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Detection of Candida Species in the Diabetes Mellitus Patient

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A B S T R A C T

Yeasts of the genus *Candida* have high genetic variability and are the most common opportunistic pathogenic, which isolated from clinical samples that cause many problems to human, frequently isolated from patient which had diabetes mellitus. The aims of study were Detection and isolation and identification of *Candida* spp. in Diyala province by routine laboratory procedures. A total of 100 cases between 20 and 90 years old, this study include patients have symptom of oral candidiasis in patient with diabetes mellitus, over a one month period (from December 2017 to February 2018), in Dialay Teaching Hospital. The oral swabs were for cultures on two media, the first media was used for primary isolation which was Sabouraud´s dextrose agar (SDA) media and the second was to differentiate *Candida* spp. according to their colours and also germ tube used for identification of *C. albicans.* Results of this study presented that the highest infection of the *Candida* spp was accounted for *C. albicans* 31 (55.35%) from the 56 (56%) positive cultures, while other species were as follows: appear *Candida glabrata* (12) (26.88%), *Candida parapsilosis* (6) (13.44%), *Candida krusi* (4) (8.96%), *Candida tropical* (3) (6.72%). Through the analysis of data that collected from patients, there were non-significant, relationships were found between *Candida* infection and age group, gender, smoking type of diabetic. But there was significant, relationship found between *candida* infection and family history. All data were statistically analyzed depending on SPSS (Statistical Package for Social Science) version 18 (2009) *C. albicans* is the most common isolated among the total *Candida* species, and *Candida glabrata* was the most frequent *non–albicans* species. In this study we found that patients with type II diabetes mellitus were more infected by *Candida albican* than type I.

Introduction

Candida infections are one of the most commonly occurring fungal infections in humans (Kumar *et al.*, 2005). Affecting mucous membrane, skin, nails and internal organs of the body, it additionally a typical opportunist infections in immune compromised patients (Makwana *et al.*, 2012; Hasan and Al-Jubouri, 2015). *Candida* species belong to the natural micro biota of an individual's mucosal oral cavity, gastrointestinal tract and vagina (Shao *et al.*, 2007). Although *Candida albicans* is considered the main agent of candidiasis and to be the most frequently isolated from oral cavity, but in recent two decades there has been important increase of other non-*Candida*

albicans species such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis* and *Candida dubliniensisas* a result of a different factors like immune-suppressants and prolonged use of broad spectrum antibiotics and antifungal drugs (Martins *et al.*, 2014; Patil *et al.*, 2015; Jain *et al.*, 2016). But the majority of fungal infections in humans are caused by the species *C. albicans* and *C. glabrata*.

The prevalence rates of *C. albicans* and *C. glabrata* infections are approximately 70% and 15%, respectively (Kolaczkowski *et al.*, 2010; Benedetti *et al.*, 2016). Diabetes mellitus is a common and growing global health problem which causes several complications. Periodontal diseases are considered the sixth complication of this disease. Diabetics have an increased predisposition to the manifestations of oral diseases like candidiasis. Diabetes mellitus is the most common endocrine metabolic disorder (Lotfi-Kamran *et al.*, 2009). Nearly 85-90% of diabetic patients are diagnosed with type II diabetes (resulting from insulin resistance) in these patients, salivary dysfunctions like dry mouth, reduced salivary function, lichen, tooth decay, and periodontal diseases are common (Ship, 2003; Vijan, 2010).

Candida infections are chronic opportunistic infections related to diabetic patients. The presence of *Candida* spp. in oral cavities of diabetics varies between (50-80 %), (Willis *et al.*, 2000; Khosravi *et al.*, 2008; Melton *et al.*, 2010). Oral candidiasis is a common profiteering infection of the oral cavity caused by an overgrowth of *Candida* species particularly *Candida albicans* (Guggenheimer *et al.*, 2000). This infection is usually accompanied by various symptoms including burning, painful sensation, change of taste, reduced saliva secretion and swallowing difficulty, but it can be also asymptomatic (Nikolic *et al.*, 2016). Among the explanations creating diabetic patients additional susceptible to oral candidiasis (Ship, 2003; Vijan, 2010). Are high levels of salivary glucose, impaired chemotaxis, low secretion of saliva and defect of phagocytosis because of polymorph nuclear white blood cell deficiency (Mohammadi *et al.*, 2016). The attachment of *C. albicans* to the crystalline hydroxyapatite produces collagen lytic protein that will increase crystal solubility and consumes chemical element of dentin albuminoid in diabetes mellitus patients (Mohammadi *et al.*, 2016).

Materials and Methods

One hundred swabs samples had been collected from patients that have symptom of oral candidiasis from patients with diabetes mellitus. All the collected samples were inoculated directly, on Sabouraud dextrose agar (SDA) containing Chloramphenicol. Than incubated for 48 hrs. Each sample of them we had made slide and stain with Lactophenol cotton blue stain for examination of *Candida* spp.

Than we used germ tube (GT) test it's used to detection of *Candida albicans.* It is Rapid diagnostic differentiates *C. albicans* from other species. In small micro centrifuge tube One ml of serum was added by using a Pasteur pipette, colony of yeast was transport by sterile wire loop and emulsified it in the serum. We incubated at 37° for 2-3 hours but no longer after we mixed than the a drop of the serum was transferred to a slide for examination, cover slip was added and examined microscopically using (40X) objective (Bhavan *et al.*, 2010).

And the only positive samples were cultured on CHROM Agar (CAC). *Candida* was resuscitated by inoculating a loop full of culture from Sabouraud Dextrose Agar into

CHROM agar media by streaking a loop full of culture and incubated at 37º for 72hours. After 72-96 hours of incubation, the *Candida* colonies were initially identified by colonial color when compared with standard color photographs supplied by the manufacturer and also presented (Mahmoudabadi *et al.*, 2000).

Results and Discussion

Culture media

After the incubation of culture for 48 hrs the morphological feature of culture on (SDA) medium was the most of culture had heavy growth, and the appearances of *C. albicans* colonies on Sabouraud dextrose agar were special it was singular and rounded and the color of the colonies were off-white to creamy and the odor of it was a characteristic yeast odor (Figure 1). Through this study from the 100 samples that cultured on (SDA) there were 56 of them gives positive result (Figure 2). The direct examination under light microscope was done to determine the shape and size of yeast, lactophenol cotton blue stain examination of *c. albicans* isolates showed spherical to oval cells, with a presence of budding and was much larger than bacterial cells (Figure 3).

Phenotype identification of *Candida* Spp

The result of germ tube (GT) formation show that from 56 samples that give positive result on SDA only 31 of them give positive for germ tube which seen as along tube - like projections extending from the yeast cells (Figure 4). And the result of CHROM agar shows that the colours of colonies of Candida spp. was similar to colour that given by protocol, green colonies of *C. albicans*. Blue colonies of *C. tropicalis*, Purple- Pink colored colonies of *C. krusei, C. glabrata* produced cream to white and *C. parapsilosis* produced pinkish to white (Figure 5). The present study shows, difference between Candida spp., 31 (55.35%) of the total isolated were identified as *C. albicans*, 25 (44.64%) isolates were identified as *non-albicans*., of which *Candida glabrata* 12 (26.88%), *Candida parapsilosis* 6 (13.44%), *Candida krusi* 4 (8.96%), *Candida tropical* 3(6.72%).

In this study show relationship between *Candida* isolated and genders in this study were displayed as it was non-significant (P>0.05) as it shown in the (Figure 7).

The females 29samples (51.8%) were more infected by candida than males 27 samples (48.2%).

According to the type of diabetes the study show that Type II of diabetes 31 samples (55.4%) higher than the Type I of diabetes 25 samples (44.6%). There were non-significant (P>0.05) differences between type diabetes and Candida spp. as shown in the (Figure 8).

According to this study *Candida* infection rates were higher in the non-smoking patients than smoking patients. There were non-significance (P>0.05) relationship between them. In the case of non-smoking 74 samples (74.00%), 41 samples (73.2%) of them give positive candidiasis while in the case of non – smoking there were 15samples (26.8%) give positive candidiasis all the result show in the (Figure 9).

Candida infection rate was higher in patient who none have history family of diabetes mellitus 51samples (51%) in compared with patient that have diabetes in their family history 49samples (49.00%). There were high significant (P>0.05) differences between history family and *Candida* spp.

In this study, there have been (100) patients from each gender suffered from signs and symptoms of oral candidiasis.

Fig.1 Colonies of *Candida* spp. Cultured on SDA at 37ºc for 48 Hrs (40X)

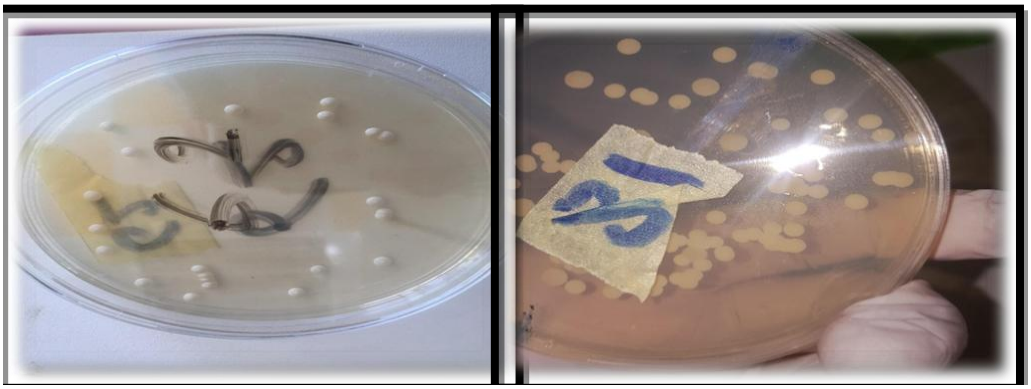


Fig.2 Infections rates *Candida* spp. among diabetes mellitus patient

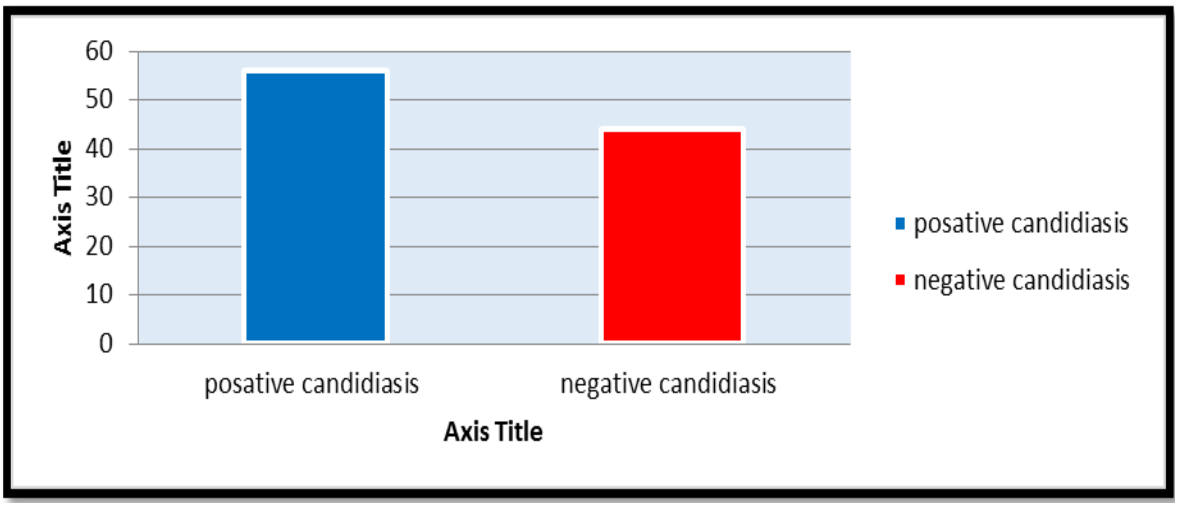


Fig.3 *Candida* spp. stained with Lactophenol cotton blue (40X)

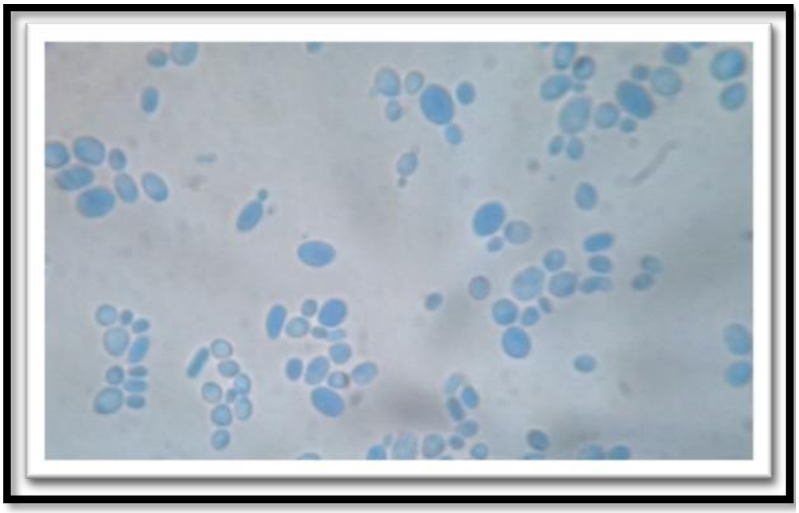


Fig.4 Germ tube formation by *C. albicans* (40X)

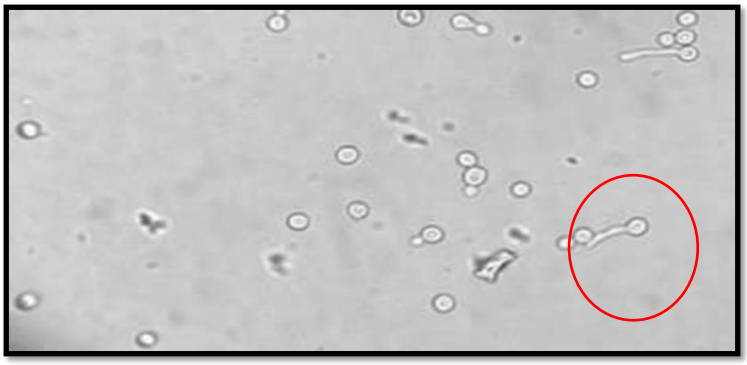


Fig.5 Colonies of *Candida spp C. albicans, C. parapsilosis, C. krusei: C. glabrata* and *C. tropicalis* cultured on CHROM agar candida at 37 C for 48 hrs appeared different colors

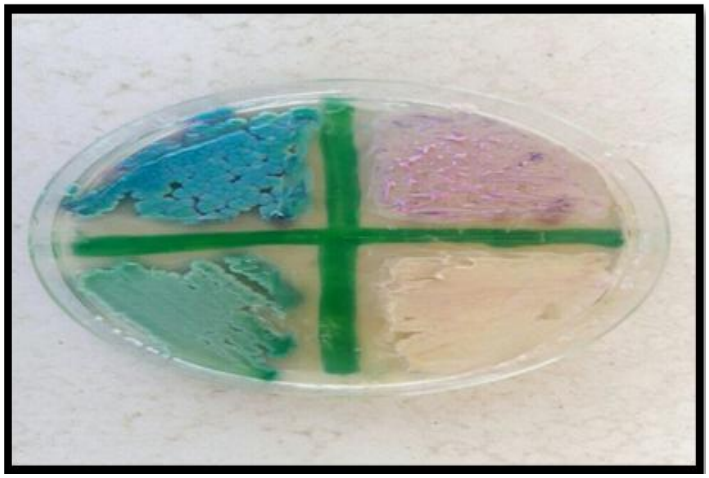


Fig.6 *Candida* spp. rate isolated from diabetes mellitus patients

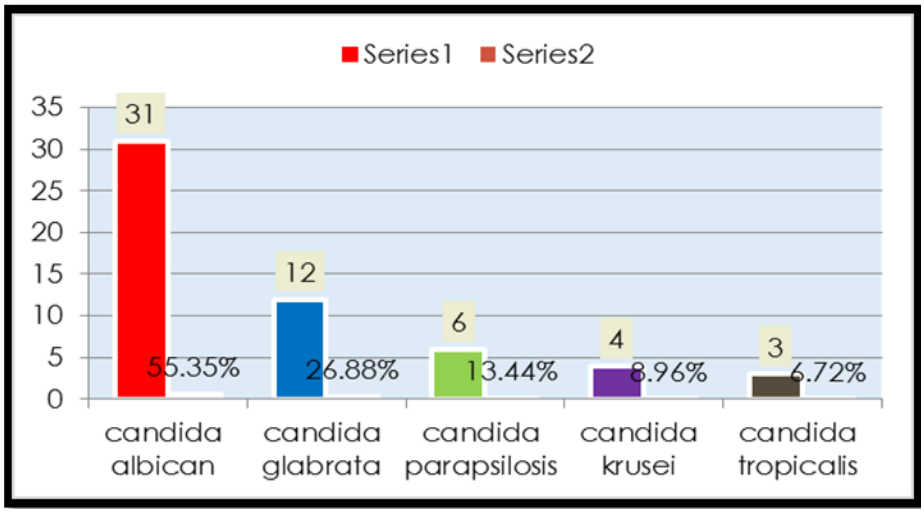


Fig.7 *Candida* infection rate among patients according to the gender

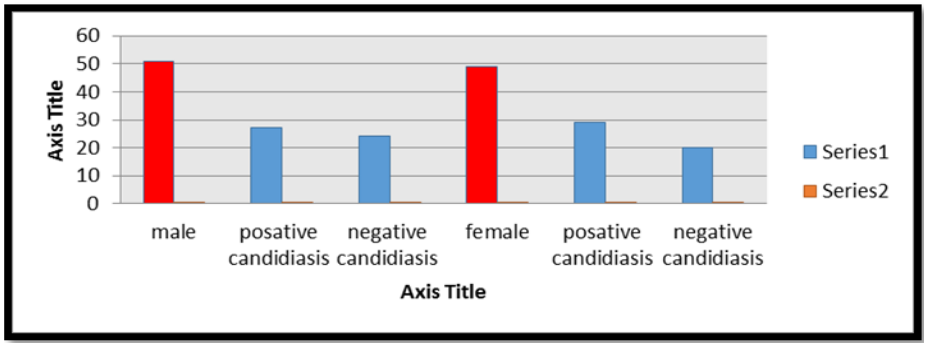


Fig.8 *Candida* infection rate among patients according to the type diabetes

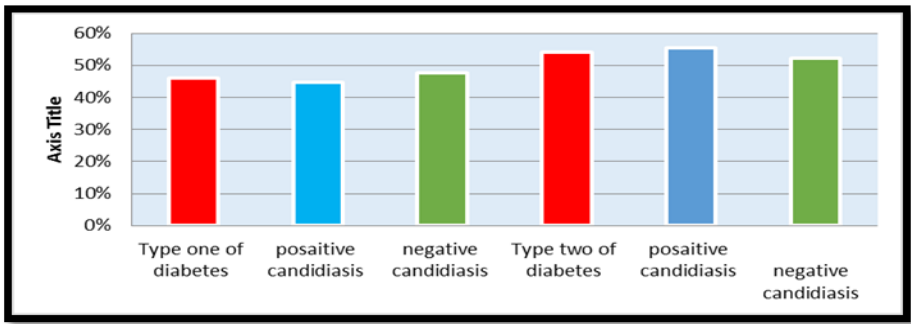


Fig.9 *Candida* infection rate among patients according to smoking

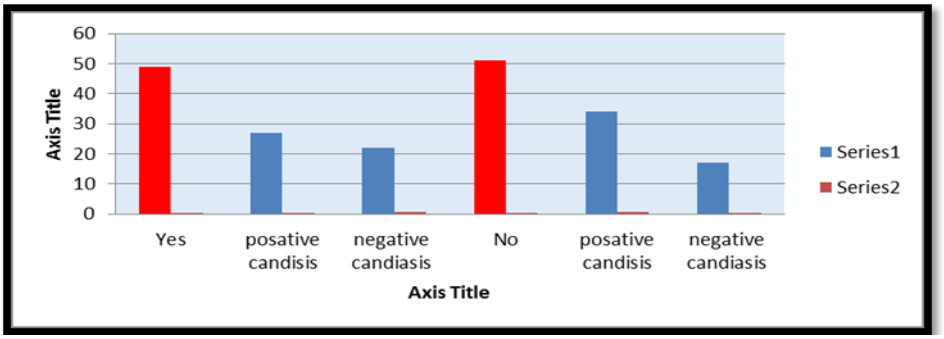
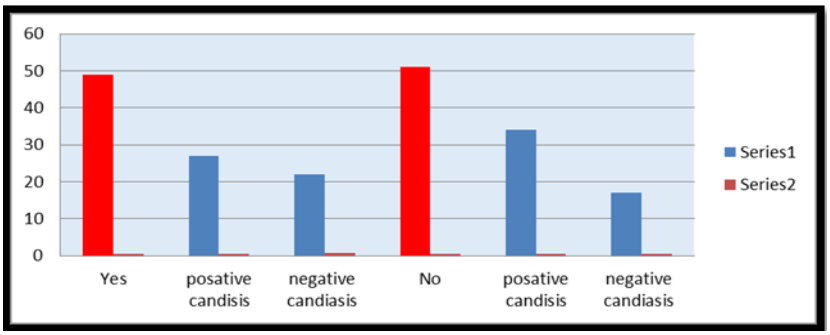


Fig.10 *Candida* infection rate among patients according to history family



The isolation rates of yeast infection were (56%) and the highest infection rates were *Candida albican* of that (55.35%), this study agree with Amin and Kasim and Jasim all found that *Candida albican* is highest infection (Amin *et al.,* 2014; Kasim and Yehia, 2006; Jasim *et al.,* 2016).

Candida spp. was phenotype known depending on the morphological features include germ tube, and chromogenic agar *candida* (CAC). The germ tubes were formed with more than two hours of incubation and this can be a singular identification characteristic of *C. albicans* differentiates it from other fungi. Other sort of yeast-like isolated gave negative result to germ tube.

The result of germ tube agreed with (Jasim *et al.,* 2016; Sudarsanam *et al.,* 2015; AL-Attraqchi *et al.,* 2017; Matare *et al.,* 2017) the germ tube was formed within two hours of incubation and it is a unique diagnostic characteristic of *C. albicans* that differentiates it from other species. Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. And it were solutions contains tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis.

It is lyophilized for stability. Germ tube is one of the virulence factors of *Candida albicans*. This is a rapid test for the hypothetical identification of C. albicans (Matare *et al.,* 2017).

The results of chromogenic agar candida (CAC) were in agreement with Mohammdi *et al.,* (2016) he found in his study that *Candida albican*was higher 34 (36.2%) and were *C. glabrata*, (0%) C. *parapsilosis*, 3 (5.2%) *C. krusei*2 (3.4%) were *C. tropicalis*. And it is in agreement with Suarez *et al.,* (2013) in the Colombia that he had found (29.0%) *C. albican*and 5 (9.0%) C. *parapsilosis*, (0 %) were *C. tropicalis* and it is also agreed with Benedetti *et al.,* (2016) that he had found *C. albicans* (39) *C. parapsilosis* (10) *C. glabrata* (1) *C. tropicalis* (5). And agree with de la Rosa-García *et al.,* (2013) in his study on Oral colonization and infection by *Candida* spp. In diabetic and non-diabetic patients with chronic kidney disease on dialysis.it was found that 22 (39.3%) *C. albicans*, *C. globate* 7 (12.5%), 6 (7.5%) *C. tropicalis* 6 (10.7%) and (0%) *C. parapsilosis,*

Chromogenic media were effective for isolating fungi and identifying *Candida* species, especially *C. albicans*, another advantage of these chromogenic media was their ease of use in the identification of non-*C. albicans* Candida species, 24–48 hrs earlier than traditional media. However, as expected and observed, performances of various chromogenic media differ from each other.

They also differ over time, because they are subject to continuous development (Davis and Rabinowitz, 2007) *Candida* spp, usually found in humans, can be isolated in about 50% of healthy population without clinical signs of infection In the case of diabetics, this prevalence is even higher (Davis and Rabinowitz, 2007).

The distribution of infection showed that males had 27 samples (48.2%) while females had 29 samples (51.8%). These results were in agreement with Matic *et al.,* (2015) at Siberia males 14 (40%) and females 16 (60%) in group B that include diabetic patients with good metabolic, and it was agreed with Al-Attas and Amro, (2010) in Saudi Arabia, males were (41.35) and females (58.7%) and it was non- significant.

It was non-significant between type of diabetes and candidiasis, and through this study Type II 31 samples (55.4%) were higher than type I 25 samples (44.6%) and the

results similar to results of Al-Attas and Amro, (2010) it found that type II 17 (73.9%) were higher than type I 14 (63.6%), and it agreed with Amin *et al.*, (2014). Also, agreed with Mohammadi *et al.*, (2016) and both found that higher infection in type II.

This higher rang of *candida* prevalence in diabetes mellitus could also be explained by the actual fact that the natural oral flora is modification by the endocrine abnormalities in diabetes mellitus.

The development of candidal formation in diabetic patients is also refer to the larger adherence of fungi to epithelial cells, assist by the increased glucose content within the saliva, genetic susceptibility to infection, altered cellular and humoral immune defense mechanisms, and native factors, as well as poor blood supply (Bremenkamp *et al.*, 2011).

Candida infection rates were higher in nonsmoking patient than smoking patients. In the case of nonsmoking there were 41 samples (73.2%) given positive for candidiasis and 15 samples (26.8%) that give positive for candidiasis in smoker patients.

This study was in agreement with Al-Attas and Amro *et al.*, (2010), In the case of non-smoking there were (89.1%) given positive for candidiasis and (10.1%) that give positive for candidiasis in smoker patient, and it agree with Matić *et al.*, (2015) it also there was non-significant with smoker patient.

While, it disagreement with Abu-Elteen *et al.*, (2006) who mentioned that smoking people is more infected with *candida* spp.

There were high significant (P 0.028) differences between history family and *Candida* spp. *Candida* infection rates were higher in patients who have no history family of diabetes mellitus 34 samples (60.7 %) of them given positive for candidiasis.

While, that have history family among them there were 22 samples (39.3%) given positive result to candidiasis. this disagreement with Sousa *et al.*, (2011) in here study Clinical study of the oral manifestations and related factors in type 2 diabetics patients it found that have history family 66 (68.8%) more than that have no history family 30 (31.1%).

References

Abu-Elteen, K. H., Hamad, M. A., and Salah, S. A. (2006). Prevalence of oral Candida infections in diabetic patients. Bahrain Medical Bulletin., 28(1): 1-8.

Al-Attas, S. A., and Amro, S. O. (2010). Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients. Annals of Saudi medicine, 30(2): 101.

AL-Attraqchi, A. A., Hassan, J. S., Dawood, H. N., and Hadab, M. A. (2017). Molecular and conventional methods for detection of Candida species isolated from a sample of immunocompromised Iraqi patients with pulmonary symptoms. Journal of Contemporary Medical Sciences, 3(10): 229-233.

Amin, A. M., Sadiq, N., and Saeed, C. H. (2014). Isolation of *Candida albicans* from oral cavity of type II diabetic subjects and its relationship to total and differential white blood cell count. Zanco J Med Sci, 18(3): 833-838.

Benedetti, V. P., Savi, D. C., Aluizio, R., Adamoski, D., Kava-Cordeiro, V., Galli-Terasawa, L. V., and Glienke, C. (2016). Analysis of the genetic diversity of Candida isolates obtained from diabetic patients and kidney transplant recipients. Memórias do Instituto Oswaldo Cruz., 111(7): 417-422.

Bhavan, P.S., Rajkumar, R., Radhakrishnan, S., Seenivasan, C. and Kannan, S. (2010). Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. Inter. J. Bio., 2(1): 84-93.

Bishara, W., Sikora, U., Mudanyali, O., Su, T. W., Yaglidere, O., Luckhart, S., and Ozcan, A. (2011). Holographic pixel super-resolution in portable lensless on-chip microscopy using a fiber-optic array. Lab on a Chip, 11(7), 1276-1279.

Bremenkamp, R., Caris, A. R., Jorge, A. O. C., Back-Brito, G. N., Mota, A. J., Balducci, I., and Koga-Ito, C. Y. (2011). Prevalence and antifungal resistance profile of *Candida* spp. oral isolates from patients with type 1 and 2 diabetes mellitus. Archives of oral biology, 56(6), 549-555.

Davis, P. J., and Rabinowitz, P. (2007). Methods of numerical integration. Courier Corporation.

De la Rosa-García, E., Miramontes-Zapata, M., Sánchez-Vargas, L. O., and Mondragón-Padilla, A. (2013). Oral colonisation and infection by Candida sp. in diabetic and non-diabetic patients with chronic kidney disease on dialysis., Nefrologia: 33(6).

Guggenheimer, J., Moore, P. A., Rossie, K., Myers; D., Mongelluzzo, M. B., Block, H. M., and Orchard, T. (2000). Insulin-dependent diabetes mellitus and oral soft tissue pathologies. II. Prevalence and characteristics of Candida and candidal lesions. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology., 89(5): 570-576.

Hasan, A. M., and Al-Jubouri, M. H. (2015). Isolation of Candida Spp. from Patients with Different Types of Leukemia Who Suffered Oral Candidiasis Due to Their Weekend Immune System. J Pharm Chem Biol Sci, 3(1): 79-83.

Jain, M., Shah, R., Chandolia, B., Mathur, A., Chauhan, Y., Chawda, J., and Bhagalia, S. (2016). The oral carriage of Candida in oral cancer patients of Indian origin undergoing radiotherapy and/or chemotherapy. Journal of clinical and diagnostic research: JCDR., 10(2): ZC17.

Jasim, S. T., Flayyih, M. T., and Hassan, A. A. (2016). Isolation and identification of *Candida* spp. from different clinical specimens and study the virulence factors. World J Pharm Pharmaceut Sci., 5(7): 121-37.

Kasim, Z. S., and Yehia, M. M. (2006). Oral candidal colonization in immune compromised patients. Iraqi Journal of Pharmacy, 13(2): 17-26.

Khosravi, A. R., Yarahmadi, S., Baiat, M., Shokri, H., and Pourkabireh, M. (2008). Factors affecting the prevalence of yeasts in the oral cavity of patients with diabetes mellitus. Journal de Mycologie Médicale/ Journal of Medical Mycology., 18(2): 83-88.

Kolaczkowski, M., Kolaczkowska, A., Środa, K., Ramalhete, C., Michalak, K., Mulhovo, S., and Ferreira, M. J. U. (2010). Substrates and modulators of the multidrug transporter Cdr1p of *Candida albicans* in antifungal extracts of medicinal plants. Mycoses, 53(4): 305-310.

Kumar, B. V., Padshetty, N. S., Bai, K. Y., and Rao, M. S. (2005). Prevalence of Candida in the oral cavity of diabetic subjects. The Journal of the Association of Physicians of India. 53: 599-602.

Lotfi-Kamran, M. H., Jafari, A. A., Falah-Tafti, A., Tavakoli, E., and Falahzadeh, M. H. (2009). Candida colonization on the denture of diabetic and non-diabetic atients. Dental research journal., 6(1): 23.

Mahmoudabadi, A. Z., Drucker, D. V., Mandall N; O'Brien, K. and Theaker, E. (2000) Isolation and Identification of Candida Species from the Oral Cavity Using CHROM agar Candida, Iranian Biomedical Journal, 4(2-3), 57-61.

Makwana, G. E., Gadhavi, H., and Sinha, M. (2012). Comparison of germ tube production by *Candida albicans* in various media. NJIRM., 3: 6-8.

Martins N., Ferreira I.C., Barros L., Silva, S., Henriques, M. (2014). "Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment". Mycopathologia., 177 (5-6):223-240.

Matare, T., Nziramasanga, P., Gwanzura, L., and Robertson, V. (2017). Experimental Germ Tube Induction in *Candida*

albicans: An Evaluation of the Effect of Sodium Bicarbonate on Morphogenesis and Comparison with Pooled Human Serum. BioMed research international, 2017.

Matić Petrović, S., Cimbaljević, M., Radunović, M., KuzmanovićPfićer, J., Jotić, A., and Pucar, A. (2015). Detection and Sampling Methods for Isolation of *Candida* spp. From Oral Cavities in Diabetics and Non-Diabetics. Brazilian Oral Research., 29(1): 1-7.

Melton, J. J., Redding, S. W., Kirkpatrick, W. R., Reasner, C. A., Ocampo, G. L., Venkatesh, A., and Mealey, B. L. (2010). Recovery of *Candida dubliniensis* and other Candida species from the oral cavity of subjects with periodontitis who had well- controlled and poorly controlled type 2 diabetes: a pilot study. Special Care in Dentistry., 30(6): 230-234.

Mohammadi, F., Javaheri, M. R., Nekoeian, S., and Dehghan, P. (2016). Identification of *Candida* species in the oral cavity of diabetic patients. Current medical mycology, 2(2): 1.

Nikolic, M., Smiljkovic, M., Markovic, T., Cirica, A., Glamoclija, J., Markovic, D., and Sokovic, M. (2016). Sensitivity of clinical isolates of Candida to essential oils from Burseraceae family. EXCLI journal., 15: 280.

Patil, S., Rao, R. S., Majumdar, B., and Anil, S. (2015). Clinical appearance of oral Candida infection and therapeutic strategies. Frontiers in microbiology., 6: 1391.

Shao, L. C., Sheng, C. Q., and Zhang, W. N. (2007). Recent advances in the study of antifungal lead compounds with new chemical scaffolds. Yao xuexuebao= Actapharmaceutica Sinica., 42(11): 1129-1136.

Ship, J. A. (2003). Diabetes and oral health: an overview. The Journal of the American Dental Association, 134: 4S-10S.

Sousa, M. G. D. M., Costa, A. D. L. L. and Roncalli, A. G. (2011). Clinical study of the oral manifestations and related factors in type 2 diabetics patients. Brazilian journal of otorhinolaryngology., 77(2): 145-152.

Sudarsanam, P., Hillary, B., Deepa, D. K., Amin, M. H., Mallesham, B., Reddy, B. M., and Bhargava, S. K. (2015). Highly efficient cerium dioxide nanocube-based catalysts for low temperature diesel soot oxidation: the cooperative effect of cerium-and cobalt-oxides. Catalysis Science & Technology, 5(7), 3496-3500.

Vijan, S. (2010). Type 2 diabetes. Annals of internal medicine., 152(5): ITC3-1.

Willis, A. M., Coulter, W. A., Hayes, J. R., Bell, P., and Lamey, P. J. (2000). Factors affecting the adhesion of *Candida albicans* to epithelial cells of insulin-using diabetes mellitus patients. Journal of medical microbiology., 49(3): 291-293.

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