Reliability of Salivary Alpha Amylase as a Biomarker of Obesity

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ABSTRACT

Obesity is generally defined as a condition of body fat accumulation in adipose tissue in excessive manner that may lead to impaired health. Saliva is an easy collected noninvasive biofluid, which contains various components like hormones and enzymes. The researches correlated to salivary α-amylase (sAA) and obesity are quite limited and not fully examined. This study aimed at finding out the reliability of salivary amylase as a biomarker to obesity. The study results showed a significant increase in anthropometric measurements (BMI, NC, WC, HC and WHR) and salivary α-amylase concentration and activity in obese subjects when compared with normal ones. In conclusion, salivary α-amylase activity was increased in obese subjects and could be an indicator to obesity. Further studies should be carried out to confirm that the salivary amylase to be a biomarker of obesity.

Keywords: *obesity, salivary amylase, BMI, NC, WC, HC, WHR*

Introduction

Obesity is a common but undervalued condition of public health and clinical importance all over the world, and is a result of fat accumulation in adipose tissue in excessive manner that may lead to impaired health [1]. The total amount of fat is not the only important issue in the risks of obesity but where it is distributed in the body [2]. Peripheral or abdominal obesity is supposed to be more risky and related morbidity and mortality because it involves more of the visceral organs [3]. Commonly Body Mass Index (BMI), is the mostly used parameter to express what is considered as an "excess" of body fat [4]. It is easy to measure by calculating the weight in kilograms divided by height in squared meters (kg/m2) in adults [5]. For children and adolescents, the BMI ranges is considered according to their sex and age and expressed as gender-specific BMI percentiles in the growth charts [6]. Waist circumference (WC) is used as simple anthropometric measurements of central obesity [2]. Neck circumference (NC) is a measurement of upper body obesity and could be a significant indicator of central adiposity and maybe of visceral adiposity and a significant risk sign of metabolic conditions [8]. Saliva is an easy collected noninvasive biofluid secreted primarily from the three pairs of major salivary glands: parotid, submandibular and sublingual, which contains hormones, enzymes, antibodies, antimicrobial component, and cytokines [9]. Salivary amylase is one of the first enzymes recognized with several mysteries remain about the molecular mechanisms contribute to amylolysis of starch and the physiological role of the salivary amylase itself. The most important function of the salivary α-amylase enzyme is to catalyze the first stage in starch digestion into dextrin and maltose [10]. The studies on salivary α-amylase (sAA) and obesity are quite limited and not fully examined. Moreover the study results that have been informed are mostly contradictory in many aspects. Limited studies have been done on sAA and its role in diabetes, proposing a close association between the salivary amylase activity and glucose homeostasis [11,12]. Studies found a positive correlation between sAA activity and obesity expressed by increased BMI in adolescent [13,14], and a significant positive association between sAA and waist to hips ratio (WHR) in women [15]. While another study revealed that average morning sAA was negatively associated with BMI [16].

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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 [5], 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 [17]. 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.

Anthropometric measurements: Height was measured by meters and weight was measured in kilograms by a digital weight scale. BMI were obtained as the weight in kilograms divided by the square of the height in meters (kg/m2) according to WHO [5]. The WC, HC and NC were measured by centimeter according to the protocol of WHO[18].

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 [19]. 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).

Salivary α-amylase measurements: Salivary alpha amylase concentration was measured according to ELISA kit procedure supplied by Elabscience - China company. The activity of sAA was determined according to Sahu *et al.*[20]

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. Bivariate correlations were performed using the Pearson correlation coefficient between the anthropometric measurements and sAA measurements[21].

Results and Discusion

Anthropometric measurements in normal weight and obese subjects: In the comparison between the obese and control groups, there was a highly significant (p≤0.01) increase in the means of BMI, NC, WC, HC and WHR (31.17 ± 0.7 kg/cm^2, 35.09 ± .67 cm, 93.21 ± 2.21cm, 101.85 ± 2.42 cm and 0.93 ± .023 respectively) in obese group as compared to the means of (18 ± 0.69 kg/cm^2, 30.00 ± .84 cm, 65.58 ± 2.59cm 80.48 ± 2.78 cm and 0.82 ± .02 respectively) in normal weight group (table 1).

The correlations between anthropometric measurements in obese subjects (table 2) revealed that BMI has highly significant (p≤0.01) positive correlation (R^2= 0.77, 0.78 and 0.65) with NC, WC and HC respectively. NC measurement was high significant (p≤0.01) positively correlated (R^2=0.85, 0.58) with WC and HC respectively, and significant (p≤0.05) positively correlated (R^2=0.33) with WHR. Furthermore WC as a measurement to central obesity had highly significant (p≤0.01) positive correlation (R^2= 0.71) with HC and positively significant (p≤0.05) correlated (R^2=0.33) with WHR (table 2).

Table 1: Anthropometric measurements in normal weight and obese subjects

Subjects	BMI (kg/m^2)	NC (cm)	WC (cm)	HC (cm)	WHR
N	18 ± 0.69	30.00 ± .84	65.58 ± 2.59	80.48 ± 2.78	0.82 ± .02
OB	31.17 ± 0.7**	35.09 ± .67**	93.21 ± 2.21**	101.85 ± 2.42**	0.93 ± .023**
P value	0.000	0.000	0.000	0.000	0.000

Data are presented as mean ± SE.

N: normal weight, OB: obese subjects, BMI: body mass index, NC: neck circumference, WC: waist circumference, HC: hip circumference, WHR: waist-to-hip ratio.

**Statistical significant at p≤0.001

Table 2: Correlation between anthropometric measurements in obese subjects

Anthropometric measurements	BMI		NC		WC		HC	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
NC	0.77**	0.000	-	-	-	-	-	-
WC	0.78**	0.000	0.85**	0.000	-	-	-	-
HC	0.65**	0.000	0.58**	0.000	0.71**	0.000	-	-
WHR	0.14	0.14	0.33*	0.000	0.33*	0.012	-0.41**	0.002

OB: obese subjects, N: normal weight, BMI: body mass index, NC: neck circumference, WC: waist circumference, HC: hip circumference, WHR: waist-to-hip ratio.

*Statistical significant at p≤0.005

**Statistical significant at p≤0.001

Salivary α-amylase measurements in normal weight and obese subjects: The analysis of results showed no significant difference in the concentration of the enzyme α-amylase in saliva of both groups of this study (37.67 ± 5.62 ng/ml in normal weight subjects and 38.61 ± 4.14 ng/ml in obese subjects) as shown in figure (1), while the α-amylase enzyme activity revealed significant (p≤0.05) elevation (figure 2) in obese subjects (335.85 ± 34.58 U/ml) as compared to normal weight subjects (285.78 ± 35.47 U/ml).

As shown in table (3), α-amylase concentration was significant (p≤0.05) positively correlated (R^2=0.37 and (0.36) with BMI and NC respectively. Meanwhile the α-amylase activity was significant (p≤0.05) negatively correlated (R2=-0.43 and 0.38) with NC and WC of obese subjects.

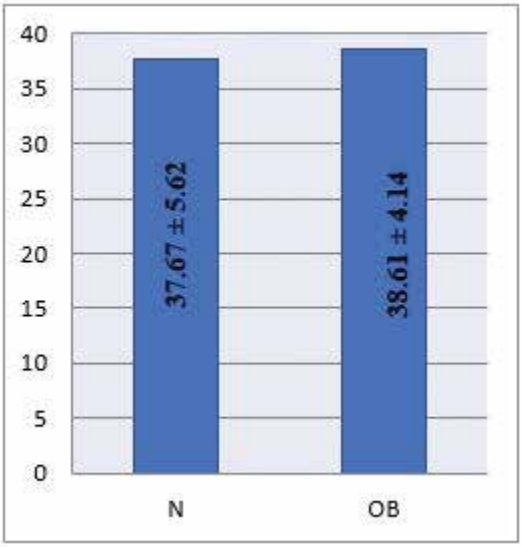

Figure 1: Salivary α-amylase concentration (ng/ml) in normal weight and obese subjects.
Data are presented as mean ± SE., OB: obese subjects, N: normal weight

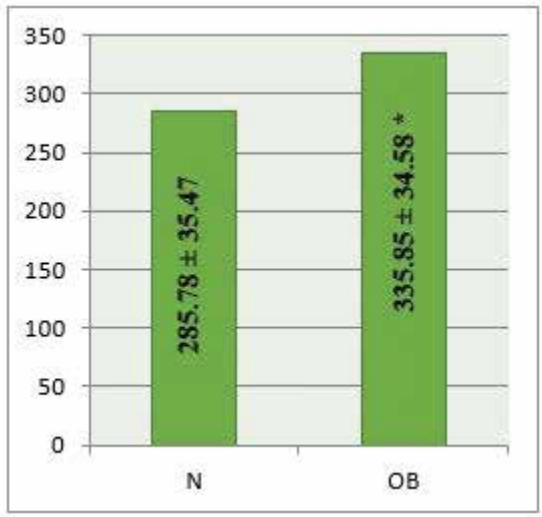

Figure 2: Salivary α-amylase activity (U/ml) in normal weight and obese subjects.
Data are presented as mean ± SE., OB: obese subjects, N: normal weight
***Statistical significant at p≤0.001**

Table 3: Correlation between anthropometric measurements and α-amylase enzyme measurements

Anthropometric measurements	α-amylase conc.		α-amylase activity	
	R^2	P value	R^2	P value
BMI	0.37*	o.o42	-0.34	0.062
NC	0.36*	0.047	-0.43*	0.016
WC	0.26	1.54	-0.38*	0.041
WHR	0.07	0.59	-0.12	0.37

BMI: body mass index, NC: neck circumference, WC: waist circumference, WHR: waist-to-hip ratio

*Statistical significant at p≤0.005

Discussion

Anthropometric measurements in normal weight and obese subjects: The present anthropometric study object was to recognize the most appropriate anthropometric measurement that best correlates with obesity in the population of this study. From table (1) all the anthropometric measurement involved in the current study were high significantly elevated in obese subjects, and correlated with each other (table 2). The BMI was strongly associated with body fat [4], but abdominal obesity is a distinct predictor of mortality and morbidity, even in people with a normal BMI [22].

The recent study showed high significant increasing in WC in obese subjects comparing to normal weight subjects. WC is used as simple anthropometric measurements for abdominal obesity. WC than 102cm in men and 88cm in women is a risk factor for diabetes mellitus, insulin resistance and cardiovascular disease [2].

NC was highly elevated in obese group of this study, it could be a significant indicator of central or visceral adiposity and a significant risk sign of metabolic conditions. Also it could be considered an important measurement in health care clinics especially pregnant women where classical measures are not meaningful [7]. NC can be used as a primary test for the prediction of obesity[23].

WHR was highly raised in obese group of this study. WHR has lately been proposed as a useful alternate method of percentage body fat assessment. But BMI is still the most commonly used method for identifying obesity in most epidemiological studies [24].

Salivary α-amylase measurements in normal weight and obese subjects: From this study (figure 1&2), sAA concentration and activity were increased in obese subjects. The concentration of enzyme in obese saliva was not significantly increased as compared with the activity of the enzyme in obese saliva. sAA catalyzes starch digestion into dextrin and maltose [10]. One study revealed a positive association between salivary α-amylase activity and increased BMI in adolescent [25]. Another study found that BMI was negatively associated with average morning sAA [16]. Lamy *et al*. mentioned that sAA activity increased in obese women as compared with normal weight women [13]. Hossain *et al*. found that sAA activity to be positively linked with BMI [14]. Most studies linked salivary amylase with diabetes and insulin [11] and cardiovascular risks [15]. A recent study proposed the association between sAA concentration and sweet taste marks in children. The favoring of sweet and high-fat foods is often described as being related to obesity occurrence. Salivary amylase may increase glucose levels in saliva. A relationship between salivary glucose levels and low-sensitivity to sweet taste was studied and explained as a higher continual stimulation of taste receptors to glucose which may lead to a desensitization of these receptors, causing the need for higher concentrations of sweet stimuli to be recognized [26]. Desensitization of taste receptors because of the constant stimulation has earlier been described [27].

Conclusion

In conclusion salivary α-amylase activity was increased in obese subjects and could be used as an indicator of obesity. Further studies should be carried out to confirm its use as a biomarker of obesity.

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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.

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