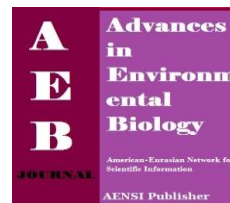




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# Evaluation of Fungal Infections and Foot Syndrome, In Diabetic Patients Referred To Health Centers in Tehran

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### ABSTRACT

**Introduction and Objective:** Diabetic foot disease is the most common complication of diabetes that results from uncontrolled diabetes and imperfect healthcare that eventually lead to particular tissue death and gangrene and amputation. Fungal infections are one of the main causes of death in patients with diabetes. The aim of this study was to investigate the incidence of fungal infection in diabetic foot syndrome in patients referred to the infection ward of Imam Khomeini Hospital in Tehran. **Methodology:** 65 patients with diabetic foot ulcers were studied from 2013 to 2014. The samples were taken from the leg wound, while the normal tissue samples were collected, too. Ten percent KOH microscopic and macroscopic culture was performed on dextrose agar medium. Diagnostic tests of culture slides and chrome agar was used to determine the type of fungus or yeast. Finally the results were analyzed. **Results:** In this study, the age of the patients was 30 to 86 years, 52.3% of whom [34 patients] were male and 47.7 percent [n = 31] were female and with ten percent KOH direct tests, 41 of them were positive [63.07%] and to estimate and study the Candida infections in diabetic foot ulcers, a cruise Candida, glabrata Candida, albicans Candida and parapsylozis candida were observed respectively. And Aspergillus was isolated in classification, Aspergillus flavous, Aspergillus fumigatus, Aspergillus niger, and Penicillium, were the types of fungus involved in diabetic foot ulcers based on the results in no fungal infection was seen less than 50 percent of the patients and 40.4% fungi were seen along with bacteria. **Conclusions:** the Results indicate a high incidence of Fusarium isolates from samples, so mycological examinations should be performed on all specimens for proper treatment of patients and to alleviate the symptoms.

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## INTRODUCTION

Diabetes is a common metabolic problem in modern societies. Diabetes mellitus is a metabolic disorder with multiple causes which is structurally defined with high levels [hyperglycemia]. A group of people with diabetes are more susceptible to infection than others.

However, there are little convincing studies and considerable discussion is done regarding the issue. Diabetes is a chronic disorder that affects a large proportion of the human population and is considered as a major problem for public health and globally 150,000,000 people have it.

Diabetes and foot problems are almost simultaneous and this will lead to lower limb sensation and these patients are not aware of their feet's small wounds. This could be result in poor nail and foot care and often lead to pathological conditions, hospitalization, and amputation. Approximately 20% of diabetic patients are infected during with foot ulcers their lifetimes. The quality of patients and their families' life is affected in the absence of effective treatment [1]. In this type of infections, a mix of aerobic gram-positive cocci, gram-negative aerobic and anaerobic micro-organisms are found. [2]. However, there are few reports in relation to the incidence of fungal diseases of deep tissue samples [3-7].

Fungal infection is important in a sense that it does not improve by antibiotic treatment and no diagnosis leads to irrecoverable results and treatment of fungal Diagnosis is essential [8-10].

According to studies done by a variety of fungal infections in patients with Aspergillus species, Candida types and other opportunistic species are created in which Yeasts share more than others. Opportunistic species are not capable of causing disease in healthy people and only when the host resistance is reduced; it can be pathogenic [11-14].

Chino and Pal studies about fungal pathogens in tissues of diabetic foot ulcers have shown that it was more in their candidates. In addition, other opportunistic infections such as Aspergillus, Candida can be seen in

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diabetic patients. Given the importance of this issue, the aim of this study was to determine and evaluate the fungal infection in diabetic foot ulcers referred to the health center of Imam Khomeini Hospital in Tehran in 2013-2014.

#### *Method:*

In this cross-sectional study, over a period of 9 months in three seasons, 65 patients with diabetic foot ulcers admitted to the infectious diseases ward of Imam Khomeini Hospital, Tehran whose wound did not receive any antifungal therapy were participated for testing samples of mycology.

Sampling was done by scraping the feet and between the toes skin in diabetic patients with diabetic ulcers. Scraping was performed by gentle scraping of the affected tissue with lesions No. 11 from affected and non-healthy tissue.

Samples were collected on the plate. Then review of the patient's records regarding the name - Age - Gender - comorbidities - medication - doctor - date of sampling and the degree of involvement [1. Superficial wound infection, 2. Deep wound infections, 3. Deep abscess, 4. Single finger wet rot 5. Full Corruption] was evaluated and was quickly moved to Mycology Laboratory.

Biopsy tissue was taken from patients for Immunology synchronously test and were transported to the laboratory for staining with PAS.

#### *Microscopic studies:*

Using KOH 10% [KOH] and adding it to the bark chips and saprophytic yeast cells [mycelial] were differentiated from each other.

Collect chips containing normal tissue and were caught were transported to the laboratory and were cultured on agar medium and were incubated at 25 ° C for one week. After this period, the colonies were observed by Wet Mount method.

In the study under the microscope, yeast cells were differentiated from saprophyte. In the case of yeast [Candida], the mass of the test tube were used. In this test Candida albicans would be reported, if the germ tubes were observed and if not, the germ tubes of medium candida species were used for detection. In case of seeing the saprophyte by KOH 10%, lactobacilli were used for the detection of phenol.

#### *Germ tube test:*

Inside the 0/5 ml tube human serum or fetal calf serum or calf serum, spills and a loop size of the colony of yeast was added to the serum. Yeast colonies, mostly in primary colors, glossy and aunt were consistency was put for 3 to 5/3 hours at room temperature and then the samples were investigated by this term. A drop of the suspension placed between slide and coverslip and a microscope lens and the creation of germ tubes was investigated.

#### *Cultured on chrome agar:*

Through the testing, a number of Candida species can be identified. After preparation of the medium chrome agar [44 g agar in 1000ml distilled water CHR], one drop of each sample was cultured on the environment. Plates were for 48 hours at room temperature and then were read.

#### *Histological examination:*

After Pasaz tissue, blocks for histological specimens were prepared and sectioned by microtome as much as 5.4 micron and pathology slides were prepared. After being fixed staining was performed by PAS. This way, the fungus was colored pink and light green fields by using either H & E, green or red.

#### *Results:*

Tissue from 65 patients who had affected tissues, 41 were positive with KOH and 24 were negative. 16 people Saprophyte [38.4%] and 25 yeasts were [61.6%] and in the study of normal tissues by KOH, 25 positive cases have been reported, 13 patients [52%], saprophytic [mycelium], 12 patients [48%] yeast was observed. nevertheless caught the texture is more prone to fungus [table 1].

Results also indicate that, in affected tissues, 6/4% were Candida albicans and 2/13% C. glabrata, 6% C. parapsilosis, and 9/15% Candida cruise and 5/26%, Aspergillus, and 8/23% Aspergillus fumigatus and 6/4% Penicillium, 3/5% Aspergillus niger has been observed. In normal tissues 1/33% lacked fungi, 2% Candida albicans, 6/10% of C. glabrata, 3/1% for C. parapsilosis 9/7% Candida cruise, 6%, Aspergillus flavus, 2/27% Aspergillus fumigatus, 6/6 Penicillium% and 3/5% Aspergillus niger were observed.

The fungus in affected tissue is much higher than in normal tissue, but the fungus has been observed in both tissues [Both normal and affected tissue]. It should be mentioned that, the fungus Aspergillus fumigatus infection in both tissue have the highest percentage.

**Table 1:** The existence of fungi by KOH in healthy and affected tissues.

[10%]kOH	healthy									collection
	Candida Albikinis	Candida glabrata	Candida para perapillousis	Candida Crusaie	Asprigillus Flavous	Asprigillus Fomiga Toos	penicillium	Asprigillus niger	Candida Albikinis	
negative	50	0	0	0	0	0	0	0	0	50
mycelium	0	0	0	0	0	9	41	0	8	58
yeast	1	1	16	2	12	0	0	0	0	32
yeast and bacteria	0	1	0	0	0	0	0	10	0	11
collection	51	2	16	2	12	9	41	10	8	151

affected Koh	affected Tissue									collection
	Candida Albikinis	Candida glabrata	Candida para perapillousis	Candida Crusaie	Asprigillus Flavous	Asprigillus Fomiga Toos	penicillium	Asprigillus niger		
mycelium	0	0	0	0	40	36	0	8		88
yeast	6	20	9	20	0	0	0	0		55
yeast and bacteria	1	0	0	0	0	0	7	0		8
collection	7	20	9	24	40	36	7	8		151

In fact, this type, affected tissue is viewed more than healthy tissue but in all cases the fungus had pandemics and about 80% of cases are caught in this type of fungus.

Type of fungus Aspergillus infection had the highest percentage in affected tissue but much lower than observed in healthy tissue.

In all the tissues involved, 7/39% were candida, 6/55% Aspergillus species and 6/4% Penicillium and in the normal tissue, 8/18% types of candidates, 5/38% all kinds of Aspergillus and 6 / 6% were Penicillium [table 2].

**Table 2:** Percent and frequency of the fungi in healthy and affected tissues.

affected tissue	frequency	percent	Total percent
Candida Albikinis	7	4.6	4.6
Candida glabrata	20	13.2	17.9
Candida para prelliosis	9	6.0	23.8
Candida Crusaie	24	15.9	39.7
Asprigillus Flavous	40	26.5	66.2
Aspiragillus fomigatous	36	23.8	90.1
penicillium	7	4.6	94.7
Aspiragillus niger	8	5.3	100.0
collection	151	100.0	

healthy tissue	frequency	percent	Total percent
negative	50	33.1	33.1
Candida Albikinis	3	2.0	35.1
Candida glabrata	16	10.6	45.7
Candida para pesillusis	2	1.3	47.0
Candida Crusaie	12	7.9	55.0
Asprigillus Flavous	9	6.0	60.9
Aspiragillous fomigatous	41	27.2	88.1
penicillium	10	6.6	94.7
Aspiragillous niger	8	5.3	100.0
collection	151	100.0	

There was a significant relationship between fungal infection and age, [Figure 1]. As well as IN pathology of the patients, 7 patients with Candida albicans [26.9%], 9 patients with C. glabrata [34.9%], 3 C. parapsilosis [11.5%], 6 cases of Candida cruise a [% 23.7] were observed [Table 3].

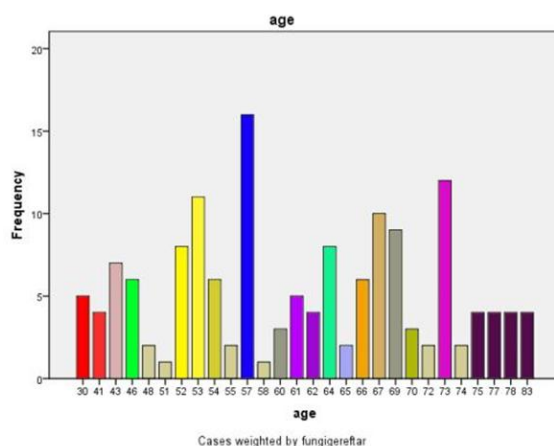
A significant relationship was found between fungal infections with patient. It was also noticed that the presence of fungi in grades 3 and 4 has been the highest.

Candida glabrata and Candida albicans had the highest value and the lowest levels were seen in grade 3 and 4 and the maximum amount of fungi belonged to Aspriragillus fomiga tous and Flavous . In Grade 4 the maximum presence was observed and minimum presence was in Grade 5.

45% of patients with grade 3 Walter, Walter, 4, and 5.3% to 49.7% with Grade 5 constitute our target population [Table 4].

#### Discussion:

In patients with diabetes since the circumstances and conditions provide the opportunity for other infectious diseases, especially fungal diseases, it has great importance.



**Fig. 1:** The relationship between fungi and person.

**Table 3:** The relation between the isolated fungi and pathobiology.

Affected tissue	Pathobiology		collection
	-	+	
Candida Albikinis	0	7	7
Candida glabrata	2	18	20
Candida para prillosis	0	9	9
Candida Crusaie	0	24	24
Aspiragillous felavous	40	0	40
Aspiragillous fomigatous	30	6	36
penicillium	7	0	7
Aspiragillous niger	8	0	8
collection	87	64	151

**Table 4:** Study of the affected tissue and Walter grade

Walter grade	Affected tissue								collection
	Candida Albikinis	Candida glabrata	Candida para perapillosis	Candida Crusaie	Asprigillus Flavous	Asprigillus Fomiga Toos	penicillium	Asprigillus niger	
3	1	4	3	8	20	24	0	8	68
4	6	16	3	16	15	12	7	0	75
5	0	0	3	0	5	0	0	0	8
Collection	7	20	9	24	40	36	7	8	151

Due to the growing weakness of the immune system, increasing the concentration of glucose in the mucosal membrane, various body tissues and fluids, increased proliferation of the normal flora of the body yeast, Diabetic patients are prone to develop fungal diseases.

Many reports concerning the increased prevalence and severity of candida infection are found in the mouth, genitals, nails, urinary tract, body parts, and tissues in diabetic patients, especially those who are partially treated.

Cleanization of the yeast in the tissues, especially damaged tissues in diabetes is due to existence of sugar including areas with high probability of injury and neuropathy in diabetic foot that is prone to yeast infections.

In this study, the highest percentage is related to the fungal infection of Candida Cruise [9/15%] and Aspergillus [5/26%] which is to the results of the study by the present investigator and colleagues [2006, Mashhad].

Samples identified in these patients include Candida albicans, and 1/9% and the 1/4% parapsilosis C. tropicalis, and Candida glabrata, and Cruise each 83/0% respectively [15].

The results of the present study is inconsistent with the study of Nir et al. In their study, 49% of Candida albicans, 23% Candida tropicalis, 18% Candida parapsilosis and 5% Candida glabrata [16].

The study of Shahin Bassiri Jahromi, Ali Asghar Khaksar in 2005 has found that the presence of fungal infections in diabetic foot syndrome includes 4. 69% Candida albicans, 9.13% Aspergillus species and% 2/4 neoformance candidate and % 8/2 Fusarium [17].

Also according to the study, Douglas and his colleagues have observed that Candida albicans isolated in 2000 in Diabetic foot ulcers has the higher binding strength and other candidates have less binding power against Candida albicans isolated.

Such as Candida tropicalis, parapsilosis Cruise. Grandol and colleagues found no empirical studies of mutated protease production in Candida yeast reduces binding to the surface of the skin. The study of Chinjolikaropal showed the existence of fungal pathogens in diabetic foot ulcer tissue as most types of candidates [18].

Hold and his long-time companion candidate with diabetic foot ulcers in our study confirm that it can improve antifungal therapy [19]. There are different types of *Candida* in diabetic patients with foot ulcers were reported by Mason [20]. Sam Peter and his colleagues in 2006 to 2007 in India selected 74 of diabetic patients with diabetic foot infections. The participants were 49 men and 25 women aged 48 to 69 years, a variety of fungi isolated from foot ulcers were 93% *Candida parapsilosis* and *Candida glabrata*.

According to this study and other studies, the need for the Mycology studies in patients with diabetic foot syndrome for early diagnosis and treatment is essential in order to effectively reduce complications.

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### REFERENCES

- [1] Khosravi, A., 1993. Medical mycology in practical ways, Jahad Tehran University Publications.
- [2] Zinati, F., M. Emami, 2006. Comprehensive Medical Mycology, Jahad Tehran University Publications.
- [3] Emilija Mlinari-Missoni, Smilija, M.V.D.S. Kalenic, 2005. *Candida* infection foot ulcer. Department of clinical Mycology, Creation National, 34: 29-35.
- [4] Elliot, J., M. Rayfield, A.I. Pect, 1982. Infection And Diabetes: The Case For Glucose Control. *Am.J.Of Ued*, 12[1-82]: 439-443.
- [5] Ajellol, R.J., Hay, 1998. Medical Mycology In To Play & Wilson's Microbiology And Microbiology In Lectian, 9th ed. Landon, Arnold.
- [6] Apelqvist, J., K. Bakker, 2008. Van Houtum Whoschaper Nc. Practical Guidelines On The Management And Prevention Of Diabetic Foot. *Diabetes Metab Res Rev.*, 24[Suppl- 1], S181- S7.
- [7] Aly F2, Blackwell Cc, Mackenzie Da, Weir Dm, Ehon Ra, Cumming Ca, Et Al M Chronic Atrophic Orod Candidiasis Amany Patients With Diabetes Mellitus – Role Of Secretor Status. *Epidemiol Infect* 1m91, 14 [Suppl 1] : 48-53
- [8] Bansal, E., A. Gary, S. Bhatia, A.I.C. Attri, J. Chander, 2008. Spectrum Of Microbial Flora In Diabetic Foot Ulcers, In *Dian J Pathol Uicrobiol*, 51:204-8.
- [9] Bassiri Jahome Shahidokht, 2005. Deep- Seated Fungal In Fection In Imnucomprom Ised Patients In Iron M Iranian Journal Of Allerey, 4[1]: 27-31.
- [10] Brossm Thalbot, Q.H., 1989. Maislin Qm Hurait2s, Strom Bl. Risk Factor For Nosocomial Candidemia: A Case Control Study In Adults With Leukemia. *Am J Med*.
- [11] Bielsa, I., J.M. Mirom, C. Herrera, E. Martin, X. Laterrem And J.M. Mascarom, 1987. Systemic Candidiasis In Heroin Abusers Cutaneous Findings. *Int. J. Dermatol*, 20: 314-9.
- [12] Calderon, R.A. *Candida An Candidiasis*, 2002. Asmm Usa.
- [13] Chinkollikar, 2002. Dam Pal Rb. Study Of Fungal And Bacterial In Fectian Of Diabetic Foot. *Indian J Pathol Microbial*, 45:15-22.
- [14] Danowslo- Toset Al, 1966. Skin Spots And Diabete, Mellitus. *Am. J. Of Med. Science*, 107: 704-105.
- [15] Denfert, C., Hubem B. Ceditor, 2007. *Candida Comparative And Functional Genomics*. Caister Academic Press. ISBN.
- [16] Dorrigg, B., A.M. Cameli, M. Trapani, D. Raspanti, M. Tprri G. Mosconi, 1995. Efficacy Of Femoral Intraarterial Administration Of Teicoplan In Diabetic Foot Infection. *Angiology*, 46: 1115-1112.
- [17] Eckhard, M., A.M. Lengler, J.M. Liersch, R.G.M. Bretzed, P. Mayser, 2007. Fungal Foot Infection In Patients With Diabetes Mellitus M Results Of Two Independent Investigations, *Mycoses*, 50[Suppl 2]:14-9.
- [18] Emilija Mlinari-Missoni, Smilija, M.V.D.S. Kalenic, 2005. *Candida* infection foot ulcer. Department of clinical Mycology, Creation National, 34: 29-35.
- [19] Elliot, J., M. Rayfield, A.I. Pect, 1982. Infection And Diabetes: The Case For Glucose Control. *Am.J.Of Ued*, 12[1-82]: 439-443.
- [20] Foot Related Compication Of Dialoetes [Http://www.Cleveland Clinic .Com](http://www.Cleveland Clinic .Com)
- [21] Gefald, P., Bodey And Victor Fainstein, 1985. *Candidiasis*. Raven Pressm New York.
- [22] Gerding, D.N., 1995. Foot Infection In Diabetic Patients The Role Of Anaerobes. *Clin Infect Dis.*, 20 [Suppl2]: S283-S8.
- [23] Hancevic, J., F.M. Coce, V. Bozиков, 2002. Eds. *Diabetic Foot*. Zagreb: Medicinska Naklada.
- [24] Hart, P.D., E. Rusell, Jr, J.S. Remington, 1969. The Compromised Host And Deep Fungal Infection. *J Infect Dis.*, 120c2: 169-97.
- [25] Heald Ahm, D.J., O'halloran, Richards Km Et Al, 2001. Fungal In Fection Of The Diabetic Foot : Two Distinct Syndromes. *Diabet Med*, 18c7:567-72.

- [26] Joshi Nm Caputp, M., 1999. Weitekamp Mrm Karchmer Aw. Infection In Patients With Diabetes Mellitus . N Engl J Med, 341:1906-12.
- [27] Johnson , S., F. Lebahn, L.R. Peterson, D.N. Gerding, 1995. Use Of An Anaerobic Colledion And Transport Swab Device To Recover Anaerobic Bacteria From Infected Foot Ulcers In Diabetics Clin Infect Dis., 20[Suppl 2]:S289-90.
- [28] Kahn, C.R., G.C. Weir, 1994. Eds, Joslin's Diabetes Memites, 13 Th Ed. Media: Williams & Willkins.
- [29] Kajeton, M., T.M. Konkoly, G. Jermendy, 1995. Experience With Microbiologic Studies Of The Diabetic Foot Orv Hetil, 13:1261-1264.
- [30] Mayfield, J.A., L.J. Reiber Gem Sanders, 1998. Janisse Dm Pogach Lm M Preventative Foot Care In People With Diabetic Care, 21: 2161-2170.
- [31] Mayser Pmhensel, J.M., Thoma Wm Et Al, 2004. Prevalence Of Fung Al Foot Infection In Patiets With Diabetes Meatus Type 1 Underestimation Pf Moccasin-Type Tinea . Exp Clin Endocrind Niabetes, 172: 264-8.
- [32] Mlinariae-Missoni, E.M., S. Kaleniae, M. Vuleeliae, 2005. De Syo Dm Belicza M. Candida Infection Diabetic Foot Ulcers. Diabetologia Evoatica, 34-1.
- [33] Mlinaric-Missoni, E., 2005. Candiada Infection Of Diabetic Foot Ulcers, Diabetologia Croatical, [1]: 29-35.
- [34] Odds, F.C., 1988. Candida and Candidosis, And Ed, London: Blliere Tindall.
- [35] Rose, H.D., 1975. Varkey Bm Deep Mycose Infection In The Hospital Aduutto A Stady Of 123 Patients. Medicine, 54[6]: 499-507.
- [36] Ross-Flanigan, Nancy, 2002. Antifungal Drays Systemic. Qale Encyaopedia Of Medicine
- [37] Saba Fata, mohammad Hadi Saeed Mohaghegh.Rabeeh Fazlizadeh, Mohammad javad Najafzadeh.M.A,M.G,2006-2008.Macotic infection in diabetic foot ulcers in Emam Reza hospital Mashhad.Jundishapur journal of Microbiology.4[1]:11-16
- [38] Schuberts, J., Heesemann, 1995. [Infection In Diabetes Mellitus ] Immun Infelct, 23-200.
- [39] Seema Nair, Abliash Sasidharan, Sujatha Sistia, 2006-2007. Incidence of Mycotic Infection in diabetic foot tissue.Journal of culture collections.pp:85-89.
- [40] Shahindokht Bassiri Jahromi, Aliasghar Khaksar, 2005. Deep-seated Fungal Infection in Immunocompromised patients in Iran.IRANIN journal of Allergy, 4: 27-32.
- [41] Georgier, S.T., 1997. V. Infection Diseases In Immunocopromised Host . Crc, 739-1148.
- [42] Tans, D.S., W.S. Joseph, 2004. Common Fungal In Fectian of Foot In Patients Whit Diabetes, 021[2]:101-12 Mellius, Drugs Agir.
- [43] Taj- Ideen S.J., J. Gent, I. Albozom, W. Buzina, J.F. Cano, J. Guarro, 2006. Gangrenous Necrosis Of The Diabetic Foot Med Mycol, 547-52.
- [44] Tekeli, A.M., I.M. Polapci, R.M. Emral, S.M. Cesur, 2004. Candida Carriage And Candida Dubliniensis In Oropharyngeal Samples Of Type 1 Diabetic Mellitus Patients. Mycoses, 47: 375-8.
- [45] Thomas, R., K. Barber, 2007. Antifungal drugs.Wikipedia.The free encyclopedia.Used by Medline
- [46] Vazquez, J.A. J.B. Sobel, 1995. Fungal Infection In Diabetics . Infect Dis .Clin. Nerth Am, 25[9-97]: 97-116.
- [47] Van Hptum Wh. 5<sup>th</sup> Inteinaltional Symposium On The Diabetic Foot, Diabetes Metab Res Rev 2008, 24[Suppl 1]: S1-S2.
- [48] Wingard, J.R., 1995. Importance of Candida Aspecies Other Than C. Albicans As Pathegens In Oncology Patients. Infect Dis., 20:115-125.