Salivary and seral sex steroid hormones variation among group of peoples with gingivitis

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ABSTRACT

Background: This study aimed to determine the level of sex steroid hormones (estradiol, testosterone) in the sera and saliva of the patients and controls, also to establish the type of correlation between the change in the biochemical inflammation markers (ALP, C-RP, IL-6, ALb) and levels of sex hormones (estradiol, testosterone) in gingivitis patients and control.

Sample and Methods: The work included ninety samples (serum and saliva) of 45 volunteers (22males & 23females), twenty one patients with gingivitis and twenty four healthy subjects as control group.

Results: the results showed a highly significant increase ($p<0.001$) in IL-6 for male in serum and saliva samples while significant increase ($p<0.05$) for females And a highly significant increase ($p<0.001$) in ALP activity in serum and saliva while significant increase ($p<0.05$) in specific activity of ALP for serum and saliva in both (male and female), and a non-significant decrease ALB Level ($p>0.05$) in serum and saliva in patients with gingivitis. The levels of serum and saliva testosterone were significantly decrease ($p<0.05$) in gingivitis than healthy group. While estradiol level was highly significant increase ($p<0.001$) in saliva and serum gingivitis patients in compared to the healthy group. The results of studying the correlation between the above parameters indicated there were a highly significant positive correlation obtained is between sera E2 with (ALP, IL-6,) and between sera testosterone with (ALP, IL-6), and between saliva testosterone with albumin .while negative correlation between sera E2 with ALB, and between saliva E2 with all three inflammation marker (i.e. ALP, albumin and IL-6), also negative association was found between sera testosterone with ALB and between salivary testosterone with (ALP and IL-6).

Conclusion: the results generated from this study could lead to improvement in gum care program for peoples and revealed an association between gingivitis progression with hormonal changes production as result to increase the inflammation marker (ie IL-6, ALP).

Also saliva analysis is better for determination the free bioavailable steroid hormones than sera analysis

Key words: Sex steroid hormones, gingival inflammation, albumin, interleukin-6 (IL-6), specific activity of alkaline phosphates (ALP). (J Bagh Coll Dentistry 2012; 24(4):103-108).

INTRODUCTION

Periodontal diseases (gingivitis and periodontitis), are two major forms of inflammatory diseases affecting the periodontium (gum). Their primary etiology is bacterial plaque, which can initiate destruction of the gingival tissues and periodontal attachment apparatus. Plaque is a sticky white colored layer which forms on the tooth surface. This layer is rich in bacteria which gets stick on teeth and slowly starts eroding the upper layer of teeth (Enamel)[1]. Gingivitis is inflammation of the gingiva that does not result in clinical attachment loss [2]. Hormones are specific regulatory molecules that have potent effects on the major determinants of the development and the integrity of the skeleton and oral cavity including periodontal tissues. It is clear that periodontal manifestations occur when an imbalance of these steroid hormones take place [3,4].

The homeostasis of the gingiva involves complex multifactorial relationships, in which the endocrine system plays an important role. Sex steroid hormones may alter immunologic factors and responses, including antigen expression, and they seem to modulate the production of Cytokines [5] in other word, Sexual hormones have been suggested to play a major role in modifying factors which influence the pathogenesis of gingival disease [4].

Androgens may play a significant role in the maintenance of bone mass and inhibit osteoclastic function, inhibit prostaglandin synthesis and reduce interleukin-6 (IL-6) production during inflammation [5] and progesterone reduce Il-6 production to 50% of that of control values [4]. This down-regulation can affect the development of localized inflammation, and gingival becomes less efficient at resisting the inflammatory challenges produced by bacteria [5].

The measurement of specific sera and saliva (ALP) activity may be valuable in the diagnosis of human periodontal disease; alkaline phosphatase has often been measured as possible indicators of gingival inflammation and bone metabolism, alkaline phosphatase levels change in relation to gingival inflammation and bone loss [6].

The levels of liver protein change during inflammation are termed acute phase proteins (APPs) [7]. Acute-phase proteins (APPs) are an evolutionarily conserved family of proteins produced mainly in the liver in response to infection and inflammation [8]. Interleukin-1 (IL-

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1), interleukin-6 (IL-6), and tumor necrosis factor (TNF) are the major cytokines that stimulate the liver to synthesize (c- reactive protein) C-RP and other positive acute-phase proteins [9]. There are large and varied groups of glycoproteins in serum released into blood stream in response to a variety of stress. All the up-regulated proteins have been called positive APP like C-reactive protein(C-RP), in order to differentiate them from the so-called negative APP that is down-regulated like albumin [8].

SUBJECTS AND METHODS

Sample collection

Ninety sera and saliva sample of 45 young persons were collected from College of Dentistry, Baghdad University (Periodontic department). Between November-2011 till March-2012, number of patients were 21(11female and 10 male) with mean age (18-40).

Twenty four healthy young volunteers (12 males and 12 females) served as control with no inflammation in their gingiva.

The study was excluded all subjects who smoking, alcohol drinking, taking hormonal therapy or antibiotic drugs, as well as individuals suffering from chronic or acute disease (e.g. diabetes mellitus, hypertension, renal disease or liver).

Specimen Collection

Serum Collection

Sera sample were collected in the morning from the patients and healthy men and women (women during 15th – 29th day of the menstrual cycle (luteal phase)). Blood samples were left for 20 minutes at room temperature. After coagulation, the sera were separated by centrifugation at (1500xg) for 10 min after collection, hemolysed samples were discarded. The sera was stored at (-20) ºC until use for different investigations. The samples were not thawed and refrozen before testing.

Saliva collection

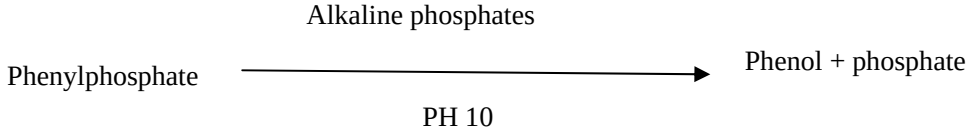
Stimulated whole saliva was collected under resting conditions in a quiet room, at least 90 minutes after the last intake of food or drink. Duration of saliva sample collecting was recorded by a stopwatch. Pre-stimulation were accomplished by chewing a piece of standard size paraffin. After 60 sec of stimulation, the participants were asked to swallow the saliva pooled in the mouth. Thereafter, whole stimulated saliva was collected for about 5 minutes into a measuring container [10].

At the end of the collection period, saliva volume was measured, the tube sealed and then frozen in dry ice until taken back to the laboratory for processing. Prior to analysis, the saliva was centrifuged at 1500xg for 10 min. The supernatant fraction was then liquated into storage vials and kept at - 20ºC until required for analysis [11,12].

- Determination of total protein concentration: The total protein concentration of all samples (serum and saliva) was determined using Hartree method (modified Lowry method), and bovine serum albumin (BSA) as standard. Protein concentrations of serum and saliva were expressed in g/dl. [13]
- Biochemical markers of Inflammation: All the laboratory tests on blood and saliva have been done in the teaching laboratories of Baghdad medical city.

1. Determination of Serum and Saliva Interluekin-6 (IL-6) Concentration: (IL-6) of sample was determined by using human ELISA (IL-6) US (ultra sensitive) KIT, (Camarillo USA), (IL-6) US kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu IL-6 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IL-6 content, control specimens, and unknowns, are pipetted into these wells.

2. Determination of activity and specific activity of Alkaline phosphatase (ALP): The activity of serum and saliva alkaline phosphatase was determined by colorimetric method [14] by using ALP kit (biomereux France) according to the following reaction:



Specific activity of ALP (U/mg) = ALP concentration/ Total protein

3. Determination of albumin concentration: The concentration of albumin was determined in the sera and saliva of each samples [15] by using albumin kit (Biolabo reagent France) for serum and saliva samples, in buffered solution at pH 4.2, bromocresol green binds albumin to form a colored compound which absorbance, measured at 630nm (620-640) is proportional to the albumin concentration in the specimen

- Hormonal analysis:

1. Determination of Testosterone levels: Testosterone was determinate by enzyme immunoassay EIA for the quantitative determination of testosterone concentration in human serum and saliva [16] by using ELISA testosterone kit (Biocheck Fostercity), the testosterone EIA based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for constant amount of rabbit anti-testosterone.
2. Determination of Estradiol levels: Estrasiol was determinate by enzyme immunoassay EIA for the quantitative determination of estradiol concentration in human serum and saliva [16] by using ELISA estradiol kit, the estradiol E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol.

RESULTS

It is clear from the results presented in the table 1 that pH and flow rate decrease significantly in the saliva sample of patients with gingivitis for male and female, compared with the healthy group.

Table 1: Means value of flow rate and pH of saliva for different groups

Groups	Sex	pH value			Flow rate ml/min		
		mean	±SD	P value	mean	±SD	P value
Controls	Male	8.1	0.2		3.048	0.384	
	Female	7.9	0.1		2.700	0.389	
Patients	Male	7.4	0.3	p<0.05*	1.385	0.282	p<0.05*
	Female	7.4	0.2	p<0.05*	1.135	0.218	p<0.05*

***significantly decrease**

The results of biochemical inflammation in serum and saliva shown in table 2, as shown in table 2 in serum and saliva in patients with gingivitis the activity and specific activity of ALP increase with highly significant difference ($p<0.001$) and significant ($p<0.05$) respectively as compared with control groups for both sexes (male and female) .

It is clear from the results observed in the table 2 that the concentration of serum and saliva albumin was decrease in both male and female patients with gingivitis non-significantly compared with healthy groups ($p>0.05$).

By using t-test the result of the present study for IL-6 showed:-

For male a highly significant difference ($p<0.001$) was founded between controls and patients in serum and saliva.

For female, a significant difference ($p<0.05$) was founded between controls and patients in serum and saliva.

As shown in table 3 the levels of sera and saliva testosterone was decreased significantly ($p<0.05$) for patients as compared to controls in contrast to E2 which increased highly significant ($p<0.001$) for both samples sera and saliva in patient than control group.

Correlation of Estradiol and testosterone with inflammation parameters

The results of the correlation coefficient in addition to p-value between E2 and testosterone with each parameter of inflammation (alkaline phosphates, albumin, and interleukine-6) in both samples sera and saliva are presented in table below. It can be noticed that all of these are highly significant correlation noticed the negative correlation between sera E2 with albumin($r= -0.279$, $p<0.001$) and between saliva E2 with ALP($r= -0.395$,$p<0.001$), saliva E2 with albumin ($r= -0.283$, $p<0.001$), saliva E2 with IL-6 ($r= -0.064$, $p<0.001$), sera testosterone with albumin($r= -0.183$, $p<0.001$), between saliva testosterone with alkaline phosphatase ($r= -0.137$, $p<0.001$) and between saliva testosterone with IL-6($r= -0.234$, $p<0.001$).

The positively correlation obtained is between sera E2 with ALP($r=0.219$, $p<0.001$) and between E2 with IL-6($r= 0.545$, $p<0.001$), sera testosterone with ALP($r=0.141$,$p<0.001$), between testosterone with IL-6 ($r=0.279$, $p<0.001$), and between saliva testosterone with albumin($r=0.343$, $p<0.001$).

Table 2: Descriptive statistics and group difference of biomarker of inflammation in serum and saliva

Group (serum)	Sex	Number	Activity of Alp U/L	Total protein mg/L10^{-1}	SP Activity of Alp U/mg	IL-6 mg/L $\times 10^{-6}$	Alb mg/L$\times 10^{4}$
Controls	Male	12	63.387 ±12.700	6.300 ±1.093	1.018 ±0.200	1.907 ±1.120	5.270 ±0.450
Patients		10	123.778 ±22.800	4.878 ±0.867	2.631 ±0.800	6.519 ±1.650	4.715 ±0.323
p-value			p<0.001**	p<0.001**	p<0.05*	p<0.001**	p>0.05
Controls	Female	12	71.045 ±15.800	6.375 ±0.722	1.139 ±0.300	1.048 ±0.406	4.823 ±0.180
Patients		11	113.418 ±20.000	4.936 ±0.645	2.403 ±0.500	2.801 ±0.645	4.241 ±0.391
p-value			p<0.001**	p<0.001**	p<0.05*	p<0.05*	p>0.05
Group (saliva)	Sex	Number	Activity of AlpU/L	Total protein mg/L10^{-1}	SP Activity of AlpU/mg	IL-6 mg/L $\times 10^{-6}$	Alb mg/L$\times 10^{4}$
Controls	Male	12	3.925 ±2.200	2.668 ±0.362	0.149 ±0.090	2.749 ±1.070	±1.237 ±0.289
Patients		10	18.394 ±5.700	1.800 ±0.299	0.929 ±0.200	9.555 ±1.716	1.185 ±0.020
P value			p<0.001**	p<0.001**	p<0.05*	p<0.001**	p>0.05
Controls	Female	12	4.548 ±3.700	2.768 ±0.169	0.153 ±0.100	2.549 ±1.169	1.199 ±0.017
Patients		11	14.923 ±4.300	1.958 ±0.202	0.764 ±0.200	4.941 ±1.632	1.167 ±0.176
P value			p<0.001**	p<0.001**	p<0.05*	p<0.05*	p>0.05

*** Significant difference**
**** Highly significant difference**

Table 3: Descriptive statistics and group difference of sex steroid hormones in serum and saliva

Groups (serum)	Testosterone $\times 10^{-3}$mg/L	Estradiol $\times 10^{-6}$mg/L	Groups (Saliva)	testosterone $\times 10^{-3}$mg/L	Estradiol $\times 10^{-6}$mg/L
Controls	2.64±0.829	62.507±10.277	controls	0.076±0.008	6.913±3.833
Patients	1.461±0.514	88.440±17.346	patients	0.046±0.023	13.128±5.650
P value	p<0.05*	p<0.001**	P value	p<0.05*	p<0.001**

***increase significantly**
****increase highly significant**

Table 4: Correlation between inflammation parameters and (testosterone, estradiol) in sera and saliva of gingivitis patients.

Estradiol			Testosterone		
Parameters in serum	**r**	**p**	**Parameters in serum**	**r**	**p**
Alkaline phosphatase	0.219	p<0.001**	Alkaline phosphatase	0.141	p<0.001**
Albumin	-0.279	p<0.001**	Albumin	-0.183	p<0.001**
Interleukin-6	0.545	p<0.001**	Interleukin-6	0.279	p<0.001**
Parameters in saliva	**r**	**p**	**Parameters in saliva**	**r**	**p**
Alkaline phosphates	-0.395	p<0.001**	Alkaline phosphates	-0.137	p<0.001**
Albumin	-0.283	p<0.001**	Albumin	0.343	p<0.001**
Interleukin-6	-0.064	p<0.001**	Interleukine-6	-0.234	p<0.001**

****highly significant**

DISCUSSION

Ionic concentrations change as saliva production is stimulated, however, and concentrations of sodium, chloride, and bicarbonate ions all increase with accelerated flow. As bicarbonate levels increase, the pH of saliva changes from slightly acidic 6-7 to slightly basic around 8 [17].

Lagerlöf and Oliveby demonstrated that salivary flow rate influences to a high degree the rate of oral and salivary clearance of bacterial substrates [18].

Alkaline phosphatase activity increases significantly with plaque accumulation and increasing inflammation. So this enzyme should be considered to be the best indicator for periodontal disease. [20] This results are agreement with Gopinath et al. [20] who showed statistically significant increased activities of ALP in serum and saliva from patients with periodontal disease compared with the control group [19].

Analyzed the saliva of patients with periodontitis and demonstrated significant increases in enzyme activity in association with cell injury and tissue cell death, salivary enzyme activity, as biochemical marker, may be useful in diagnosis, prognosis, and monitoring of periodontal diseases [19,21].

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and may play a central role in host defense mechanisms [22].

A local increase of pro inflammatory cytokines (IL-6) in the patients with different stages gingivitis, comparing to healthy subjects [23].

(Table-2)The increased level of the marker is expression of the inflammatory response in gingivitis. In both male and female and that agreement with Gurban et al. [17], Anna Dongari-Bagtzoglou [22] and Costa et al. [24] who reported that: the increased level of the marker is expression of the inflammatory response in periodontitis and gingivitis. The intensity of the immune response correlates with the increase of these mediators that represent evolution and monitoring markers of the periodontitis progression.

Because the gingivitis is mild inflammation and albumin decrease non-significantly so the albumin may be an indicator of chronic or acute periodontitis (the progressive of gingivitis) and that's agreement with Abhijit et al. [25] and Moshage et al. [26] who observed that Severe periodontitis was associated with low serum albumin compared with individuals without severe periodontitis disease .

Androgens play a significant role in the maintenance of bone mass and inhibit osteoplastic function, inhibit prostaglandin synthesis and reduce interleukin-6 (IL-6) production during inflammation. Furthermore, testosterone stimulates bone cell proliferation and differentiation and therefore has a positive effect on bone metabolism, where testosterone has an effect on periodontal tissues by increasing matrix synthesis [27].

Parkar et al. [28] demonstrated that increasing (dihydrotestosterone) DHT concentrations progressively reduced IL-6 production by gingival cells isolated from normal individuals and patients with gingival inflammation and gingival hyperplasia.

Estrogen receptors present in the human gingiva are believed to be responsible for the increased accumulation of these hormones in gingival tissues. Cyclic increases in the production of female sex steroid hormones often alter the composition of biofilm microbiota, the biology of gingival tissue and vasculature, and recognition by effector cells of the local immune system. Throughout women's hormonal cycles (menstruation) alterations on the periodontal tissues may be easily observed [29,30].

Lindhe and Attström [31] noted that during the menstrual cycles, women without clinical gingivitis showed no increase in gingival fluid, whereas those with gingivitis showed increases in gingival fluid. It is generally accepted that increased sex hormones during the menstrual cycle modulate the development of localized gingival inflammation [32].

Systemic endocrine imbalances may have an important impact on periodontal pathogenesis, and, vice versa, changes in periodontal conditions might be associated with variations in sex hormone levels. This association is evident in the recent periodontal disease classification which includes the following hormone related disease categories: puberty-associated gingivitis, menstrual cycle-associated gingivitis and pregnancy-associated gingivitis [33].

As a conclusion, the search of new parameters that affected it and vice versa is still an interesting field of research especially if the parameters were studied in saliva instead of serum, the results generated from this study could lead to improvement in gum care program for peoples and revealed an association between gingivitis progression with hormonal changes production as result to increase the inflammation marker (ie IL-6, ALP). Also saliva analysis is better for

determination the free bioavailable steroid hormones than sera analysis.

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