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INVESTIGATION OF ZINC CONCENTRATIONS IN SERUM AND SALIVA OF OBESE

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ABSTRACT

Obesity is rapidly growing epidemic worldwide, influenced by both genetic and environmental factors. The onset of obesity is due mainly to low energy expenditure (such as from exercise) combined with high caloric intake. In this case, control study, serum and saliva of 22 obese with (18-39 years) and 22 healthy person, who matched with the case group in respect of age and gender was gathered and analyzed in order to evaluate serum and saliva Zn levels. Blood and saliva samples obtained from AL-kendy Hospital in Baghdad and are compared with that of control group. The data was analyzed using SPSS and independent t-test. The Zn levels were determined by an atomic absorption spectrophotometer. Our results demonstrated that serum Zn levels were significantly increased in total, male (p = 0.0001, 0.012), respectively, while non-significantly increased in female of obese as compared with control subjects. In saliva, Zn levels were decreased non-significantly in total, male and female of obese as compared with control subjects. The correlation study between serum and saliva Zn levels indicated that there were significant lower levels (r = 0.972, p = 0.001) in male of obese.

Key words: Serum zinc, Saliva zinc, Obesity.

INTRODUCTION

Medically defined, obesity is a physiological dysfunction in humans with environmental, genetic and endocrinological causes[1]. Imbalance between taking food and consuming calorie leads to overweight and obesity[2]. It's widely prevalent and over 1.5 billion adults worldwide are classified as either overweight or obese, with rates continuing to

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increase[3]. According to the World Health Organization (WHO), there were one billon adults overweight and about 400 million obese people in 2005 while in 2015, the number will become 700 million people. The incidence of obesity in the Arab world (mostly Arab gulf countries) was approximately similar to that found in developed countries[4]. Obesity classification depend on the body mass index (BMI), that equal to weight (in kilograms) divided by the square of height (in meters)[5]. In obese people, the metabolic disturbances are decompensate. Although, overweight is a preclinical condition, obesity is the clinically manifested metabolic disorder, including mineral imbalances[6]. Women seemed to be more at risk for toxic metal exposure than men and at the same time, more vulnerable to micronutrient deficiency[7].

The clinical significance and evaluation of trace elements remain controversial and many questions still remain unanswered. The essential trace element zinc (Zn), an intracellular signaling molecule and an anti-inflammatory agent, is instrumental in immune response and serves important functions in the body with its antioxidant properties[8]. Moreover, it is important for several bodily functions such as vision, taste perception, cognition, cell reproduction, growth and immunity. It plays a vital role in metabolisms, particularly as a cofactor of many enzymes, required for natural metabolic processes[9]. In addition it, is present in small amounts in all tissues and body fluids, including saliva. Saliva consists mainly of water (98%), electrolytes, mucus, antibacterial constituents, and various enzymes. It is well known that the composition of saliva depends on a number of factors related to physiological, pathological, environmental factors, and the humoral agents[10]. It is known that saliva could offer an excellent alternative to serum as a biological fluid analyzed for diagnostic purposes. This would be of great biomedical importance, because it is easy to collect, offering a cost-effective approach for screening of large populations, and represent an alternative for patients, whose blood is difficult to obtain or when compliance is a problem, which can be repeated more frequently[10,11].

Therefore, in the present study, we have investigated serum and saliva Zn levels and correlations between them in obese as compared with control subjects.

EXPERIMENTAL

Materials and methods

Ethics

Informed parental consent was gained to be eligible to enrollment to our work, which was done to the requirement rules of the Local Ethics Committee of Chemistry Department, College of Science, Al-Mustansisiyah University, Baghdad, Iraq.

Subjects

A total of 22 obese, attending obese treatment and research center in AL-kendy Hospital in Baghdad city were included in this study. The obese diagnosed as having hepatitis, diabetes mellitus were excluded. The samples were collected during the period 11-2014 till 1-2015. They were compared to a control group of 22 healthy individuals, who matched for age and gender (Table 1).

Table 1: The information of all obese and control subjects

Groups	No.	Serum & saliva samples	Gender	No.	Age range (year)
Controls	22	44	M	11	18-38
			F	11	19-38
Obese	22	44	M	11	19-39
			F	11	18-39

M: Male, **F**: Female

Blood collection

Blood samples (5) mL were collected from the healthy donors and obese. Blood samples were centrifuged at (1500 xg) for 10 min. after blood coagulation, serum thus separated into two eppendorf tubes and frozen at -20ºC until being used. The samples were not thawed and refrozen before using.

Saliva collection

The study of saliva secretion was performed without any stimulus in the morning (9 h-11 h). All subjects refrained from eating, drinking and smoking for a minimum of 2 h before saliva collection. They rinsed their mouths several times by water, then waited for 1-2 min. (for water clearance). Unstimulated saliva was collected in sterilized clear containers. Therefore, the saliva was collected for 5 min. into a measuring container. The saliva was centrifuged at (1500 xg) for 10 min., and the supernatant was kept frozen at -20ºC until the time of assay[12].

Estimation of BMI

Body Mass Index (BMI) is a measure of someone's weight in relation to their height. BMI is equal to a person's weight divided by their height square. It is calculated as:

$$\text{BMI} = (\text{Weight in kilograms} / \text{Height in meters}^2) \quad \ldots(1)$$

The BMI is used to screen persons for weight categories that may lead to health problems. Equal or less than 18.5 (Kg/m^2) indicate underweight, between 18.5-24.9 indicated a person of normal weight, 25-29.9 is overweight, while equal or more than 30 is obese[13].

Measurement of Zn concentrations

Zn concentrations were determined using atomic absorption spectrophotometer (Shimadzu, AA-7000f). For the determination of Zn in serum or saliva, the slit width was 0.4 nm, lamp flow was 3.0 mA, and wavelength was 213.9 nm. The linear range of Zn standard curve was 0.0-0.6 μg/mL (ppm). Detection limit of Zn was 0.009 μg/mL. All of the reagents were of analytical grade and the water used was deionized distilled water. Just before analysis, the samples were allowed to defrost at room temperature and vigorously shaken in a vortex for a half of min to rehomogenize it just before analysis. Aliquot (1 mL) of serum or saliva samples were diluted with 2 mL of 1% nitric acid immediately before the assay. The levels of serum or saliva Zn were calculated from a suitable calibration curve (r = 0.9995). A concentration of Zn element was calculated as μg/mL mixed serum or saliva volume.

Statistical analysis

The results were expressed as mean ± SD. Student’s t-test were used for the evaluation of data. Statistical analysis was performed with SPSS version 22.0 software. The correlations between variables were performed by Pearson's correlation test. Differences were considered significant at a probability level of $p < 0.05$.

RESULTS AND DISCUSSION

The determination of Zn Serum and salivary levels were carried out by atomic absorption spectrometry using flame, and are shown in Table 2 and Fig. 1. According to the results, serum Zn levels were significantly higher in total, male (p = 0.0001, 0.012), respectively, while non-significantly increased in female of obese as compared with control subjects. On the other hand, saliva Zn levels were non-significantly lower in total, male and female of obese as compared with control subjects.

Table 2: Serum and saliva Zn levels with characteristics of obese and control subjects

	Obese (Mean ± SD)	Control (Mean ± SD)	*p*-Value
No.	22 (11M, 11F)	22 (11M, 11F)	-
Age (year)	28.45 ± 8.50 (Range 18-39)	24.50 ± 5.28 (Range 18-38)	0.071
BMI (Kg/m^2)	38.63 ± 6.36	23.14 ± 2.90	< 0.0001**
Saliva Zn (µg/mL)	T 0.949 ± 0.306 M 1.029 ± 0.124 F 0.868 ± 0.446	T 1.062 ± 0.048 M 1.051 ± 0.014 F 1.073 ± 0.072	0.392 0.779 0.477
Serum Zn (µg/mL)	T 1.655 ± 0.178 M 1.734 ± 0.082 F 1.575 ± 0.232	T 1.165 ± 0.157 M 1.147 ± 0.216 F 1.183 ± 0.117	0.0001* 0.012* 0.059

**Highly Significant p < 0.0001, *Significant p < 0.01, T: Total, M: Male, F: Female

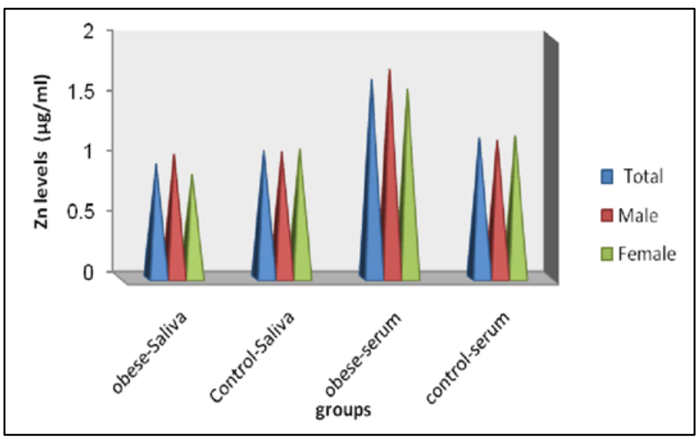

Fig. 1: Serum and saliva Zn levels of obese and control subjects

The correlation study between serum and saliva Zn levels indicated that there were significant lower levels (r = 0.972, *p* = 0.001) in male of obese (Table 3).

As serum levels of a variety of electrolytes are correlated to saliva levels of these electrolytes, it is possible that some of the factors that affect serum Zn levels will also affect saliva Zn levels. It has been reported that various factors including diet, stress, infection, age, pregnancy, medications and oral contraceptives affect Zn levels[10]. It was revealed that body fat deposition can be aggravated by zinc supplementation in both types of obese mice. Zinc may be allied with the energy homeostasis of obesity via its interaction with dietary fat consumption[14].

Table 3: Correlation between serum and saliva Zn levels of the groups

	Obese	**Control**
Total Zn levels (µg/mL)	r = 0.440 *p* = 0.153	r = 0.840 *p* = 0.001*
Male Zn levels (µg/mL)	r = 0.972 *p* = 0.001*	r = 0.990 *p* = 0.0001**
Female Zn levels (µg/mL)	r = 0.773 *p* = 0.072	r = 0.569 *p* = 0.238

**Highly Significant p < 0.001, *Significant p < 0

There are a few researches in the literature investigating the level of serum and saliva Zn in obese. Although there are some conflicting findings. However, Zakya et al.[15] have found that the zinc in serum, no statistically significant difference between the control and the obese groups, the same of our results in female subject. Weisstaub et al.[16] found that the plasma zinc was not associated with body composition as in overweight preschool children. The results of the present study were disagreement to those of Yerlikaya et al.[17], who found decrease of serum Zn levels were significant as for women as compared with healthy controls. Trace elements and minerals influence the pathogenesis of obesity and diabetes and their complications, mainly through their involvement in peroxidation and inflammation. In obese people, the metabolic disturbances are decompensated. Although, overweight is a preclinical condition, obesity is the clinically manifested metabolic disorder, including mineral imbalances. Also, in non-accordance with other study, Mohammed et al.[18] show that the analysis of salivary Zn concentration was highly significant higher among the overweight females than that among the normal weight. Moreover, other study has also shown that obese individuals have low concentrations of zinc in plasma, erythrocytes and serum, and that it is associated with alterations in the metabolism of the adipose tissue of obese[19].

CONCLUSION

Our results show that serum Zn levels are affected by obesity. Also, further studies involving larger number of subjects with parallel measurement of Zn both in serum and saliva may provide additional information about this element in obese subjects.

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