Inflammatory Adiopcytokines in Saliva as an Indicator of Obesity

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ABSTRACT

Obesity is a non-communicable and multi-factorial disease that may define as excessive accumulation of fats. Usually blood is most frequently used as a source of biomarkers measurements. Saliva may be used as an alternative to blood since many elements pass from the blood to saliva. Visceral adipose tissues secrete a diversity of adipocytokines that are contributed in inflammatory processes and alter immune responses. This study aimed to find out the changes in the levels of salivary adipocytokines due to obesity. The study results reveal a high significant decrease in ADP, significant decrease in GZm B and high significant increase in TAC. In conclusion the study revealed significant changes in the levels of most adiopcytokines in the saliva of obese subjects, which can be used as an indicator to the obesity.

Keywords: *obesity, saliva, APD, Il-1ra, GZm B, TAC*

Introduction

Obesity might have negative impact on health [1]. The obesity epidemic is a worldwide problem and increased over time [2]. Traditionally blood serum or plasma is most frequently used as a source for biomarkers measurements. Saliva may be used as an alternative to blood since many elements pass from the blood to saliva, and most components found in blood are existed in saliva too [3]. Adipose tissue have been studied intensively in the regulation of energy balance because of their role in calorie storage [4]. Visceral adipose tissues secrete a diversity of adipocytokines that are contributed in inflammatory processes and altered immune responses [5].

Adiponectin may have various immune roles in many biological systems. It has an anti-inflammatory, insulin-sensitizing and anti-atherosclerotic effects when it secreted into the circulation [6]. Despite that, adiponectin may directly contributes in the control of energy metabolism [7].

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The interleukin-1 receptor antagonist (IL-1ra) is a unique naturally occurring cytokine that binds to IL-1 receptors without prompting a cellular response, thus it competitively antagonizes the inflammatory effects of IL-1α and IL-1β [8]. It has been found that white adipose tissue is a main provenance of IL-1ra [9].

Natural killer cells (NK) are a subgroup of innate lymphocytes that capable of performing the cytotoxic actions against tumor cells without previous exposure. Their Numbers are reduced and display functional impairment in obese subjects [10]. NK secrete Granzyme B (GZm B)that acts to induce inflammation by promoting the release of cytokine. The levels of Granzyme B were high in serum and saliva of obese subjects [8].

Obesity is linked with an elevation in oxidative stress, due to excessive production of reactive oxygen species (ROS) [11]. When the adipose tissue is hypertrophied, the blood supply becomes unable to keep up with the growth, and hypoxia of the cells occur. In an attempt to keep the cells alive, the mitochondria of the adipocytes are put under a very much oxidative stress because of the increased catabolism [12]. Endogenous antioxidant enzymes (like superoxide dismutase, and glutathione and catalase) and food-derived antioxidants characterizes the total antioxidant capacity (TAC) of the extracellular fluids [13]. Saliva may establish the first defense line against free radical mediated oxidative stress [14].

Materials and Method

Study Population: This study was carried in the laboratories of Biology Department, College of Science, Babylon University, Iraq. The volunteers were recruited from October to December 2018 in the clinics of College of Dentistry, Babylon University. The study was performed on 89 subjects (56 obese subjects and 33 normal subjects) aged from 6 to 50 for both genders (40 males and 49 females). Each subject was given a questionnaire included age, gender, diseases, family history and last medical check.

According to the World Health Organization [15], normal weight subjects were with BMI 18.5-24.9 kg/m^2 and obese subjects were with BMI of >30 kg/m^2. Children (aged 6-10 years) and adolescents (aged 11-18 years) were also grouped into normal weight group and obese group depending on their BMI percentile. Subjects with BMI of <85th % for age and gender were included in the normal group and subjects with BMI of ≥95th % for age and gender were considered obese subjects [1].

Subjects with diabetes, hypertension, or other diseases, or having any medicine during the last three months were excluded from the study. Smokers, alcohol abusers, pregnant and nursing women were not involved in the study.

Collection of saliva: Unstimulated whole saliva (4 ml) was collected by draining method in the morning at 8:30 to 11am after fasting for at least 8 hours. Before that subjects were told to rinse their mouth with 10 ml of water (preferably distilled) for 30 seconds to remove debris and moisturize the mucosa [16]. Immediately after saliva collection the samples were centrifuged at 3000 rpm for 15 minutes. The supernatant were removed and transferred to the eppendrof tubes and stored at -20ºC until analysis (not for more than one month).

1. **Physiobiochemical measurements:** Salivary ADP, Il-1ra and GZm B concentrations were measured using ELISA kit procedure supplied by Elabscience - China company. Salivary TAC assay was determined according to [17].

2. **Statistical analysis:** Statistical analysis was performed by using SPSS version 23. All data were analyzed using descriptive statistics for normal distribution and homogeneity of variance by the Kolmogorov–Smirnov tests before statistical analyses were managed. For comparison between obese and normal weight groups, independent t-test or Mann-Whitney U test was performed. Data were expressed as the means ± standard error. P value (P≤0.05) was considered statistically significant [18].

Results and Discussion

In the comparison between the obese and control groups of this study, there was a high significant (p≤0.01) decrease in the mean of ADP concentration (15.38 ± 1.88 ng/ml) in obese subjects compared to (28.7 ± 2.93 ng/ml) in normal weight group (figure 1). The study reveal in figure (2) no significant differences in Il-ra concentration (15.8 ± 1.3 pg/ml) of obese subjects as compared to (17.7 ± 2.29 pg/ml) in normal weight subjects. The analysis of results (figure 3) showed significant decrease in GZm B concentration in obese subjects as compared to (0.36 ± 0.02 and 0.42 ± 0.04 pg/ml respectively) in normal weight subjects. As shown in figure (4), there was a highly significant (p≤0.01) increase in the mean of TAC concentration in obese subjects as compared to (798.3 ± 7.92 and 692.41 ± 11.05 µM respectively) in normal weigh group.

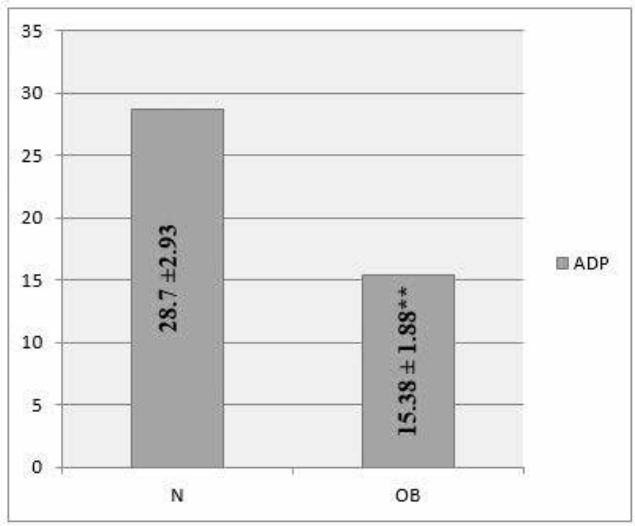

Figure 1: Adiponectin concentration (ng/ml) in normal weight and obese subjects

Data are presented as mean ± SE., OB: obese subjects, N: normal weight

**Statistical significant at p≤0.001

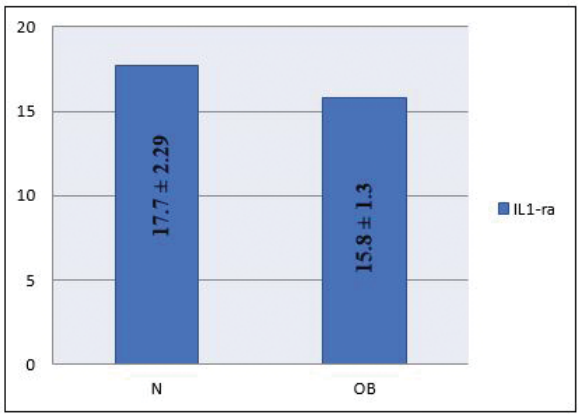

Figure 2: Interleukin-1 receptor antagonist (Il-1ra) concentration (pg/ml) in normal weight and obese subjects.

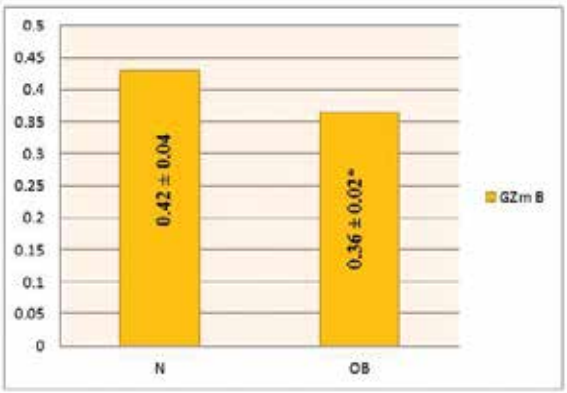

Figure 3: Granzyme B (GZm B) concentration (pg/ml) in normal weight and obese subjects.

Data are presented as mean ± SE., OB: obese subjects, N: normal weight

*Statistical significant at p≤0.005

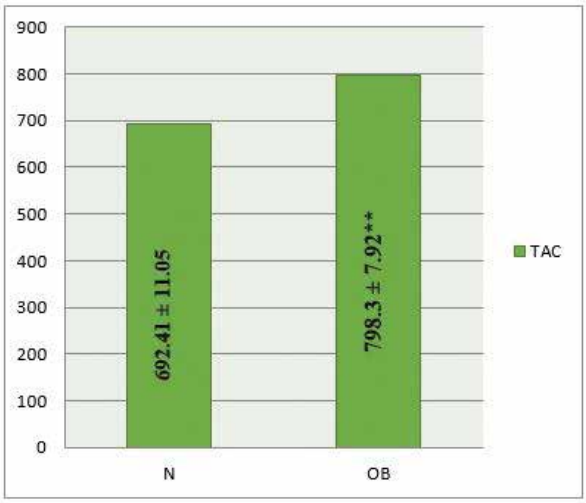

Figure 4: Total antioxidant capacity (TAC) concentration (µM) in normal weight and obese subjects.

Data are presented as mean ± SE., OB: obese subjects, N: normal weight

**Statistical significant at p≤0.001

Currently it is accepted that adipose tissue function is not only the storage for excess calories, but it also has essential secretory functions. Even though leptin, adiponectin, and resistin are peptides secreted by adipose tissue with recognized metabolic actions, white adipose tissue also secretes a number of cytokines and chemokine that take part in insulin resistance [9]. In this study four adipocytokines were measured in saliva to find out the changes in their levels in obese subjects. Three of them were significantly changed in obesity. ADP level in the saliva of obese subjects was highly significantly (p≤0.01) decreased as compared with normal weight subjects (figure 1). It has been found that the salivary ADP level was highly correlated with plasma concentrations[19,20], the plasma ADP concentration is negatively regulated in obesity [21]. It seems to help in the modulation of glucose and lipid metabolism by stimulating fatty acid oxidation, reduction of hepatic glucose production and increasing sensitivity to insulin [22]. ADP is inhibited by inflammation and oxidative stress of obesity [6]. The study reveals no significant decreasing in Il-ra levels in obese saliva (figure 2). Although it has been found that Il-ra was highly associated with obesity and chronic periodontitis [23]. Other study accorded with our study [8] showed no significant changes in Il-ra levels in the saliva of obese subject compared to normal weight subjects. In other study Serum IL-1Ra was elevated in the obese subjects [24]. IL-1Ra is an anti-inflammatory cytokine that is obviously up-regulated by IL-1β, IFN-β, and IL-4 in human obesity and it represents a promoter of obesity [9]. IL-1ra inhibits the leptin-induced reduction in food intake [25]. Although previous study [8] showed significant increase in salivary GZm B levels in obesity, this study demonstrates that GZm B levels in the saliva of obese subjects was significantly decreased (p≤0.005) in compares with normal weight subjects. NK secrete Granzyme B that acts to induce inflammation by promoting the release of cytokine [8]. NK cells frequency was changed due to severity of obesity, and was reduced in obese subjects [26]. This may affect the minimizing the GZm B levels in blood and consequently in the saliva of the obese subjects. The study showed a highly significant (p≤0.001) increase in TAC levels in the saliva of obese subjects as compared with normal weight subjects. Obesity is linked with an elevation in oxidative stress

[11]. Endogenous antioxidant enzymes and food-derived antioxidants characterizes the total antioxidant capacity (TAC) of the extracellular fluids [13]. When obesity continues for a long time, antioxidant sources can be exhausted, reducing the activity of antioxidant enzymes [27]. On the other hands studies showed elevation in TAC levels in obese people [28]. Although body has an advanced and supportive group of endogenous antioxidant defenses, consumption of antioxidant-rich diets may minimize the risk of non-communicable diseases like obesity via increasing TAC [29].

Conclusion

In conclusion the study revealed significant changes in the levels of most adiopcytokines measured in the saliva of obese subjects, and can be used as indicator to the obesity.

Source of Funding: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

REFERENCES

1. WHO. World Health Organization. Obesity and overweight. Fact Sheet. Available from: http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. 2018.
2. Zammit C, Liddicoat H, Moonsie I, Makker H. Obesity and respiratory diseases. International Journal of General Medicine. 2010; 3: 335–343.
3. Lee YH, Wong DT, Saliva: an emerging biofluid for early detection of diseases. Am J Dent. 2009;22(4): 241-248.
4. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature*, 2006; *444*(7121):847.
5. Sarhat ER, Mohammed IJ, Hamad AI. Biochemistry of Serum and Saliva in Obese Individuals with Periodontitis: Case-Control Study. *Journal of Oral and Dental Research*, 2017;*23*(52):1-10.
6. Tremblay M, Loucif Y, Methot J, Brisson D, Gaudet D. Salivary pH as a marker of plasma adiponectin concentrations in women. *Diabetology & metabolic syndrome*, 2012; *4*(1):4.
7. Lee B, Shao J. Adiponectin and energy homeostasis. *Reviews in Endocrine and Metabolic Disorders*, 2014;*15*(2):149-156.
8. Recker EN, Brogden KA, Avila-Ortiz G, Fischer CL, Pagan-Rivera K, Dawson DV, Elangovan S. Novel biomarkers of periodontitis and/or obesity in saliva—An exploratory analysis. *Archives of oral biology*, 2015*;60*(10): 1503-1509.
9. Juge-Aubry CE, Somm E, Chicheportiche R, Burger D, Pernin A, Cuénod-Pittet B, Meier CA. Regulatory effects of interleukin (IL)-1, interferon-β, and IL-4 on the production of IL-1 receptor antagonist by human adipose tissue. *The Journal of Clinical Endocrinology & Metabolism*, 2004; *89*(6): 2652-2658.
10. Tobin LM, Mavinkurve M, Carolan E, Kinlen D, O'Brien EC, Little MA, O'Shea D. NK cells in childhood obesity are activated, metabolically stressed, and functionally deficient. *JCI insight*, 2017; *2*(24).
11. Chielle EO, Casarin JN. Evaluation of salivary oxidative parameters in overweight and obese young adults. *Archives of endocrinology and metabolism*, 2017; *61*(2):152-159.
12. Abdali D, Samson SE, Grover AK. How effective are antioxidant supplements in obesity and diabetes?. *Medical Principles and Practice*, 2015; *24*(3):201-215.
13. Tinahones FJ, Murri-Pierri M, Garrido-Sánchez L, García-Almeida J M, García-Serrano S, García-Arnés J, García-Fuentes E. Oxidative stress in severely obese persons is greater in those with insulin resistance. *Obesity*, 2009*;17*(2):240-246.
14. Peluso I, Raguzzini A. Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. *Pathology research international*, *2016*.
15. WHO EC. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet (London, England)*, 2004;*363*(9403): 157.

16. Nunes S, Alessandro L, Mussavira S, Sukumaran Bindhu O. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systematic review. *Biochemia medica: Biochemia medica*, 2015*;25*(2): 177-192.

17. Apak R, Güclü K, Özyürek M, Celik SE. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*, 2008; *160*(4): 413-419.

18. Machin D, Campbell MJ, Walters SJ. Medical statistics. a textbook for the health sciences. 4th edn. John Wiley & Sons. 2007.

19. Browne RW, Kantarci A, LaMonte MJ, Andrews CA, Hovey KM, Falkner KL, Van Dyke TE. Performance of multiplex cytokine assays in serum and saliva among community-dwelling postmenopausal women. *PLoS One*, 2013*;8*(4):e59498.

20. Thanakun S, Watanabe H, Thaweboon S, Izumi Y. Comparison of salivary and plasma adiponectin and leptin in patients with metabolic syndrome. *Diabetology & metabolic syndrome*, 2014*;6*(1):19.

21. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *The Journal of Clinical Endocrinology & Metabolism*, 2001*;86*(5):1930-1935.

22. Apovian CM. Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care*, 2016*;22*(7 Suppl): s176-85.

23. Baidaa TA, Maha SH. Assessment of Salivary Interleukin-1 Receptor Antagonist and Obesity Measures of Patients with Chronic Periodontitis in Comparison to Healthy Control. *International Journal of Medical Research & Health Sciences*, 2017;*6*(9):173-178.

24. Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin?. *The Journal of Clinical Endocrinology & Metabolism*, 2002; *87*(3):1184-1188.

25. Perrier S, Darakhshan F, Hajduch E. IL-1 receptor antagonist in metabolic diseases: Dr Jekyll or Mr Hyde?. *FEBS letters*, 2006*;580*(27): 6289-6294.

26. Lynch LA, O'connell JM, Kwasnik AK, Cawood TJ, O'farrelly C, O'shea DB. Are natural killer cells protecting the metabolically healthy obese patient?. *Obesity*, 2009*;17*(3):601-605.

27. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women?. *Arya Atherosclerosis*, 2010*;2*(4).

28. Gunjalli G, Kumar KN, Jain SK, Reddy SK, Shavi GR, Ajagannanavar SL. Total salivary anti-oxidant levels, dental development and oral health status in childhood obesity. *Journal of international oral health: JIOH,* 2014;*6*(4): 63.

29. Peluso I, Raguzzini A. Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. *Pathology research international*, 2016.