RESEARCH PAPER

Prevalence of opportunistic infection in hyperglycemia condition: A comparative cross sectional fungal case study

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Abstract

Background: Due to the impaired immune responses the opportunistic fungal infection are serious cause of morbidity mainly in the diabetes mellitus individuals. Fungal infection require prompt diagnosis for the appropriate clinical management, direct microscopy and fungal culture plays a crucial role in identifying the organism.

Objective: To determine, isolate and identify the fungal pathogens from diabetic and non-diabetic individuals.

Methods: An illustrative cross sectional study was carried out in Indore, Madhya Pradesh. A total of 85 volunteers were enlisted for the study, involving 45 non-diabetic and 40 diabetic individuals. Volunteers were selected irrespective of age and gender. Respiratory samples, including Broncho alveolar lavage (BAL), sputum and endotracheal secretion were collected from the individuals who were having any clinical sign or symptoms indicating the respiratory tract infection. The specimens were collected in the leak proof sterile container, aseptically. Direct microscopy was done of the samples by using 10% Potassium Hydroxide (KOH) solution. . Gram staining of the specimens were also performed where required to observe the presence of any fungal filament or the budding yeast cells. To obtain fungal isolates all the specimens were inoculated on the sabouraud dextrose agar (SDA) medium by using a sterile inoculating loop. After inoculation the inoculated Petri plates were kept inside the incubator at 25° C for 7 to 15 days and observed daily for the presence of any fungal growth. Cultures were examined upto 15 days and the specimens showing no growth were reported negative.

Results: All the specimens collected were processed using conventional mycological techniques. The 10% Potassium Hydroxide (KOH) wet mount revealed the preliminary presence of fungal filament or the budding yeast cells. The culture method on sabouraud dextrose agar (SDA) confirmed the isolation of the fungal species from the positive samples. The study showed the heavier load of the fungal isolates in the diabetic individuals showing hyperglycemia a predisposing factor for the colonization of the fungus.

Conclusion: Diabetes individuals are more susceptible to the fungal infection. Hyperglycemia condition act as an enrichment medium for the fungus to proliferate. Elevated glucose concentration inside the body facilitates fungal growth, on the other hand impaired neutrophil function and reduced host immune response promotes fungal colonization and virulence.

Keywords: Diabetes mellitus, Respiratory fungal infection, Sabouraud Dextrose Agar (SDA). Potassium hydroxide (KOH), Broncho alveolar lavage (BAL), Aspergillus, Candida, Hyperglycemia, Neutrophil, Immune system.

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Introduction

Universally, about 300 million individuals are at high risk and around 25 million people are at risk of dying because of fungal infection (1). Diabetes mellitus is an endocrine disorder, increasing globally and causing several health problems and complications (2). It is characterized by increase glucose level in the blood which is due to the presence of inadequate insulin secretion, defect in insulin action or both condition. The prevalence of diabetes is increased because of the unhealthy diet, obesity, lack of physical activity which in turn increases the serum glucose level. In 1964, an evaluation was done which stated that 30 million individuals were living with diabetes mellitus. The prevalence of diabetes mellitus has substantial rise in the recent past studies. In 2015, evaluation was made that 415 million individuals of age in between 20-79 had diabetes, analysing 8.8% prevalence, globally. Assumptions have been made that the count might increase to 642 million by 2040 making the rate of prevalence to 10.4%, globally (3).The disease prevalence have grown rapidly in middle and low income countries than in the high income countries. Estimation says that in 2019 the diabetes was the direct cause of death of 1.5millions, and in 2012 hyperglycemia became the reason of death for 2.2 million individuals (WHO 2021) (4, 5, 6).

The disease is categorized into three types: Type 1, Type 2 and gestational diabetes. Type 1 diabetes mellitus develops as an autoimmune response against the insulin producing cells leading to insulin deficiency. Type 2 diabetes mellitus is characterized by varying degrees such as impaired insulin secretion, insulin resistance, and elevated glucose production in hepatic cell. Deficiency in insulin secretion or action results in hyperglycemia

condition in Type 2 diabetes mellitus (T2DM). Gestational diabetes occurs during pregnancy as a result of carbohydrate intolerance. The prevalence of gestational diabetes is 2 to 5% of all pregnancies (7, 8).

Insulin is vital anabolic hormone produced by the beta cells of pancreas located inside the pancreatic islets of Langerhans. The hormone regulates the blood glucose level inside the body by enhancing the intake by various cells. The absorbed glucose is either stored in the form of glycogen by liver or it is used as energy source for the other cells. Therefore, resistance or insufficient insulin level elevates the blood glucose level (9). The increased glucose level can lead to the cellular damage, reduced neutrophil function, dysfunction of humoral immunity (10).

Effective glycemic management can be achieved by appropriate monitoring of blood glucose, adherence to prescribed treatment and also the insulin therapy when needed. Although the glycemic control alone is insufficient for the diabetes management. There is necessity of including associated risk factors, particularly hypertension which significantly increase the cardiovascular disease. As obesity and sedentary lifestyle are established risk factor for Type 2 diabetes mellitus. Accordingly, comprehensive lifestyle management emphasizing on regular exercise, healthy diet and body weight are essential to minimize the risk of chronic complications (11).

T2DM account for 90% cases of diabetes worldwide. Apart from metabolic dysfunction, T2DM is associated with chronic dysfunction of innate immune response including defects in the functions of NK cells (natural killer cells), neutrophils, and macrophages. These abnormalities in the host defense system, thereby, increases the susceptibility to opportunistic infections (12, 13).

Fungal infections are silent killers and are not taken seriously, leading to severe chronic infection. Diabetic patients, due to impaired immune responses, are susceptible to infections, including fungal infections. Common fungal infections include mucormycosis, aspergillosis, and candidiasis. Due to a weakened immune system, diabetic individuals can be exposed to these pathogens either by inhaling fungal spores present naturally in the environment or by close contact (14). The most common mycotic infection worldwide is candidiasis, with *Candida albicans* as the etiological agent. Other Candida species, such as *Candida kruseii*, *Candida tropicalis*, and *Candida glabrata*, are opportunistic pathogens (1). Gastrointestinal, genitourinary, skin, and respiratory infections occur predominantly in individuals with diabetes mellitus (15). Respiratory fungal infections are particularly significant due to the high number of immunosuppressed individuals and the emergence of antifungal-resistant fungal pathogens. Candida and Aspergillus species are commonly isolated from respiratory specimens and are responsible for colonization and progression to invasive candidiasis and aspergillosis (16, 17, 18).

1.1 Candida Species

Candida is an oval, gram-positive, polymorphic fungus capable of undergoing multiple morphological transitions between pseudo hyphal and yeast forms. The infection caused by the polymorphic fungus is termed as candidiasis. In a healthy person, Candida are generally harmless commensals and colonizes the oral cavity, vagina mucosa and the gastrointestinal tracts. Candidiasis is an opportunistic infection capable of colonizing where the host immune response is impaired. Variety of factors are involved such as cancer, AIDS, organ transplantation, and diabetes mellitus for the immunocompromised conditions. Candida spp is frequently isolated from respiratory specimens and is identified as the normal gut flora. However, compromised host immunity or any other underlying factor may result in their proliferation causing opportunistic respiratory infections. Life threatening infection can occur if they enter inside the blood stream, a condition known as candidemia or colonizes inside the internal organ termed as invasive candidiasis. *Candida albicans* is the commonly isolated species in human species followed by *Candida glabrata*, *candida tropicalis*, *candida parapsilosis* and *candida krusei* (19-23).

Candida infection may be chronic or acute and involves the oral cavity, lungs, skin, bronchi, and gastrointestinal tract. In immunocompromised individual's disruptions in the epithelial or the mucosal barrier promotes the invasion of candida into the blood stream and disseminate to various internal organs. The pathogenesis of candida involves sequential series of steps beginning with adhesions to epithelial cell by incorporating the glycosylphosphatidylinositol cell glycoprotein. Upon adhesions the hyphae grows by thigmotropism and the expression of invasins (E-cadherins, N- cadherins) promotes endocytosis which enables the fungi to invade into the host cell (23, 24).

1.2 Aspergillus

Aspergillus is saprophytic, filamentous fungi found naturally in dead decaying vegetation, construction sites and soil. Aspergillus in present in both the environment indoor and outdoor where it disperse abundant conidia. Although the conidia are inhaled by most individuals, clinical symptoms are seen only in individuals with impaired immune system and hence such individuals are susceptible for aspergillosis. Upon inhalation of spores by the host lacking effective immune response, they germinate and becomes enlarged inside the alveoli. The geminated hyphae invades the tissues and surrounding blood vessels which promotes the fungal proliferation and dissemination of infection. Aspergillus infection may cause broad spectrum of acute or chronic pulmonary disease including chronic pulmonary aspergillosis, aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis (23, 25-29).

2. Materials and Methods:

2.1 Study Design:

An observational hospital based cross sectional study was conducted in Indore, Madhya Pradesh, India. During the study period, 85 individuals were enrolled, consisting 40 diabetic individuals and 45 non- diabetic individuals. Individuals were taken irrespective of age and gender. Patients suggesting clinical symptoms of respiratory tract infection meeting the inclusion criteria were enrolled in

the study. The study aimed to compare the prevalence of opportunistic infection in diabetic and non-diabetic individuals.

2.2 Study Population:

The study population included patients of the selected hospital visiting outpatients, inpatients or the intensive care unit. The enrolled participants were categorized into two groups: diabetic individuals (n=40), non- diabetic individuals (n=45). Participants were enrolled irrespective of demographic variable of age and gender, to ensure broad representation of the study population.

2.3 Sample Collection.

Respiratory samples, including Broncho alveolar lavage (BAL), sputum and endotracheal secretion were collected from the individuals who were having any clinical sign or symptoms indicating the respiratory tract infection. The samples were collected inside the sterile leak proof container, upon collection immediately transferred to sterile cool condition to maintain sample integrity and to prevent any kind of contamination prior to sample processing.

Inclusion criteria:

1. Patients with all age group and gender visiting outpatient department (OPD), inpatient department (IPD), and intensive care unit (ICU) were enrolled, showing signs and symptoms of respiratory tract infection.
2. Patients whose diabetic and non-diabetic condition is confirmed by its previous medical record.
3. Patients from whom the respiratory samples can be obtained from the selected hospital for the further examination.

Exclusion criteria:

1. Patients who were on anti-fungal drugs were excluded.
2. Patients whose respiratory sample was inadequate, contaminated were excluded.
3. Patients with limited or incomplete medical record were excluded.
4. Duplicate Sampling of the same patient were excluded.

2.4 Ethical Consideration:

The proposal was evaluated by the university ethical committee and with due permission of the chairperson, it was approved for ethical clearance by the committee.

2.5 Sample Processing:

For the isolation of fungi, collected specimen were inoculated on the sabouraud dextrose agar (SDA) supplemented with chloramphenicol. After inoculation the cultured samples were incubated at 25° C for 7 to 15 days. Daily monitoring was made to examine any fungal growth and any visible appearance was further recorded for morphological identification (30).

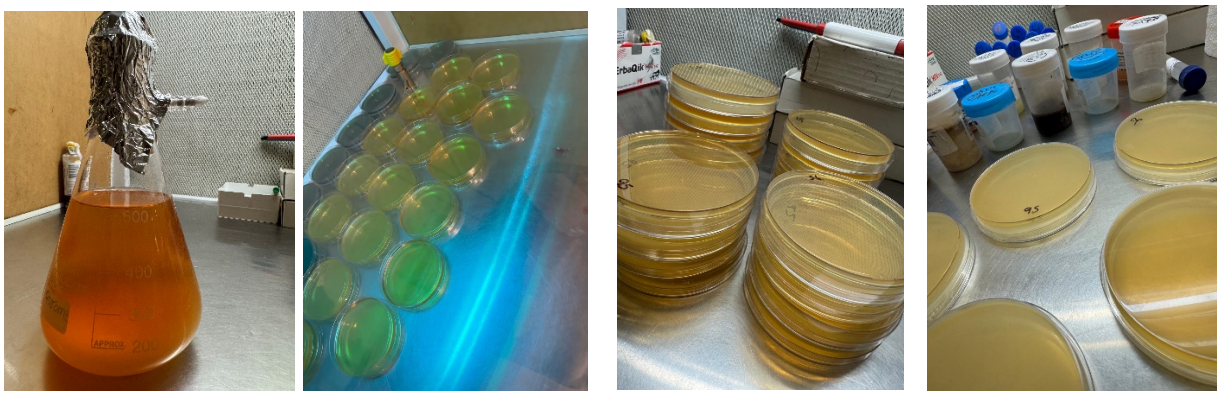

Figure1: Sterile SDA plates for the isolation of the fungal pathogens.

2.5.1 Direct microscopic examination:

10% KOH or Potassium Hydroxide wet mount

Direct examination of the collected specimen was performed using 10% KOH solution. Few drops of sample was mixed with the 1-2 drops of KOH, after mixing of both solution coverslip was placed. After allowing the mixture to incubate for 10 – 15 minutes, it was observed under 10X and 40X lens of the microscope for the presence of budding yeast cell, pseudo hyphae, hyphae and other fungal elements. The wet mount is reliable method for the preliminary presence of fungal infection inside the specimen (31, 32).

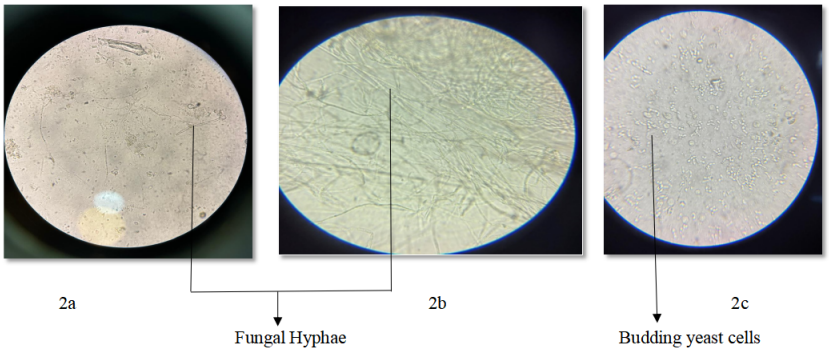

Figure 2a, 2b and 2c: Direct microscopic visualization of the specimen under 10X, appears to show fungal hyphae, budding yeast cells suggestive of fungal infection.

2.5.2 Gram Staining:

The specimen were subjected to gram staining whenever indicated to detect the presence of hyphae , budding yeast cells, especially in the case of candida infection. Slides were prepared by following standard gram staining protocol (33, 34, 35). The prepared slides were observed under oil immersion (100X objective)

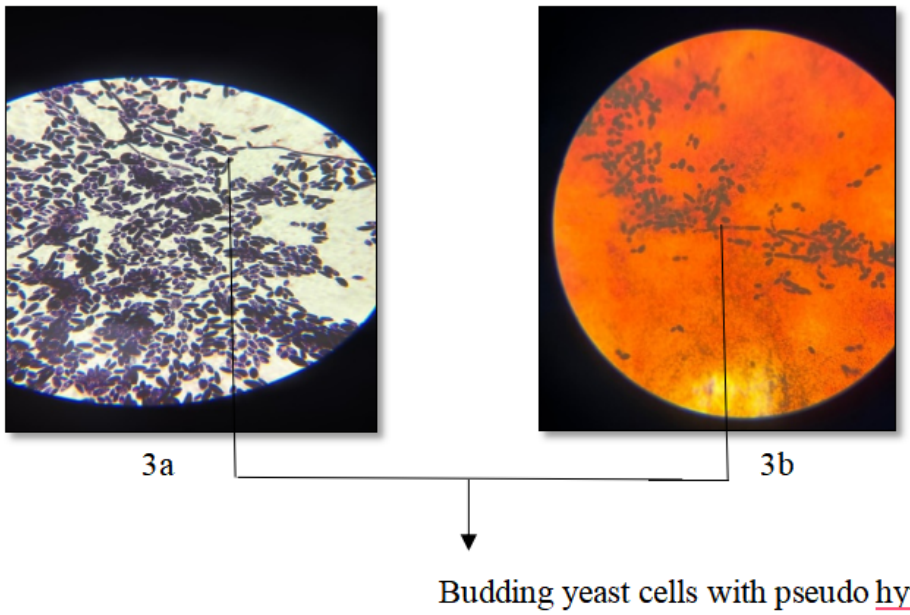

Figure3a, 3b: Gram stain visualization of the specimen under 100X showing budding yeast cells with pseudo hyphae suggestive of candida spp.

2.6 Identification of molds:

Isolated fungi was identified by using the conventional microbiological techniques based on morphological and microscopic characteristics. The colony characteristic include shape of conidia (spores), colony pigmentation, colony shape and presence of septa (36).

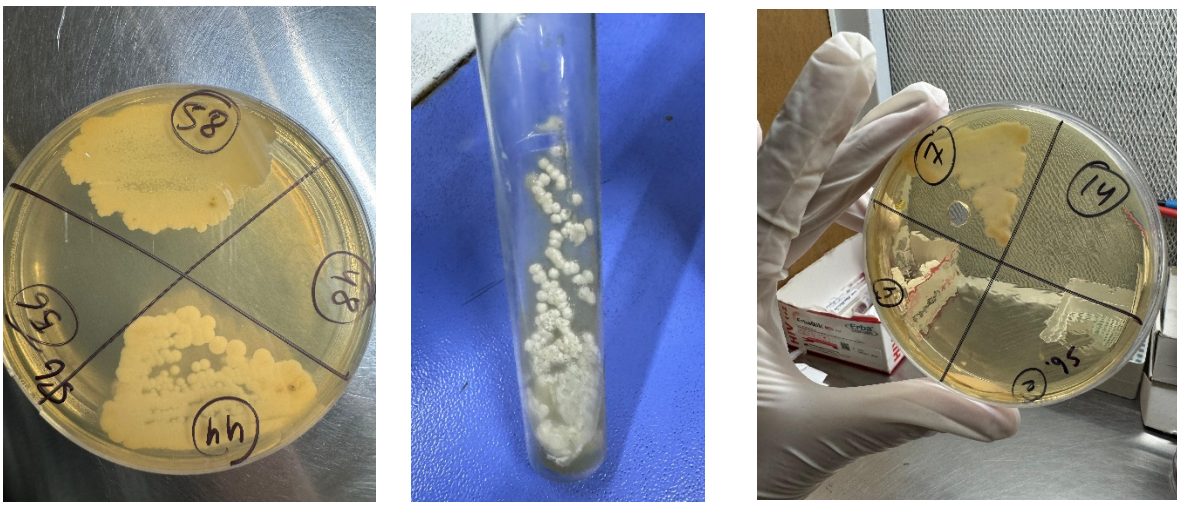

Figure4: Petridish showing colonies for Candida spp.

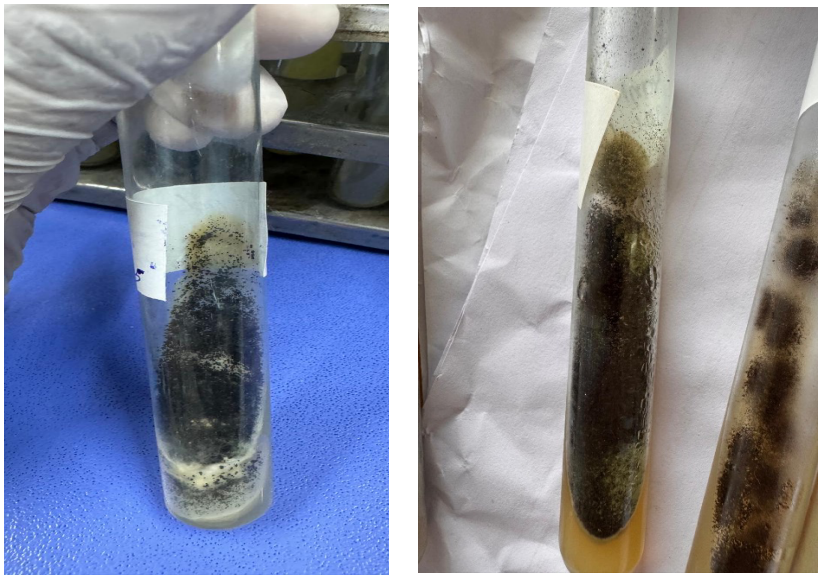

Figure5: SDA slants showing colonies of mold *Aspergillus niger.*

2.6.1 Lacto phenol cotton blue (LPCB Mount)

On a clean glass slide by the inoculating needle a small fragment of fungus was placed, followed by mixing it with lacto phenol cotton blue. A coverslip was placed over the slide to prevent any bubbles and for evenly distribution of the fungal filament. The prepared slide was observed under the microscope. It involves mainly three reagents:

i.Phenol: acts as a disinfectant

ii.Lactic acid: preserves the fungal structures

iii.Cotton Blue: stains the fungal chitin wall and other structures

LPCB acts as a staining reagent and imparts a blue color to fungal structures including spores, hyphae and conidiophores. Hence, enhances the visualization of the fungal isolates under the microscope (37, 38, 39).

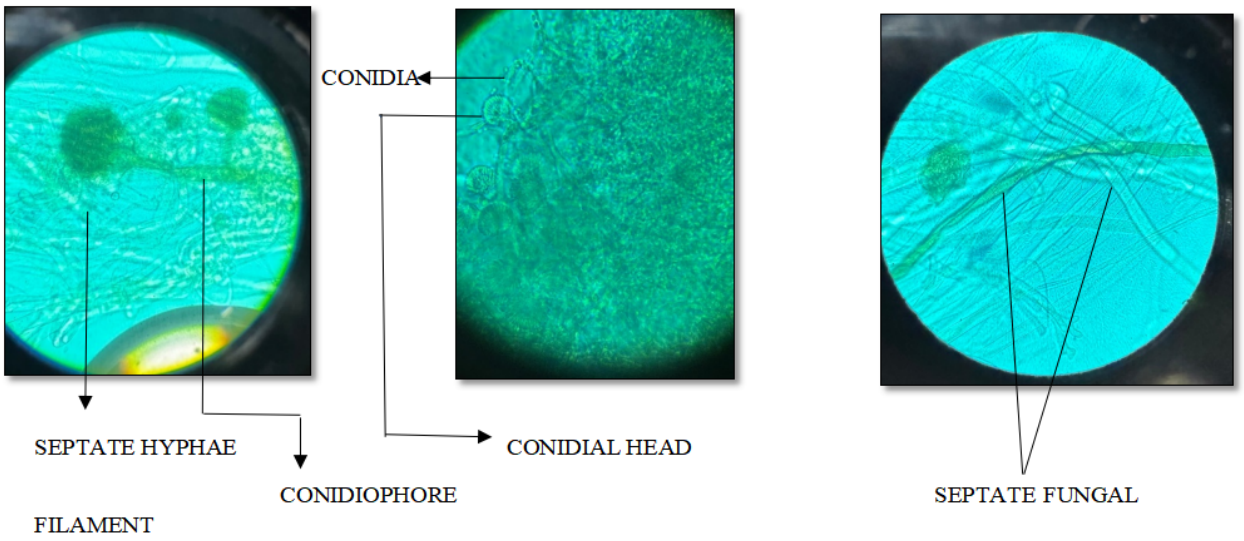

Figure6: Lactophenol cotton blue mount of mold demonstrating septate hyphae, conidia heads.

2.6.2 HiCrome Candida differential agar:

The isolates of candida was further sub cultured on the Hicrome TM agar for species identification. Upon incubation, the candida species showed characteristic colony colors.

Table1: Differential identification of Candida species by chromogenic appearance on the Hicrome agar (32, 40).

Species	**Characteristic Colony Color**
Candida albicans	Light green colonies
Candida tropicalis	Blue to metallic blue colonies
Candida glabrata	Cream to white colonies
Candida krusei	Purple fuzzy colonies

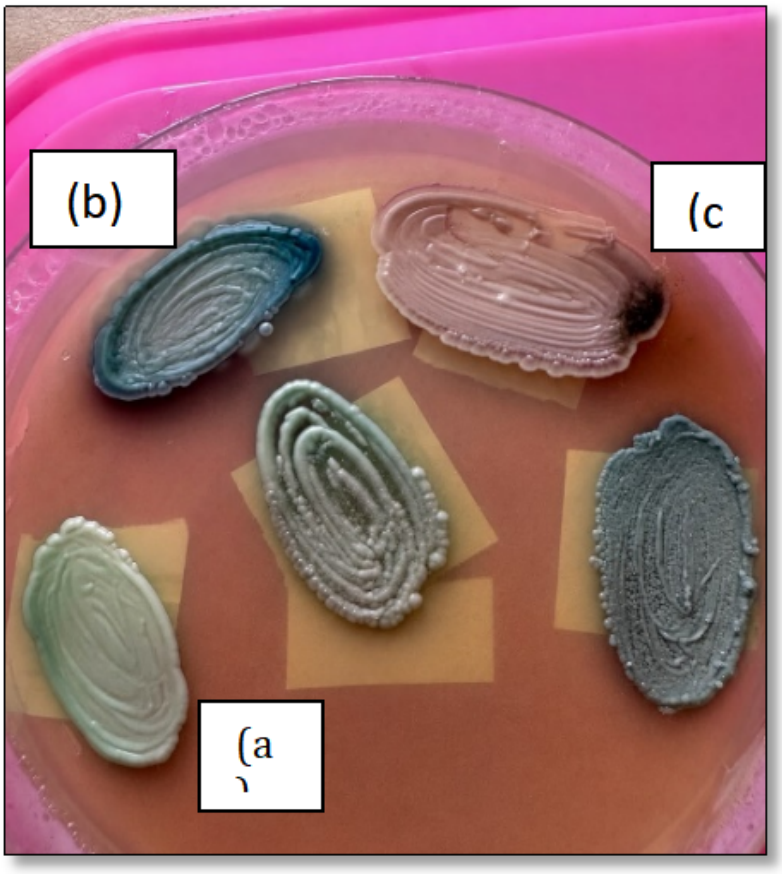

Figure7: Different colors of candida spp on Hicrome agar, (a) *Candida albicans.* (b) *Candida tropicalis*, (c) *Candida glabrata*

3. Results:

3.1 Demographic Distribution:

The study analyzed a total of 85 respiratory specimen's from diabetic and non-diabetic. The collected samples undergone direct and indirect examination for the detection of fungi.

3.2 Culture positivity:

Of the 85 specimens, the fungal growth was observed in 25(29%) of specimens whereas 60 (70%) of specimen were reported as negative cultures. Among diabetic specimens, 16(40%) recorded for fungal growth, in comparison, 9(20%) of non-diabetic specimens recorded for fungal growth, whereas 36(80%) were culture negative. Overall negative cultures were more recorded in non-diabetic individuals indicating increase prevalence among the diabetic individuals.

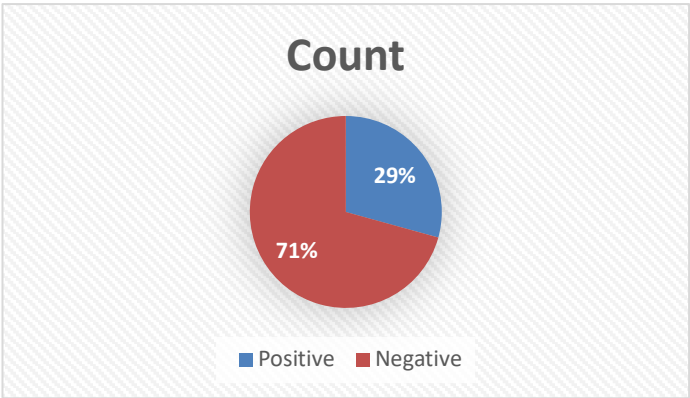

Fig8: Pie chart showing the total percent of the positive negative results among the study population.

Table2: Overall distribution of fungal culture results among the study population

Study Group	Positive (n)	Positive %	Negative (n)	Negative %	Total (n)

Diabetic	16	40	24	60	40
Non-diabetic	9	20	36	80	45

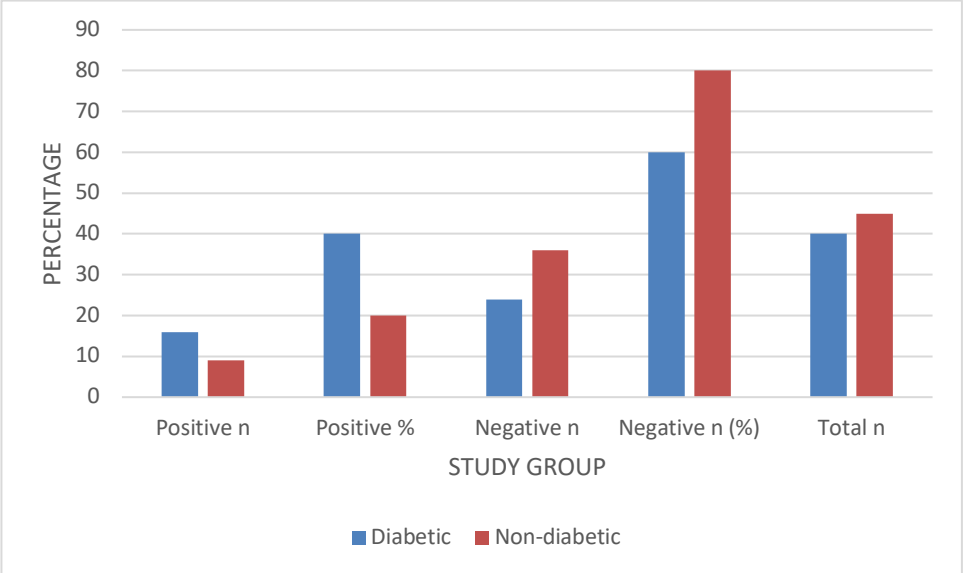

Figure9: Distribution of culture positivity according to the study group.

3.3 Distribution of fungal isolates:

Among all the 28 fungal isolates, *Candida albicans* was the predominant fungal isolate accounting for 14(50%) followed by other species.

Table3: Distribution of fungal culture isolates among the positive specimens.

Fungal Species	Number (n)	Percentage of Positive Isolates (%)
Candida albicans	14	50
Aspergillus niger	7	25
Candida glabrata	5	18
Candida tropicalis	1	3.5
Aspergillus fumigatus	1	3.5
Total	28	100

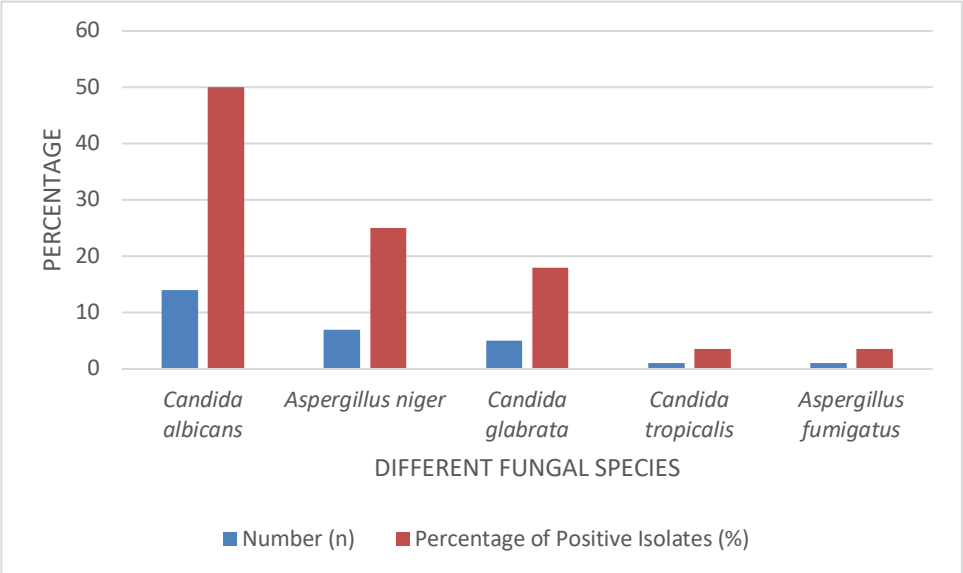

Figure10: Prevalence of fungal species isolated from the study population.

4. Discussion

The present study focused on the prevalence of fungal colonization in respiratory tract infection in both diabetic and non-diabetic individuals. The fugal pathogen was isolated from both the group, an increase percent of growth was observed in diabetic individuals. The observed findings support the established findings that the increase glucose level (hyperglycemia), acts as enrichment medium for the fungus colonization and infection. Diabetic mellitus individuals are predisposes to opportunistic infection due to impaired neutrophil function, reduced cell mediated immunity and alterations in the mucosal defense mechanism. The elevated glucose level promotes the fungus adhesion, and increase glucose with low pH, poor oral hygiene stimulates the growth of candida species in the oral cavity (1, 41). According to different studies, the colonization rate of fungi in diabetic and non-diabetic was 84% and 27%, respectively (42). Among all isolates *Candida albicans* became the predominant isolate. The finding is consist with previous finding stating that candida as frequent opportunistic yeast colonizing the respiratory tract especially in individuals with underlying metabolic disorders. Other non-albicans candida such as *Candida glabrata* and *Candida tropicalis* were also isolated. *Candida glabrata* have emerged as an opportunistic pathogen in diabetic individuals with its decreased susceptibility to fluconazole drug (43).

The second most common pathogen for causing oral mycosis after candida is aspergillus, commonly isolated species are *aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus*. In immunosuppressive individuals these species can have life threatening effects (45, 46, 47).

The other common isolate in the study was the *Aspergillus Niger.* Although worldwide, *Aspergillus fumigatus* is reported as the cause of invasive aspergillosis, *Aspergillus Niger* have also been identified as the respiratory pathogen mainly in immunocompromised condition (43). The predominance of *Aspergillus Niger* observed in the study may reflect the geographical and environmental factors including climatic conditions, environmental exposure and local epidemiological characteristics. Thus the predominance of *Aspergillus niger* in the study shows the local epidemiological characteristics rather than the universal fungal pathogen distribution. Consequently, clinicians should include respiratory fungal infection in the diagnosis of diabetic patients with symptoms of respiratory infection particularly when there is no response to conventional antibacterial therapy.

5. Conclusion:

The present study highlighted a higher prevalence of fungal infection in diabetic group than among the non-diabetic individuals, reinforcing diabetes mellitus as the risk factor for the opportunistic infection. Chronic hyperglycemia and the impaired immune response creates a favorable environment for the fungus to proliferate. Although, the isolates were also found in non-diabetic individuals but their occurrence was low. *Candida albicans* was the most common pathogen followed by *Aspergillus niger* in the given study population. The occurrence *of Candida albicans* in most positive specimens highlights its clinical importance as an opportunistic fungi especially in case of immunocompromised condition. Early diagnosis became a necessity through mycological examination in individuals with persistent respiratory infection symptoms for proper patient care and appropriate management of the disease. Overall the study emphasize on incorporating mycological investigation for timely management of the disease in diabetic individuals mainly when they fail to respond the conventional antibacterial therapy.

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