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Comparison of salivary interleukin-6, interleukin-8, C-reactive protein levels and total antioxidants capacity of obese individuals with normal-weight ones

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Objective: Obesity is a worldwide concern that may lead to type 2 diabetes, cardiovascular diseases, etc. Several serum biomarkers have been identified in the saliva of obese individuals, including inflammatory cytokines, adipokines, insulin, and cortisol. The present study aimed to compare salivary interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP) levels and total antioxidants capacity (TAC) of obese individuals with normal-weighted ones.

Methods: In this case–control study, 92 participants matched in terms of age and gender were placed into two groups according to the body mass index (BMI); case group: BMI>30 and control group: 18.5<BMI<24.99. Unstimulated saliva was collected. ELISA and FRAP method were used to determine IL-6, IL-8, CRP and TAC. Data was analyzed using SPSS 24, at the significant level of 0.05.

Results: Mean salivary levels of IL-6 and IL-8 in the normal individuals were 53.36 and 421.25 ng/mL, with 86.09 ng/mL and 510.19 ng/mL in obese individuals, respectively. There were significant differences in the mean salivary levels of IL-6 and IL-8 between two experimental groups.

The mean salivary levels of CRP of control and case group was 2.84 and 2.63 ng/mL and the total salivary antioxidant levels in the normal and obese individuals were 0.29 and 0.36, respectively which had no significant different.

Conclusion: According to the results of the present study, salivary levels IL-8 and IL-6 was significantly higher in obese individuals than in those with normal weight. However, the mean salivary CRP and TAC were not significantly different between the obese individuals and normal-weighted ones.

Key words: obesity, C-reactive protein, interleukin-6, interleukin-8, antioxidants, saliva, obesity markers.

INTRODUCTION

Obesity is a global problem due to an imbalance between the intake and consumption of energy. According to the World Health Organization (WHO), obesity has become an epidemic, and over two billion people worldwide are obese [1]. Obesity is diagnosed based on BMI, an index based on weight and height, categorizing individuals into low-weight, normal weight, overweight, and obese groups [2].

Many studies have shown that obesity and inflammation are related. As an endocrine gland, the adipose tissue secretes proteins called adipokines or adipocytokines that play a role in systemic inflammation, in addition to its role as a reservoir for fats [3]. Chronic low-grade obese-related inflammation is called metabolic inflammation, which plays an important role in inducing various medical conditions such as type 2 diabetes and atherosclerosis, which is associated with increased plasma levels of CRP and adipokines such as IL-1, IL-8, IL-6, IL-1b, TNF-α, and IL-12 [4, 5]. Recently, many studies have investigated saliva as an adjunctive noninvasive diagnostic tool. Saliva has a complex chemical composition, consisting of water, electrolytes, proteins, enzymes, and antimicrobial components. Cytokines enter the saliva through passive diffusion or active intracellular mechanisms or intercellular spaces from the circulation. In overweight and obese individuals, several serum biomarkers have been identified in saliva, mainly focusing on inflammatory cytokines, adipokines, insulin, and cortisol. Some of the cytokines identified in saliva that affect oral health include IL-6 and IL-8, which are related to the metabolic inflammation resulting from obesity [6, 7].

 DOI: 10.2478/rjim-2022-0013

IL-6 has both proinflammatory and anti-inflammatory activity, which might cause the growth and differentiation of lymphocytes. A strong correlation has been reported between the serum and salivary levels of IL-8. It is predominantly secreted by macrophages and epithelial cells under inflammatory conditions and plays a significant role in activating neutrophils and the induction of acute inflammation [8]. CRP is an acute phase protein secreted by the liver in response to inflammatory factors released from macrophages and adipocytes [9]. High CRP levels indicate the presence and severity of inflammation. CRP levels are used as a clinical parameter to evaluate the inflammatory status in many diseases, including diabetes and the relevant conditions and coronary artery accidents [10].

Cytokines comprise a vast field in the pathophysiology studies of obesity. It has been widely reported that IL-6 is involved in this pathological condition. However, only a small number of studies have investigated its salivary levels in obese individuals [11]. The salivary levels of IL-8 in obese individuals have not been widely investigated, and the results of its serum levels are contradictory [12, 13].

Reactive oxygen species (ROC) are produced in response to infections or cytokines. Oxidative stress is an imbalance in the production of free radicals that can be measured by the total antioxidant capacity (TAC) [14]. One of the defense mechanisms to eradicate ROC is antioxidant enzymes found in all the body fluids, including the saliva [15]. It has been suggested that saliva can be considered the first line of defense against oxidative stress resulting from free radicals [16].

Considering the important role of the saliva in the early diagnosis and prognosis of oral diseases and the ever-increasing use of the saliva to determine the general health of the body, including overweight and the associated conditions, and the limited number of studies on changes in salivary composition during obesity, the present study was undertaken to compare the salivary levels of IL-6, IL-8, C-reactive protein, and total antioxidant capacity (TAC) between obese individuals and those with a normal weight.

MATERIAL AND METHODS

Ethical considerations

First, all the participants were provided with an adequate explanation about the reasons for undertaking the study and its procedures. The participants signed informed constant forms, and their demographic data and medical history were recorded. The protocol of the study was approved by the Ethics Committee of Hamadan University of Medical Sciences under the code IR.UMSHA.REC.1399.576.

Participants and the relevant factors

In the present case–control study, the subjects in the case group consisted of 46 obese individuals, and the subjects in the control group consisted of 46 individuals with normal weight, who were referred to the Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran, in 2021.

Inclusion criteria

The case group consisted of individuals with a BMI of >30(only primary obesity), considered obese based on the WHO definition. The control group consisted of healthy individuals with a BMI ranged between 18.5 and 24.99 (in the normal weight range). Also, the case group was matched with the participants in the control group in terms of age and sex.

Exclusion criteria

The exclusion criteria consisted of immune system deficiencies, xerostomia, AIDS, a history of radiotherapy and chemotherapy during the past month, cardiovascular diseases, smoking and use of medications that decrease the salivary flow and systemic disease: hypothyroidism, Cushing disease. Also, those subjects who presented chronic or genetic diseases that could alter their nutritional status were excluded, the two groups were matched in terms of age and gender.

Salivary sample collection method

The salivary samples were collected from 8 to 10 a.m. The participants were asked to refrain from eating, drinking, and smoking one hour before the sample collection session. Unstimulated salivary samples were collected using the spitting method. The subjects sat on a chair comfortably in a calm environment, bending their head slightly forwards after initially swallowing the saliva. In 2–5 minutes, each participant evacuated his/her saliva into 5-mL capped sterile microtubes every 60 seconds [17]. Then the microtubes were placed in ice and frozen at -80ºC.

IL-6 and IL-8 analysis

Il-6 and IL-8 levels were determined in ng/L using an ELISA kit (Human Interleukin ELISA Kit, Hangzhou Eastbiopharm, Co., China) with LOD: 1.03 ng/l and LOQ: 2 ng/l. ELISA reader TECAN (sunrise, Switzerland) was used for determination.

CRP analysis

Salivary CRP levels were measured using the Novin Bio kit (NegarinTeb Behnam, Iran) in mg/L with the ELISA method.

Total antioxidant analysis

Salivary total antioxidant levels were determined using the FRAP method using the Zellbio kit (GmbH, Germany) in mM. Then the color intensity was determined by measuring the absorption of the samples using ELISA.

Statistical analysis

First, the data were reported using descriptive statistics. T-test was used to compare the means of the salivary markers in question between the two groups. In the case of non-normal data distribution, Mann-Whitney

test was used the normal distribution of data of the marker in each group was analyzed with the Shapiro-Wilk test. The data was analyzed with SPSS 24(P-value ≤ 0.05).

RESULTS

Ninety-two participants were included in two groups of obese and normal-weight individuals in the present study. The obese group consisted of 16 males and 30 females, and the control group consisted of 13 males and 33 females. The descriptive statistics of demographic variables, demonstrated in Table 1, showed more females participants in each group; however, the mean age in both groups was almost the same.

Table 1

The distribution of gender and the means and standard deviations of the participants' age

		18.5<BMI<24.99	**BMI>30**	
		Frequency (%)	**Frequency (%)**	P-value
Gender	Male	13 (28.2)	16 (34.7)	0.587**
	Female	33 (71.7)	30 (65.2)	
Age		**Mean (SD)**	**Mean (SD)**	
		35.58 (10.33)	35.09 (10.06)	0.827*

*: Two-sample t-test; **:Chi-squared test

Two-sample t-test showed that salivary CRP levels were not significantly different between the two groups (P=0.208). Mann-Whitney test showed that although the mean salivary TAC in obese individuals was higher than that in normal-weight individuals, the difference was not significant (P=0.172). Mann-Whitney test showed that the mean salivary levels of IL-6 and IL-8 in the case group were significantly higher than those in the control group (P<0.05) (Table 2).

Table 2

The means and standard deviations of salivary levels of CRP, IL-6, IL-8, and TAC separately for the two study groups and P-values of the Wilk-Shapiro test

Variable	**Group**	**Mean**	**SD**	**P-value**	**Mean differences between the groups**
CRP	18.5<BMI<24.99	2.84	1.03	0.280	0.209
	BMI>30	2.63	0.69		
IL-6	18.5<BMI<24.99	53.36	13.99	**<0.001**	32.73
	BMI>30	86.09	46.06		
IL-8	18.5<BMI<24.99	421.25	108.09	**0.038**	88.94
	BMI>30	510.19	162.22		
TAC	18.5<BMI<24.99	0.29	0.27	0.172	0.07
	BMI>30	0.36	0.31		

Shapiro-Wilk test was used to check the normality of the data for the obese and normal groups, whose significance probability value is reported in Table 2, and according to this test, the salivary levels of IL-8 in the normal body mass index group and levels Salivary CRP was normalized in both groups. The results of Pearson's correlation coefficient in determining the intensity of the relationship between BMI and IL-6, IL-8, CRP and total antioxidants are given in Table 3.

Table 3
The correlation between BMI, IL-6, IL-8, CRP, and TAC

	CRP	**IL-6**	**IL-8**	**TAC**
BMI	-0.134	0.316	0.340	0.129
P-value	0.226	0.003	0.001	0.241

There were significant positive correlations between the salivary levels of IL-6 and IL-8 with BMI; however, there was no significant correlation between BMI, salivary CRP level and antioxidant capacity.

DISCUSSION

Currently, obesity is increasing, resulting in a higher risk of cardiovascular diseases, cancers, and diabetes [18]. Obesity can lead to changes in salivary composition and affect oral health status. The search for new diagnostic methods is important for the early evaluation of obesity and the relevant metabolic disorders so that preventive and therapeutic measures can be undertaken for optimal outcomes. The analysis of patients' saliva might be a valuable innovation in this respect. Evaluating salivary parameters might be helpful due to its speed of collection, low cost, and noninvasive nature [19]. The present study aimed to compare salivary levels of IL-6, IL-8, CRP, and TAC between obese individuals and normal weight ones. Based on the results, IL-6 and IL-8 salivary levels in obese individuals were significantly higher than those in individuals with a normal weight. However, salivary CRP and TAC levels were not significantly different between the two groups. Obesity results in chronic inflammation. It appears that the accumulation of the adipose tissue leads to local inflammation and the activation of immune cells, and the release of proinflammatory cytokines such as IL-6 and IL-8 [20]. IL-6 is a cytokine produced by the adipose tissue and is involved in inflammatory signaling pathways and insulin signaling in tissues. Its high circulatory levels are related to insulin resistance and type 2 diabetes [21]. Increased circulatory levels of IL-6 have been reported in obese individuals. Several studies have reported a positive and strong correlation between plasma IL-6 concentrations and salivary levels [6, 22]. Increased IL-6 levels in obese individuals are due to the secretion of this cytokine by adipocytes, which results in the aggregation of macrophages in the adipose tissue [23]. There is evidence that the host's response mediated by IL-6 exacerbates periodontitis and endothelial injury through inflammatory reactions mediated by oxidative stress [24]. In addition, the secretion of IL-6 during obesity results in a concomitant production of CRP in the liver [25].

Consistent with the present study, Pirseam *et al.* showed significantly increased salivary IL-6 levels in obese individuals, with 4.5-fold that of the control group in obese children, which correlated with BMI. The highest salivary IL-6 levels were detected in children with the highest BMI [26]. Some studies have shown a positive correlation between salivary IL-6 and BMI and dental caries, which might be a predictive factor for dental caries development [27, 28]. A study showed increased salivary IL-6 levels in patients with periodontitis [29]. However, studies by Goodson *et al.* (2014) and Al-Hamoudi *et al.* (2018) showed no significant differences in IL-6 salivary levels between obese individuals and those with normal weights [7, 30].

IL-8 is predominantly secreted by macrophages and epithelial cells after inflammatory stimulation and has an essential role in the inflammatory response by activating neutrophils [31, 32]. Consistent with the present study, Ostrowska *et al.* showed significantly higher IL-8 salivary levels in obese women than in women with a normal weight. In addition, there was a positive correlation between the salivary levels of IL-8 and BMI [33]. However, Goodson *et al.* did not report significant differences between the two groups [7].

Some studies have shown a relationship between IL-8 and obesity; however, the results have been contradictory. These studies have only evaluated serum samples, and salivary samples have not been evaluated [12, 13, 34]. Therefore, it is recommended that their relationship be evaluated in future studies with larger samples sizes.

TAC is the best parameter to evaluate the antioxidant condition in obese individuals and is widely used to diagnose systemic diseases. It shows the overall ability of a biological system to destroy free oxygen radicals. It has been reported that antioxidants are effective in diagnosing diseases destroying nerves, cancers, and metabolic conditions such as resistance to insulin, type 2 diabetes, nonalcoholic steatohepatitis, hypertension, and chronic cardiac insufficiency [35–37].

Choromanska *et al.* reported higher TAC levels in obese individuals [38]. In addition, studies on TAC in children and adolescents have shown higher salivary levels of TAC in control groups [39, 40].

Despite increased TAC levels in obese individuals in previous studies, there were no significant differences in TAC levels between the two groups in the present study. Increased TAC levels indicate an increased ability to eliminate free radicals and to protect against oxidative stress in obese patients effectively. It can be hypothesized that antioxidant systems become activated in the early stages of obesity. In long-term obesity, antioxidant reservoirs decrease gradually, resulting in

decreased activity of the antioxidant barrier [38]. Therefore, higher oxidative injuries to proteins, lipids, and DNA in patients with morbid obesity can be explained. In addition, the reason for such an increase in previous studies can be attributed to differences in age between the control groups in previous studies and the present study because, based on previous investigations; there is a direct relationship between deficiencies in the antioxidant system and the duration of obesity. In addition, age, gender, diet, and lifestyle can affect the salivary levels of TAC [41].

C-reactive protein is produced by the liver in response to IL-6 and TNF-α and significantly affects inflammatory response. It is considered an inflammatory factor related to obesity and a predictor of diabetes and cardiovascular accidents [42–45]. A study in 2021 showed that CRP levels in obese children were six folds higher than in children with a normal weight [46]. All the previous studies have shown a significant relationship between CRP and obesity; however, such a relationship was not detected in the present study, which might be attributed to the small sample size in the present study.

Other reasons for differences in the results of studies arethe severity and different levels of obesity in subjects in different studies. In addition, factors such as gender, age, the duration of obesity, and serum and salivary levels of CRP might explain the difference in theresults of studies. In addition, some studies have shown the effect of fat accumulation location on inflammatory factors. In this line, the accumulation of adipose tissue in the middle part of the body is effective in increasing the serum levels of proinflammatory factors such as hs-CR and IL-6 [47].

Study limitations: One of the limitations of this study is the small sample size. Therefore, some of the collected data may be underrepresented. Interpretation of our data may be limited due to overlap in the pathophysiology of the various conditions analyzed and their complex interactions.

CONCLUSION

According to the results of the present study, IL-6 and IL-8 are adipokines that increase in the saliva of obese individuals. However, there were no significant differences in TAC and CRP levels. Based on the results of the presentstudy, it can be concluded that salivary IL-6 and IL-8 can be considered biomarkers of obesity for the early diagnosis of obesity. However, further studies are necessary on the salivary levels of TAC and CRP.

Introducere: *Obezitatea este o patologie ce duce la diabet zaharat tip 2, boli cardiovasculare, etc. Mai mulți biomarkeri serici au fost identificați și la pacienții obezi. Studiul și-a propus să compare nivelurile salivare ale IL-6, Il-8, CRP și ale capacității totale antioxidante (TAC) la pacienții obezi comparativ cu cei normoponderali.*

Metode: *92 de participanți au fost împărțiți în 2 grupuri: obezi și normoponderali după BMI. Au fost utilizate ELISA și FRAP pentru analiza biomarkerilor salivari din salivă colectată fără stimulare. Datele au fost analizate cu SPSS 24, cu un nivel de semnificație de 0,05.*

Rezultate: *Nivelurile medii ale IL-6 și IL-8 la normoponderali au fost de 53,35 respectiv 421,25 ng/mL comparativ cu 86,09, respectiv 510,19 ng/mL la obezi, valori diferite semnificativ. Nu s-au observat diferențe semnificative între nivelurile CRP sau TAC.*

Concluzii: *Nivelurile salivare ale IL-6 și IL-8 au fost mai mari, dar nu s-au observat diferențe între CRP sau TAC.*

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Conflict of interest disclosure: The authors declare no conflict of interest.

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Received 6th May 2022