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DETECTION OF ORAL FUNGAL MICROBIOME (MYCOBIOME) OF DIABETIC PATIENTS IN POLYCLINICS OF BENGHAZI - LIBYA

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Abstract:-

This study was conducted in Benghazi city, Oral fungal microbiome were isolated from oral cavities of 92 diabetic patients and 40 non diabetic volunteers. Swab taken from their oral cavities then inoculated in Sabauruod Dextrose Agar (SDA) and incubated at 27 °C for two to three days for colony study after that make subcultures using Potato dextrose agar (PDA) for microscopic study. Identification of oral fungi shows that Out of 132 subject, 38 shows no fungal growth , 63 samples of candida (53 pure candida and 10 cases candida mixed with other fungi), 7 fungal species other than Candida have been identified with conformation from Assiut University Mycology Centre (AUMC) in Egypt which were : 7 samples of Asperogillus spp (6 in diabetics &1in non diabetics), 8 samples of Alternaria .alternate (5 in diabetics & 3 in non-diabetics), 3 samples of Fusarium foetens (2 in diabetics & 1in non diabetics), 4 samples of Cladosporium herbarum. (Diabetics), 2 samples of Stemphylium botryosum (in diabetics), 2 samples of Geosmithia lavendula. (Diabetics), 5 samples of Penicillum crustosum (diabetics). The Candida species identified by Quyfia chest hospital Laboratory (Phoenix 100 Apparatus – BD, USA), .candida species were identified as : Candida albicans (44 cases), Wangilla dermatitidis (Saccaromycets) (7cases Candida dubliniensis (4 cases), Geotricum species (4cases),Candida sake (4 cases). Asperogillus species were: 2 samples Asperogillus.nigger, 3 samples A. fumigatus and 2 samples A. flavus.

Keywords: - *Diabetics, oral mycobiome, Benghazi*

INTRODUCTION

The oral microbiome are critical components of health and disease. Disruption of the oral microbiome has been proposed to indicate trigger or influence the course of oral diseases especially among immune compromised patients (e.g.HIV-infected, Diabetic and cancer patients). Oral colonization with Candida species occurs more frequently in diabetic patients compared with non-diabetic individuals [1]. Although fungi, particularly Candida are important components of oral microbiota and are influenced by the immune status and therapy of affected individuals. Studies of oral microbiota have focused largely on the bacterial components, only few studies of oral microbiome that included some fungal profiling reported the presence of *Candida albicans* and *Saccharomyces cerevisiae* in the subgingival plaque microbiota of HIV-infected patients [2]. Normally human blood have antifungal activity. fungal infection is not common put in case of Diabetic people fungal infection is common due to high blood sugar level , and low salivary secretion specially in case of removable denture wearer[3] . There are more than 100,000 known fungi, but only few invade human tissues. Oral candidiasis is an opportunistic infection of the oral cavity; it affects various sectors of the world population irrespective of age or health status (4)

Oral candida infection usually involves a compromised host and the compromise may be local or systemic. Local compromising factors include: decreased salivation, poor oral hygiene, wearing dentures, while systemic factors include diabetes mellitus, nutritional deficiency, HIV infection, patients receiving chemotherapy treatments for malignant diseases and others. So such patients should take local antifungal drugs as a protective doses against oral fungal growth [5]. The non-candida oral fungal infections are less common than oral candidiasis, they commonly produce subclinical infection, especially pulmonary infections [6]. In rare cases these infections can produce clinical disease in healthy persons, including oral lesions [7]. Purpose of this study was to determine the prevalence, species distribution of oral fungi in oral cavities of diabetic patients in Benghazi City.

MATERIALS AND METHODS

The subjects (Study population):

A total of 132 individuals were enrolled in the study, 92 were diabetics from different places of Benghazi polyclinics between January and August 2014, and all patients were receiving treatment for diabetes mellitus. The control group composed of 40 healthy volunteers. All were matched for age (age groups from 25 to 80), sex, dental status, and smoking habits. Patients and control were examined for signs or symptoms of oral fungal infection. Swab taken from their oral cavities and inoculated in Sabauruod Dextrose Agar (SDA) and incubated at 27 °**C** for two to three days .after that made subcultures using Potato Dextrose Agar (PDA).

Isolation of fungi:

Fungi were isolated from oral cavities of diabetic patients and non-diabetic volunteers in different places of Benghazi city, some of which are identified by using Phoenix 100, BD, USA apparatus, others identified with confirmation in AUMC in Egypt. In this study used Sabouraud Dextrose Agar (SDA) for colony study , and use Potato Dextrose Agar (PDA) for microscopic study. Cotton swab used to isolate samples from patient's oral cavities (diabetic patient) or non-diabetic people , then inoculated them within 9 cm dishes of Sabouraud dextrose agar (SDA) and incubated for two to three days at 27 ºC after that examined the **colonies** then made subcultures using PDA media, then slides were made and examined under microscope**.**

Statistical analysis:

Clinical and Laboratory data are recorded in special formats and entered in statistical computer program **(SPSS)**. Descriptive and analytical statistical analysis performed and final results are plotted in tables.

RESULTS

Identification of oral fungi:

The results shows that out of 132 subjects (92diabetic patients and 40 non diabetics people) 38 shows no fungal growth (18diabetics and 20 non diabetics people) , while 63 samples are Candida (10 are mixed with other Fungi, pure *Candida* are 53), 31samples are non candidal fungal growth. 7 fungal species other than Candida have been identified with confirmation from Assiut University Mycology Centre (AUMC) in Egypt Table (1). Others such as *Asperogillus. Nigger* and *Asperogillus flavus* have been identified in Benghazi University. 7 samples of *Asperogillus*, *8* samples of *Alternaria* .spp. (*A .alternate*), 3 samples of *Fusarium foetens*, 4 samples of *Cladosporium herbarum*, *2* samples of *Stemphylium botryosum*, *2* samples of *Geosmithia lavendula*, 5 *Penicillum crustosum* Table (2). Candidal growth was more in females and mixed growth more in males table (3)

Table1. Identification of some of non Candidal fungi in AUMC:

Isolate No.	Identification	No
1	Geosmithia lavendula Pitt	2
2	Candida sp. + Fusarium foetens	1+1
3	*Fusarium foetens* Schroers, O'Donnell, Baayen, Hooftman	2
4	Penicillium crustosumThom	5
5	Stemphylium botryosum Wallroth	2
6	Alternaria alternata(Fries) Keissler	8
7	Cladosporium herbarum(Persoon) Link	4
8	Aspergillus fumigatus Fresenius	3

Table 2. Oral fungal species.

	Frequency	Percent %
Candida	48	33.6
Asperogillus spp.	7	5.3
Alternaria spp.	8	6.1
Fusarium spp.	3	2.3
Penicilum spp.	5	3.8
Stemphylium botryosum	2	1.5
Geosmithia , Lavendula	2	1.5
Cladosporium .herbarium	4	3
mixed infection	15	14.2
No growth	38	28.8
Total	132	100

Table (3) Relationship Between sex and fungal infection.

			Sex		Total
			F	M	
y2	No growth	Count	17	21	38
		% within Sex	27.0%	30.4%	28.8%
	Candida	Count	29	24	53
		% within Sex	46.0%	34.8%	40.2%
	Other fungal infection	Count	14	17	31
		% within Sex	22.2%	24.6%	23.5%
	Mixed	Count	3	7	10
		% within Sex	4.8%	10.1%	7.6%
Total		Count	63	69	132
		% within Sex	100.0%	100.0%	100.0%

Candida species identified by Quyfia chest hospital laboratory in Benghazi (Phoenix 100 Apparatus – BD , USA) .Out of 132 subjects **63** have candidal growth, **53** samples were pure candida and **10** were Candid mixed with other fungi , Candidal species which were identified are:

1- *Candida albicans* (44 cases).
2- *Wangilla dermatitidis (Saccaromycets*) (7 cases).
3- *-Candida dubliniensis (4cases).*
4- *Geotricum species (4cases).* Asperogillus species were: 2 samples *Asperogillus. Nigger , 3 samples A.fumigatus and 2 samples A. flavus*
5- *Candida sake (4 cases).*

Mixed samples:

a. in case of diabetic patients five genera had been found *Asperogillus , Alternaria ,Candida, Fusarium* and Geosmithia lavendula**.**

- 1 sample *Fusarium spp.+Alternaria spp.+ Candida*
- 1 sample *Fusarium. spp + Candida spp*
- 1 sample *Alternaria spp+Candida spp*
- 1 *Asperogillus spp + Candida spp*
- 1 sample *Geosmithia lavendula spp + Candida spp*.

b. in case of non-diabetic volunteers:

- 1sample *Candida spp +Asperogillus* spp.
- 1 sample *Candida spp+ strepto coccus (Bacteria*).
- 1 samples *Alternariaspp+Candida* spp.

There are relation between oral hygiene and oral fungal growth: In case of good oral hygiene there is no fungal growth, but in case of fair and poor oral hygiene there are oral fungal growth or infections in different percentages:
Candidal.spp in case of fair oral hygiene are 39.4%, while in case of poor oral hygiene are 50%.
Asperogillus spp in case of fair oral hygiene are 0 %, while in case of poor oral hygiene are 6.4 %.
Alteraria spp in case of fair oral hygiene are 5%, while in case .of poor oral hygiene are 6.4%.
Fusarium. spp in case of fair oral hygiene are 1.8% , while in case of poor oral hygiene are 5%.
Penicillum.spp in case of fair oral hygiene are 1.8%, while in case of poor oral hygiene are 15%.
Stemphylium. botryosum in case of fair oral hygiene are 1.8% , while in case of poor oral hygiene are 2%. *Geosmithia Lavendula* in case of fair oral hygiene are 0.9%, while in case of poor oral hygiene are 5.0%. *Cladosporium. Herbarium* in case of fair oral hygiene are 2.8%, while in case of poor oral hygiene are 5.0%. Mixed infection in case of fair oral hygiene are 7.3 % while in case of poor oral hygiene are 10%. 31.2% of fair oral hygiene haven't fungal growth , while in case of poor oral hygiene only 5% haven't fungal growth as showed in Table(4).

Table 4. Relationship between Oral hygiene and fungal growth:

			Oral hygiene			Total
			good	fair	poor	
y3	Candida spp.	Count	0%	43	10	53
		% within oral hygiene	0%	39.4%	50.0%	40.2%
	Asperogillus spp.	Count	0%	7	0	7
		% within oral hygiene	0%	6.4%	.0%	5.3%
	Alteraria spp.	Count	0%	7	1	8
		% within oral hygiene	0%	6.4%	5.0%	6.1%
	Fusarium spp.	Count	0%	2	1	3
		% within oral hygiene	0%	1.8%	5.0%	2.3%
	Penicillum spp.	Count	0%	2	3	5
		% within oral hygiene	0%	1.8%	15.0%	3.8%
	Stemphylium botryosum	Count	0%	2	0	2
		% within oral hygiene	0%	1.8%	.2%	1.5%
	Geosmithia ,Lavendula	Count	0%	1	1	2
		% within oral hygiene	0%	.9%	5.0%	1.5%
	Cladosporium herbarium	Count	0%	3	1	4
		% within oral hygiene	0%	2.8%	5.0%	3.0%

	Mixed infection	Count	0%	8	2	10
		% within oral hygiene	0%	7.3%	10.0%	7.6%
	No growth	Count	3%	34	1	38
		% within oral hygiene	100.0%	31.2%	5.0%	28.8%
Total		Count	3%	109	20	132
		% within oral hygiene	100.0%	100.0%	100.0%	100.0%

DISCUSSION

Out of 92 Diabetic patients 74 have fungal growth.(84.43% have fungal growth) , and Out of 40 non diabetic volunteers only 20 have fungal growth. (50% have fungal growth).

This result agreed with Ghannoum et al., 2010 who found that Candida species were the most frequent oral fungi (75%) in this study 56.38% pure candida +10.63% mixed with other fungi (67%). Also Presence of Cladosporium spa , Aspergillus spp , Fusarium. spp and Saccharomycetales (Wangilladermatitidis). Also this result agreed with Grimoud A. M et al.,2005 that Candida is most frequently present in 67% of oral cavities of elderly people in hospitals and the most frequently identified strain of Candida spp is Candida.albicans .

This study also agreed with Abu-Elteen, H et al.,2003 who made study on diabetic patients and isolated Candida from oral cavity sites of diabetic patients and C. albicans was the most prevalent species in both diabetic and non-diabetic people . [1]

This study agreed with Scully .c et al 2002 that recorded that diabetes mellitus is the main risk factor for oral fungal infection eg: Asperogilosis and Candidiosis.

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