

In vitro comparison the anti-Candida properties of Smoker and nonsmoker's saliva

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ABSTRACT

Aim and Objectives: Candida species are the most prevalent commensal fungi of human mouth. **Materials and Methods:** In this case-control study, a sum of 60 adult male, who referred to the oral medicine department of Shahid Sadoughi University of medical sciences, participated. The health status of all subjects was verified by a meticulous interview and questionnaire, so that none of them had any systemic or local risk factor for candidiasis lesions, After a 90-minute fasting period, unstimulated whole saliva samples were collected and send to the laboratory. Then samples were sterilized and stored at -20° C. All samples were tested by Broth microdilution method to find out the degree of Candida colonization. Resulted data was analyzed by SPSS 16 software and the tests were applied are T-test, Chi-Square and Spearman correlation test. **Results:** Fifteen cases out of 30 smoker males and 11 cases out of 30 nonsmoker ones had positive colonization of Candida species. But the difference between two groups was not statistically significant (Pvalue: 0.297) same as the differences in regard to colony counts and type of cultured subspecies (Pvalue: 0.746). However, this did not keep an increasing linear correlation as the colony counts dropped in heavy smokers. **Conclusion:** Candida colonization may be influenced in an exaggerated manner, in response to a stimuli like smoking. Lower colony count in heavy smokers may be related to more keratinized mucosa which traps Candida cells. In this regard, swab samples are recommended for further investigations.

Keywords: Anticandidal properties, saliva, smoker, Candida.

Introduction

Candida is a type of mushroom from the category of Yucaritic yeast 1 Extracellular wall and plasma membrane. Except for some macroscopic and microscopic characteristics, Candida species are similar in culture ^[1]. Although many Candida species can be the cause of the disease, in most cases, the organism is isolated from clinical cases of Candida albicans. In fact, Albicans and other Candida species are saprophytic organisms. Under normal conditions, their number is influenced by environmental

conditions such as other microorganisms, IgA levels, intestinal motility, and ... control And thus have little pathogenicity for their host. There is a clear relationship between the occurrence of oral candidiasis and effective public and local predisposing factors. Local predisposing factors are able to stimulate Candida's growth or affect the immune response of oral mucosa. General factors (generalized) are often related to the immune and endocrine status of the patient. The immune status can be affected by drugs and diseases that suppress primary or adaptive immunity. Also, false membrane candidiasis, with infections The fungus is associated with young children whose immune system has not completely evolved, denture induced stomatitis, corneal inflammation and inflammation of the middle form of the tongue are known as Candida-associated infections, as these lesions In addition to candida, they can also be created by bacteria^[2]. The presence of Candida as a living organism in the natural flora complicates the differentiation of the natural condition of the infection. This is essential that the clinical and laboratory findings both coincide in order to arrive

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at an accurate diagnosis. sometimes treatments antifungal to contribute to the process dueiagnostic done.

Discover the organisms fungal, as a sign of infection taken.this technique,especially when candidiasis membrane false and inflammation corner lip suspected be useful list. In order to increase the sensitivity, the second smear of the lesion is transmitted to a medium carrying the carrier and subsequently cultured on a subgoagar. To differentiate between different types of Candida, test Excess Pagano-Levin Agar can be done.

Saliva is one of the most important, fluid and complex liquids that provides a wide range of physiological needs. In the gastrointestinal tract, saliva plays an important role in esophageal physiology, digestive process and protects gastrointestinal cells. In the oral cavity, saliva plays a role in chewing, talking, swallowing, watching, protecting the mucus, acting as an antiviral, antifungal and antibacterial activity. An important role is played [3].

Saliva has been shown to be an important mediator for Diagnosis and evaluation of some systemic diseases. Currently, saliva is commonly used to detect viral infections, blood alcohol and hormones, as well as screening for addictive drugs, and it has been shown that saliva for the screening of cancers and other systemic diseases It is useful [2].

Saliva contains many antifungal proteins such as Histamine, lysozyme, lactoferrin and immunoglobulin are secretion type A. Many studies on the association between oral candidiasis and Have shown the concentration of histamine and salivary lysozyme [4].

The most important role of histatines is their antifungal activity against Candida albicans and Cryptococcus neoformans, but its mechanism of action on candida albicans is unknown, but it seems different from antifungal drugs the azoles are bases that inhibit the synthesis of ergosterol[5]. These proteins also exhibit strong antifungal activity in vitro. Parethylated salicylic acid histidine polypeptides have antifungal strength similar to that of the antibiotic imidazole. The complete control of Candida Albicans by Hyssydindrkdv's polypeptide [6, 7].

It seems that the use of some tobacco, including cigarettes, is also Can reduce the inhibitory effect of saliva and thus increase the possibility of candidiasis colonization [8-14].

Table 1: The association of the number of candidate counts from saliva culture with age, the number of years of smoking, the number of cigarettes pulled per day

Number of Samples	P-Value	Correlation Cefficient	Variation
60	0/730	./045-	Age
30	./518	./123-	Number of Smoking Siggarrets Per Day
30	./494	./130-	Years of Smoking

Premiss

Candidate a fungal category yeasts is ukariotic with extracellular and plasma membrane.Except for a few exceptions features

macroscopic and microscopic,species candidate in media similar are.

Although many species candidates can factor disease are , but in most cases the organism isolated from clinical cases candida albiccansis.In fact albicans as well as other species candidates organisms such SUPROFITE are in under natural , the number of them by condition on the environment , such as there other microorganism , valves IgA ,peristalsis intesting and control has and so pathogenesis much for your host not.

It's a clear between the occurrence of oral candidiasis and factors perdispoising

Public and local effective there.

Predisposing factors local able to growth of candida stimulate or immune responsethe oral affect.factors general(generalized) ofthen associated with the status safety and endocrine are sick.

Safety situation can by drugs and disease that the immune system primary or adaptive suppress the affect the.

Also candidiasis membrace false with fungal infections in children young immune system quite evolution not, related is.

Stomatitis caused by denture ,inflammation , corner lips and inflammation Diamond shape the language as infection associated with the candidate know as the wase in addition to candidates can by bactria also occure.

The presence of candidate as a member symbotic in flora natural differentiate natural state of infectin complex is.

It is essential that the finding of clinical and laboratory both to achieve a correct diagnosis coordinated.

In order to increase the sensitivity .smear latter of the lesion in a matter by carrier transferred and subsequent culture on SABOURAUD AGAR played.to differentiate between the different types of candida , experiments additional on AGAR PAGONA-LEVIN can be done.

Saliva one of the liquid important,mental and complex body is a wide range of the needs of physiological provides.in the gastrointestinal tract saliva an important role in physiology the process of digestion and protection oesphagus of cells in the gastrointestinal tract plays.

In the oral cavity,saliva in chew, talk, swallow,tasting,protect the mucosa,activity anti-viral and anti-fungal and anti-bacteria an important role plays.

Most recently shown that saliva a by important to recognize and evaluation of some of the disease system is.

Saliva includes a lot of protein anti- fungalnd like the histatin ,the lysozyme lactoferrineandImmunoglobulin secretory type A is.many studies relationship between candidiasis oral and concentration of histatin and lysozyme of saliva to have shown.

The most important role histatin anti-fungal activity cryptococcusneoformensis.the mechanism of action On candida albicans is un know,but it seems different from the anti-fungal drugs with base AZOL the synthesis of the ergostrol inhibit.

This protein in the environment in vitro also anti fungal activity strong show.

Polypeptits rich of histedin saliva parotid power anti fungal similar antibiotics imidazole are.

Inhibition full candida albicans by polypeptids of hystatin in the opacity 106cfu/ml has been reported.

It seems use some use tobacco including smoking can reduce inhibitory effect and disinfection saliva and thus increasing the possibility colonization candidate is.

Though smoking is widespread all over the world,oral studies related to smoking are rare, this study was designed as a case-control study since it was not feasible to design a cohort study because of the moral principals. Most of the similar studies chased the same procedure [9, 10, 14, 15-18].

Considering the strict criteria and limited available course of time in this single-center study, having a sample greater than 60 was not practical. The studies with longer available time or the ones which were multi-center had normally larger population in their sample [14, 16-19].

At the beginning a comprehensive questionnaire about the studied patients was conducted in order to examine their health and reject their any systematic diseases which puts this study in the same classification with same students [20, 21] and superior to same others [14, 16, 17, 19, 22].

One of the most unique aspects of this study was examining the pattern of smoking along with examining the effect of habit itself with variables of the years of smoking and the numbers of cigarettes smoked.

This aspect can to some extent decrease the effect of unwanted inevitable factors on the study too.

This was done on males. The reason to select males was the cultural context of the region.

Albeit the statistics show that the number of female smokers has risen in recent years, it is not accepted in society and thus could lead to denial of the habit by female patients.

So the sample was purpose fully selected from men. Not having women in the sample of study eliminates the effect of female hormones on the operation of sputum.

Based on the statistical analysis, the two studied had no considerable difference in age range which reduces possible effect of age factor on current study to the least (pvalue=0.905).

Another aspect to be mentioned is the age range of the sample which was 19-62 with a mean of 30.

The younger lower limit of the sample in Iran compared to similar studies in western countries declares the fact of early start to smoke in this country which requires a more comprehensive study (24,29). Overall, the positive and negative effect of candida culture on the two groups didn't have a meaningful difference. (pvalue=0.297)

These results are similar to Oliver et al [16], Andorf et al., and Rasool et al [18] which had showed that the candida prevalence in both smoker and non-smoker groups were the same. This can be the result of the strict criteria and same Limitations of the studies.

One of the controversial findings of our study was the numbers of candida colonies which didn't have a meaningful difference between age two groups of smokers and non-smokers (pvalue=0.746).

By focusing on the reason of this phenomenon in which the anti candida feature of sputum remained harmful even after adding the smoking factor, a astonishing feature of life biology appears. Overall, normally the dispersal of natural qualities is in a way that people with the same quality are in the center of this distribution. Now, when a new exciter enters an environment, each person reflects to this environmental change according to their genetic make-up.

These reflections are so diverse that make the people dissipated on normal distribution diagram.

The achieved diversity guarantees new and adaptive qualities and surviving of the living creatures. In a smaller scale, this phenomenon happens at all living systems without stop. Mouth as a biologic environment is not an exempt. If we consider candida as a natural quality, adding the exciter of cigarette smoke which can ...

Table 2: The relationship between the number of smoking cigarettes used per day and the positive and negative culture of smoker's salivary candidates

50%	16/7%	16/7%	50%	Percent	Negative
30	6	6	18	Count	Total
100%	100%	100%	100%	Percent	

*P value:0/069

*Test:Chisquave

Table 3: Relationship between mean and standard deviation of candida colony count made of saliva

The equivalents	Clony Count		Variable title
	Deviation from Count	Mean	Number of cigarette
7/039	18	5/833	Low
9/460	6	9/5000	Average
0/816	6	0/333	High
7/314	30	5/466	Total

*P value:0/057

*Test: Kruskal_wallis

The relation between the number of cigarettes used per day and the number of colonies slaughtered by smoker was not statistically significant.

In the study of various species of candida in two groups of smokers and nonsmokers, the result showed that 15 of the non smokers, candidate species in the saliva sample were not cultured.

In seven samples (23/3) candida Albicans, in 6 samples (20) non aligned candidates and in both samples (6/7) both Candida Albicans and non aligned candida were obtained.

In 19 cases of non smokers (63/3), no candida was born.

Candida albicans was found in 5 cases (16/7) and in 5 cases (16/7%) were found non aligned candida. But in one case (3/3%) both types of candidiasis were obtained.

In general, there was no significant difference between the two groups in terms of separation of candida species (albicans and non albicans) in saliva.

Table 4: comparison of different candida species isolated from saliva culture in two groups.

Total		Positive culturing of both types		Positive culture of non-latic candidate type		Positive culture of candida type		Candida's negative culture		Group
Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	
100	30	6/7%	2	20%	6	23/3%	7	50%	15	Smoker
100	30	3/3%	1	16/7%	5	16/7%	5	63/3%	19	Non-smokers
100	60	5%	3	18/3%	11	20%	12	56/7%	34	Total

*The distribution of the trait examined in two groups was compared to the normal cure/shown in Fig.1.

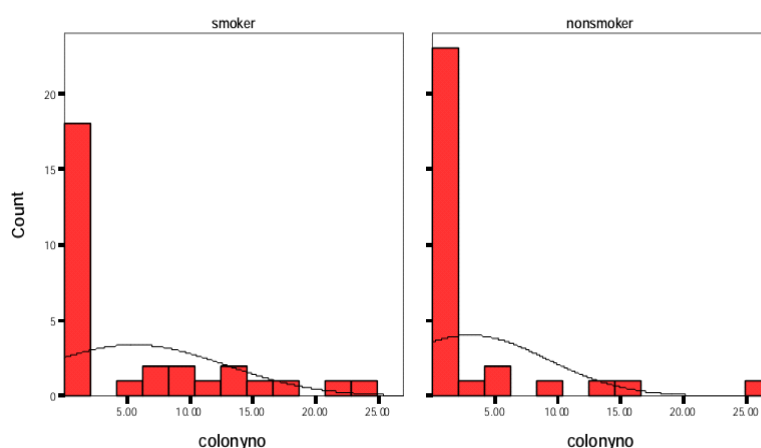


Figure 1: Comparison of the distribution of data in two groups based on the number of candidate colony

Discussion

In the present study, the mean age of samples variation from 30 to 50 years, which is consistent with the non-elderly adult group in the Odds study. Regarding the result of this study, it seems that one of the reason for no significant difference in the control group is the ability to compensate for non recurrence in this time period. Apparently, the body's compensatory capacity at this age is still at a level that has ability to cope with the effects of isolated agents such as smoking, and main tains the anti-candidiasis of the saliva in a normal range.

Kadir and Colleagues in 2002 study examined candida colonization in 55 diabetic patients and 45 non-diabetic patients. In this study, there was no significant difference between the incidence of candida colonization in diabetic and non diabetic patients; however, no study was conducted on such cases as smoking, drinking Alcohol, anti microbial therapy and denture use, etc...

Since the latter factor play a significant role in candida colonization, the similarity of candida colonization in the two groups is not unexpected. They confirmed the decrease in salivary flow in diabetic patients. Saliva has secreted immunoglobulin (SIgA), which prevents candida adherence to epithelial cells, so reducing the salivary flow, which is expected

to reduce (SIgA) increase the candida colonization occurs in diabetic patients.

AHADANI and his colleagues compared the anti-drug properties of saliva in diabetic type 2 and non-diabetic patients in 2010 and showed that the mean number of candida colonies was significantly higher in diabetic patients than non-diabetic subjects ($P=0/0001$). This difference was significant between diabetic subjects with FBS above 200 and below 200 ($P=0/512$). Based on these two studies, one of the strengths of the present study, the rigorous elimination of all systemic.

Diseases involved in candida colonization, which makes the results more reliable. In a study, in addition to the role of normal saliva in preventing candida growth, shows the superiority of the effect of systemic immunity in preserving oral microbial ecology. It seems that the weakening of the immune system is more severe in general, the effect of saliva will be more quantitative and qualitative and its compensatory capacity will be reduced [23]. Our rigorous emphasis on the removal of systemic diseases in patients under study, in this regard, is one of the positive points in the design of the study. In addition, other systemic diseases each have the ability to change quantitatively and qualitatively the saliva, for example: 55% of people with hypertension are not aware of their illness this

condition causes vascular disorders and effects the characteristics in saliva ^[22].

Can change the dispersion in the studied attribute in normal distribution curve significantly. These results match with the study on electrical resistance of the unstimulated saliva of the patients with contact lens lesions by AkhavanKarbasi et al. Also, in this study, electrical resistance of the healthy people with Amalgama repair in comparison with the patients with contact lens lesions didn't show significant difference (Pvalue = 0.253). This insignificance, is due to the dispersion in the resulting data from the effect of an external factor like Amalgama in the mouth. It is undisputable that with the significant increase in the studied population, the pattern of this dispersion will be distracted to a certain result.

The relationship between the number of smoked cigarettes per day and cultivation as well as the number of the colonies has a negative coefficient but it is very close to the significant values (respectively Pvalue = 0.069, Pvalue = 0.057). In grouping the smoker samples to three groups of low smokers, medium smokers, and heavy smokers; the highest number of cultivated colonies were related to the group that they reported an average daily usage of drugs. While often it is expected that with an increase in the drug usage the number of colonies gets higher. It seems that one of the reasons for decrease in the number of colonies was that saliva of heavy smokers was more hyperkeratinitis. This makes the amount of collected cells in the saliva samples less by adding up to the adhesion in the saliva of the candidates. It seems that simultaneous usage of swap sample compensates the reduction in this result difference. Other notable and unexpected note was that the relationship between the number of the smoking years and age of the candidate with the number of the colonies has a negative correlation coefficient (Pvalue = 0.494). Apparently the adaptability of the body before reaching aging – more than 65 years – passing through time, makes it possible that the live creature to maintain its performance in the natural environment by expanding their storage capacities and overcoming the destructive effects of environmental factors. seems that the more years pass from cigarette usage or the more the person's age gets higher, concerning the presence of pathologic factors caused by smoking in person, adaptability is generated. After determining the abundance of the candidate colonies in two groups of saliva, it was time to study the microbial ecology in the cultivated samples. Albicanstype in both smokers and non-smokers group was predominant and it included 40% of the total number of samples. The percentage of the people that were infected with Albicanswere more than the non-smoker people. Also, non-Albicanstypewere observed more in smokers than non-smokers. In 6.7% of smokers and 3.3% of non-smokers both type of Albicans and non-Albicans were observed, but none of these results made sense statistically. (Pvalue = 0.746)

Conclusion and Recommendations

Study results could not be successful in emphasizing the anti-candidacy properties of smokers and non-smokers. However,

the existence of this difference was confirmed implicitly, so highly dispersion of the studied attribute in between the case group samples, prevented the meaningful difference between the control group and the case group. The strict criteria for carrying out this study and time limitation practically prevented excessive sampling. Thus, it is recommended that this study be used for later extensive studies for bigger populations.

It is concluded from the results of this study that saliva has multifactorial performance and under certain effects including salivary gland injuries, tissue damage and blood injuries.

The question that why smoking in different people causes different effects contributes to the vulnerability of the person as a pathological factor. The reason that causes more candidacy colonies some smokers – which is a criterion for anti-candidacy of the saliva- become more or less, is the same for variety in saliva performance in response to Amalgam mercury and it is not far from mind.

At the end by considering the candidate's secondary mucosal injuries and increase in oral cancer risk in exposure to products of the metabolism of this fungus; it is recommended that in smokers

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