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Research Article,

Study of the relationship between Cigarette Smoking and fungi isolated from the oral cavity

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Abstract:

Background: Smoking is associated with a variety of changes in the oral cavity. Cigarette smoke has effects on saliva, oral commensal bacteria and fungi. Fungi are capable to produce opportunistic infections within the oral cavity. This study was done to determine the effect of cigarette smoking on the oral cavity.

Materials and Methods: In this case-control study, the study included 20 cases of smoker men and 20 cases of non-smoker were compared. All of the samples were collected by swab by culture on sabouraud dextrose agar and microscopically examined.

Results: Out of the 20 smoker individuals, 14(70%) had fungi colonization in their oral cavity while 7(35%) of the 20 non-smoker individuals had developed this fungi colonization. A significant difference was observed between the two groups ($p<0.05$).

Conclusion: Cigarette smoking can act as an important underlying factor in oral growth of fungi.

Keywords: Fungi, Cigarette, Smoking, Oral cavity

Introduction:

Fungi are eukaryotic unicellular or multicellular organisms producing various infections in man [1]. Infections of the different structures in oral cavity can be caused by bacteria, viruses and fungi [2] [3]. Different practices like wearing dentures, cigarette smoking and immunosuppressive conditions like HIV infection and inhalational steroid use predispose individuals to oral infection by fungi [4] the oral cavity is the first organ in contact with cigarette smoke which is responsible for many oral diseases, including cancer. Oral cancers were found to increase 3 to 5 times more among smokers than in non-smokers and also in a dose-depending manner (number of smoked cigarettes/day and smoking years) [5]. Smoking is associated with a variety of changes in the oral cavity. Cigarette smoke has effects on saliva, oral commensal bacteria and fungi, the fungi most common opportunistic microorganism infection in human[6]. One of the most relevant factors in this regard is smoking habit, which is quite common in the global world society and is associated withrelevant oral diseases, such as periodontitis and oral cancer. The higher prevalence and greater severity of oral disease in smokers might be explained by the altered host immune response as disturbances in immunoglobulin and cytokine levels, altered lymphocyte counts, and impairment of oral neutrophil function demonstrated in smokers [7][8].

Normal microbial flora of the oral cavity:

The environment being in the human mouth allows the growth of characteristic microorganisms found there. It gives a source of water and nutrients, as well as a moderate temperature.[9] Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid [10]. Anaerobic bacteria in the oral cavity include: Actinomyces, Arachnia, Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus, Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacterium, Selenomonas, Treponema, and

Veillonella.[11] In addition, there are also a number of fungi found in the oral cavity, including: Candida, Cladosporium, Aspergillus, Fusarium, Glomus, Alternaria, Penicillium, and Cryptococcus.[12]

Impact of smoking on microbiome:

obacco smoking has been noted to affect the peripheral immune system on several levels, including a lessening in the activity of natural killer cells, rise in white blood cell counts, and a higher susceptibility to infection [13]. Smoking raises the number of macrophages, neutrophils, eosinophil's, and mast cells, lowers the number of airway dendritic cells, and alters macrophage and neutrophil function [14, 15]. Expanding macrophages and neutrophils demonstrated impaired phagocytic functions to the efficient clearance of bacteria or pathogen, as evidenced by reduced bacterial-stimulated production of superoxide and surface receptor expression, (e.g. TLR2) which is important for the recognition and response to gram-positive bacteria [16, 17]

Agents causing fungal infections of the oral mucosa:

a. fungi like Blastomyces spp., Histoplasma capsulatum and Cryptococcus neoformans can also cause oral disease, as a part of disseminated fungal infection[18] .

b. Histoplasmosis, caused by Histoplasma capsulatum, a dimorphic fungus, can produce verrucous or granulomatous lesions [indurated and painful ulcer) in any area of the mouth, especially tongue, gingiva or palate, usually as a component of systemic infection, although primary affection has also been documented [19].

c. Aspergillus spp. has been implicated in sinusitis and further extension into the oral cavity can cause involvement of the hard palate, resulting in manifestations like loosening of teeth. Oral Aspergillosis has been graded from Grade I to Grade V according to severity [20].

d. Similarly mucomycosis can extend into the oral cavity from Maxilla and nasal sinuses, manifesting mainly as spreading sinusitis or facial cellulitis with palatal ulcer [21]. Rhizopus spp. is the principal agent associated with this disease entity, and the jaw is almost always involved [21].

e. Rarely fungi, previously considered as saprophytes, e.g. Rhodotorula spp. can cause infection of the oral mucosa, usually in the immunocompromised host (HIV infected patient)[22] .

Material and Methods:

Samples included in the study:

The study included 20 cases of smoker men and 20 cases of non-smoker in Baqubah city, Iraq.

Collection of Samples:

Forty (40) oral cavity swabs collected from (20) smoker and (20) non-smoker and culturing immediately.

Culturing of Samples:

Sabouraud dextrose agar [SDA) standard medium for isolation fungi, it allows the growth of fungi and represses the expansion of many bacteria because of the low pH. Therefore, SDA was cooled down to 45-50ºC then chloramphenicol antibiotic (250mg /liter) it was added then poured into sterile Petri dishes. SDA medium was inoculated with specimen with a sterile swab. The plate was incubated at 37 °C for 1 weak. and prevent contamination when saved in the refrigerator [23].

Identify isolated fungi:

Isolated fungi were described and diagnosed according to their culture characteristics (shape, consistency, colors, and pigment secretions) .Then, a part of the fungal colony was taken and placed on a glass slide loaded with a drop of lacto phenol cotton blue and its characteristics described its microscopes according to the nature of hyphae, Conidiophores and the presence or absence of spores [24].

Results:

Out of the 20 smoker individuals, 14(70%) had fungi colonization in their oral

Cavity while 7(35%) of the 20 non-smoker individuals had developed this fungi of colonization as show in Table 1. In smoker .The most common molds isolated were Rhizopus spp 3(21.4%), Aspergillus niger 2(14.2%), Aspergillus niger and Rhizopus spp. 1(7.14%) and Aspergillus fumigatus 2(14.2%) while yeasts were Rhodotorula spp. 2(14.2%) and Candida spp. 4(28.5%). In non-smoker, the most common molds isolated were Rhizopus spp 1(14.2%), Aspergillus niger 1(14.2%), Aspergillus fumigatus 3(41.8%)and while yeast was Candida spp.
2(28.5%) as show in Figure 1 and Figure 2.
Morphological, colonies of fungi colonies on sabouraud dextrose agar, as it shown in Figure 1 and Figure 2.

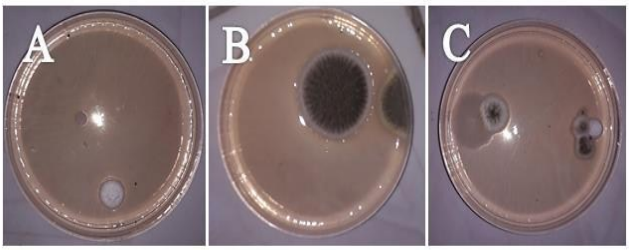

Figure 1: Molds growth on Sabouraud Dextrose Agar, A: Rhizopus spp., B: Aspergillus fumigatus, C: Aspergillus niger and Rhizopus spp.

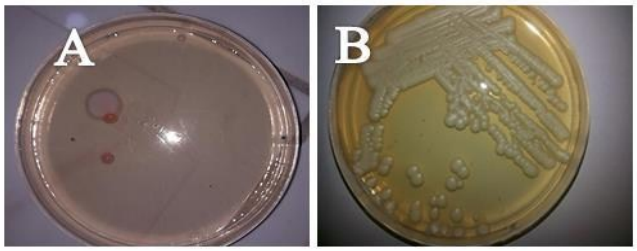

Figure 2: Yeasts growth on Sabouraud Dextrose Agar, A: Rhodotorula spp., B: Candida spp.

Table 1: Fungal isolated from smokers and non-smokers

Factor	Total samples	Fungi growth*		Fungi growth	No.(%)
		(-ve)	(+ve)		
Smokers	20	6(30%)	14(70%)	**Mold**	
				Rhizopus spp.	3(21.4)
				Aspergillus niger	2(14.2)
				Aspergillus nigerand Rhizopus spp.	1(7.14)
				Aspergillus fumigatus	2(14.2)
				Yeast	
				Rhodotorula spp.	2(14.2)
				Candida spp.	4(28.5)
Non-smokers	20	13(75%)	7(35%)	**Mold**	
				Rhizopus spp.	1(14.2)
				Aspergillus niger	1(14.2)
				Aspergillus fumigatus	3(42.8)
				Yeast	
				Candida spp.	2(28.5)
Total	40	19	21		21

* p-value: 0.026 , significant($p<0.05$)

Discussion:

A study conducted at the University of Porto showed that most commonly molds were Penicillium, Cladosporium and Aspergillus. Smokers presented significantly higher levels of yeasts and pathogenic molds than did non-smokers[25]. Ghannoum et al. found the yeasts Rhodotorula,Candida spp. and a variety of mold genera, our results are consistent with those taken recently[26]. Bennett and Klich found that the greatest fungal found in the oral mucosa, especially Penicillium spp. [27].

Conclusion:

A number of researchers have found that smoking either alone or with other factors act to be an important predisposing factor for fungi, although this relation or its pathogenic impact on oral fungi is remote from determined. However, the influence of smoking on local and oral immune mechanisms, and the mechanisms by which yeast proliferate intraoral as a result of tobacco smoking, requires investigating to explain these unresolved concepts.

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