Oral Mycotic Flora and Its Association with pH, OHI-s & DMFT indexes in students of Mazandaran University of Medical Science in 2015

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ABSTRACT

Candida fungi is grouped in the fermenting fungi strain. Candida types are usually the normal flora and mostly get pathogens in immune system deficiency. The most common place of candidiasis infection is in the mouth. One of the effective factors of colonization intensity of this fungi is the mouth hygiene. The goal of this study is to determine the mouth fungi flora of dentistry students of Mazandaran University of medical science and to determine the correlation of this flora with the indexes of OHI-s, DMFT and saliva pH of this individuals. 119 individuals of dentistry students of Mazandaran participated in this analytic descriptive study. Clinical examinations for DMFT and OHI-s indexes were conducted. Saliva and plaque samples which were cultured in the saborodextrose *and type-determined in chromagar. After gathering data they were imported to SPSS22 and were analyzed. In total, 61 cases were albicans typed. DMFT index average was calculated to be 5.31±3.44, pH was 7.07±0.77 and OHI-s was 1.33±0.67. DT in individuals with colonization is meaningfully more than individuals who had colonization lower than 50 (P-value≤0.05). No meaningful differences between the growth number of lingual plaque and mouth saliva were observed. Among the sub-indexes of DMFT, DT had the lowest value. Considering OHI-s, students were placed in the medium level. The DMFT would be decreased down by increasing teeth brushing, mouth washing and flossing times. A meaningful correlation between gender and average of pH and OHI-s indexes was observed.*

Keyword: Candida; Dental plaque; Dental caries

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INTRODUCTION

The fungi of Candida is grouped in fermenting fungi genus [1]and is also a Deuteromycete, pathogen and opportunist[2].At normal state, many kinds of Candida don`t cause disease in human body and are not dangerous but along with immune system weakening they can be dangerous and cause disease[3].In 1 cc sample of carrier and non-

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symptomatic Individuals, 200-500 candida are detected which increase in the growth of this fungi leads to Candidiasis infection[4].

Candidiasis mostly occurs in the mouth, vagina, outer ear, skin and fingernails which the most incident place of infection is in the mouth[5].Candidiasis is one of the world`s considerable infection diseases which includes 5-18 percent of all of clinical infections and is also reported from 25 to 50 percent in CCU which is really considerable[6].Candida has several kinds, Candida Albicans, Candida Tropicalis, Candida Glabrata, candida Krusei and Candida Parapsilosis are important kinds of candida which the most incident one is candida albicans[7].In mouth cavity Candida albicans is colonized in places like tongue, teeth decayed surfaces and teeth plaque[4].

The incident of C.albicans is reported 45% in newborns` mouth[8],45-65% in non-symptomatic children[9],30-45% in non-symptomatic teenagers[10],50-65% in individuals using removable dentures, 90% in cancer patients under chemotherapy[11]and 95% in HIV infected people[12].The extremity and tensity of colonization of this fungi is determined by factors like age, gender, food habits and oral care[13].The health staff(14),diabetes mellitus patients[15],HIV+ individuals[16],people with O blood type[17],Sjogren syndrome, people using dentures[13]taking drugs[18],disorders in saliva pH[19],and radiotherapy patients are high risk for this disease[20].

The influence of candida infections on the number of decayed teeth has been proven. Ghasempour conducted a research on dentistry students of Babol University of Medical Science and concluded that average number of decayed teeth in individuals having candida albicans in their plaque and saliva samples is considerably higher than individuals without candida albicans. Also it was reported in some studies that the average index of DMFT in people with positive culture of candida is higher[21]. Oral thrush, Erythmatosis candidiasis, angular chelitis inflammation and chronic candidiasis hyperplastic are different kinds of infections with candida source[7].

To treat the candidiasis, medicines and drugs like Nystatin, Amphotericin B, Itraconazole and Fluconazole are used which some of these drugs have side effects on kidneys and liver and also cause drug-resistance to azole-group drugs and lower the anti-fungi effect of drugs[22].

In a cohort study which was conducted by Negroni and et al. dentistry was stated as a risk factor of mouth contamination with candida because of its close professional association with mouth[23].

Aerndorf TM et al (18)reported the smoking as a risk factor of Candidiasis infection in mouth cavity. But this issue doesn`t necessarily mean the increase in the number of candida albicans colonies in mouth cavity.This conclusion doesn`t get along with Rindum JL study which reported the smoking as a factor of increase in the number of candida colonies and development of candidiasis infection in mouth.

In accordance to the higher rate of candidiasis danger in health staff compared to others and considering this fact that dentistry students visit different patients in their education period and are high risk, the dentistry students of Mazandaran University of Medical Science are chosen as the studied community.

Considering the priority of prevention to cure policy and side effects of medicines taken to treat the candidiasis infection, expenses that this disease imposes to the patients and contradictions found in some studies, this study proceeds to determining the mouth fungi flora of dentistry students of Mazandaran university of medical science and also determination of association of this flora with OHI-s[Oral health Index- Simplified], DMFT and pH indexes which the results can be helpful to improve the knowledge in the mentioned field and will also be useful to determine the health policy this community requires[24].

MATERIALS AND METHODS

This study is applying due to reaching practical results and is descriptive-analytic considering the fact that it gets into determining the oral fungal flora in students of Mazandaran University of Medical Science in 2015 and determining the correlation to DMFT, OHI-s, pH indexes and it is cross sectional because it was conducted in a special year (2015).

The study population of this study has been all the dentistry students of Mazandaran University of medical science which were sampled based on Cochran sampling formula with acceptable error coefficient of 0.05,119 samples were

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taken. Sampling method was random and simple. Not having systemic diseases and not taking steroidal drugs were standards for the participants to be included in this study. After explaining the goals and the method of conducting the project and guarantying the identity safe keeping, the students orally stated their satisfaction to participate in the study. A 7-question questionnaire and a check list consisted of two parts were used to record the data. The first part of the questionnaire included the demographic data like gender, age and university entrance year. In the second part, 4 questions about number of tooth brushing times, using dental floss, number of using mouth wash times and smoking habits were asked. The first part of the check list associated to DMFT index, the second part associated to OHI-s and the third part which was completed in the laboratory associated to pH of saliva sample.

Samples were examined on the dental unite and under the unite light. Teeth surfaces were precisely examined after air drying and removal of debries to diagnose caries and tooth colored and non-tooth colored restorations. In this study teeth that missed because of decay were grouped in Missed Teeth (MT). Lesions in smooth surfaces or in pit and fissures of teeth that undermined the enamel with chalky appearance or had soft sense in examining with explorer, teeth with frank cavities or had temporary restorations and also restored teeth with recurrent caries were grouped in Decayed Teeth (DT). Restored teeth which their appearance, function and size were normal, were considered filled teeth (FT) and it included teeth with crowns or filling or had endodontic therapies[25].

To study the OHI-s, the facial surface of teeth 9 and 25, and lingual surface of teeth 3, 12, 19 and 28 were divided into five subdivision including mesial, distal and gingival, medial and Inscisal or occlusal. For each part of tooth whether for plaque or for calculus, score 1 in case of presence and score 0 for absence were assigned to that section. So for each tooth minimum score of 0 and maximum score of 3 for plaque or calculus would be possible. The difference between OHI and OHI-s is that instead of checking 12 surfaces, 6 surfaces will be checked. The existence of calculus in the CI or Debri in DI were inserted to the check list by the project team mate while examining based on their development on the mentioned surfaces. Then the average of these two indexes were calculated as OHI-s[26].

To determine the fungi flora in the students` mouth, the saliva and plaque were sampled. Thus the students were asked to pour their saliva samples in single-use plates given to them. To sample the plaque, a sterilized swap which was placed in physiologic serum in a numbered test tube was used to sample from the upper surface of tongue. Then the saliva and plaque samples were immediately taken to Mazandaran Science and Technology Park laboratory to culture the fungi in solid saborodocstrose agar beside fire flame and under hood. Also to determine the pH of students` mouth from saliva samples, pH determining paper of Merck Inc. Germany were used. Then the cultured samples were placed in $37°$ centigrade incubator for 24 to 72 hours and fermenting growth of this fungi was evaluated by counting the number of colonies. Then samples were taken from culture media the fungi cultured in and were immediately cultured in chrome agar culture media and again placed in $37°$ incubator for 24 to 48 hours and were kind-determined based on colorometric principles [4]. In other words, they were kind-determined based on the color the colonies got in their culture media. The Albicans kind got bright green, Tropicalis got blue, Krusei got pink to violet and other kinds got gray. After gathering the data, they were inserted to SPSS 22 software and then they were analyzed using descriptive statistics methods and quiddity variants using average± and qualitative variants using descriptive statistics. The records were analyzed using Pierrson correlation coefficient test and independent T-test and Chi-square and variance analysis. All test were conducted in *P-value* of 0.05.

RESULTS

In this study, 119 students participated and were examined and sampled of plaque and saliva which 52(43.7%) of them were male and 67(56.3%) were female. A total of 71(59.7%) cases were Candida positive which, 61 cases (85%) of them were Candida Albicans, 5 cases (7.5%) were Krusei and 5 cases (7.5%) were other types of Candida. Also the existence of Tropicalis was checked but no growth of this type was detected.

Table 1.Teeth brushing, flossing and mouth washing times in participants

	At least once a day	2 or 3 times a day	Once a week	Once a month	Never
Teeth brushing times	106(89.1%)	10(8.4%)	2(1.7%)	0(0%)	1(0.8%)
Flossing times	49(41.2%)	30(25.2%)	12(10.1%)	5(4.2%)	23(19.3%)
Mouth washing times	5(4.2%)	5(4.2%)	20(16.8%)	10(8.4%)	79(66.4%)

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Among the studied samples, 106 individuals (89.1%) brushed their teeth daily, 10 individuals (8.4%) brushed two or three times a week, 2 individuals (1.7%) brushed once a week and one person which was less than 1% didn`t brush. This data besides flossing and mouth washing times are mentioned in the table 1.

Only 7 individuals smoked which included the 5.9% of participants. The average of DMFT index was 5.31 ±3.44 (females 5.47±3.37 and males 5.09±3.54). The average value for DT is 2±1.8, 0.18±0.6 for MT and 3.12±2.95 for FT. Among different entries, considering DMFT index, the entry of 2014 with the average of 3.8±2.74 had the best condition and after that the entries of 2009 and 2011 were in the next places with the average of 4.22±2.53 and 4.76±3.64 and totally, 11 individuals (9.2%) had DMFT of zero, 52 individuals (43.7%) had DMFT of 1 to 5, 46 individuals (38.7%) had DMFT of 5 to 10 and 10 individuals (8.4%) had DMFT of more than 10. 33 individuals (27.7%) didn`t have any decayed teeth.81 individuals (68.1%) had 1 to 5 decayed teeth and 5 individuals (4.2%) had more than 5. About the number of teeth extracted because of decay it must be mentioned that only 12 individuals (10.1%) had extracted teeth witch 4 of them only had one, 7 of them had two and 1 of them had four extracted teeth. Also about the restored teeth because of decay, 33 individuals (27.7%) had no restored teeth, 64 individuals (53.8%) had 1 to 5 and 22 individuals (18.5%) had more than 5 restored teeth.

The total average of pH index is 7.07±0.77 witch for the female is 6.94±79.0 and for the male is 7.25±0.71.

Results show that the average of OHI-s is 1.33±0.67 (1.18±0.67 in female and 1.51±0.64 in male). In a comparison between different entrances, the entrance of 2009 with the OHI-s average of 1.03±0.88 had the lowest value among all the other studied students. The numeric value of OHI-s, DI and CI indexes is shown in the figure 1 based on each entrance and in the table 2 based on the molar and central teeth.
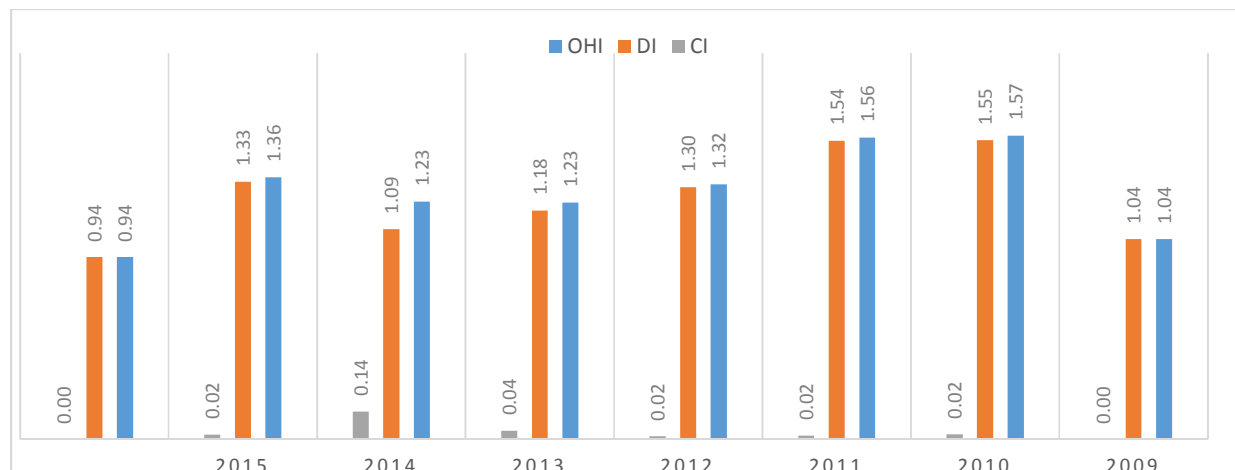

Figure 1. The numeric values of OHI-s, DI and CI indexes in each entrances.

Table 2.The numeric values of CI, DI and OHI-s indexes in molar and central teeth

	Molar		Inscisal		Molar	Inscisal
Index	CI	DI	CI	DI	OHI	
Mean	.0315	1.4958	.0672	.8739	1.5273	.9412
Std. Deviation	.17392	.68888	.21515	.80311	.72690	.87618

Table 3. The average of DI and CI in each jaw

	Maxilla		Mandible	
Index	DI	CI	DI	CI
Mean	1.1148	.0364	1.4622	.0504
Std. Deviation	.73318	.20717	.69041	.16026

In this study, for the OHI-s, 57 individuals (47.9%) with the average of 0.75±0.3 were considered Good (0 to 1.2), 61 individuals (51.3%) with the average of 1.83±0.4 were Medium (1.2 to 3) and 1 individual (0.8%) was Bad (3.1 to 6). The highest value of OHI and the DI along with mandibular right first molar and CI is mandibular fist incisor.

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Also the average of OHI in maxillary arch teeth is less than mandibular teeth. The average of DI and CI based on each jaw is shown in the table 3.

The number of cultured saliva simples was 61 (%51.2) cases witch in 27(22.6%) cases 1 to 10 colonies, In 13 (10.9%) cases 5 to 10 colonies and in 21 cases more than 50 colonies were formed. So 21 cases had colonization. Also the results of sampled cultures of tongue plaque indicate that a total of 40 cultures were positive witch 23 individuals (19.3%) had 1 to 10 colonies, 9 individuals (7.5%) had 10 to 50 colonies and 8 individuals (6.7%) had colonization.

In statistical reviews the relationship between the number of colonies and age, gender, entrance year, number of brushing, flossing, mouth washing times and smoking were considered and no meaningful statistical relationships were discovered.About the DMFT index it must be mentioned that the average of this index among men and women is the same and has no meaningful relationship with age and entrance year. Statistical analyzes show relationship between DMFT and number of teeth brushing, flossing and mouth washing times, so that by the increase of these the DMFT decreases down.

The number of decayed teeth in people who had more than 50 colonies was meaningfully more than other people who had less than 50 colonies (P- value≤0/05). Also an assumption was occurred about the meaningful difference of growth number of colonies which were sampled from tongue plaque and mouth saliva. This assumption was rejected after statistical analysis of relevant data.

It must be mentioned that after analytic reviews, a meaningful connection between gender and average pH index was observed as the male have more alkali saliva than female(P- value≤0/05).

Findings show that there is a meaningful connection between gender and OHI-s (P-value≤0.05) so that the average of OHI-s in men is higher than in women. Further to that, using mouthwash causes decrease in OHI-s (P-value≤0.05). Also no association between this index and variants like age and entrance year were observed. Correlations between pH, DMFT, OHI-s were evaluated but no connections were observed.

DISCUSSION

89.1% of participants brushed their teeth daily which is good. 41.2% of participants flossed their teeth daily. In other words half of the participants who brushed, didn`t floss before that and this can be one of the causes of teeth decay. Because brushing can`t clean the plaque off all the teeth surfaces especially proximal surfaces only by itself. About 80% of participants didn`t use mouthwash at all which is not acceptable.

In this study, saliva of women meaningfully had more acidic pH than men. Also the total average of this index is 7.07±0.77 which is lower than Ghasempour et al study (7.13±.075). DMFT index has an average of 5.31±3.44 which is lower than Ghasempour study (7.2±4.03) [4].

Basir et al studied the OHI and DMFT indexes in thalassemia patients in 2003 which reported DMFT as 9.5±5.5 that is considerably higher than the present study[27].

In Khalillinejad`s study which was conducted in Khuzestan to determine BMI and DMFT in 1500 individuals, the average of DMFT index was reported to be 2.41[28].

Pourshams et al in a prospective cohort study titled "teeth and oral hygiene condition in north-eastern Iran" after studying 45862 individuals with age average of 54.14±9.1 which was consisted of 42.2% men and 57.8% women, reported that the average of teeth number in people`s mouth was 13.3±9.4, extracted teeth was 18.4±9.5, decayed teeth was 4.7±6 and filled teeth was 0.23±1.1which the average of sub-indexes of DT, MT and FT are higher than the present study[29].

Deyhimi et al in a descriptive cross-sectional study, evaluated the DMFT index and teeth and oral health care in all of the general dentistry students of Isfahan dentistry school who were counted 202 people and reported the average of DMFT index 5.54 in men and 5.33 in women which is very similar to DMFT average in this present study. Also the correlation between variants of gender and DMFT was studied but no correlations were observed [30]which this

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finding is commensurate with the statistical reviews of the present and Ghasempour's study[4]and is also in conflict with Ramezani`s study titled "prevalence of DMFT and fluorosis in the students of Dayer city (Iran)" which reported the DMFT 1.65±1.76 in men and 1.98±1.67 in women[31].The OHI-s average is 1.33±0.67 which is 1.18±0.67 in women and 1.51±0.64 in men and a meaningful statistical difference was observed which can be due to better teeth and oral health care in women rather than men.

Babaiee et al chose 300 twelve year-old students based on cluster sampling method and checked their teeth and oral health condition and reported their OHI-s average as 1.38±0.46. Also according to the findings of this study, gender is a factor of oral health index difference[32].Some of the studies report that concerns about teeth and oral hygiene[33],injuries because of trauma[34]and the difference in hygiene functions are different in men and women which is commensurate with the findings of the present study[35].

Researchers in other countries have also studied the OHI-s and reported different values which Almerich Silla study in Spain that reported 1.84[36]and a study in Nepal on rural and urban students that reported the OHI as 0.98 for urban students and 1.34 for rural students can be mentioned[37].The OHI average in molar teeth was higher than centrals (1.52±0.72 compared to 0.94±0.87). This is because of high value of DI in the mentioned teeth which it`s probable that because of lips movements, the DI value of central teeth is lower and another reason for this high value in molar teeth is the presence of parotid duct in that area. Also the CI value, unlike the DI and OHI, is higher in central teeth which is mostly because of smoke contact with these teeth. In addition to that, the OHI in the teeth of maxilla is lower than the teeth of mandible.

The number of decayed teeth in individuals with colony number higher than 50 is meaningfully higher than individuals with colony number lower than 50 which can be due to more possibility of adhesion of Candida fungi to microbial plaques of teeth surfaces which are more present on decayed surfaces.

Moalic et al reviewed the fungi flora of 353 dentistry students in 2000 which after culturing on culture media, 58.6% of individuals were candida positive which is slightly higher than the present study(38). In the Ghassempour et al study on 121 dentistry students with age average of 24.61±5.2 in 2006 the total number of cultures having candida albicans in saliva was 53 cases (43.8%) and in plaque was 51 cases (42.1%) which like the present study, the colony growth rate in mouth is higher than dental plaques but with this difference that the total colony growth rate in the studied statistical population was lower than the present study [4].Negroni et al in most of their studies used the posterior surface of the tongue of their subject individuals to sample from which out of the whole studied population which had no systemic diseases (70 individuals), 15 individuals (21.4%) were candida carriers which is still lower than the present study[39].According to some of former studies, about 20 to 60% of people have candida fungi in their mouth[40].So we can see that the presence of this fungi in different populations and different regions of the world is variable. In Matlabnejad et al study one of the effective factors of being a candida carrier is reported to be professional careers and dentistry students are reported to be 2.85 times more high-risk than the other students[41].The dominant kind of candida in the studied population of this study is candida albicans which is commensurate with the findings of other studies[8, 42, 43].

CONCLUSION

In this study a high percentage of individuals brushed their teeth (99%) but lower percentage of individuals flossed their teeth. But about mouthwash it must be said that the percentage of individuals who use mouthwash is very low and they should consider some time in their personal hygiene plan to enhance the usage of mouthwash. The DMFT average is 5.31 which is not a high value but it must be lowered by enhancing the teeth and oral hygiene to reduce the treatment expanses imposed to the patient and community due to lack of health care. It must be mentioned that among the sub-indexes of DMFT, the MT index has a really low percentage or in other words, the percentage of extracted teeth because of decay has a really low percentage which according to the present statistical population that is dentistry students can be because of higher knowledge in the field of teeth and oral hygiene which leads to in time diagnostic which is one of the factors of decrease in expanses and harms done to the body. So it is recommended that one of the preventive strategies to enhance the community`s hygiene level be enhancing the people`s knowledge in the fields of prevention and in time visit to therapeutic centers for check-up and if necessary, treatment before serious harms.Findings show that considering the OHI-s division time periods, students are placed in the medium level (OHI average is 1.33). Also considering the correlation between using mouthwash and OHI decrease, it is recommended that using mouthwash should be taken more seriously because presence of tooth

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plaques on teeth surfaces can cause calculus during calcification mechanism which literally if not treated it leads to gums (gingival) erosion and periodontal diseases and in more severe cases it can cause alveolar bone erosion.Also lack of formation of proper adhesive substrate for different organisms leads to decrease in decay occurrence possibility. Also according to fact that DI is higher in molar teeth rather than centrals, it is recommended that the right way of teeth brushing which is also along with flossing teeth in posterior teeth be considered.The number of candida fungi carrier individuals is 71 cases (59.6%) but the colony growth rate is not high in this study. Also no special correlations between the number of colonies sampled from saliva and colonies sampled from posterior surface of tongue was observed.

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