

Comparison of Salivary Micro Flora of Fasting and No Fasting Persons

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Abstract: Dental caries and periodontal diseases are originated from complex relationship between diet and natural oral micro flora. The fasting period in holy month of Ramadan is presenting with changes in diet oral hygiene, time and amount of food intake and it seems these modifications induce some changes in oral micro flora. So, the present study was designed to comparison of saliva micro flora in fasting and non fasting persons. The study was descriptive, case-control type. About 100 high school students between 15-20 years old were selected. The persons fasted at least 20 days of holy month of Ramadan were entered the study. They were requested to collect their saliva in the container. Samples were stained by Gram method. Number of gram-positive and negative cocci, gram-positive and negative bacilli, gram-negative spindle shaped bacteria, gram-negative diplococci and yeast were counted by microscopic examination. The mean frequency of each group was obtained. About 1 month after holy month of Ramadan, they were examined again. Paired t-test was used for statistical analysis. The $p = 0.05$ were considered significant. The difference between gram-positive cocci, gram-positive bacilli, gram-negative bacilli, gram-negative spindle shaped bacteria frequency in fasting and no fasting persons was significant ($p = 0 < 0.05$, $p = 0.01 < 0.05$; $p = 0 < 0.05$, $p = 0.03 < 0.05$). Diet, time and amount of food intake modifications in holy month of Ramadan cause saliva micro flora change.

Key words: Saliva micro flora, gram staining, microorganism, fasting, Gram method

INTRODUCTION

The oral cavity is presenting with microbial colonies in different, unstable physical and chemical conditions. In spite of temporary changes, it has been shown that oral cavity is an existence place (Mergenhagen *et al.*, 1987). Dental caries and periodontal diseases are originated from complex relationship between diets and natural micro flora. The patients are usually suffered from pain and tissue damage in management of dental problems and sometimes the condition is very expensive. So, the major part of microbiological researches are focused on bacterial procedures involve in tooth decay and periodontal diseases (Listgarten, 1992) to prevent and reduce them. Different physical, chemical and mechanical factors control the microbiologic distribution and ecologic system of oral cavity normal micro flora (Richardson *et al.*, 1977). At birth, the oral cavity is sterile (Socransky and Manganiello, 1971) but in next few days, the colonization of normal microbial flora will begin. About >40 different of normal micro flora in oral cavity has been found. The major part of oral micro flora is anaerobic. The *Streptococcus salivarius* and *Streptococcus mitis*,

vionella and other anaerobic bacteria immerge in oral cavity in 1st week after birth (Davis and Stegeman, 1998). With tooth eruption and after 1 year, the oral micro flora will be almost as same as adults. Unlike, the host and micro flora relation balance theory oral cavity is probably the only place that survival of micro flora constantly relates to diet and local diseases (Krasse, 1965). Periodontal diseases and dental caries are originated from complex relationship between diet and natural oral micro flora. The relationship between diet and dental caries has been shown too (Beckers *et al.*, 1984; Firestone *et al.*, 1988).

In holy month of Ramadan, fasting cause diet changes and studying of oral micro flora in this month will probably reveal the effect of diet, food consumption hours and oral hygiene on normal micro flora. So, the present study was designed to compare the salivary micro flora of fasting and no fasting persons.

MATERIALS AND METHODS

The study was descriptive and case-control type. About 100 high school students between 15-20 years old

were selected from Shemiran area, Tehran. The students fasted at least 20 days of holy month of Ramadan were entered the study. The exclusion criteria were antibiotic therapy in recent 2 months; patients were involved by systemic diseases or periodontal problems and smokers were excluded from study too.

All persons were examined in same day. The fasted persons were requested to collect their saliva in container. The collection was completed in 11 am. About 50 mL of saliva were speared over a slide and fixed by heat. All samples were stained by gram as follows: sections were stained with crystal violet for 1 min and then were rinsed with water. In the next step, sections were treated with iodine for 1 min and rinsed with water again. The 3rd step was using of acetone for 5-10 sec. After rinsing the sections with water safranin stain were used. All prepared sections were examined by light microscopy at $\times 100$ magnifications. Number of gram-positive and negative cocci, gram-positive and negative bacilli, gram-negative spindle shaped bacteria, gram-negative diplococci and yeast were counted in one microscopic field.

The mean numbers of each group were obtained. About 1 month after Ramadan, same persons were examined again as no fasting persons. Paired t-test was used for statistical analysis. $p = 0.05$ were considered significant.

RESULTS

Mean frequency of different bacteria in fasting and no fasting persons are shown in Table 1 and 2. Based on the data, results were as follows:

- The difference between gram-positive cocci frequency in fasting and no fasting persons was significant ($p = 0 < 0.05$)
- The difference between gram-negative cocci frequency in fasting and no fasting persons was not significant ($p = 0.39 > 0.05$)
- The difference between gram-positive bacilli frequency in fasting and no fasting persons was significant ($p = 0.01 < 0.05$). Numbers of gram-positive bacilli were more in fasting
- The difference between gram-negative bacilli frequency in fasting and no fasting persons was significant ($p = 0 < 0.05$). Numbers of gram-negative bacilli were more in fasting
- The difference between gram-negative spindle shaped bacteria frequency in fasting and no fasting persons was significant ($p = 0.03 < 0.05$). Numbers of gram-negative spindle shaped bacteria were more in no fasting

Table 1: Mean frequency of different bacterias in fasting and no fasting persons

Bacteria	No fasting	Fasting
Gram-positive cocci	32.07	23.37
Gram-negative cocci	2.10	2.67
Gram-positive bacilli	46.49	52.58
Gram-negative bacilli	3.02	7.30
Gram-negative spindle shaped bacteria	8.07	4.65
Gram-negative diplococci	8.88	7.42
Yeast	0.42	0.33

Table 2: Mean frequency of different bacterias in fasting and no fasting males and females

Bacteria	No fasting		Fasting	
	Female	Male	Female	Male
Gram-positive cocci	30.44	32.19	23.92	22.48
Gram-negative cocci	1.81	2.34	3.14	2.57
Gram-positive bacilli	43.91	46.79	50.26	54.36
Gram-negative bacilli	4.45	2.65	7.02	8.28
Gram-negative spindle shape bacteria	8.58	8.07	5.23	4.11
Gram-negative diplococci	8.12	9.06	7.63	7.3
Yeast	0.40	0.44	0.35	0.33

- The difference between gram-negative diplococci frequency in fasting and no fasting persons was not significant ($p = 0.2 > 0.05$)
- The difference between yeast frequency in fasting and no fasting persons was not significant ($p = 0.73 > 0.05$)

DISCUSSION

Franklin and Skoryna (1966) studied the bacterial flora of fasting human subjects in 1966 and it was probably the first study in this condition (Franklin and Skoryna, 1966). Their study was based on throat swabs and gastric secretions instead of saliva after 12 fasting period and reported bacterial species in this group without comparing them with no fasting group. On the other hand, Mofid and Mehr (2003) evaluated the comparison of *S. mutans* and *A. viscosus* colonization before and after Ramadan month on dental surface.

They reported no significant difference in these colonization so, concluded that fasting in Ramadan month could not induce dental caries and periodontal diseases. In the present study, results show the difference of mean frequency in gram-positive cocci. Gram-positive and negative bacilli and gram-negative spindle shaped bacteria were significant in fasting and no fasting persons.

bacteria in no fasting persons. The difference of gram-negative cocci gram-negative diplococci and yeast were There were higher frequency gram-positive and negative bacilli in fasting time and gram-positive cocci, spindle shaped e not significant in fasting and no fasting persons. It seems that different sampling volume and also

method of study can probably explain the situation. In no fasting persons because of food intake, there was higher frequency of gram-positive cocci. These microorganisms are the most important bacteria of oral cavity.

The study of gram-negative cocci in male and female persons showed that fasting had not strict effects on this group. On the other hand, there was higher frequency of gram-positive and negative bacilli and it was more obvious in males.

The difference between gram-positive and negative bacilli frequencies was not significant in females. This result probably reflects the difference between male and female diet.

Comparison of gram-negative spindle shaped bacteria has been shown that the frequency of these bacteria was different in fasted and no fasted males. In fasting males, the number of gram-negative spindle shaped bacteria was decreased. In females no any difference was shown.

This result can be originated from different diet favorites in males and females. Diet, time and number of food intake changes in holy month of Ramadan cause the changes in salivary microorganisms. In holy month of Ramadan because of lesser time of food intake, producing time of microorganisms is shorter. On the other hand, fluid reducing and sweet increasing consumption causes microorganisms producing.

Study of male and female in separate groups has been shown that diet pattern, sweet food consumption and oral hygiene habit were different in males, these differences effect on salivary microorganism content of males in holy month of Ramadan.

CONCLUSION

Diet, time and amount of food intake modifications in holy month of Ramadan cause saliva micro flora change.

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