4^{0}ര-ൽ സൂക്ഷിക്കുക.
- 5000 ആർ.പി.എം.ൽ 5 മിനിറ്റ് സെൻട്രിഫ്യൂജ് ചെയ്ത ശേഷം ലഭിക്കുന്ന പെല്ലറ്റ് ക്രോം അഗാറിൽ പ്ലേറ്റ് ചെയ്യുന്നു. ഇവ 37^{0}ര-ൽ 3-4 ദിവസം ഇൻകുബേറ്റ് ചെയ്യുന്നു.
- 24 മണിക്കൂറിനു ശേഷവും 72 മണിക്കൂറിനു ശേഷവും 7 ദിവസം വരെയും കോളണി വളർച്ച ഉ—ാ എന്ന് പ്ലേറ്റുകളിൽ പരിശോധിക്കുന്ന ക്രീമീ കോളണികളുടെ നിറവ്യത്യാസമനുസരിച്ചാണ് കാൻഡിഡയുടെ വിവിധ ഇനങ്ങളെ തിരിച്ചറിയുന്നത്.
- പി.സി.ആർ. ഉപയോഗിച്ച് ഈ ഇനങ്ങളെ തരംതിരിച്ച് തീർച്ചപ്പെടുത്തുന്നതിനായി കർണ്ണാടകയിലെ മാഹേ മണ്ഡൽസ് എൻ.ജി.എച്ച് ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസ് ആൻഡി റിസർച്ച് സെന്ററിൽ അയക്കുന്നു.

ഏറ്റവും സാധാരണമായ കാൻഡിഡ ഇനങ്ങളെ വളരെവ്യക്തമാക്കി തരം തിരിച്ചറിയാൻ പി.സി.ആർ. പരിശോധന സഹായിക്കുന്നു.

6. പഠനത്തിൽ പങ്കെടുക്കുന്നവർക്ക് വരാവുന്ന ദോഷഫലം

- പഠനം മൂലം പങ്കെടുക്കുന്ന വ്യക്തു—ാകുന്ന നേട്ടം
- പഠനത്തിൽ പങ്കെടുക്കുന്ന വ്യക്തിക്ക് നേരിട്ട് പ്രയോജനം ഇല്ല. ശേഖരിക്കുന്ന ഉമിനീർ
- ഗവേഷണത്തിന് ഉപയോഗിക്കുന്നതാണ്. പഠനത്തിൽ പങ്കെടുക്കുന്ന വ്യക്തിയുടെ വിവരങ്ങൾ രഹസ്യമായി സൂക്ഷിക്കുന്നതാണ്.
- ടൈപ്പ് 2 പ്രമേഹ രോഗികളിലെ കാൻഡിഡ അണുബാധയെക്കുറിച്ചുള്ള വിവരങ്ങൾ ശേഖരിക്കാൻ ഈ പഠനം സഹായകമാകും.

7. പഠനം മൂലം പങ്കെടുക്കുന്ന ആൾക്ക് ഉ—ാകുന്ന നേട്ടം ?

ഇതിന് പണമൊന്നും നൽകേ—തില്ല നിങ്ങളുടെ ദന്തനിരകളെക്കുറിച്ച് ചോദിച്ച് മനസ്സിലാക്കാവുന്നതാണ്.

8. പഠനത്തിന്റെ സഹസ്യ സ്വഭാവം ?

നിങ്ങളെക്കുറിച്ചുള്ള വിവരങ്ങളും, വൈദ്യശാസ്ത്രവിവരങ്ങളും രഹസ്യമായി സൂക്ഷിക്കുന്നതാണ്. ഗവേഷണ സംഘത്തിലെ അംഗങ്ങൾ, ഈ സ്ഥാപനത്തിലെ എതിക്സ് കമ്മറ്റി, നിയമം അനുശാസിക്കുന്ന മറ്റ് ഏജൻസികൾ എന്നിവർക്കുമാത്രം. ആവശ്യമെങ്കിൽ ഗവേഷണ വിവരങ്ങൾ പരിശോധിക്കാം. നിങ്ങളുടെ മെഡിക്കൽ രേഖകളിൽ ഗവേഷണ വിവരം രേഖപ്പെടുത്തും. ഗവേഷണഫലങ്ങൾ പ്രസിദ്ധീകരിക്കുകയോ, ശാസ്ത്രവേദികളിൽ അവതരിപ്പിക്കുകയോ ചെയ്യുമ്പോൾ നിങ്ങളുടെ വ്യക്തിഗത വിവരങ്ങൾ വെളിപ്പെടുത്തുന്നതല്ല.

9. എന്നെ എന്തുകൊ—് ഈ പഠനത്തിൽ ഉൾപ്പെടുത്തിയത് ?

പഠനത്തിന് ഉൾപ്പെടുത്താൻ യോഗ്യനായതിനാലും, കൂടുതൽ പങ്കാളികൾ പഠനത്തിന് ആവശ്യമായതിനാലും ആണ് താങ്കളെ ഉൾപ്പെടുത്തിയത്.

10. എത്ര സാംബിളുകളാണ് ഈ പഠനത്തിൽ ഉൾപ്പെടുത്തിയിട്ടു—്. 200

11. നഷ്ടപരിഹാരത്തെക്കുറിച്ചുള്ള താരണ ?

പഠനത്തിൽ പങ്കെടുക്കുന്നവർക്ക്പഠനവുമായി ബന്ധപ്പെട്ട് എന്തെങ്കിലും പ്രശ്നങ്ങളു—ായാൽ ആവശ്യമായിവന്നാൽ ചികിത്സയും മറ്റും ഈ സ്ഥാപനത്തിൽ നിന്ന്നൽകുന്നതാണ്.

12. താന സഹായം? - ഇല്ല

13. പഠനത്തിനിടയിൽ എനിക്ക് പിന്മാറാൻ കഴിയുമോ ? -

സ്വമേധയാ ആണ് താങ്കൾ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നത്. താങ്കൾക്കിഷ്ടമുള്ളപ്പോൾ ഇതിൽ നിന്ന് പിന്മാറാനും അവകാശമു—്. പിന്മാറുമ്പോൾ ഗവേഷണ സംഘത്തോട് വിവരം പറയുക.

14.പഠനത്തന്റെ കാലാവധി? ഒരു ദിവസം

15. കൂടുതൽ വിവരങ്ങൾക്കായി താഴെ പറയുന്നവരെ നിങ്ങൾക്ക് ബന്ധപ്പെടാവുന്നതാണ്.

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സ്ഥലം: പ്രഥമ അന്വേഷകന്റെ ഒപ്പ്

തീയതി :

പങ്കെടുക്കുന്ന ആളിന്റെ ഒപ്പ്