



SALIVARY TNF- α AND ALP RELATED TO THE GENDER OF CHILDREN

Suha T. Abd

Department of Basic Sciences, College of Dentistry, University of Baghdad, Iraq.

ABSTRACT

The compositions of saliva are an important marker to reflect healthy physiological state of the body. The gender is very effective factor in many physiological aspects even in children. The aim of this study was to show if there is any difference between male and female of children in concentration of TNF- α and ALP in saliva. Sixty primary school children were selected for this study. The sample was unstimulated whole saliva. Estimation of salivary TNF- α concentration was done by Elisa kit. While the salivary Alkaline Phosphatase activity was spectrophotometrically determined. The non-parametric tests for this study showed significant difference between male and female children in concentration of salivary TNF- α . While non-significant difference for the activity of salivary ALP. The concentration of salivary cytokines TNF- α is greater in male children as compared with female children. While the activity of salivary ALP is greater in female children in comparison to male children. There are physiological differences between male and female children lead to difference in some composition of saliva.

KEY WORDS: Salivary compositions, TNF- α , ALP.

INTRODUCTION

Saliva, an essential physiologic fluid including a very complicated mixture of substances, is quickly gaining attractiveness as a diagnostic tool. Saliva, as a mirror of oral and systemic health, is an important source for clinically appropriate information because it contains biomarkers specific for the distinctive physiologic aspects^[1]. Tumor necrosis factor alpha (TNF- α), the cytokines belong to a family of low-molecular weight, cell-to-cell mediators that regulate many immune and inflammatory responses. It is an imperative mediator in inflammatory reactions and appears to play a fundamental role in the pathogenesis of severe chronic inflammatory diseases^[2]. Variations in the rate of production of TNF have been verified and a familial ability to produce lower or higher cytokine levels appears to occur^[3]. Salivary proteins for instance CD44, defensin-1, TNF alpha, IL-1, 6 and 8 and CEA displayed increase in their detection in patients with oral cancer^[4]. Alkaline phosphatase (ALP) is a broad-spectrum hydrolase enzyme present in all bodily tissues, but is predominantly concentrated in the liver, bone and kidney^[5]. ALP is a catalyzing enzyme that quickens the removal of phosphate groups in the 5 and 3 positions from a variety of molecules, including nucleotides, proteins, and alkaloids. Although ALP is nearly present in all tissues, but it is likely to be largely derived from the periodontal tissues^[6]. The major source of ALP within gingival crevicular fluid is host derived and in early inflammatory disease is likely to be of polymorphonuclear leukocyte origin^[7]. Salivary ALP is one of the sensitive markers for the early detection of oral diseases like carcinoma. Diagnosis of saliva can be achieved in all the dental institutions to assess the malignant risk

potential of potentially malignant disorders and thus quality of life of patients can be enhanced^[8]. This study aimed to show if there is any difference between male and female in children in concentration of salivary TNF- α and activity of ALP in saliva.

MATERIALS & METHODS

Subjects of the study

Sixty primary school children were selected for this study from schools of Baghdad city. Their ages ranged between (6-8) years. Thirty children were male and thirty children were female. All the children seemingly healthy and does not undergo any systemic diseases.

Sample collection

Three milliliters of un-stimulated whole saliva was collected from the children. Collection of saliva was done according to the standard method for collection saliva cited by Tenovou and Lagerlof^[9]. Collection was performed 2-3 hours after the volunteer usual breakfast time and after thoroughly rinsing the mouth with water. Saliva was collected by standard spitting method from both groups: male and female group then saliva collected in a plane tube, centrifuged 10 minute at 3000 xg, and the centrifuged supernatant liquid was stored in deep freeze at (-20°C) until the time of chemical analysis.

Estimation of TNF-in saliva

The detection of TNF- α by ELISA kit at 570 nm wave length. The principle of detection by using double-sandwich Elisa technique. The pre coated antibody is human TNF- α monoclonal antibody and the detecting antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody are added into ELISA plate wells and

washed out with phosphate buffer solution. Then A Vidin-peroxidase conjugates are added to ELISA wells in order. Use Tetramethylbenzidine substrate (TMB) for coloring after reactant thoroughly washed out by PBS. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated. Figure 1 shows the standard curve of TNF- of saliva sample.

Estimation of ALP in saliva

Alkaline phosphatase activity was measured to determine the best saliva volume for this experiment by using different volume of sample (20, 40, 60, 80 and 100) μ l. The salivary ALP activity was spectrophotometrically determined according to the recommendation of the German Clinical Chemistry Association (using the kit of Human Company, Germany). In the presence of alkaline phosphatase (100 μ L of saliva), p-Nitrophenylphosphate is reduced to p-nitrophenol. The wave length of absorbance at 405 nm is directly proportionate to the ALP activity in the sample of saliva.

Statistical analysis

The results were statistically analyzed using SPSS version 14 descriptive statistics (number of the samples, mean and standard deviation) were used in this study. The mean rank and Mann-Whitney U nonparametric tests were used. P values over 0.05 were considered statistically significant.

RESULTS

The Shapiro-Wilk test of normality show the results of all data in this study was not normally distributed < 0.05 (Table 1). So the statistical analysis for all data was non-parametric tests. Descriptive statistics (mean, standard deviation with the number of the samples) for the TNF- and ALP in two groups (male and female) in children. Table 2 and table 3.

Figure 2 and figure 3 show the difference in concentration of TNF- and activity of ALP respectively in saliva of children between male and female. Table 4 display mean rank and Mann Whitney U test for the non-parametric data of this study to estimate the significant level between male and female for the salivary TNF- and ALP. The significant level for TNF- was significant while for the ALP was non-significant.

TABLE 1: Tests of Normality for the data of study

| | Groups | Shapiro-Wilk | | |
|-----|--------|--------------|----|-------|
| | | Statistic | df | Sig. |
| TNF | male | 0.700 | 30 | 0.000 |
| | female | 0.686 | 30 | 0.000 |
| ALP | male | 0.819 | 30 | 0.000 |
| | female | 0.818 | 30 | 0.000 |

TABLE 2: Descriptive statistics for salivary TNF- in two groups (male and female)

| Concentration of TNF- in saliva (pg/ml) | N | Mean | Std. Deviation |
|--|----|-------|----------------|
| Male | 30 | 7.272 | 4.718 |
| Female | 30 | 6.340 | 4.4387 |

TABLE 3: Descriptive statistics for salivary ALP in two groups (male and female)

| Activity of ALP in saliva (U/L) | N | Mean | Std. Deviation |
|---------------------------------|----|-------|----------------|
| Male | 30 | 1.373 | 1.289 |
| Female | 30 | 1.742 | 1.460 |

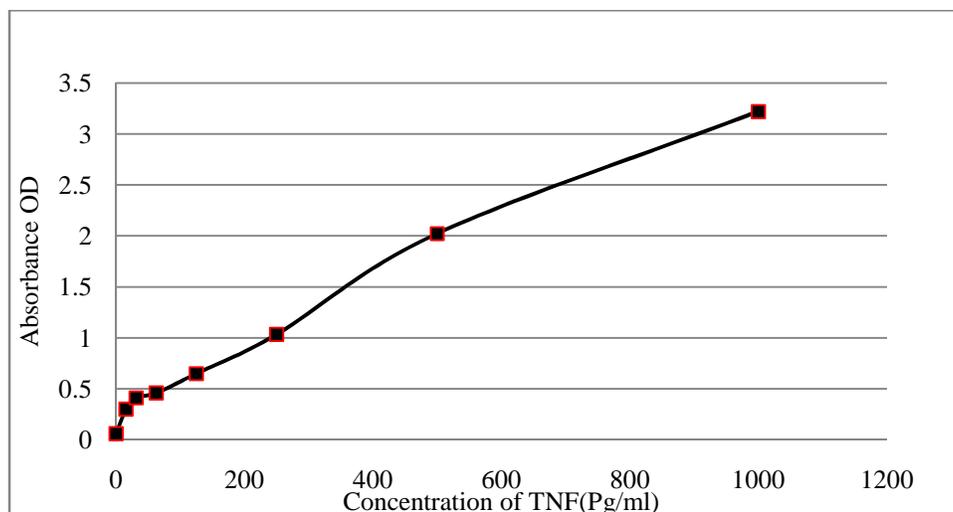


FIGURE 1: Standard curve of TNF-

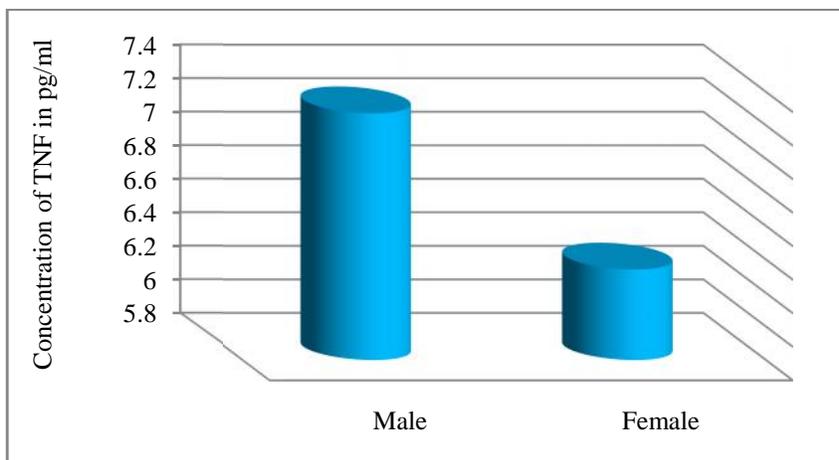


FIGURE 2: Difference in salivary concentration of TNF- between male and female

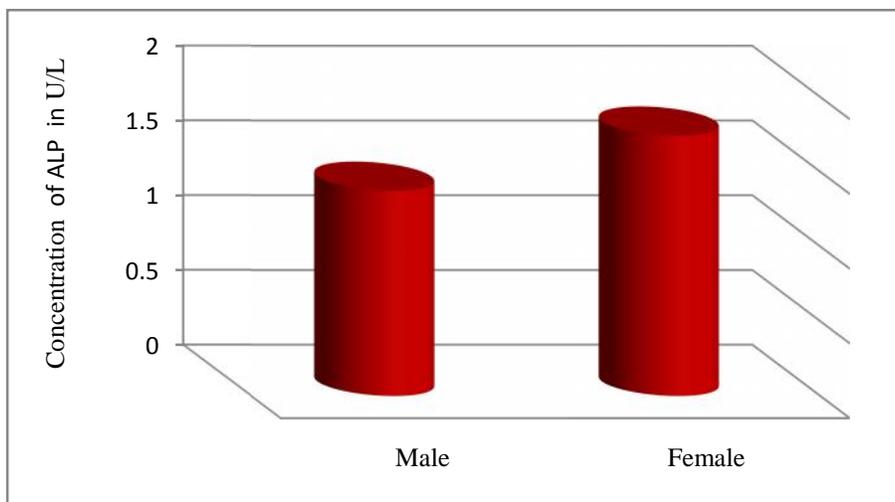


FIGURE 3: Difference in salivary concentration of ALP between male and female

TABLE 4: Significant level by Mean Rank and Mann Whitney U test for TNF- and ALP between the two groups

| Groups | N | Mean Rank | Mann-Whitney U | p-value | Sig. (2-tailed) |
|--------|--------|-----------|----------------|---------|-----------------|
| TNF- | male | 30 | 34.90 | 318.000 | 0.048 |
| | female | 30 | 26.10 | | |
| ALP | male | 30 | 27.52 | 360.500 | 0.184 |
| | female | 30 | 33.48 | | |

DISCUSSION

The results of this study show significant increase of salivary TNF- in male related to female. The mean concentration of TNF- is 7.00 pg. /ml in male and 6.80 pg. /ml in female. These results resemble the results of Yousefimanesh *et al.*, 2013^[10] in which concentration of salivary TNF- in male and female was 9.7 and 8.5 pg/ml respectively but the simple difference in this value may be related to age difference between the two studies. The explanation for increasing of salivary TNF- in male in comparison to female may be due to physiological nature of the children. Or may belong to activity of children in male in comparison to female lead to released greater amount of TNF- in male.

As shown in result of Timothy *et al.*,1999^[11] there is an increase of systemic inflammatory cytokines IL-6, TNF- , and IGFBP-1due to response to active exercise prone to be encountered in daily life activities of many American children. Hormonal changes between male and female children may lead to this difference in concentration of TNF- in saliva. All these results are in contrast to the result of the study of Nao *et al.*, 2016^[12] in which there was opposite relationship between TNF- level in saliva and vigor scores. But the difference between this study and study of Nao was the saliva sample collected in study of Nao from healthy adult's smoker not children as in this study. In concern to the ALP in saliva the non-significant increase of salivary

alkaline phosphatase in female as compared with male children, there was not found any study to compare with other results. This slightly increase concentration of ALP in saliva may be due to physiological difference between male and female.

CONCLUSION

This study conclude that the concentration of salivary TNF- α was higher in male than female in children while the concentration of salivary ALP was higher in female than male although the difference between them was non-significant. There is a physiological difference between male and female children in composition of saliva.

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