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Evaluation of Serum Homocysteine and Nitric Oxide Levels in Women with Polycystic Ovarian Syndrome and Periodontal Diseases

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Key words
Polycystic ovarian syndrome, periodontal diseases, homocystiene and nitric oxide.

Abstract

Polycystic ovary syndrome (PCOS) and periodontal diseases are common chronic inflammatory disorders. PCOS is a complicated condition affects overall health and causes broad spectrum changes that affect periodontal health status. Available evidence suggests that oxidative stress might comprise a link for the association between periodontal diseases and components of the metabolic syndrome. Both homocysteine (Hcy) and nitric oxide (NO) are considered to reflect the strength of oxidative stress. The aims of the study were to compare the periodontal health condition among the study groups (gingivitis, gingivitis +PCOS, chronic periodontitis (CP) and CP + PCOS group) by measuring the clinical periodontal parameters (Plaque Index (PLI), Gingival Index (GI), Bleeding on Probing (BOP), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL), and measure serum (Hcy) and (NO) and compare their levels between study groups , then correlate between these parameters with each other and with clinical periodontal parameters in order to determine the effect of PCOS on periodontal health status and levels of serum Hcy and NO.

Introduction:

The periodontal diseases are a family of chronic inflammatory diseases, including gingivitis and periodontitis that involve the periodontium. Gingivitis, an inflammation of the gingiva which is a reversible condition while, periodontitis is a more severe and irreversible condition that causes loss of attachment and loss of bone that supports the teeth[1], resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both[2]. PCOS is a series of signs due to hormonal disturbances[3]. It is the most familiar endocrine disorders detected among women between ages of (18 to 44) years. It influences about (5% to 10%) of this age group[4]. PCOS represents a state of Chronic inflammatory disorder[5]. Hence, chronic inflammation could be originated by another inflammatory conditions such as periodontal disease, which is an ordinary pathology observed in patients with PCOS so, it seems logical to presume a relationship with hormonal disturbance, like PCOS[6]. (Hcy) is an amino acid created through the body's natural function, which plays several important roles in human physiology[7]. Preliminary investigation suggested that such serum biomarker is abnormal in women with PCOS[8]. Also, other study showed that there were rise and descent of serum Hcy levels with periodontal inflammation and therapy respectively, which indicated a direct relationship of Hcy with CP [9].NO is a physiological messenger molecule involved in various physiological processes such as immune response[10]. NO has an essential effect in the progression of periodontal diseases[11]. In

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addition, this molecule is well documented as a local inflammatory generator and included with the factors introduced as those responsible for the ovulatory processes and the PCOS as well[12]. In blood, the serum contains all proteins, electrolytes, antibodies, antigens and hormones. Serum is used in numerous diagnostic tests[13], and this has encouraged us to use it in this study.The purpose of this study was to determine the effect of PCOS on periodontal health status and the severity of periodontal diseases, as well as on the levels of serum Hcy and NO as there was no previous study concerning the estimation of the serum levels of Hcy and NO and their relation with the periodontal health status among women with PCOS.

Materials and method:

In this study, (80) females with an age of (25-35) years were employed; they were attending Babylon Hospital/ Infertility Clinic. All these persons were notified regarding the purpose of these investigations and agreed to its protocol. The subjects were divided into four study groups each group include (20) patient.
Group (1): The patients were with gingivitis. The data of this group considered as a base for the levels of serum Hcy and NO.
Group (2): The patients with gingivitis and PCOS (Gingivitis + PCOS).
Group (3): The patients with CP.
Group (4): The patients were with CP and PCOS (CP + PCOS).

Patients with (PCOS) were diagnosed by the gynecologist according to Rotterdam criteria [14]. Patients in group (1) and (2) were collected from those who are relatives to attending patients or working at the same hospital where the study done, they were with regular menstrual cycles and with no clinical or biochemical features of hyperandrogenism and ultrasound exclusion of polycystic ovary (without PCOS). Patients with group (1) must have signs of gingival inflammation [15] with no pockets or loss of attachment. Patients with group (3) should have at least four surfaces with probing pocket depth (≥4mm) and clinical attachment loss of (1-2) mm or more[16].
Exclusion criteria included:
Smoking.
Pregnancy.
Patient undergone periodontal therapy at the previous three months prior to the study.
A course of anti-inflammatory or anti-microbial therapy during the last three months prior to the study.
Administration of contraceptives or hormonal medications.
Patients on medications for PCOS.
Patients with other systemic diseases (e.g. diabetes, hypertension, cardiovascular disease) which could affect periodontal health condition.
Evaluation of clinical periodontal parameters was performed by using Michigan O periodontal probe at the four sides (mesial, buccal/ labial, distal and lingual/ palatal) of all teeth excluding the third molar tooth; at minimum (20) teeth should be present for each one of the participants. The data collected included:-
1. Evaluation of soft debris by the Plaque Index system (PLI)[17].
2. Evaluation of gingival inflammation by the Gingival Index system (GI)[15].
3. Evaluation of Bleeding on Probing (BOP): the periodontal probe introduced to the base of the gingival sulcus or pocket with slight movement around the root surface. If the bleeding observed within (30 seconds) after probing, the surface was given a score (1), and a score (0) was given for the non-bleeding surface[18].
4. Evaluation of Probing Pocket Depth (PPD): It represents the distance measured from gingival margin to the most apical insertion of the periodontal probe at the the gingival sulcus or periodontal pocket [19].
5. Evaluation of Clinical Attachment Level (CAL): It is the distance from the cementoenamel junction to the most apical position of the inserted probe at the base of gingival sulcus or periodontal pocket[19].
After the clinical periodontal parameters examination, (5ml) of blood was gathered from the patients of the study groups. The blood centrifuged for (15) minutes at 1000

rpm to separate serum samples, which then were kept frozen at (-80) °C. Levels of serum (Hcy and NO) were demonstrated by mean of (ELISA) test, using (MYBioSource human Hcy ELISA kit) for quantifiable identification of serum Hcy levels and (USBiological NO ELISA kit) for quantifiable identification of serum NO levels. Statistical analysis was evaluated by employing t-test, Analysis of variance (ANOVA) test, least significant difference (LSD) and Pearson's coefficient of correlation (r).

The fallowing levels of significance (Sig) were used in the statistical assessment:

Non-significant	(NS)	$P > 0.05$
Significant	(S)	$0.05 \geq P > 0.01$
Highly significant	(HS)	$P \leq 0.01$

We verify that 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:

The current results revealed that mean value of PLI was highest at group (1). While the mean values of GI and BOP score 1 were highest among group (2). The highest mean values of the PPD and CAL were recorded in group (4). The mean values of serum levels of Hcy and NO were found to be highest in group (4), as shown in Table (1). All of the clinical periodontal parameters as well as serum biochemical parameters revealed highly significant differences in comparisons among the study groups, as summarized in Table (1). Highly significant differences were demonstrated regarding the inter groups comparisons of mean values of clinical periodontal parameters between all pairs of the study groups, except for the PLI, they were non-significant in comparisons between group (1) with group (2) and between group (3) with group (4) as well as, for GI and BOP in comparisons between group (3) with group (4) while, it was a significant difference in comparison between group (2) with group (4), as shown in Table (2). The inter groups comparisons of mean values of serum Hcy as well as NO between all pairs of the study groups displayed highly significant differences, except, for serum Hcy comparisons between group (2) with both group (1) and group (3) in addition, for serum NO in comparison between group (2) with group (3), the results presented significant differences as demonstrated in Table (3). For the correlations between serum Hcy and clinical periodontal parameters, the results revealed significant moderate positive correlation with PLI at group (1) and highly significant moderate positive correlation with GI at group (3). While non-significant correlations of Hcy which were positive existed with other clinical periodontal parameters at group (1), group (2) and group (3) but they were negative at group (4) for PLI, BOP and PPD, as illustrated in Table (4). Non-significant correlations were observed between NO and clinical periodontal parameters among all study groups as demonstrated in Table (5). The results revealed non-significant correlations between serum Hcy with serum NO among the study groups, as shown in Table (6).

Discussion

The findings from the current study indicated that the mean value of the PLI was highest at the group (1). This may be due to the leading effect of plaque in the pathogenesis of periodontal diseases when taking in consideration, the lack of other risk factors of periodontal diseases such as smoking and systemic diseases, which excluded from all study. The highest mean values of GI and BOP sites score (1) were among group (2). The hormonal disturbances in PCOS are believed to affect the levels of salivary periodontal pathogens, or their antibody systemic responses, mainly when connected with inflammation of gingiva [(20)].Estrogen decreases keratinization while increasing epithelial glycogen that results in the diminution in the effectiveness of the epithelial barrier [(21)] as well as suppress leukocytes and inhibits production of

proinflammatory cytokines by human marrow cells [22]. From this study, the mean values of PPD and CAL were found to be higher at group (4). In women with hormonal disturbance, estrogen and progesterone inhibit proliferation of gingival fibroblast [23], also collagen and non-collagen synthesis in the fibroblast of periodontal ligament [24], and increases the metabolic breakdown of folate which is necessary for tissue maintenance and repair [23]. In addition, number of testosterone receptors on fibroblasts may be increased in swollen or inflamed gingival tissues, which may lead to increase matrix synthesis [25]. Hence, increased sex hormone levels in serum could induce tissue destruction by activating matrix metalloproteinases proteins, proteolytic enzymes as well as by osteoclast formation [26]. These hormones may alter immunologic factors and responses of periodontal tissues including antigen expression and presentation, and cytokine production [27]. The data of this study revealed that the highest mean values of serum Hcy and NO concentrations were found at group (4). Hence, numbers of studies revealed an increase in the levels of serum Hcy in subjects with CP in comparison to control group [9, 28]. Hyperhomocysteinemia is the result of vitamins deficiency (B6, B12) or folate, or a combination of them [29]. During inflammatory processes, such as CP, the pro-inflammatory cytokines, like interleukin- 6, released from inflamed tissue of periodontal pockets, interacts with vitamin B6 result in elevation of serum Hcy concentration [30]. Moreover, interleukin-6 strongly stimulates the generation of reactive oxygen species (ROS) by monocytes as well as macrophages. Accordingly, other oxidative-sensitive molecules, such as tetrahydrofolate and vitamin B12 (which are required for the metabolism of Hcy), become a target for ROS and this may result in significant accumulation of Hcy [31]. Hcy is identified to have a significant effect on bone modulation mechanism. Available evidence suggested that slightly elevated concentrations of Hcy increase osteoclast activity and bone resorption [32]. On the other hand, there is a study showed that Hcy induces oxidative stress by promoting ROS and its concentrations were increased in PCOS patients compared with control women [33]. Moreover, it has been found that insulin resistance will further increase the level of Hcy among women with PCOS [34] as they have similar pathogenic effects on vascular endothelial cells; this is agreed with other study [35]. Regarding the level of serum NO, available records indicated that there was a statistically significant increased level of serum NO among the CP group as contrasted to those in the control group [36]. The increased levels of NO in the periodontitis patients were attributed to the fact that there were increased levels of inducible nitric oxide synthase enzyme releasing cells during the inflammation of the periodontal tissue. Hence, redness of gingival tissues may be attributed to the vasodilatory action of NO, since the gingival enlargement which was caused by the vascular permeability, increase the influence of NO [37]. In addition, the preventive effect of NO on platelet aggregation and the adhesion-inhibitory action of NO may lead to increased bleeding ability of the gingival tissues even with slight probing [37]. Also alveolar bone resorption will be enhanced by the stimulatory effect of NO on the efficacy of the osteoclasts cells [37]. On the other hand, research introduced for evaluating oxidative damage products, support the elevated serum level of serum NO in patients with PCOS [38]. Findings reported that the NO molecule, as a proinflammatory element, may induce PCOS by activating inflammatory factors in ovaries that affects the endocrine and metabolic measures relevant to PCOS [39]. The present study illustrated that there was a significant moderate positive relationship between the mean values of PLI and serum Hcy concentration at group (1) also it demonstrated a highly significant moderate positive correlation with GI at group (3). The elevated plasma Hcy in these groups may be a consequence of the persistent immunoinflammatory activation by periodontal pathogens as the elevated levels of plasma Hcy in patients with CP could be a marker for systemic inflammation [9]. Also there is a study

reported that marked platelet aggregation may be secondary effects of increased level of Hcy [40], which in some way may explain the negative correlation of this marker with BOP reported at group (4). Non-significant correlations between serum NO concentration and clinical periodontal parameters of the study groups was revealed in this study. Previous research found significant relationship between CAL and PPD with the level of NO in saliva in patients with CP compared to healthy subjects [41]. Moreover, other study revealed significant positive correlations among the clinical periodontal parameters (PLI, GI, BOP, PPD) and NO levels in the gingival crevicular fluid among patients with PCOS [6]. It seems that, local/periodontal NO metabolism is more influenced than the systemic one in PCOS; this may be due to the cumulative effects of both PCOS and periodontitis in the periodontal region[6]. Regarding the correlation between serum Hcy with NO, the present study revealed non-significant weak correlations between them. Hence, prolonged exposure of endothelial cells to Hcy impairs production of NO [40]; while, other study suggested that, during inflammation, NO have the ability of binding to vitamin B12, which in turn will raise the level of circulating Hcy, since inflammation increases the synthesis of NO, therefore, it further will raise the level of Hcy [42]. To some extent, the sample size may affect the results of the correlation among clinical periodontal parameters and the serum Hcy and NO concentration.

It could be concluded that hormonal disturbances play an important effect in modifying and may alter the response of periodontal tissues to microbial plaque, and thus directly may contribute to periodontal diseases and that both serum Hcy and NO may be used as additional early diagnostic tools in the investigation for periodontal diseases and PCOS.

Table (1): Descriptive statistics of clinical periodontal and biochemical parameters among study groups

Parameters	Group (1)		Group (2)		Group (3)		Group (4)		ANOVA	P-Value Sig.
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.		
PLI	1.236	0.080	1.207	0.080	1.130	0.047	1.159	0.075	8.689	0.000 HS
GI	1.293	0.063	1.371	0.065	1.211	0.058	1.216	0.038	34.492	0.000 HS
BOP score 1	23.250	4.077	28.950	4.799	17.950	2.417	18.550	3.236	27.488	0.000 HS
PPD					4.79	0.53	6.21	0.59	t-test -8.021	0.000 HS
CAL					2.75	0.44	3.84	0.52	t-test -7.157	0.000 HS
Hcy	17.425	1.360	21.055	2.573	24.620	3.529	57.230	7.643	338.979	0.000 HS
NO	11.090	0.802	24.215	1.092	21.700	0.912	43.100	6.069	359.580	0.000 HS

Table (2): Inter groups comparisons of mean values of clinical periodontal parameters between all pairs of study groups

Groups	PLI		GI		BOP score 1	
	Mean Difference	P-Value Sig.	Mean Difference	P-Value Sig.	Mean Difference	P-Value Sig.
Group (1) X Group (2)	0.029	0.213 NS	-0.079	0.000 HS	-5.700	0.000 HS
Group (1) X Group (3)	0.106	0.000 HS	0.082	0.000 HS	5.300	0.000 HS
Group (1) X Group (4)	0.077	0.001 HS	0.077	0.000 HS	4.700	0.001 HS
Group (2) X Group (3)	0.077	0.001 HS	0.160	0.000 HS	11.00	0.000 HS
Group (2) X Group (4)	0.048	0.038 S	0.155	0.000 HS	10.400	0.000 HS
Group (3) X Group (4)	-0.029	0.205 NS	-0.005	0.783 NS	-0.600	0.664 NS

Table (3): Inter groups comparisons of serum Hcy(nmol/ml) and NO(mml/L) mean concentrations between all pairs of study groups

Groups	Hcy		NO	
	Mean Difference	P-Value Sig.	Mean Difference	P-Value Sig.
Group (1) X Group (2)	-3.630	0.012 S	-13.125	0.000 HS
Group (1) X Group (3)	-7.195	0.000 HS	-10.610	0.000 HS
Group (1) X Group (4)	-39.805	0.000 HS	-32.010	0.000 HS
Group (2) X Group (3)	-3.565	0.013 S	2.515	0.013 S
Group (2) X Group (4)	-36.175	0.000 HS	-18.885	0.000 HS
Group (3) X Group (4)	-32.610	0.000 HS	-21.400	0.000 HS

Table (4): Correlations between serum Hcy mean concentrations (nmol/ml) with clinical periodontal parameters at study groups

Clinical periodontal parameters	Descriptive Statistics	Group (1)	Group (2)	Group (3)	Group (4)
PLI	r	0.505	0.249	0.278	-0.115
	P	0.023(S)	0.289	0.236	0.629
GI	r	0.297	0.273	0.596	0.143
	P	0.204	0.245	0.006(HS)	0.549
BOP score 1	r	0.258	0.421	0.317	-0.207
	P	0.273	0.064	0.174	0.380
PPD	r	/	/	0.267	-0.102
	P	/	/	0.256	0.668
CAL	r	/	/	0.249	0.005
	P	/	/	0.290	0.984

Table (5): Correlations between serum NO mean concentrations (mml/L) with clinical periodontal parameters at study groups

Clinical periodontal parameters	**Descriptive Statistics**	Group (1)	Group (2)	Group (3)	Group (4)
PLI	r	0.072	-0.276	0.047	0.157
	P	0.763	0.254	0.846	0.509
GI	r	-0.117	-0.171	-0.051	0.009
	P	0.624	0.470	0.830	0.971
BOP score 1	r	0.098	-0.365	0.112	0.035
	P	0.682	0.114	0.638	0.885
PPD	r			-0.357	-0.078
	P			0.122	0.742
CAL	r			-0.159	0.283
	P			0.503	0.227

Table (6): Correlations between Hcy with NO at study groups

Parameters	Descriptive Statistics	Group (1)	Group (2)	Group (3)	Group (4)
Hcy with NO	r	0.073	-0.324	0.248	-0.318
	P	0.759	0.163	0.292	0.172

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