Evaluation of serum levels Superoxide dismutase in women with polycystic ovarian syndrome and gingivitis

Omar Husham Ali, B.D.S, M.Sc.[1]
Zainab J. Raheem, B.D.S, M.Sc.[2]
Nada K. Imran, B.D.S, M.Sc. [2]
Ali .Y.Majid, M.B.Ch.B., M.Sc., F.I.C.M.S[3]
Maha A. Ahmed, B.D.S, M.Sc.[4]
Azza Wala AL-deen Khairi, B.D.S.[5]

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women, there is an increasing evidence for an oxidative stress in PCOS that induce genomic and mitochondrial deoxyribonucleic acid damage that leads directly to reduced fertility. The objectives of this study are to assess and compare the periodontal health status by measuring clinical periodontal parameters (PLI, GI and BOP)as well as serum levels of superoxide dismutase at gingivitis ,gingivitis with PCOS and healthy periodontium groups, then correlate between clinical and biochemical parameters.

Materials and Methods: 60 females with an age range between (25-40) years old had been tested and divided into3 groups ,the control group consists of (20) females with healthy periodontium, group of (20) females with gingivitis and group of (20) females with gingivitis and PCOS. After completion of clinical periodontal parameters recording (PLI, GI& BOP), blood samples were collected and biochemical analysis of serum samples were carried out by using [Super oxide dismutase Assay kit] to evaluate serum super oxide dismutase levels.

Results: The highest mean values of PLI,GI and BOP score1 were found in gingivitis+PCOS group. Highly significant difference was revealed among the groups regarding mean values of Superoxide dismutase with the highest mean value at gingivitis+ PCOS followed by gingivitis groups. Non-significant correlation were demonstrated between clinical and biochemical parameters except the significant moderate positive correlation of BOP at gingivitis+PCOS group.

Conclusion: It could be certified that severity of gingivitis may increase in patients with PCOS. The concentration of serum SOD increased with the severity of gingival inflammation as well as the presence of PCOS. Serum SOD may be useful biochemical marker for early detection of periodontal disease and PCOS.

Key words: polycystic ovarian syndrome, gingivitis, superoxidedismutas. **(J Bagh Coll Dentistry 2018; 30(2): 29-33)**

INTRODUCTION

Periodontal disease (PD) is a chronic inflammatory condition characterized by destruction of supporting structures of the teeth. It is one of the most considerable health problems because it leads, if not treated, to loss of teeth in its terminal stages [1, 2]. Gingivitis involves a limited inflammation of the unattached gingiva and is a relatively common and reversible condition [3].

The first line of defense done by neutrophils, produce free radicals responding to the plaque biofilm by formation of lipid peroxidation products and several reactive oxygen species (ROS) that destroy the microorganisms, the ROS are required in physiological quantities by the human body but over production could lead to periodontal tissue destruction caused by an inappropriate host response to the plaque biofilm[1]. The human body contains non-enzymatic include Vitamins E and C, and reduced glutathione while enzymatic include superoxide dismutase (SOD), catalase, and glutathione peroxidaseantioxidant defense mechanisms to counter this excessive production of harmful ROS as soon as they are formed to prevent their deleterious effects.[4].

The most common endocrine abnormality is polycystic ovary syndrome (PCOS) which mainly occur in reproductive-age women, its etiology remains unclear, an oxidative stress (OS) considered as the most common cause of PCOS [5,6]. OS may be systemic, affecting the whole body, or it maybe localized, affect only specific site as in oral soft tissues. It can cause micro damage to the cell membrane, (DNA) damage, protein deactivation, in addition to stimulation of cell signaling molecule-induced tissue damage inflammation. OS are common causative factors for many chronic diseases, such as periodontitis, atherosclerosis, rheumatoid arthritis and diabetes.

(1) Lecturer, Department of Periodontics, College of Dentistry, University of Baghdad.
(2) Assistant Lecturer, Department of Periodontics, College of Dentistry, University of Baghdad.
(3) Consultant. Drugs and poisoning center. Iraq.
(4) Professor, Department of Periodontics, College of Dentistry, University of Baghdad.
(5) Dentist, Department of Periodontics, College of Dentistry, University of Baghdad.

Furthermore, gingivitis and periodontitis considered as contributing factors to OS [7].

Dismutation is a reaction between two identical molecules in which one is reduced and the other oxidized, it is the process in which the superoxide transformed to elemental oxygen and hydrogen peroxide, SOD is responsible for catalyzing the conversion of superoxide,there are three forms of superoxide dismutase present in humans (SOD1, SOD2 & SOD3),SOD1 is a dimer (consists of two units) and it's located in the cytoplasm,SOD2 is a tetramer (four subunits) and located in the mitochondria,while SOD3 is also a tetramer but it's extracellular. SOD1 and SOD3 contain copperand zinc, whereas SOD2, the mitochondrial enzyme, hasmanganese in its reactive center [8]. For normal cell function, it is necessary to keep SOD at reduced concentrations within the follicular fluid milieu (FF), which provide the appropriate balance of superoxide anion and hydrogen peroxide. Hydrogen peroxide is the first line of defense in antioxidant reactions against ROS and SOD, which responsible for dismutation of the superoxide anion to hydrogen peroxide. A thresholdlevel of ROS in the FF may be correlated with fertilization,embryo quality, pregnancy rate and outcome [9].

In PCOS patients, serum SOD activity has been reported with mixed results [10, 11]. The FF provides a very important micro environment for the development of oocytes and it's easily available during oocyte pick-up.The extracellular secreted isoform of SOD is responsible for SOD activity, the cytosolic copper/zinc SOD (Cu/Zn-SOD) are located within both granulose and theca cells [12, 13].

This study was conducted to determine the effect of PCOS on periodontal health condition and serum levels of SOD.

MATERIALS AND METHODS

The human sample consisted of 60 females with an age range between (25-40) years old, all of them from subjects attended College of dentistry/University Of Baghdad and Baghdad Hospital/ infertility center. The participants in this study were informed about the purpose of the investigation to confirm their agreement for participation in the study, then the case sheet was filled with patient's name, age, full medical and dental history and medications. The subjects enrolled in this study should be apparently healthy without history of any systemic diseases (e.g., diabetes mellitus, hypertension and cardiovascular disease) which could affect periodontal health condition, non-smoker, non-pregnant, while females under administration of contraceptives or hormonal medications, course of anti-inflammatory or anti-microbial and patients undergoing periodontal treatment in the last three months prior to the study should be excluded.

The subjectsincluded in this study were divided into 3 groups:

Control group: consists of (20) females with healthy periodontium.

Gingivitis group: consists of (20) females with gingivitis

Gingivitis+PCOS group: consists of (20) females with gingivitis and PCOS (females with PCOS were diagnosed by gynecologist according to Rotter dam criteria [14]. Females in groups 1 and 2 were with regular menstrual cycles and without clinical or biochemical features of hyper androgenism and ultrasound exclusion of polycystic ovary (without PCOS). Patients with gingivitis must have signs and symptoms of gingival inflammation [15], without pockets or loss of attachment.

All subjects examined clinically and full examinations of clinical periodontal parameters (PLI[16], GI[15] and BOP[17]) were carried out by using Michigan O periodontal probe for all teeth except the third molar at four site (mesial, distal, buccal or labial and lingual or palatal) with the presence of not less than 20 teeth.

Blood sample collection:

After completion of clinical periodontal parameters recording, blood samples were collected, under a strict aseptic condition three milliliters of venous blood were collected from each female from ante-cubital fossa by venipuncture using 20-gauge needle with 5 ml syringes. Blood sample was transferred into jell tubes, which help to obtain blood clot rapidly and easy subsequent separation of serum, then immediately transferred to laboratory. Blood samples were allowed to clot at room temperature for 30 minutes before centrifugation for 15 minutes at 1000 rpm to separate serum from blood and collected in eppendrof tubes and kept in the deep freeze at -80 ˚C till used for subsequent biochemical analysis of SOD.

Biochemical analysis

The biochemical analysis of serum samples were carried out at theTeaching Laboratories of Baghdad teaching hospital. For the analysis of serum SOD we used [Super oxide dismutaseAssay kit (SZA Kit)] which consist of {Crbonate buffer (50Mm, PH80) and Ethelendiaminetetraacetic acid sodium salt buffer (10Mm, PH=10.2)}and followed the products manual protocols for the test procedure rigorously.

We certify that all subjects included in this study in accordance with the Declaration of Helsinki of 1975 [18].

Data were analyzed using the following statistics: {mean standard deviation (SD), t- test, F-test and Pearson correlation coefficient (r)}.

RESULTS

Table (1) revealed descriptive analysis (mean and standard deviation)for the clinical periodontal parameters (PLI, GI, BOP score 1) for the three groups .The highest mean values of PLI, GLI and BOP score 1 were belong to (Gingivitis + PCOS) groupthey were (1.306±0.272, 1.411±0.149, 0.325±0.138) respectively. Comparison between Gingivitis group with (Gingivitis +PCOS) regarding clinical periodontal parameters demonstrated significant difference for GI while they were non -significant differences for PLI and BOP score 1 ,table(2).The mean values and standard deviation for serum levels of SOD present in table (3), hence the highest mean value was (60.627±11.019) at (Gingivitis+PCOS) group,followed by (45.887±9.79) at Gingivitis group while Control group had the least mean value was (21.12±2.48).Comparisons among the three groups concerning mean values of serum SOD detected highly significant difference ,as noticed in table (4).Also highly significant differences were shown in table (5),when comparing mean values of serum SOD between all pairs of groups using t-test .Table (6) revealed non-significant weak correlations at Gingivitis group which was negative for PLI and were positive for GI and BOP score 1with serum levels of SOD, on the other hand the correlations at (Gingivitis+PSOC) group between SOD serum levels with PLI and GI were non-significant weak hence it was positive at the former and negative at the latter, while it was significant moderate positive correlation with BOP score1.

Table 1: Descriptive statistics of clinical periodontal parameters for groups

Groups	PLI		GI		BOP Score 1	
	Mean	± SD	Mean	± SD	Mean	± SD
Control	0.44	0.06	0.31	0.06		
Gingivitis	1.304	0.125	1.3105	0.079	0.26	0.11
Gingivitis + PCOS	1.306	0.272	1.411	0.149	0.325	0.138

Table 2: Comparisons of mean values of clinical periodontal parameters between Gingivitis group with Gingivitis +PCOS group

Clinical periodontal parameters	t-test	*P-value*	Sig.
PLI	-0.019	0.98	NS
GI	-2.737	0.01	S
BOP	-1.64	0.1	NS

Table 3: Descriptive statistics for serum levels of SOD (U\ml) for groups

Groups	SOD	
	Mean	± SD
Control	21.12	2.48
Gingivitis	45.887	9.79
Gingivitis + PCOS	60.627	11.019

Table 4: Comparison of mean values for serum SOD among groups

Source of Variation	F	*P-value*	Sig.
Between Groups	107.0568	<0.001	HS

Table 5: Comparisons of mean valuesfor the serumSOD between all pairs of groups

Groups	t-test	*P-value*	Sig.
Control & Gingivitis	10.96	<0.001	HS
Gingivitis & Gingivitis + PCOS	-4.47	<0.001	HS
Control & Gingivitis + PCOS	15.64	<0.001	HS

Table 6: Pearson correlationbetweenserum levels of SOD and clinical periodontal parameters at study groups

Groups	Clinical Periodontal parameters	r	*P-value*	Sig.
Gingivitis	PLI	-0.14	0.56	NS
	GI	0.11	0.64	NS
	BOP	0.12	0.61	NS
Gingivitis + PCOS	PLI	0.05	0.83	NS
	GI	-0.15	0.53	NS
	BOP	0.48	0.03	S

DISCUSSION

This study was carried out to evaluate the serum level of SOD in group of patients with Gingivitis, (Gingivitis +PCOS) group and control group, additionally, to assess the periodontal health status in women with PCOS and to study the correlation between clinical periodontal parameters and the serum level of SOD.The results showed that the mean values of clinical periodontal parameters (PLI, GI and BOP) were higher in

(Gingivitis+PCOS) group than comparing gingivitis group. These findings are compatible with several studies [19-21] that showed higher periodontal disease indices among women with PCOS. High risk of developing PD in PCOS patients contributed to the hyperandrogenism status, which results in menstrual and fertile abnormalities [22], over production of steroid hormones whichis associated with exacerbation of gingivitis. Estrogen and progesterone are affected on essential elements that contribute in developing and progression of PD, which are gingival epithelium , collagen synthesis, osteoblasts, and bony tissue, the capillary system, inflammation, and angiogenesis processes, so excessive proliferation of vascular endothelial cells and epithelial keratinization in gingival tissues will occur[23,.24].The salivary levels of periodontal pathogens and their systemic antibody responses, especially when gingival inflammation is presented, are affected by hormonal abnormalities in PCOS patients. Moreover, intensified oxidative stress in affected periodontal tissues play a role in the pathology of PCOS by mechanisms like increasing glucose intolerance and dyslipidemia [25, 26].

The periodontal tissues had the SOD enzyme, which has biological protection against ROS, especially oxygen (O) during the inflammatory response .Bacterial lipopolysaccharidewas also shown to stimulate O release from gingival fibroblast, suggesting that the induction of SOD may represent an important defense mechanism of the fibroblast during inflammation [27]. In the present study, increased serum SOD levels in both study groups appear to assist the above findings. Furthermore, elevated serum levels of SOD in study groups than control group may indicate that polymorph nuclear leukocytes, which attack the diseased tissue, produce large amount of O.The OS occur as a result of over production of O, which in turn increased need for production of SOD to make the ROS/ antioxidant balance to protectthe tissue[27-.29].The accumulative effect of both PCOS and gingivitis explained the elevated serum levels of SOD in (Gingivitis + PCOS)group than (Gingivitis group). Many studies showed that the mean values of SOD were higher in presence of gingival inflammation and PCOS [20, 27-29].

Regarding the correlation between serum SOD levels and clinical periodontal parameters of study groups, this study revealed non-significant weak correlation ,and this may be due to small sample size and also that SOD levels measured in serum , while other studies that measured SOD in saliva and gingival crevicular fluid, found significant correlation between SOD levels and clinical periodontal parameters [30-.32]. In conclusion, hormonal disturbances have modifying and exaggerating roles in the periodontal tissue response to microbial plaque, and thus directly may affect and contribute to PD as well as SOD may be used as additional early diagnostic toolin diagnosis of PD and PCOS.

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الخلاصة

خلفية: متلازمة المبيض المتعدد الكيسات هو واحد من اضطرابات الغدد الصماء الاكثر شيوعا لدى النساء .وهو احد الاسباب الرئيسة لقلة الخصوبة لدى النساء و يزيد الاكسدةفيالجسم.الهدف من هذة الدراسة هو تقييم و مقارنة مؤشرات اللثة السريرية (مؤشر الصفيحة الجرثومية ,مؤشر اللتهاب اللثة,موشر نزف عند التسبير) اظافة الى مستويات (SOD) المصلية لدى كل مجموعة من مجاميع الداخلة في الدراسة .ثم ايجاد العلاقة بين المؤشرات السريرية و مستويات (SOD)

المواد و الطرق : المجموعة الداخلة في الدراسة 60 انثى , مع معدل عمري يتراوح بين (25-40) سنة . تم تقسيمهم الى ثلاث مجاميع , المجموعة الضابطة تتالف من (20) انثى يملكن انسجةماحول الاسنان صحية سريريا.و مجوعة تتالف من (20) انثى مصابات بالتهاب اللثة فقط و مجموعة تتالف من (20) مصابات انثى بالتهاب اللثة مع متلازمة المبيض المتعدد الكيسات . بعد قياس مؤشرات اللثة السريرية (موشر الصفيحة الجرثومية , موشر اللتهاب اللثة , مؤشر النزف عند التسبير) تم سحب عينات الدم من ثم قياس مستويات (SOD) المصلية .

النتائج:اظهرت النتائج ان قيم المتوسط الحسابي لكل من مؤشر الصفيحة الجرثومية و مؤشر التهاب اللثة و مؤشر النزف عند التسبير كانت اعلى لدى مجموعة التهاب اللثة و متلازمة المبيض المتعدد الكيسات. و وجدت فروقات معنوية عالية لمستويات (SOD) بين مجاميع الدراسة .و قيم حسابية عالية لمستويات (SOD) المصلية لدى مجموعة اللتهاب اللثة ومتلازمة المبيض المتعدد الكيسات .لم نجد اي ارتباط معنوي بين مستويات (SOD) المصلية ومؤشرات اللثة السريرية باستثناء مؤشر النزف عند التسبير فوجد ارتباط معنوي متوسط طردي .