Measurement of serum Superoxide dismutase levels in women with polycystic ovarian syndrome and chronic periodontitis

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is one of the most important reproductive and endocrine disorders in women at reproductive age. It's associated with metabolic disorder, obesity, insulin resistance and oxidative stress chronic periodontitis and PCOS both of them associated with low chronic grade of inflammation. The prevalence of periodontal disease seems to be higher in women with PCOS. Superoxide dismutase enzyme (SOD) is an important circulating marker and protecting enzyme helping the body tissues to get rid of reactive oxygen species (ROS) that damage the tissue.

Aim of the study: The aim of this study was to measure and compare the levels of (SOD) among group of chronic periodontitis patients with PCOS, group of chronic periodontitis without PCOS and a third group who were systemically and periodontally healthy.

Material and Method: This study consist of (60) women at reproductive age ranged between (25-40) years old. They divided into three groups Group I consist of 20 women systemically healthy and with healthy periodontium, group II consist of 20 women with chronic periodontitis and systemically healthy and Group III consist of 20 women with chronic periodontitis and (PCOS). We evaluated the periodontal health of the groups through measuring these important indices: Plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss. SOD antioxidant marker was measured colormeterically for the three groups.

Results: this study showed higher means of periodontal parameters (plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss (1.275±0.246, 1.295±0.239, 0.24±0.16, 6.47±0.345, 4.125±0.328 respectively). Highly significant differences were found using t-test in inter group comparison. (P≤0.001) regarding pocket depth and clinical attachment loss .Higher mean of (SOD) level was found for G3 (137.72±29.769) U/mL . F-test was used for intragroup comparison and highly significant difference was found (P≤0.001). Positive but weak correlation where found among (SOD) level, bleeding on probing in Group I and Group II , also among (SOD) level, probing pocket depth and clinical attachment loss.

Conclusion: (PCOS) associated with oxidative stress and more prone to periodontal diseases with high level of antioxidant agent like (SOD) level to compensate the high level of (ROS)

Key words: superoxide dismutase enzyme, chronic periodontitis, polycystic ovarian syndrome. **(J Bagh Coll Dentistry 2017; 29(4): 76-81)**

INTRODUCTION

Chronic Periodontitis is one of the most important chronic inflammatory disease resulting from accumulation of dental plaque on the tooth surface that cause destruction in periodontal attachment and adjacent alveolar bone, Its recently defined as "an infectious disease resulting in inflammation with in supporting tissues of the teeth, progressive attachment loss and bone loss"(1)

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Chronic periodontitis can occur when there is imbalance between host response and microbial biofilm and their product. Specifically when loss of balance between antioxidant defense systems that protect and repair vital tissue cells (ROS).(2)
Chronic exposure to ROS can initiate pathologic reactions like periodontal disease. The body has evolved certain defense and repair systems inherently to prevent the accumulation of oxidatively damaged molecules that are toxic by producing the antioxidant agents. Antioxidants are defined as those substances that protect body tissues and balance the damaging oxidative effect. (SOD) is one of the antioxidant enzymes that protect the cell against the deleterious effects of (ROS). (SOD) convert the superoxide anion to hydrogen peroxide and it's the first line of defense in antioxidant reactions against (ROS).(3-5)
Studies have found that gingival SOD activity is significantly higher in chronic periodontitis,

which suggested that SOD activity increases with the progression of inflammation.[6] (PCOS) It is one of the most common hormonal disorder affecting women at reproductive age (18-44).years old [7]. According to national institute of health [NIH] criteria the prevalence of (PCOS) ranging from 6.5% - 8%.[8] It considered as metabolic syndrome with cardiovascular, insulin-dependent diabetes (IR), dyslipidemia and endothelial dysfunction and visceral obesity[9] risk factors. Because both periodontitis and metabolic syndrome are associated with systemic inflammation and IR, these two disorders may be linked through a common pathophysiologic pathway[10]

(PCOS) is like chronic periodontitis associated with chronic inflammation.[11]

Importantly, the effect of female steroid hormones on the composition of oral microbiota has been reported in puberty, menstruation, pregnancy and with oral contraceptive usage[12] Taking into consideration that periodontal diseases are chronic infections that cause a low-grade chronic systemic inflammation[13]it is important to consider an association with hormonal disorders, such as (PCOS).

Kuçu *et al.* (2009) demonstrated that (SOD) levels were significantly higher in a (PCOS) group compared with a control group (8.0±0.7 vs 7.28±0.8, p=0.001).

The aim of this study was to measure and compare the levels of (SOD) among group of chronic periodontitis patients with (PCOS), group of chronic periodontitis without (PCOS) and a third group who were systemically and periodontally healthy.

MATERIAL AND METHODS

Study design:

Sixty females were participated in this study with an age range between (25-40) years old. They were patients who attended the teaching hospital of college of dentistry, university of Baghdad and Baghdad Hospital/ infertility clinic. Participants enrolled in the study should be healthy with no history of any systemic diseases, non-smoker Non pregnant, not take antibiotic or other medication in the last three months. Patients undergo periodontal treatment in the last three months should be excluded.

In the study the participants were divided into three groups

- Group 1 (G1) consist of (20) females with healthy periodontium.
- Group 2 (G2) consist of (20) females with chronic periodontitis
- Group 3 (G3) consist of (20) females with chronic periodontitis and PCOS.

Participants in G1and G2 should be with regular menstrual cycles and with no clinical or biochemical features of hyperandrogenism and without PCOS improved by ultrasound. G2 & G3 should have at least four surfaces with probing pocket depth (≥ 4mm) and clinical attachment loss of (1-2 mm) or more[15].

Participants that have PCOS were diagnosed by the gynecologist according to Rotterdam criteria [16].

Periodontal examination:

Measurement of clinical periodontal parameters were performed by using Michigan O periodontal probe at the four sides (buccal or labial, lingual or palatal, mesial and distal) of all teeth excluding the third molar tooth; participants should have at least (20) teeth. The data collected included:-

Plaque index (PlI) [17], gingival index (GI) [18], bleeding on probing (BOP)[19], probing pocket depth (PPD)[20], and clinical attachment level (CAL)[20].

Collection of blood samples:

Blood sample of 3ml of was taken from each participant of the three groups. The blood was transferred into gel tubes and allowed for 30 minutes at room temperature to help clot formation and separation of serum subsequently, then centrifuged at 1000 rpm for (15 minutes) to separate the serum and kept frozen at (-20 °C).[21] the level of (SOD) was measured colormetrically by spectrophotometer.

(SOD) Assay Kit

Reagents:

Carbonate buffer (50 mM, pH 8.00): Ethylenediaminetetraacetic acid sodium salt buffer (10 mM, pH=10.2):

Epinephrine Indicator:

Procedure:

Sample µ L	Carbonate buffer (50 mM, pH 8.00) µ L	Ethylenediaminetetraacetic acid sodium salt buffer (10mM, pH=10.2) µ L	Epinephrine µ L
100	1800	1000	100

Read the samples at a wave length 480 nm immediately (A1) and after 5 minutes (A2).

Calculation of (SOD) enzyme activity:

One unit of SOD was defined as the amount of enzyme that inhibit the oxidation 50%.

Blank sample was used in order to exclude different spontaneous degree of oxidation. The absorbance of blank sample was subtracted from

the absorbance of the sample to calculate the real absorbance of each sample. The activity (units/ml) is given by the following equation. One unit is the amount of enzyme that catalysis the reaction of 1 µmol of substrate per minute.

Inhibition % = (A1-A2)/A1

Superoxide dismutase Activity (U/ML) = (I%/2/t) xD

Superoxide dismutase Activity (micromole/min/ML) = (I%/2/5) x300

I%= Inhibition, t= time= (5), D= Dilution factor= 300.

Statistical analysis was evaluated by employing t-test, Analysis of variance (ANOVA) test, and Pearson's coefficient of correlation (r).

Significant (S) = $0.05 \geq p > 0.01$

Highly significant (HS) = $P \leq 0.01$

Non-significant (NS) = $P > 0.05$

This study implicating human subjects is in accordance with the Helsinky declaration of 1975 as revised in 2000 and that it has been approved by the relevant Institutional Ethical Committee.

RESULTS

Table (1) revealed descriptive analysis, mean and standard deviation for the periodontal parameters of (PlI, GI, BOP score (1), PPD, CAL) for three study groups. The highest mean of PlI and GI were belong to G3, they were (1.275±0.246 & 1.295±0.239) respectively. For BOP score (1), PPD and CAL, highest mean in G3 they were (0.24±0.16, 6.47 ±0.345, 4.125 ±0.328) respectively. Table (2) revealed the intergroup comparison regarding the periodontal parameters T test was used, highly significant differences were found for PPD and CAL (P value < 0.001) Table (3) revealed mean and standard deviation of superoxide dismutase (U/mL) for GI, G2, G3. For G1the mean value was (21.12±2.48) U/mL. It was (56.51±13.574) U/mL for G2 and It was 137.72±29.769 U/mL for G3. F- Test was used to show the intragroup comparison, highly significant differences were found (P<0.001). Table (4) was shown inter groups comparisons regarding SOD level and highly significant differences (P<0.001) were found between each pairs of groups. Table (4) revealed the correlation between the periodontal parameters (PlI, GI, BOP score (1), PPD, CAL) and (SOD) for G2 and G3. For G2 positive but non-significant correlation was found for SOD level with the GI and BOP. For G3 positive but non-significant correlation was found for SOD level with PlI, PPD and CAL.

Table 1: describe mean and standard deviation for the periodontal parameters of the three groups

Groups	PlI		GI		BOP Score 1		PPD		CAL	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
GI	0.44	0.06	0.31	0.06	--------	------	------	------	------	---------
G2	1.205	0.234	1.24	0.084	0.215	0.15	5.025	0.535	3.26	0.298
G3	1.275	0.246	1.295	0.239	0.24	0.16	6.47	0.345	4.125	0.328

Table 2: Intergroup comparison for the periodontal parameters with significant differences using T- test

	T-test	P-value	Sig
PlI	-0.92	0.36	NS
GI	-0.97	0.34	NS
BOP	0.47	0.6	NS
PPD	-10.14	<0.001	HS
CAL	-8.73	<0.001	HS

Table 3: describe mean, standard deviation of SOD Levels (U/mL) of the three groups and intragroup comparison with significant differences using the F- test

Groups	SOD (U/mL)		F test	P-value	Sig
	Mean	± SD			
GI	21.12		2.48		
G2	56.519	13.574	199.1633	<0.001	HS
G3	137.72	29.769			

Table 4: Intergroup comparison of the SOD level (U/mL) with significant differences using T-test

Groups	T-test	P-value	Sig
G1&G2	-11.47	<0.001	HS
G2&G3	11.09	<0.001	HS
G1&G3	17.4	<0.001	HS

Table 5: Pearson's coefficient of correlation (r) between SOD levels (U/mL) and periodontal parameters for G2 and G3 with significant differences

		r	P-value	Sig
G2	SOD & Pl.I	-0.23	0.33	NS
	SOD & GI	0.29	0.21	NS
	SOD & BOP	0.17	0.47	NS
	SOD & PPD	-0.12	0.61	NS
	SOD & CAL	-0.41	0.07	NS
G3	SOD & Pl.I	0.36	0.12	NS
	SOD & GI	-0.32	0.17	NS
	SOD & BOP	-0.03	0.9	NS
	SOD & PPD	0.22	0.35	NS
	SOD & CAL	0.21	0.34	NS

DISCUSSION

The current study was compared important periodontal parameters, antioxidant marker (SOD) among women with healthy, chronic periodontitis and chronic periodontitis with PCOS Till now few studies related to these subjects are present. Our results revealed higher periodontal parameters in G3, these finding are compatible with these studies [22,23,24,25] that showed higher periodontal indices among women with PCOS.

The effect of steroidal hormones imbalance during puberty, pregnancy and menopause on the periodontal flora and health had been reported [(26)], women with PCOS have hormonal imbalance, hyperandrogenism with elevated total testosterone. Hyperandrogesim status in those women resulting in infertility, disturbance in menstrual cycle and increased risk to periodontal diseases. Testosterone will convert to estrogen, high level of estrogen and testosterone are exist.[(27)] Increased in estrogen and progesterone level associated with increased in gingival inflammation, capillary changes and excessive proliferation of vascular endothelial cells.[(28)] Increased susceptibility of PCOS women to periodontal diseases due to influence of altered circulating steroidal hormones in the periodontal tissues. These changes impact gingival tissues through changes in oral flora and proinflamatory cytokines affecting the bone and enhanced oxidative stress in periodontal tissues of patients with PCOS. PCOS appeared to have an enhancing effect on the levels of P.gingivalis and F. nucleatum and their association with gingival inflammation, hinting towards a microbial specificity[(6)] In this study we exclude smoker patients and those under antibiotic treatment or having systemic diseases that had effect on periodontal health to minimize the effect of these risk factors. In agreement with other previous studies [(22)] BOP is highest in patients with PCOS due to the effect of hyperandrogenism on vascular flow rate but with none significant difference in comparison between the groups. Also the increased in CAL and PPD in G3 might attributed To increased susceptibility to inflammatory process and gingival inflammation that progress to more sever periodontal destruction and subsequent increased in gingival sulcus, Attachment loss and bone loss.

Inflammation that associated with periodontitis involving increased in production of oxygen free radical manly from inflammatory polymorphnuclear cells (PMNs) these cells are important in first line of defense mechanism. These free radicals recently known as (ROS), although the (ROS) have short half-life they cause damage to the host cells through production free radical chain reactions.

PCOS associated with oxidative stress and metabolic disorder and insulin resistance

Oxidative stress can define as imbalance between production of Antioxidant markers (AO) and oxidative agents (ROS)

Protection against these species provided through production (AO) from PMNs and other cells. These agents when present they will protect the tissues against the free radicals and inhibit or delay oxidation of the substrate.[(29,30,31).] Superoxide dismutase enzyme is one important AO that protect the tissues against the ROS.[(32)] In this study the concentration of (SOD) was higher

in the G3 those women suffering from chronic inflammation in the periodontium and PCOS associated with oxidative stress and metabolic disorder and insulin resistance, these protective enzyme converting superoxide anion radical to H2O2 and O2, may be explained as a compensatory mechanism in response to the increased production of oxidant molecules mentioned above. Increased (SOD) level may indicate increased in $^{-}O_2$ free radical from inflammatory cells at the diseased site. This is in agreement with these studies [33,34,35] but disagree with others[36,37] Positive but weak correlation where found among SOD level, BOP and GI in G2 and among SOD level , PPD and CAL these results might be due to small sample number and different techniques for SOD measuring.

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