Investigations of the Prevalence of *Candida* Infections in Diabetes Mellitus Patients in Tripoli

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Abstract

This study was conducted on patients with diabetes who are suffering from immune deficiency, and are most susceptible to opportunistic fungi, including *Candida*. In this study, number of isolates of *Candida* were isolated from 104 diabetic cases between June and September 2019 in the Diabetes Hospital in Tripoli. The study showed that 66.34 % of patients suffer from the presence of *Candida*. Samples were taken with sterile cotton swabs and cultured in Petri dishes containing the nutrient medium of Sabouraud Maltose Agar (SMA). The dishes were incubated at 37^{o} Ċ for a period of 72 hours. The fungal growth samples were examined under a microscope to identify species of *Candida*. The highest number of *Candida* was observed in vaginal swabs (32) followed by toe swabs (24) and nail swabs (13).

Keywords: *Candida* infection; Diabetes Mellitus; Patients; Nails; Toes; Vagina; Libya.

المستخلص

تضمنت هذه الدراسة الاصابات الفطرية التي يسببها فطر المبيضات للمرضي المصابين بالسكري والذين يعانون من قصور في المناعة. اخذت 104 مسحة من اصابع القدمين واظافر الاصابع والمهبل. عزل فطر المبيضات من هذه المسحات التي اخذت في الفترة ما بين شهر يونيو واكتوبر2019 من المرضي الذين يترددون علي مستشفى السكر بطرابلس. اظهرت نتائج هذه الدراسة ان 66.34% من المصابين يعانون من الاصابة بفطر المبيضات. كما اوضحت ان اكثر الاصابات كانت في المهبل (32) يليها اصابيع القدمين (24) والاظافر (13). اخذت العينات بواسطة مسحات قطنية معقمة وزرعت في اطباق تحتوي على الوسط المغذي سابرويد مالتوز اجار، وحضنت الاطباق عند درجة حرارة 37 درجة مئوية لمدة 48 ساعة.

Introduction

Candidiasis is a common opportunistic infection that occurs in immune-compromised individuals. It is also known as thrush and can cause yeast infections in many areas of the body. Candidiasis is one of the most common diseases of human caused by several species of *Candida*. These species live commensally on the skin, gastrointestinal tract in the genitor-urinary tract and they are harmless in their human host when they do not overgrow and interrupt the human immune system (Brown et al., 2007).

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Under certain conditions, some species of *Candida* exploit the host body and cause infection to several places in the human body, including the skin, nails, eyes, vagina, lungs and toes. *Candida* takes advantage of the immune deficiency of some people such as pregnant women, HIV-infected people, chemotherapy patients, diabetics, people who use drugs and the user of immunosuppressive drugs and cancer patients (Kabir et al. 2012). *Candida albicans* is an opportunistic fungal pathogen that is responsible for candidiasis in humans host, and it is responsible for about 70% of fungal infections around the world (Morad, et al. 2018). *Candida albicans* is considered to be the most important species among the other clinically significant species of *Candida*; *accounting* for more than 70% of all yeasted isolated clinical samples (Eggimann, et al. 2003). *Candida albicans* exists in two forms: in the form of yeast-like cells when incubated at a temperature of 37° C, and as mycelium at a temperature of 25° C (Sudbery et al. 2004) . *Candida albicans* can also form chlamydospores, round spores with thick walls (Chaffin et al. ,1998). The pathogenicity of Candida species is attributed to certain virulent factors, such as the ability to invade host defences, adherence, biofilm formation (on host tissue and on medical devices) and the production of tissue-damaging hydrolytic enzymes such as proteases, phospholipases and haemolysin (Silva et al., 2011). People with diabetes are more likely to develop candidiasis because the elevated level of sugar in the body provides nutrients for *Candida* and encourage its overgrowth (Barnett, 2004). Diabetes mellitus is a chronic disease that can affect any organ of the body. One of the problems associated with this condition is infection (Malazy et al., 2006).

Materials and Methods

This study was carried out in the Department of Botany, Faculty of Science, University of Tripoli. Samples were collected through regular visits to the Diabetes Hospital in Tripoli-Libya during the period from June to September 2019. 104 swab samples were collected from vaginas, toes and nails from patients with diabetes. Samples were transported to the Mycology lab. at the Department of Botany, Faculty of Science, University of Tripoli. In the laboratory, 65 g of the test medium of Sabouraud Maltose agar (SMA) was dissolved in 1000 ml distilled water and sterilized by autoclaving at 121ºC for 15 min. Antibiotic agent (Chloramphenicol) was added to the medium as pure powder before sterilization of medium. Swabs were inoculated under aseptic conditions on SMA. The plates were then placed in the incubator for 72 hours at a temperature of 37Ċ. Specimens of fungal colonies were stained by lactophenol and then mounted on microscope slides. Identification of *Candida* species colonies were sub-cultured on CHROM Agar medium (Willinger et al., 1999), and incubated at 37°C for 48 hours. *Candida* isolates were identified by the detection of various colour characteristics on CHROM agar plates. Species of *Candida* were identified as *C. albicans*, C. *glabrata* and *C. tropicalis.* The data was subjected to statistical analysis using Statistical Package for Social Science (SPSS).

Results

We investigated 104 diabetic patients. The results were analysed according to their sex, *Candida* and non-*Candida* infection, age, site of infection and species of *Candida* isolated. Among these diabetic patients, 52 were male and 52 were female patients, 69 patients showed positive *Candida* infection and 35 patients did not (Table 1). The results showed that *Candida* infection was higher in both female and male patients (66.345 %) than non-*Candida* infection (33.652%) (Fig.1)**.**

Table 1. Distribution of *Candida* and non-*Candida* infections among diabetic patients.

Gender	*Candida* infection		Non- *Candida* infection	
	number	%	number	%
Female	44	42.3	11	10.5
Male	25	24.0	24	23.0
Total	69	66. 4	35	33.6

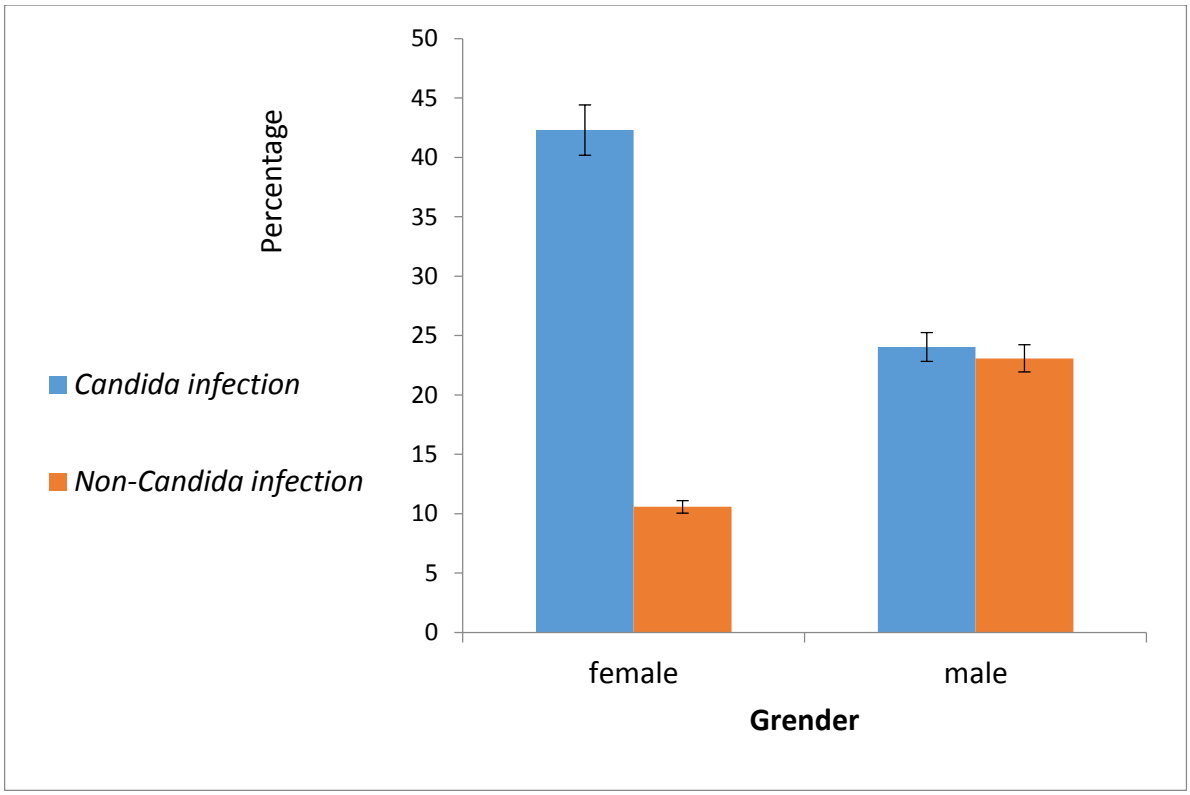


Fig. 1. Percentage of infection of *Candida* and a non-*Candida* in diabetic patients.

Table 2. Prevalence of *Candida* infection among patients with different ages.

age	No. of *Candida* infection	Percentage
30- 45	20	28.98 %
46 – 61	26	37.68 %
>61	23	33.33 %

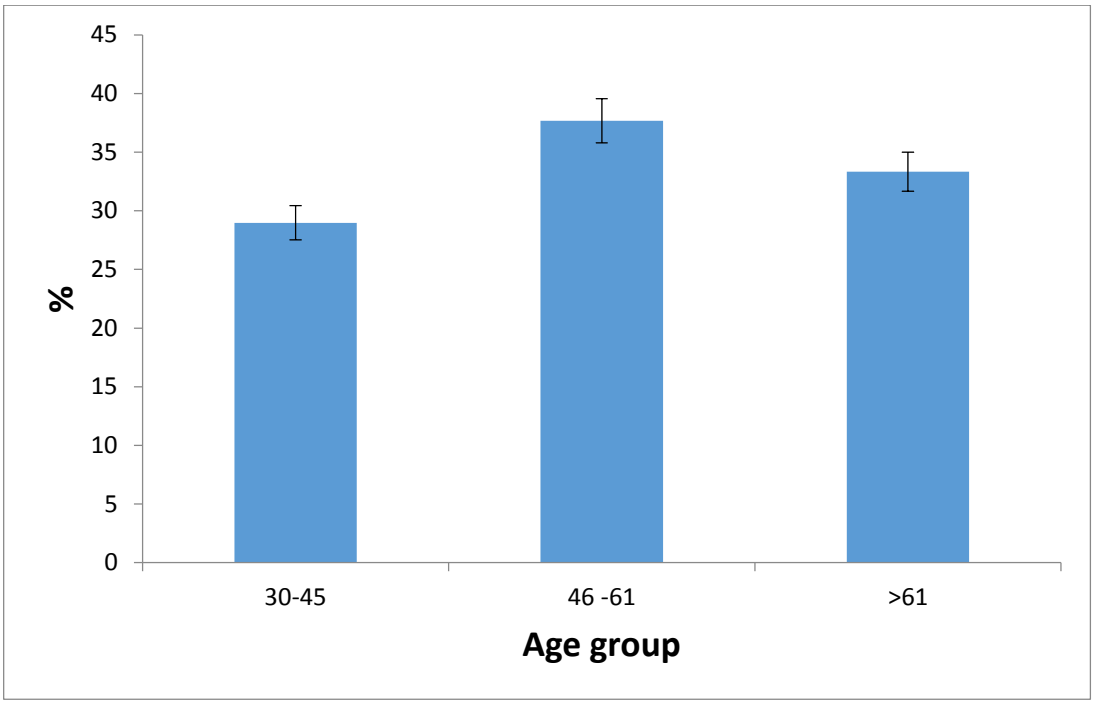


Fig. 2. Percentage of *Candida* infection between age groups.

Among diabetic patients, the highest number of *Candida* was observed in vaginal swabs (32) followed by toe swabs (24) and nail swabs (13). The value was not, however, significantly higher in samples collected from vagina compared with ones collected toes and nails as shown in Table 3 and Fig. 3.

Table 3. Frequency of *Candida* species isolated from diabetic patients

Isolated species	**Vaginal samples**	**Toa samples**	**Nail samples**
Candida albicans	21	13	8
C. tropicalis	7	6	4
C. glabrata	4	5	1
Total	32	24	13

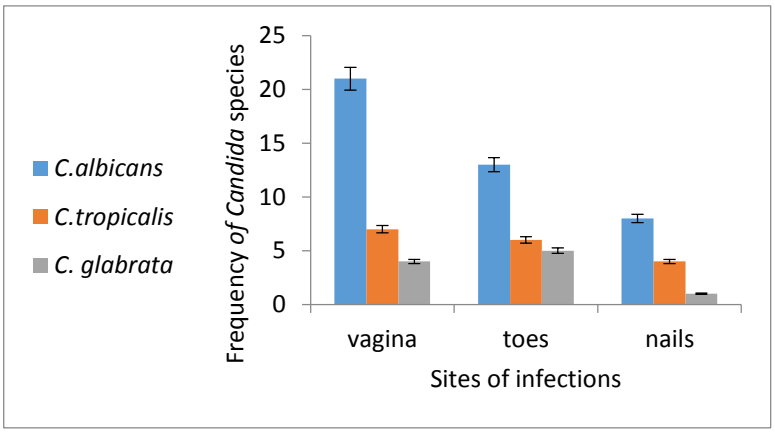


Fig. 3. Number of *Cndida* species isolated from infected sites.

As shown in Fig. 4. *Candida albicans* was the most prevalent species (73.91%) followed by *Candida tropicalis* (15.94%) and *Candida glabrata* (10.14%). Statistical analysis has revealed that the number of *C. abicans* was significantly higher than *C.tropicalis* and *C. glabrata* (Table 4)**.**

Table 4. Percentage of *Candida* species

Candida species	Number	Percentage
Candida albicans	51	73.91 %
Candida tropicalis	11	15.94 %
Candida glabrata	7	10.14 %

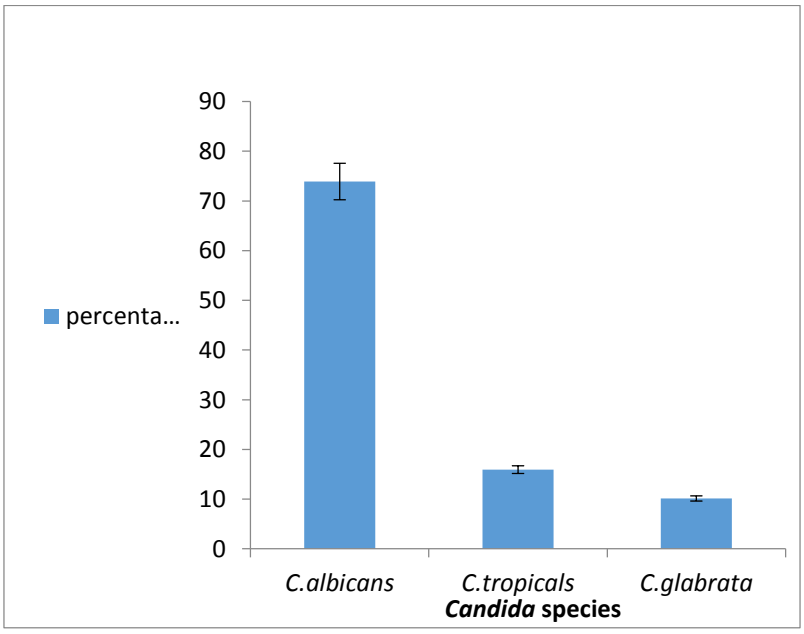


Fig. 4. Prevalence of *Candida* species.

The identification of *Candida* colonies is based on colour of colonies, light green as *C. albicans*, blue as *C.tropicalis* and cream to white as *C.glabrata* (Fig .5).

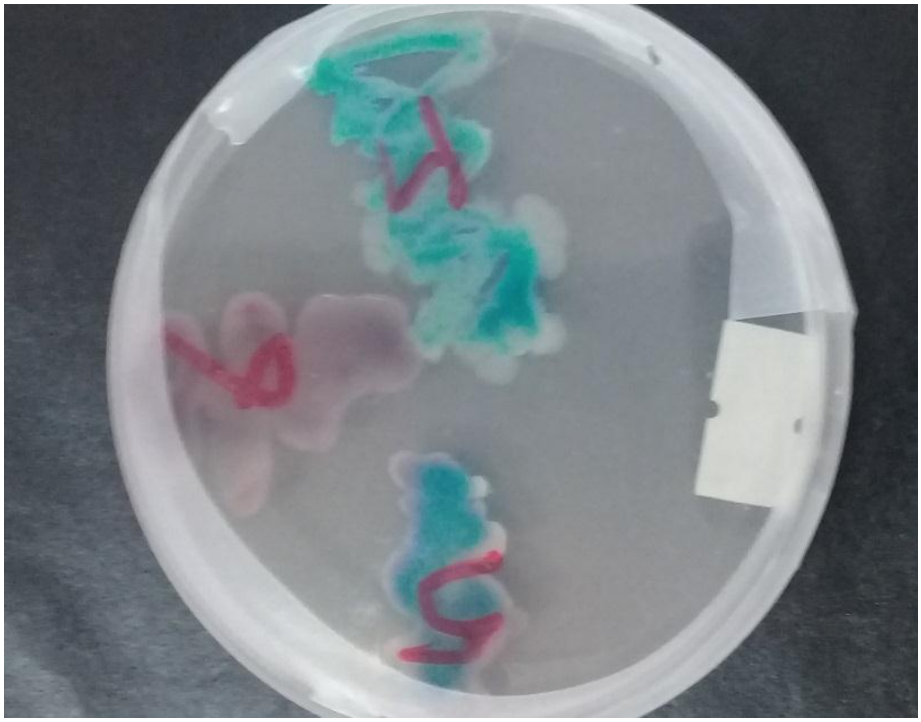


Fig. 5. Growth of *Candida* species in CHROM agar medium *C. albicans* (4), *C.tropicalis* (5) and *C. glabrata* (6).

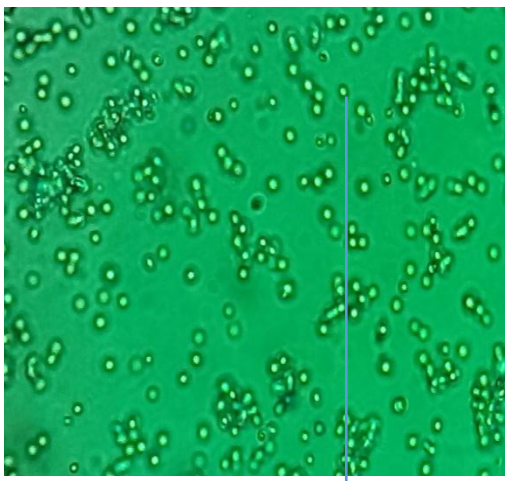
A  B 

Fig. 6. Colonies of *Candida albicans* (A), germ tube formation of *C.albicans* (B)

Germ tube

Discussion

Candidiasis is the most common fungal infection and is responsible for 90% of the cases of infectious vaginitis (Hedayati and Shafiei, 2010). Distribution of *Candida* species from three different sites were also observed in this study. In study group 104 samples (Table 5) were collected from three different sites of the body of diabetic patients. Out of 69 positive samples, 71.01% of *Candida* was found in vaginal swabs, 15.94% in toe swabs and 13.04% in nail swabs. In vagina acidic pH favours the growth of *Candida* and increased glucose levels in genital tissues enhance Candida adhesion and growth. Vaginal epithelial cells bind to *Candida* with greater propensity in diabetic patients than in non-diabetic patients. The results of this study showed the relationship between prevalence of *Candida* and age groups. We found that group age between 46 and 61 years and >61 were higher because they are more susceptible to *Candida* infection, which is due to the decline in the ability of their immune system. The study, revealed also that *C.albicans* was more prevalent (73.91%) followed by *C. tropicalis* (15.94%) and *C. glabrata* (10.14 %). Similar findings were observed in many previous studies (Muvunyi and Hernandez 2009; Nelson et al., 2013; Faidh, 2013; Zisova et al., 2016; Abourghiba and Alghadem, 2020; Tapper-Jones, et al., 1981).

Table 5. Details of the status of patients

No.	**Age**	**blood glucose Levels**	**Sex**	**Sample 's site**	**Type of diabetes**	**Result**
1	70	177	male	nails	Type 2	*Candida*
2	60	261	male	toes	Type 1	*Candida*
3	55	174	female	vagina	Type 1	*Candida*
4	48	344	male	nails	Type 1	*Candida*
5	55	177	male	nails	Type 2	-
6	61	180	female	vagina	Type 1	*Candida*
7	54	186	female	vagina	Type 1	-
8	50	214	female	vagina	Type 2	*Candida*
9	60	274	male	toes	Type 1	-
10	57	277	male	toes	Type 1	*Candida*
11	65	272	male	toes	Type 1	*Candida*
12	60	260	female	toes	Type 1	*Candida*
13	50	161	male	toes	Type 1	-
14	35	181	female	vagina	Type 1	-
15	66	135	female	vagina	Type 1	*Candida*
16	65	577	female	vagina	Type 1	*Candida*
17	50	224	female	vagina	Type 2	*Candida*

Table 5. (Cont.)

18	43	136	male	nails	Type 2	*Candida*
19	52	181	male	nails	Type 2	-
20	55	165	male	toes	Type 2	-
21	48	230	male	toes	Type 1	-
22	66	345	female	toes	Type 1	*Candida*
23	39	221	female	vagina	Type 1	*Candida*
24	48	229	female	vagina	Type 1	*Candida*
25	37	309	female	vagina	Type 1	*Candida*
26	60	218	female	toes	Type 1	-
27	59	430	female	vagina	Type 1	*Candida*
28	37	173	female	vagina	Type 1	*Candida*
29	70	380	male	toes	Type 1	-
30	65	121	male	nails	Type 1	-
31	39	60	male	toes	Type 1	*Candida*
32	*44*	*158*	female	vagina	Type 1	-
33	33	199	female	vagina	Type 1	*Candida*
34	64	70	female	vagina	Type 1	*Candida*
35	40	71	male	toes	Type 1	*Candida*
36	77	100	male	toes	Type 2	-
37	63	170	female	toes	Type 2	*Candida*
38	55	150	female	vagina	Type 2	*Candida*
39	62	263	Female	nails	Type 2	*Candida*
40	42	183	Female	vagina	Type 2	*Candida*
41	46	293	male	toes	Type 1	*Candida*
42	60	217	male	toes	Type 1	-
43	34	148	female	vagina	Type 1	*Candida*
44	56	355	male	nails	Type 1	-
45	30	238	female	vagina	Type 1	*Candida*
46	60	337	male	nails	Type 1	*Candida*
47	65	333	male	toes	Type 1	*Candida*
48	40	280	female	vagina	Type 1	-
49	71	278	male	nails	Type 1	*Candida*
50	63	230	female	toes	Type 1	*Candida*
51	37	229	female	vagina	Type 1	*Candida*
52	65	337	male	toes	Type 1	*Candida*

Table 5. (Cont.)

53	42	263	female	toes	Type 1	*Candida*
54	55	271	male	nails	Type 1	-
55	67	73	female	vagina	Type 2	-
56	60	149	male	toes	Type 1	-
57	51	287	female	toes	Type 1	*Candida*
58	45	334	female	toes	Type 1	-
59	71	203	male	toes	Type 1	*Candida*
60	35	229	female	vagina	Type 1	*Candida*
61	56	373	female	vagina	Type2	*Candida*
62	43	171	male	toes	Type 1	-
63	70	235	female	vagina	Type 1	*Candida*
64	60	217	male	nails	Type 1	-
65	53	337	female	vagina	Type 1	-
66	73	100	male	nails	Type2	*Candida*
67	42	150	female	vagina	Type2	*Candida*
68	55	71	male	toes	Type 1	-
69	41	280	female	toes	Type 1	*Candida*
70	35	361	female	vagina	Type 1	*Candida*
71	61	225	male	toes	Type 1	*Candida*
72	40	351	female	toes	Type 1	*Candida*
73	51	462	female	vagina	Type 2	*Candida*
74	62	552	male	Fingers	Type 2	*Candida*
75	38	221	male	nails	Type 1	*Candida*
76	66	120	female	toes	Type 2	*Candida*
77	63	351	female	vagina	Type 1	*Candida*
78	63	212	female	vagina	Type 1	-
79	75	462	male	toes	Type 1	-
80	64	641	male	nails	Type 2	-
81	41	170	female	toes	Type 1	*Candida*
82	65	346	male	nails	Type 2	*Candida*
83	55	221	female	vagina	Type 1	*Candida*
84	71	174	male	toes	Type 1	-
85	66	354	male	toes	Type 1	-
86	52	163	female	vagina	Type 1	*Candida*
87	39	81	female	toes	Type 1	*Candida*

Table 5. (Cont.).

88	75	136	male	toes	Type 2	*Candida*
89	48	577	female	toes	Type 1	*Candida*
90	31	272	female	vagina	Type 1	-
91	50	186	female	vagina	Type 1	*Candida*
92	43	372	female	toes	Type 1	*Candida*
93	57	166	male	nails	Type 1	*Candida*
94	61	231	female	vagina	Type 2	*Candida*
95	32	415	male	nails	Type 1	-
96	48	174	female	vagina	Type 1	*Candida*
97	60	297	male	toes	Type 1	*Candida*
98	63	153	male	toes	Type 2	-
99	30	246	male	toes	Type 2	-
100	41	310	male	nails	Type 1	-
101	40	413	male	nails	Type 1	*Candida*
102	59	153	female	vagina	Type 1	*Candida*
103	32	170	male	nails	Type 1	*Candida*
104	67	195	male	toes	Type 1	-

(-) Absence of *Candida* infection

Conclusion

High prevalence of *Candida albicans* was observed in this study. This investigation has shown that factors such age of the patients with diabetes responsible for prevalence of *Candida*. Our finding should be taken into consideration in further studies regarding presence of *Candida* among diabetic patients. Moreover, it is important for diabetic patients to maintain good control of level of glucose in blood and maintenance of adequate hygiene of body which are critical in the prevention of *Candida* infection.

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