

Candida in patients with diabetes mellitus type II on insulin with metformin vs. metformin only: randomized clinical trial

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ABSTRACT

Introduction: *Candida albicans* can cause multiple illnesses affecting the oral mucosa, especially in immunocompromised patients. Diabetes mellitus type II may reduce immunity. Wearing dentures will violate the normal flora by harbouring more plaque

Objectives: The aim of present study was to investigate the prevalence of *C. albicans* in the oral cavity among type II diabetic patients on insulin with metformin vs. those on metformin only with or without an acrylic plate. The effect of the level of oral hygiene on candida growth was also assessed.

Methods : The study was carried out on 100 (50 male, 50 female) patients who had been diagnosed with type II diabetes mellitus at least 1 year previously.

An upper acrylic plate was constructed for Test group which allocated of 50% of the sample (25% male, 25% female), consisting of an acrylic plate covering the upper denture, clasps and Adams clasps for molars and premolars.

We asked the patients to wear the plate full time and gave them instructions on how to clean it regularly, and to remove it at night.

The glycohaemoglobin (HbA1c, A1C) test was performed on all patients at beginning of study.

Samples were collected from the hard palate of dentate patients and from the tissue-bearing area of the upper denture by scraping with a sterile swab. The swabs were processed for microbiological examination by immersion in 5 ml of sterile 0.9% physiological saline. This was vortexed for 1 minute to disperse the adhering bacteria. A loopful of the suspension was plated on Sabouraud's dextrose (SD) agar containing gentamycin (2 mg/dL) and chloramphenicol (5 mg/dL) and incubated for 48 h at 37°C.

Results: The sample consisted of 100 (50 male, 50 female) participants aged 20–68 years (mean, 49.1 ± 10.7 years; men, 47.5 ± 8.4 years; women, 50.6 ± 8.8 years).

C. albicans was isolated from 32 (32%) patients. The mean HbA1c level was 8.27% ± 1.41 in those positive for *Candida* and 8.38% ± 1.58 in those negative for *Candida*. Patients on metformin only had a mean HbA1c of 8.33% ± 1.02 and those on insulin with metformin had a mean HbA1c of 8.28% ± 1.80. In the control group the mean HbA1c was 8.42% ± 1.47 and in the test group with the acrylic plate it was 8.19% ± 1.45.

The prevalence of *C. albicans* was higher in patients on metformin only than in patients on insulin with metformin.

The prevalence of *C. albicans* was higher in the test group with the acrylic plate than in the control group without the acrylic plate.

Poor oral hygiene had an influence directly on the prevalence of *C. albicans* in both groups. Males had poorer oral hygiene than females.

Conclusion: Treatment Protocol for Treating Diabetes type II using Insulin with Metformin or Metformin only and the use of an acrylic plate had no effect on increasing or decreasing the candida albicans prevalence. On the other hand the level of oral hygiene had more influence on the prevalence of *C. albicans*, with males being more susceptible than females. HbA1c test cannot be used as an indication

of the possibility of colonization by *C. albicans* among type II diabetic patients, because the level of control of glucose found in current study to be not the major factor. Oral Hygiene level proved to be important factor affecting the *Candida albicans* growth .future investigation recommended for other diabetic medications and effect of glucose level in saliva on prevalence of oral candida albicans.

Keywords: *Candida albicans*, Type II diabetes, Insulin, Metformin

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RMS April 2021; 28(1): 10.12816/0058879

INTRODUCTION

The human oral cavity can support approximately 700 different species of microorganism, including 20 *Candida* species.(1,2) Previous studies have shown a high prevalence of *Candida* spp. in the oral cavities of patients with denture stomatitis (94%) and healthy people who wear dentures (75%).(3)

Candida can be present in healthy hosts and is not considered harmful unless certain conditions are met, creating opportunities for virulence that causes candidiasis.(4) Oral candida was reportedly found in 34.4% of healthy subjects without symptoms and in 54.7% of hospitalized subjects.(5) However, candidal infections are considered a major problem globally, particularly for people with immunosuppression.(6–12)

Different types of *Candida* that are associated with both clinical and nonclinical conditions can be present on the surface of the oral cavity, such as *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. stellatoidea*, *C. krusei* and *C. kefyr*, but only a few cause candidiasis.(13–17) Specifically, *C. albicans* is commonly isolated from the oral cavity.

Diabetes mellitus is a chronic metabolic disorder that is becoming one of the most common chronic diseases worldwide. The recent increase in the number of adults with diabetes is particularly surprising; an estimated 300 million adults are expected to have diabetes by the year 2025.(18) In Jordan, there has been a significant increase in the prevalence of type II diabetes mellitus during the last 10 years, with 31.5% of the population affected.(19) Metformin and insulin are the most common treatment for type II diabetes (20).

Patients with diabetes have a higher prevalence of candida than patients without diabetes,(21–23) and clinical manifestations of candida infection occur more frequently and severely in patients with diabetes than patients without diabetes.(24–27) *C. albicans* is one of the most common species isolated from patients with diabetes, with a prevalence of up to 80%,(21,24,25,28) and is the most common virulent fungal pathogen of the *Candida* species.(29) Changing in salivary glucose levels in patients with diabetes can causes an alteration of yeast growth. (30)

However, the results are controversial based on contradictory results in other studies.(24,31–33)

The prevalence of *Candida* infection is also higher in people who use dentures, especially in patients with diabetes.(27,28,34,35) Owing to the increase in diabetes within the population, especially in the elderly, there has been an increased need for dentures. As the use of dentures is becoming more common, this could increase the risk of candida infection in this population, owing to the effects in the oral cavity and altered oral mucosa in addition to systemic complications.(21,26–28,35)

The aim of present study was to investigate the prevalence of *C. albicans* in the oral cavity among type II diabetic patients on insulin with metformin vs. those on metformin only with or without an acrylic plate. The effect of the level of oral hygiene on candida growth was also assessed.

Does Diabetic type II patients on Metformin and insulin, wearing an upper denture associated with higher prevalence of candidiasis?

Does Patients with poor oral hygiene had higher prevalence of candidiasis?

METHODS

The present study was approved by the Royal Medical Services ethics committee. All of the participants provided verbal consent and permission for the procedures.

The study was conducted in multiple diabetes care clinics in the Royal Medical Services (RMS), Ministry of Health (MOH) and private sector, on patients who had been diagnosed with type II diabetes mellitus at least 1 year previously and were on medication protocol with either Metformin only or Insulin with Metformin and follow the protocols for Diabetes type II treatment for each clinic. Patients who were currently taking or who had been administered antibiotics, antifungal medications, steroids, or immunosuppressive drugs in the past 3 months were excluded. All patients who used removable denture complete or partial were excluded.

A list of case numbers was randomly generated from 1 to 50 for male patients and 1 to 50 for female patients. Half of each list (25) was randomly assigned to be fitted with an acrylic plate (test group) and the rest formed the control group.

Personal information (name, age, sex) and the medical history (medication use) of the patients were recorded by a registered nurse. A case number was assigned to each patient.

An alginate impression of the upper arch was taken from patients in the test group and sent to the lab. An upper acrylic plate was constructed for each patient, consisting of an acrylic plate covering the upper denture, clasps and Adams clasps for molars and premolars.

We asked the patients to wear the plate all day, and to remove it at night. They were given instructions on how to clean it regularly.

The dental history, oral hygiene of the patient and acrylic plate were graded as good, average, or poor based on the dentist's report.

Tissue samples were collected from the upper palate of patients without an acrylic plate and from the tissue-bearing area of the upper acrylic plate by scraping with a sterile swab. The swabs were processed for microbiological examination by immersing them in 5 mL sterile 0.9% physiological saline. This was vortexed for 1 min to disperse the adhering bacteria. A loopful of the suspension was plated on Sabouraud's dextrose (SD) agar containing gentamycin (2 mg/dL) and chloramphenicol (5 mg/dL) and incubated for 48 h at 37°C. which produce creamy white pasty ,followed by using CHROMagar for identification of candida albicans which show light green colour.

All patients underwent a glycohaemoglobin (HbA1c) test.

Statistical analysis

Data were entered and coded using SPSS version 17.0 (Chicago, IL, USA). Values are reported as frequencies and mean \pm standard deviation. Pearson's r was used to test the correlations between variables. P values <0.05 were considered statistically significant.

RESULTS

The sample consisted of 100 (50 male, 50 female) participants aged 20–68 years (mean, 49.1 ± 10.7 years; men, 47.5 ± 8.4 years; women, 50.6 ± 8.8 years).

C. albicans was isolated from 32 (32%) patients: 21 men, 11 women; 20 patients on metformin only, 12 patients on insulin with metformin; 12 patients with poor oral hygiene, 9 patients with average oral hygiene, and one patient with good oral hygiene. The remaining 68 (68%) patients tested negative for *C. albicans* (Table 1).

The mean HbA1c was 8.3 ± 1.5 (male 8.4 ± 1.6 , female 8.2 ± 1.4). Candida-positive patients had a mean HbA1c of 8.3 ± 1.4 and Candida-negative patients had a mean HbA1c of 8.4 ± 1.6 . The mean HbA1c in patients on metformin only was 8.3 ± 1.0 and in patients on insulin with metformin it was 8.3 ± 1.8 . The mean HbA1c in the control group was 8.4 ± 1.5 and in the test group with the acrylic plate it was 8.2 ± 1.5 .

The data revealed that there was no significant difference in prevalence of Candida according to age, HbA1c test result, type of medication or presence of acrylic plate ($p = 0.84, 0.73, 0.09$ and 0.40 respectively), but it was significantly affected by gender and oral hygiene ($p = 0.03$ and 0.00 respectively). The prevalence of Candida spp. was higher in males than in females (Fig. 1). The prevalence of *C. albicans* was higher in patients on metformin only than in patients on insulin with metformin (Fig. 2). The prevalence of *C. albicans* was higher in the test group with the acrylic plate than in the control group (Fig. 3). Poor Oral hygiene had direct proportion influence on the prevalence of *C. albicans* in both groups (Fig. 4). Males had poorer oral hygiene than females. Oral hygiene was rated as poor in 32% of the participants (*C. albicans* positive 34.6%), average in 26% of the participants (*C. albicans* positive 26.0%), and good in 42% of the participants (*C. albicans* positive 2.4%) (Table I).

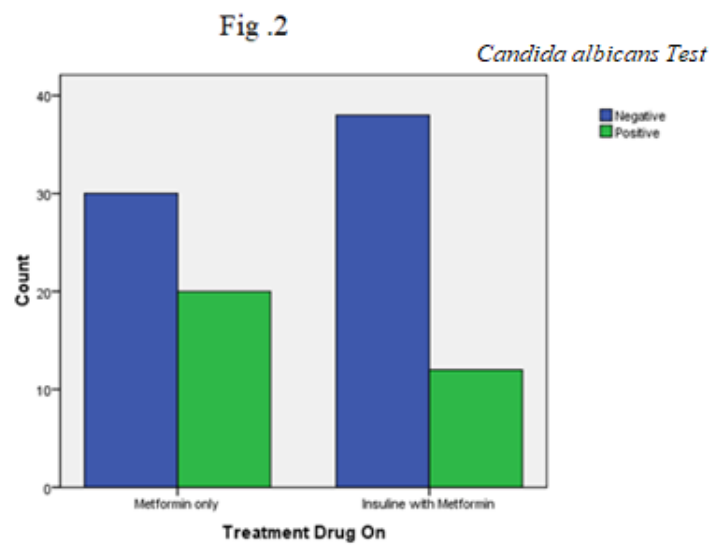
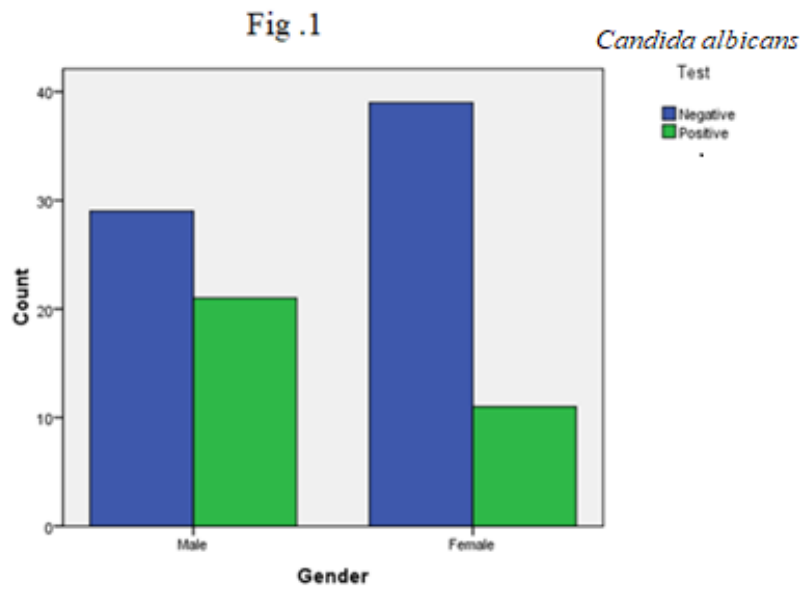


Fig .3

Candida albicans Test

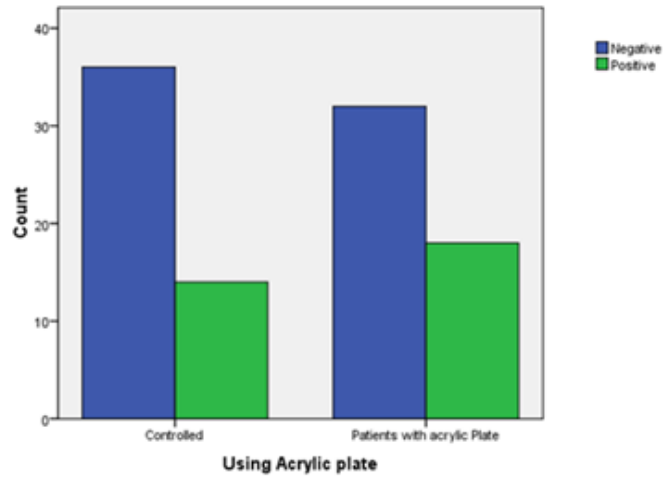


Fig .4

Candida albicans Test

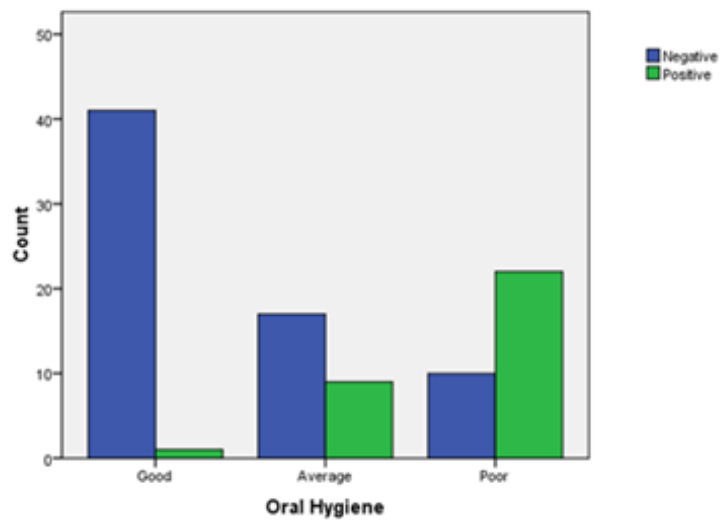


Table I Candida albicans: Gender, Treatment Regime, Use of Acrylic Plate and Oral Hygiene.

		Candida albicans Test	
		Negative Count	Positive Count
Gender P value = 0.03	<i>Male</i>	29	21
	<i>Female</i>	39	11
Treatment Drug P value = 0.09	<i>Metformin only</i>	30	20
	<i>Insulin with metformin</i>	38	12
Using Acrylic Plate P value = 0.40	<i>Control</i>	36	14
	<i>Patients with acrylic plate</i>	32	18
Oral Hygiene P value = 0.00	<i>Good</i>	41	1
	<i>Average</i>	17	9
	<i>Poor</i>	10	22

Age P value = 0.84, HbA1c test P value = 0.73

DISCUSSION

In the present study, 32 of the 100 (32%) patients tested positive for *C. albicans* in their oral cavity, a lower prevalence than in previous studies testing diabetic patients but higher than in healthy patients ($p < 0.05$)(36–38). There was a significantly higher prevalence of colonization in males compared with females, unlike in Kadir et al. (33) and Sahin et al. (32), who found no significant effect of age or sex on the presence of *C. albicans* ($p > 0.05$). Diabetes mellitus affects the composition and amount of saliva, which influences the microorganism population in the oral cavity. Increased salivary glucose levels in patients with diabetes causes yeast growth owing to an increased number of candida receptors. (30) Similarly, reducing salivary flow also increases colonization by candida and plays a role in candidiasis.(33) Furthermore, immunosuppression can occur with diabetes mellitus, which could increase the susceptibility to oral infections with *Candida* spp.(6,7,21,22,24,31–33)

Regarding the association between *C. albicans* colonization and the degree of diabetic control, as indicated by HbA1c, there was no difference in control between patients who tested positive and those who tested negative for *C. albicans*. The mean HbA1c was 8.3 ± 1.4 for *C. albicans* negative and 8.4 ± 1.6 for *C. albicans* positive subjects.

Regarding treatment regime patients who only used metformin had a higher prevalence of *C. albicans* (40%) than patients who were using insulin with metformin (24%), but the difference was not significant

(P=0.09). patients on Metformin had higher glucose level in saliva due to difference in control potency between Insulin and Metformin.(39)

The presence of an acrylic plate decreases the salivary pH and flow rate and impedes the mechanical cleaning of the soft tissue of the oral cavity.(15) This increases infective virulence and aggravates previously existing infective conditions. The use of an acrylic plate changes the physiology and normal flora of the palate. The tissue in contact with the surface of the acrylic plate is disturbed less often, which favours the colonization of microbes, especially acidogenic bacteria and Candida.(40) Wearing an acrylic plate induces plaque formation, favouring an increased population of potentially pathogenic bacteria and Candida spp.(41,42) The current investigation is consistent with previous studies that showed an increase in Candida colonization in a group with an acrylic plate (36%) compared to the control group (28%) (28,43), although the difference was not significant.

In the present study, 34.6% of patients with poor oral hygiene had candida, 26% of patients with average oral hygiene had candida, and 2.4% of patients with good oral hygiene had candida; these rates are higher than in the normal population but lower than those reported by Muzurovic et al., who reported Candida spp. in 83.4% of patients with teeth and poor oral hygiene; the most frequently isolated type was *C. albicans*.(44)

CONCLUSION

Treatment Protocol for Treating Diabetes type II using Insulin with Metformin or Metformin only and the use of an acrylic plate had no effect on increasing or decreasing the candida albicans prevalence. On the other hand the level of oral hygiene had more influence on the prevalence of *C. albicans*, with males being more susceptible than females. HbA1c test cannot be used as an indication of the possibility of colonization by *C. albicans* among type II diabetic patients, because the level of control of glucose found in current study to be not the major factor.

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