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Opportunistic Fungal Infections in Diabetes Patients

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ABSTRACT

Uncontrolled diabetes is a major health problem with more than million of death occurs due to opportunistic fungal infection annually. The risk of opportunistic infections increases with high sugar level in diabetic patients causes high morbidity and mortality. These fungal infection increases with geographical location, which varies from region to region. This research was carried out to view the occurrence of fungal infections among diabetes patients in central Nepal. this is a tertiary care centre based descriptive study carried out in the Department of Microbiology, CMS-TH, Bharatpur, Nepal over a period of two year from (January 2018 to December 2020). A total of 300 clinically fungal infection suspected uncontrolled diabetes samples were enrolled for our study. Informed written consent was taken from individual patients for the research. Fungal isolation and identification was done as per standard Microbiological procedure. Out of 300 clinically suspected opportunistic fungal infection in diabetes patients only showed 60 patients fungal culture positive, giving an incidence of (20.0%). The majority of infections were by saprophytic fungal species of *Candida* 37(61.7%) followed by *Aspergillus* 9(15.0%), *Cryptococcus* 5(8.3%) and *Mucor* 3(5.0%) in that order. Only 2(3.3%) *Trichosporon* and *Fusarium* each; and 1(1.7%) *Pseudoallescheria boydii* were also isolated from skin and soft tissue infections of diabetes patients. One 1(1.7%) dematiaceous unidentified fungus was also identified. The opportunistic fungi mostly isolated from diabetes patients were *Candida*, *Aspergillus*, *Cryptococcus* and *Mucor* species. *Trichosporon*, *Fusarium*; and *Pseudoallescheria boydii* were least isolated from different clinical samples.

Introduction

Opportunistic fungal infections have become an important cause for sickness and death in immunosupressed and diabetes patients.[1] The incidence of invasive fungal infections is increasing and new species are emerging as important pathogens. In diabetic patients, the main risk factor for the development of systemic fungal infection is severe, due to high sugar level in serum of diabetes patients.[2] Other factors like minor or major accidents and other co-morbid conditions like prolonged wound infection central venous line, immunosuppressive therapy and broad spectrum antibiotics are the main contributing factors.[3] These type of diabetes patients are at high risk for infection with opportunistic fungal infection namely *Aspergillus*, *Candida*, *Cryptococcus*, *Zygomycets*, *Pencillium*, *Trycosporon*, *Malassezia*, *Fusarium* and numerous other molds and yeasts other than the above, fungal pathogens that unusually infect otherwise healthy person exposed to endemic fungi such as *Histoplasma capsulation*, *Coccidioides immits*, *Blastomyces dermatidis* could also be the causative pathogens.[4]

Morbidity, mortality and healthcare cases associated with fungal infections are high. Addressing the emergence of fungal disease will require increased surveillance coupled with the availability of rapid non invasive diagnostic tests, monitoring the development of resistance to antifungal agents and research focused on understanding prevention and control of fungal infections in such cases.[5]

Materials and Methods

This study was conducted at the department of microbiology, Collage of Medical Sciences, Bharatpur, Nepal over a period of January 2018 to December 2020. A total of two hundred patients receiving care at CMS-TH at who were diagnosed as diabetes patients were selected to enter in the present research.

Questionnaire eliciting epidemiological information was completed and also asked to sign completely filled form contents from patients or their attendant. At the 1st visit, the appropriate samples were collected based on the presenting symptoms and clinical feature. The samples investigated were sputum, blood, urine cerebrospinal fluid, stool, pleural fluid, ascetic fluid, synovial fluid, throat swab, cervical or vaginal swab, cunula, nasal discharge, skin scrapings and tissue biopsies from different sites.

Sample transportation:

All the samples were collect under taking aseptic precautions and reached in the lab within 15-30 min.

Identification:

Direct microscopy was done by 10.0% potassium hydroxide preparation and slides were examined under low and high power of microscope. Some of the samples smear

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grams staining was done and observed under oil immersion. All CSF samples, India ink wet mount preparation was done to observe capsulated yeast cells under low power objective.

Culture:

All the fungal infected suspected samples were cultured on Sabouraud's Dextrose Agar with or without chloramphenicol (0.05%). Brain heart infusion agar with 5.0% sheep blood and caffeic acid agar for *Cryptococcus* were used.[6]

Colonial morphology and microscopic examination of isolates:

The fungal isolates were presumptively identified by rate of growth of colonies, type of colonies pigment, colour and texture. After the microscopic examination of culture, fungal identification was done by tease mount stained with lactophenol cotton blue. The yeast or yeast like cells was further identified by combination of morphological and physiological and serological tests.

Germ tube:

This test was done by inoculating 0.5ml of serum with yeast and incubated it at 37^{o}C for 2 hours. At the end of 2 hours one drop of suspension was kept on microscopic slide and covered it with coverslip and observed under 40X objective to see germ tube formation.

Chlamydospores production:[7]

Germ tube negative yeast like cells were cultured on Cornmeal agar with 0.3 % tween 80. The streaked line were covered with coverslip and incubated at 22^{o}C for 2 days and plates were directly observed under the microscope for morphological features.

Physiological test:

Carbohydrate assimilation by auxanographic technique:[8]

This test was performed by seeding the Yeast Nitrogen Base Agar plate with test organisms fallowed by addition of five sugar disc of glucose, sucrose, lactose, trehalose and raffinose at appropriate distance and incubated at room temperature for 48 hrs. The assimilation of a particular sugar was detected by growth around that particular sugar disc.

Carbohydrate fermentation:[8]

This test was performed by inoculating the test organisms into each of the 2.0% sugar media of glucose, sucrose, lactose and maltose and incubated at room temperature for 48-72 hrs. The ability of yeast to ferment a sugar was shown by change of the colour of sugar media to pink and gas in Durham's tube.

Urease production:[9]

This test was done for identification of *Cryptococcus.* The suspected yeast colonies were streaked on to surface of christens urea agar slant and incubated at room temperature. The test was positive when colour changed to pink.

Serological test:[9]

Reverse passive agglutination test: This test was done on cerebrospinal fluid (CSF) for *Cryptococcus* antigen detection.

Results

A total of 300 clinically suspected opportunistic fungal infection in diabetes patients were enrolled for the present study. The fungus was isolated from 60 patients giving an incidence of (20.0%). Most of the isolates were found to be saprophytic fungus that is *Candida*, *Aspergillus* and *Mucor*.

All 60 cases were reviewed for predisposing conditions and clinical severity. Different types of predisposing conditions were found in 59 out of 60 cases which are listed vide table-1.

Table 2. Diabetes conditions associated with the occurrence of fungal infections.

Samples	Number	Percentage
Blood	21	35.0%
Eye scrapings	10	16.6%
Hivaginal swab (HVS)	6	10.0%
Gastro intestinal tract (GIT)	6	10.0%
Respiratory tract (RT)	3	15.0%
Miscellaneous	8	13.3%

Table 3. Shows age wise case distribution among diabetes patient.

Fungal species	Number	<10 year	11-40 year	>40 year
Candida	37	6(16.2%)	9(24.3)	22(50.5%)
Aspergillus	9	-	-	9(100%)
Mucoraceae	3	-	-	3(100%)
Cryptococcus	5	-	-	5(100%)
Fusarium	2	-	-	2(100%)
Trichosporon	2	-	2(100%)	-
Psedoallescheria boydii	1	-	1(100%)	-
Unidentified fungus	1	-	-	1(100%)
Total	60	6(10.0%)	12(20.0%)	42(70.0%)

The majority of the patient (70.0%) were above 40 years of age and was found least only (10.0%) in age group below 10 years. The number and percentage of fungal infection in different age group is given in (Table-3).

Table 4: Shows the incidence of opportunistic fungal infection in relation to sex. The incidence of opportunistic fungal infection in Diabetes patients was more among males (71.7%) when compared to female (28.3%). The male and female ratio was found to be 2.5:1

Sex	No	Percentage
Male	43	71.7%
Female	17	28.3%
Total	60	100%

Table 5. Shows the summary of fungal isolated from different clinically confirmed diabetes patients.

Fungus	Number	Percentage
Candida	37	61.7%
Aspergillus	9	15.0%
Mucoraceae	3	5.0%
Cryptococcus	5	8.3%
Fusarium	2	3.3%
Tricosporon	2	3.3%
Psedoallescheria boydii	1	1.7%
Unidentified	1	1.7%

Clinically suspected 300 diabetes patient were taken for fungal infection where we found 60 cases of fungal infection. Most of the isolates were found to be saprophytic fungi that

Table 1. Predisposing factors for fungal infection in clinically confirmed diabetes patients since >5 years.

FS/PP Average Sugar	Predisposing factors	Number	Percentage
140/400 mg/dl	Trauma	28	46.6%
110/200 mg/dl	Surgery	10	16.7%
120/300 mg/dl	Previous fungal infection	8	13.3%
120/300 mg/dl	Steroids	6	10.0%
120/400 mg/dl	Systemic illness	4	6.7%
120/150 mg/dl	Viral infections	2	3.3%
120/200 mg/dl	Non identified	1	1.7%
120/200 mg/dl	Malnutrition	1	1.7%

is, *Candida*, *Aspergillus* and *Mucor*. The majority of infections were by species of *Candida* (61.7%) followed by *Aspergillus* (15.0%), *Cryptococcus* (8.3%) and *Mucor* (5.0%) in that order. *Candida* species infection was found more frequently than *Aspergillus* in the present study.

Discussion

There is no doubt that there has been an increase in the incidence of myotic infection in diabetes patients. The high incidence of mycosis in patients with diabetes in this study has been noted by other workers as well.[10] the incidence of fungal infection in complicating diabetes has been reported to vary from 14-30%,[11] this report is consistent with our report.

Fungi are ubiquitous;[12] in the diabetes patients predisposing factors *viz* trauma, surgery and high dose steroids for long period of time use had high chances to cause fungal infections. The present report also revealed that trauma 28(46.6%), surgery 10(16.7%) and patients taking steroid 6(10.0%) showed high rate of fungal infections in diabetes patients. Here, trauma causes fungal deposition and steroid use decreases the diabetes patient immune status. Therefore, these are compounding factor that increases the chances of fungal infection.

In this present research poverty geographical area and the (may-august) season was noticed as the main factor increasing chances of fungal infections in uncontrolled diabetes patients. This type of poverty and seasonal trend (with high temperature and humidity) related mycosis is increasing day-by-day among diabetes patient. These type of research reports were also observed in research done by Bhuvan *et al*.[10] It could be due to no money for buying medicine, not maintaining proper diabetic food habit and increase blood sugar level and fungal species present in that particular area are the main compounding factors heightens the chances of fungal infections.

It was quite interesting to observe that highest number of patients 21(35.0%) had blood infection and 2nd highest was fungal eye infection compared to other sites infection were very less. Our results were in agreement with data reported earlier by (Santhosh and Ramanath, 2011)[13] where they found immunity of the host is the main factor which enhances the fungal infections in patients.

Demographic data of the patients reveled that majority of them belong to the (70%) age group of >40 years and was found least 10% in age below 10 years. Thus it shows a large group of people belong to working age group has more chances to get trauma. This was the major factors observed in the present research; similar type of observation was also shown by Felix *et al*[14] who showed as high as 56.0% of fungal infection among diabetes patient occurs in the age group 31-40 years

Out of 60 patients studied, 43(71.7%) were male and 17(28.3%) were female. The fungal infection ratio of male: female was found to be 2.5:1. Similar type of observation was also noted by Bhuvan *et al*[10] in a study from Nepal. However in few researches from another country showed female had more fungal infection compared to diabetic male. It could be due to females are more farm worker than male in that particular area.

Forty five 45 (75.0%) of the subjects were office workers by profession. As we know Nepal is an agricultural country even then, only 15 (25.0%) were farmers by occupies. It shows that three (I) infection, injury and immunity status of diabetes patient are the main factors that increases the chances of fungal infection. Other co-factors like long term and high dose use of steroids and antibiotics also increases the chances of fungal infections.[15-16]

Clinically suspected 300 diabetic fungal infected patient samples were selectively taken, where we found only 60 cases of fungal infection in such patients. Most of the isolates were found to be saprophytic fungi like *Candida* spp., *Aspergillus* spp and Mucur species as shown in table-5. The majority of fungal infections were by *Candida* 37(61.7%), followed by *Aspergillus* 9(15.0%) and *Cryptococcus* 5(8.3%). Among all fungal infection *Candida* were mostly causing blood and nail infections whereas, respiratory tract infections were mainly caused by *Candida* and *Aspergillus* species. *Cryptococcus* species were only causing meningitis that's why it was mostly isolated from CSF in diabetes patients. This type of results were totally corroborates with the reports of Sah *et al*.[17] In some research it was also shown that *Candida* is the main agent causing lung infection in diabetes patients.[18]

The least isolated fungi like *Mucoraceae* 3(5.0%), *Fusarium* and one unidentified 1(1.7%) fungi causing mostly eye infection in diabetes patients. In present research *Mucor* 3(5.0%), *Fusarium* 2(3.3%) and *Aspergillus* 2(3.3%) were isolated from eye infections causing fungal keratitis in diabetes patients. These types of reports were also documented from different parts of Nepal and India.[19] in a few research it was also reported that *Fusarium* species as a main responsible fungi causing keratitis in diabetes patients.[20]

Yogesh *et al*[20] also found *Fusarium* as the main fungus isolated from different cases of keratitis like (32.0%) fallowed by unidentified dematiaceous fungal species in (22.0%). As we know that *Mucor*, *Aspergillus* and *Fusarium* are also present in plant, soil and dead decade materials of house host food items. The high rate of these fungi isolation were due to geographical location and ecological conditions where it is predominantly found favors and flourish these fungi. Only 2(3.3%) *Trichosporon* and 1(1.7%) *Pseudoallescheria boydii* were isolated from skin and soft tissue infections of diabetes patients, similar types of report was also documented by Meltem *et al*. [21] showed these opportunistic fungi causes life threatening infections in immune compromised hosts.

Conclusion

This present investigation showed that trauma, surgery and steroids use were the most common predisposing factors for fungal infections in diabetes patients. Fungal etiology only accounted for more severe uncontrolled diabetes patients. In the present trend of pandemic mycosis treatment, it is very much important to know the emerging and reemerging patterns of fungal infections in diabetes patient and their treatment policy.

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