The Comparison of Enterococcus faecalis Pollution Amount in Saliva of People with Obesity and Normal Weight

Marziyeh Aghazadeh¹, Zahra Aghazadeh²,³, Sahar khademneghad⁴, Fahimeh Kabiri⁵, Hosein Eslami⁶

¹Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, IR Iran
²Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
³Department of Oral Medicine, Faculty of Dentistry, Zanjan University of Medical Sciences, Zanjan, IR Iran

*Corresponding Author:
Hosein Eslami
Email: eslamihosein56@yahoo.com

Abstract: The worldwide explosion of overweight people has been called an epidemic. In this climate of concern over the increasing prevalence of overweight conditions in our society, we focus on the possible role of oral bacteria as a potential direct contributor to obesity and this study designed for comparison of Enterococcus faecalis pollution amount in saliva of people with obesity and normal weight. In this study saliva of 90 patients was collected with body mass index between 18.5 and 25 for normal weight people and over 30 for obesity people, and were measured bacterial populations of their saliva. The data were analyzed by descriptive-statistically and chi-square test. In this study, 59 people of 90 investigated people had obesity and 31 people were in normal weight. The number of enterococci in the saliva of people with normal weight and obesity were mostly low. Analysis of data suggests that the composition of salivary bacteria changes in people with normal and overweight overweight people but this change is not significant statistically. Finally, we can conclude.

Keywords: Enterococcus, obesity, obesity

INTRODUCTION

Obesity and its complications are a leading cause of metabolic diseases in the developed and developing countries [1]. Several factors are involved in obesity, such as genetic factors, hormonal and environmental factors such as excessive food intake, and decrease or lack of physical activity [2].

Intestinal flora contains millions of bacteria with useful and harmful effects on health of the human body. More than 35,000 species of bacteria have been identified in the intestinal flora [2]. Recent studies have pointed to the relationship between intestinal flora and body weight [3]. Studies on mice have shown that intestinal flora plays a role in weight gain. The deposition of fat was 60% higher in mice with intestinal flora containing bacteroides and firmicuitc compared to the control group. These studies emphasized on the impact of the intestinal bacterial flora in the absorption and release of energy.

Based on studies in humans, as well, intestinal flora is different in individuals with overweight. These studies suggest that human intestinal flora in childhood can predict the risk of obesity or overweight in the future [4].

The most obvious results indicated the association of bacterial flora with the weight in a study by Di Baise in 2008. It was shown in this study that intestinal bacterial flora of the mice is involved in uptake of monosaccharides and lipogenesis and in some of them less weight change occurred even with consuming more calories than the control group. This study and similar studies led to the formation of the new term “infectobesity” meaning “infectious obesity” [4].

Bacteroids, feedmicous and enterococci exist in a significant number in the intestine [5]. Based on studies, the amount of bacteria increases in the intestinal flora of obese people [3].

Studies by John. K. Di Baise and colleagues in 2012 showed that the intestinal flora is involved in how the food is stored in the body and its consumption and can lead to obesity [6].

The results of some studies suggest that some bacterial species in the intestine can absorb higher energy and calories from foods and pass the calories to the body and lead to more fat production [7]. Some of the bacteria are involved in the microbial homeostasis and in weight loss as well [3].
Although these studies have focused on the bacterial flora of the intestine, all intestinal bacteria have passed from the mouth and some of them may remain and be localized in the mouth. It is estimated that approximately 1gram of bacteria is ingested in each 500 to 1500 mL of saliva per day. So the microbial flora of the mouth may influence the microbial flora of the gastrointestinal tract [8].

A study by Goodson and his colleagues was conducted on the bacterial flora of saliva in 313 obese patients that showed that the bacterial flora of obese patients is different from the control group. In this study, 40 different species of bacterial infection was assessed in women with overweight and normal weight and in a large number of bacterial species, the saliva contamination in the two groups was different. The study also showed that Sulfomonones species increase in 98.4% of obese patients [9].

In a study by Shilite and colleagues on patients with obesity and type 2 diabetes, it was found that obese patients with diabetes with HbA1c higher than 6.5 have different salivary bacterial flora than healthy individuals and bifid bacteria in saliva of these patients is less than healthy individuals [10].

Enterococci exist in the gastrointestinal tract, mouth and flora of the vagina. These bacteria are not normally assessed in saliva, but increase in chronic periodontitis [11], endodontic failure and chronic apical lesions [10].

Kian Wang and colleagues found in a study that enterococci increase significantly in infectious dental roots and teeth requiring root canal treatment and also in the saliva of these patients [12] and this bacteria was not identifiable in mouth of the patients without root infection or root treatment [13].

Rams TE et al found that enterococci, especially Enterococcus faecalis, is involved in subgingival periodontal infections, early-onset periodontitis and refractory periodontitis [14-16].

Numerous studies have mentioned the positive impact of Enterococci on weight gain. According to the European food security (Efsa) enterococci Faesium in the diet of 4,000 chickens causes weight gain over 32 days. This study examined three different stages and the results show positive effects of Enterococci at each stage on weight gain in the studied groups [17].

Lojanica and colleagues showed the positive impact of enterococci in weight gain of hamsters [18]. Samli and colleagues also reported similar results in increasing the body weight with increasing enterococci in the gastrointestinal tract in chicken [19].

Body mass index (BMI) is a value calculated by dividing body weight in kg to the square value of height in meters. BMI over 25 was considered as overweight and a BMI over 30 as obese and a BMI between 18.5 and 25 known as normal weight [20]. According to studies, as obesity changes the oral and intestinal flora and regarding the relationship between obesity and oral floral change and the positive effect of enterococci on weight gain and lack of similar studies on the possible bacterial contamination of saliva by enterococci in obese patients, this study aimed to assess the presence or absence of enterococci bacteria in the saliva of patients and oral flora changes with weight changes.

METHODS

In this study, 59 obese patients and 31 healthy controls were examined for the saliva culture. Male and female obese (BMI over 30) and normal weight (18.5> BMI> 25) patients without any systemic disease [9] who did not use antibiotics, antifungal or PH-changing agent during the past 2 weeks, were recruited into the study.

The saliva sample of patients were taken under the following conditions: the patients were checked for not taking any food, not having their teeth brushed and not smoked during the past 2 hours before sampling and not performing any dental treatments during the past 24 hours. The sampling was performed at 9-11 AM, during which 2 cc of the patients’ saliva was taken and transferred to the laboratory. In the laboratory, samples were collected under sterile conditions and cultured and incubated at 37°C for 24 hours, then the plates were examined and in case of positive growth of micro-organisms, Enterococcus colonies were identified and counted.

With the possibility of presence of bacteria in the study groups P=0.5 (50%) and α=0.05 and 10% difference in bacteria existence, at least 30 samples was estimated for each group.

Saliva samples (n = 90) were added to falkole 15, early in the morning for 3 minutes with at least 2 cc saliva and saliva was collected according to the standard protocol.

Saliva was homogenized for 2 minutes by a vortex mixer and transferred to the laboratory as soon as possible. Sterile swab of 0.05 was taken from saliva samples and added to the plate containing blood agar and EMB and spread in the culture beside flame. Considering the sterilization points, the plates were incubated at 37°C for 24 hours and colonies’ grown were checked after 24 hours and were identified through the differential environment and growth in bile esculin and 6.5% NaCl and biochemical characteristics.
RESULTS

According to Table 1, the number of enterococcus is low in obese patients and people with normal weight. According to Table 1, the number of enterococcus is low in people with normal weight, as well as the results of the above table, the number of enterococcus is low in obese patients.

Table 1: Participants’ weight and its relationship with the number of Enterococcus bacteria in saliva samples

<table>
<thead>
<tr>
<th>Total</th>
<th>Many</th>
<th>Medium</th>
<th>Few</th>
<th>Number of bacteria Patients’ weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td></td>
<td></td>
<td>23</td>
<td>Normal</td>
</tr>
<tr>
<td>59</td>
<td>13</td>
<td>16</td>
<td>30</td>
<td>Obese</td>
</tr>
<tr>
<td>90</td>
<td>16</td>
<td>21</td>
<td>53</td>
<td>Total</td>
</tr>
</tbody>
</table>

Inferential statistics

In this section, Chi-Square test was used to compare the number of bacteria in both normal-weight and obese patients, based on data, obtained from the samples of the study.

Results of Chi-Square test

<table>
<thead>
<tr>
<th>p-value*</th>
<th>Pearson Chi-Square</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.962</td>
<td>0.077</td>
<td>The number of enterococcus in obese patients and people with normal weight</td>
</tr>
</tbody>
</table>

The results of Chi-Square test show the rejection of the H₀ assumption, as the significance level was more than 0.05, meaning that the amount of enterococcus contamination in saliva is not related to their obesity.

DISCUSSION

Overweight and obesity are a major nutritional and health problem that increase the percentage of mortality and reduces the life expectancy [21]. WHO describes obesity as a chronic disease that is replacing many previous health problems, such as infectious diseases [22].

The cause of obesity is grossly related to rapid widespread change in lifestyle, physical activity and diet. Bacteria in the body are also another cause of obesity that it is entitled “infectobesity” meaning "infectious obesity" [4, 21]. Several studies have examined the role of bacterial flora in obesity. In our study, also, there was a non-significant difference in contamination volume of saliva with enterococci bacteria between the obese and normal weights. Sakransky and Hafajy also studied populations with and without periodontitis and concluded that 70% of those with periodontitis are overweight or obese, which shows that the bacteria is associated with weight gain [23].

Intestinal flora contains millions of bacteria with useful and harmful effects on the health of the human body. More than 35,000 species have been identified in bacterial intestinal flora [2]. Recent studies have pointed to the relationship between intestinal flora and body weight [3].

Numerous studies have mentioned the positive impact of Enterococci on weight gain [17-19]. The results of the present study, conducted on the saliva of normal weighted versus obese patients, showed that the number of enterococci bacteria in saliva of people with normal weight and obese patients is low, but in the obese patients, medium and high bacteria is more than people with normal weight, although this difference was not statistically significant.

On the one hand, various studies show that oral bacteria leads to obesity at least through three mechanisms: increasing the efficiency of metabolism, weight gain by increase in appetite, and guiding energy metabolism by oral bacteria (by facilitating the insulin resistance by increasing the levels of TNF-α or decrease in adiponectin levels), and with each of these mechanisms, even a slight increase in calorie intake without a change in diet and exercise can lead to an unacceptable additional weight. On the other hand, with the same logic, people who are infected may reduce their calorie intake or the amount of exercise to compensate weight gain [6, 7, 9]. In the present study, due to lack of controlling diet and exercise, the confounder may possibly affect the final results. Thus, it is recommended that future studies on weight gain related to infection consider food intake and exercise, as well, so that it would not interfere with the results.

Finally, it can be concluded that the reasons for the association between obesity and oral bacteria are undoubtedly complex and diverse and this association may be environmental (such as the relationship with diet) or opportunistic (like proliferation of metabolic changes in the host’s body) [9].
CONCLUSIONS
Considering the data analysis, it can be concluded that the number of enterococci bacteria in saliva of people with normal weight and obese is low, but in obese patients, the number of medium and high bacteria is higher than normal weighted people, although the difference was not statistically significant. So, there is no statistically significant relationship between obesity and the number of salivary Enterococcus bacteria.

SUGGESTIONS
1. In case of controlling diet and exercise in further studies, obesity will be more manageable.
2. Studies with a greater sample size will give us more accurate results.
3. Checking the bacterial infection of oral and intestinal flora at the same time will give us more practical and more scientific results.

REFERENCES
2. Microbe Wiki, microbiology resource. Link Between Microbes and Obesity
17. European food safety Authority (EFSA), Parma, Italy .(2010). Scientific opinion on Enterococcus faecalis as a feed additive for chickens for fattening, EFSA Journal, 8(6), 1636.