Salivary Free Testosterone and Gingival Health Condition among a Group of Women with Polycystic Ovary Syndrome

AndalusAbid HassanTahir, B.D.S. (1)
Baydaa Hussien,B.D.S, M.Sc. (2)

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women in their reproductive age.It is characterized by anovulation or oligo-ovulation and hyperandrogensim.Androgen excess is the central defect in polycystic ovary syndrome. It is a complex disorder affects general health in addition to oral health.This study aimed to assess the gingival health status among a group of women with polycystic ovary syndrome as well as to estimate the levels of salivaryfree testosterone in unstimulated saliva in relation to gingival health condition.

Materials and methods: Sixty two women with an age range 20-25 years old and with a body mass index range18.5-24.9 (normal weight) were included in this study. They were divided into two groups; the study group which included thirty one women with polycystic ovary syndrome, those women.

nattended Babylon Teaching Hospital/ Infertility Center and the control group which included thirty one women with regular menstrual cycles,without clinical or biochemical features of hyperandrogenism and ultrasound exclusion of polycystic ovary (without polycystic ovary syndrome). Collection of unstimulated salivary samples was carried out under standardrizedconditions, salivary free testosterone levels were estimated. Plaque index of Silness and Löe (1964) was used to assess dental plaque. While gingival index of Löe and Silness (1963) was used to assess gingival inflammation. Data analysis was conducted through the application of the (SPSS version 18). The analysis of data included: Mean, Standard deviation, t-test, Pearson Correlation (r), Receiver Operative Characteristics Curve (ROC curve).

Results: Results showed that the mean value of plaque index was found to be higher in the control group (1.18±0.03) than that in the study group (1.00±0.08) with statistically highly significant difference (p<0.01).While the mean value of gingival indexwas higher among the study group (1.52±0.06)than that in the control group (1.31±0.05)with statistically highly significant difference (p<0.01).Salivary levels of free testosterone among women with polycystic ovary syndrome (44.12±1.37 pg/ml) were found to be higher than that of control (18.50±0.87) with statistically highly significant difference (p<0.01).The results in this study showed that the correlations between salivary free testosterone with the oral variables (plaque index, gingival index) were statistically not significant (P>0.05).

In order to assess the specificity and sensitivity of the use of salivary free testosterone as a diagnostic tool, statistical analysis of Receiver Operative Characteristics Curve (ROC) was used and predictive Value Measurmentswere done in this study. The results showed that the area under the curve for salivary free testosterone test was (1.000) with highly significant p-value (p<0.01). The best optimal cut off point for salivary free testosterone was (30.86) with (100%) sensitivity and specificity, indicating a very good predictive capacity for using salivary free testosterone as a marker for the diagnosis of polycystic ovary syndrome.

Conclusions : It was concluded that gingival inflammation was high among women with polycystic ovary syndrome. Furthermore, salivary levels of free testosterone were found to be high among women with polycystic ovary syndrome.

Key words: polycystic ovary syndrome, salivary free testosterone, plaque index, gingival index. **(J Bagh Coll Dentistry 2017; 29(1):165-169)**

INTRODUCTION

Polycystic ovary syndrome (PCOS) is now recognized as a common, heterogenous, heritable disorder affecting women throughout lifetime(1) and it is the leading cause of female anovulatory infertility (2) .The exact cause of PCOS is unknown, however, it results from a combination of genetic and environmental factors(3). The precise prevalence of PCOS is unknown as the syndrome depends on the diagnostic criteria used, however, World Health Organization (WHO) estimates that it affected 116 million women worldwide in 2012 (3.4% of women)(4).PCOS is chronic condition of anovulation or oligovulation with clinical or biochemical hyperandrogenism which occurs in the absence of other underlying condition(5).

(1) Master student , Department of Pedodontic and Preventive Dentistry, College of Dentistry, University Baghdad.
(2) Assistant Professor, Department of Pedodontic and Preventive Dentistry, College of Dentistry, University Baghdad.

It was found that the majority of women with PCOS demonstrated elevated circulating androgen levels(6). Serum level of free testosterone, and not total testosterone, are more frequently elevated in women with PCOS (7).

It has become evident over the past 30 years that PCOS is more than a reproductive disorder(8). PCOS shows a link with oral health in addition to its effect on general health (9).Periodontal diseases and PCOS are the most common disorders in women with significant public health impact(10).This syndrome have an impact on gingival inflammation or vice versa(9).It was found that the salivary free testosterone level was high in gingivitis patients (11).

Salivary diagnostic approaches have been developed to monitor oral diseases such as periodontal diseases(12,14).Saliva is said to be a "mirror of the body" because it provides vital clues to systemic health (14); therefore, saliva has become

useful as an alternative for blood in medical diagnosis and research[15].Besides, the development of new technologies may promote a wider use of salivary assay in the near future [16].

As far as it is known, there was no previous Iraqi study concerning the estimation of the salivary levels of free testosterone and their relation with gingival health condition among women with PCOS. Furthermore, in order to gain knowledge regarding the gingival health status and salivary free testosterone for this target group in which saliva may provide a simple, inexpensive and noninvasive measure, therefore, this study was designed and conducted.

MATERIALS AND METHODS

The total sample composed of sixty two females aged(20-25) years with normal weight (body mass index range of 18.5-24.9). Those were divided into two groups: thirty one women newly diagnosed with PCOS who referred to Babylon Teaching Hospital/ Infertility Clinic as a study group and thirty one women with regular menses,without clinical or biochemical features of PCOS and ultrasound exclusion of polycystic ovary syndrome from the relatives of those women as a control group. This study was carried out during the period from the end of December (2014) till the end of May (2015). The study group was diagnosed according to Rotterdam criteria[17].Exclusion criteria included: smocking,pregnancy,previous diagnosis of condition with hormonal disturbance,presence of systemic disease(diabetes,hypertention,cardiovascurdisease),medication affecting periodontium (antiepeliptic,immunosuppresent, antihypertensive, corticosteroid), confounding medications(contraceptive pills, steroid hormone), use of antibiotic and /or inflammatory drugs within the last months,the presence of less than 20 natural teeth (less representive for periodontal condition).Collection of unstimulated salivary samples was carried out under standardrized conditions following the instruction cited by (Navazesh and Kumer,)[18],the salivary samples were collected and stored in -20C until analysis, salivary free testosterone level (pg/dl) was measured by ELISA method using DEMEDITEC ELISA kit[19]. Plaque index of Silness and Löe[20]was used to assess dental plaque. While gingival inflammation was evaluated using gingival index of Löe and Silness [21].Data analysis was conducted through the application of the (SPSS version 18). The analysis of data included:Mean, Standard deviation, t-test, Pearson Correlation (r), Receiver Operative Characteristics Curve (ROC curve).

RESULTS

Table (1) shows the mean values of plaque index and gingival index among the study and control groups. It was found that the mean value of plaque index was higher in the control group than that in the study group with statistically highly significant difference ($p<0.01$).While the mean value of gingival index was higher among the study group than the control group with statistically highly significant difference ($p<0.01$).

Table (2) shows the mean values of salivary free testosterone for both the study and control groups. The result revealed that the salivary level of free testosterone was higher among women with PCOS than their level in the control group with statistically highly significant difference ($p<0.01$).

Table (3) shows the correlation between salivary free testosterone with oral variables (PlI and GI). It was found that all correlation between salivary free testosterone and oral variables were statistically not significant($P>0.05$).

Table (4) shows the best optimal cut off point for salivary free testosterone as a diagnostic tool. It was found that the optimal cut off point for salivary free testosterone was (30.86) with (100%) sensitivity and specificity and the area under the curve was (1.00).

Table1: Plaque index and gingival index(mean±SD) among the study andcontrol groups.

Variable	Group				Statistical Difference	
	Study		Control			
	Mean	±SD	Mean	±SD	t-test	p-value
PlI	1.00	0.08	1.18	0.03	12.30	0.00**
GI	1.52	0.06	1.31	0.05	15.09	0.00**

****=Highly significant (P<0.01). df=60**

Table 2: Salivary free testosterone (mean±SD) among the study and control groups.

Variable	Group				Statistical Difference	
	Study		Control			
	Mean	±SD	Mean	±SD	t-test	p-value
Salivary free testosterone (pg/ml)	44.12	1.37	18.50	0.87	88.08	0.00**

****=Highly significant at P<0.01 df=60**

Table 3: Correlation coffecient between salivary free testosterone with plaque index, gingival index among the study and control groups.

Variable	Study				Control			
	PlI		GI		PlI		GI	
Free testosterone (pg/ml)	r	P	R	P	r	P	r	P
	-0.01	0.98#	-0.08	0.69#	-0.11	0.54#	0.15	0.43#

 # = not significant at P>0.05

Table 4: Cutoff point, sensitivity, specificity and area under curve of salivary free testosterone.

Variable	Test Result Variable(s)	Cut off	Sensitivity %	Specificity %	Area under curve	P-value
Salivary free testosterone	Optimal	30.86	100	100	1.00	.000**

****=Highly significant at P<0.01**

DISCUSSION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women in their reproductive years [22]. It affects general health as well as oral health [9].

The results of the current study showed that the mean value of the plaque index was found to be higher in the control group compared to that in the study group with statistically highly significant difference (p<0.01). This may be due to oral health negligence in the control group and this could be attributed to lack of motivation about dental plaque control. This resultwas inconsistent with the result reported by previous studies[9,10,23]which found that the mean value of dental plaque was lower in the control group.

The result of present study showed a highly significant increased gingival inflammation represented by higher gingival index among PCOS group. This result was also reported by other researchers[8, 23]who found thatgingivitis was higher in the PCOS group than that in the control group. This could be explained by the fact that gingival inflammation may be linked to systemic inflammation [24].It was reported that PCOS is considered as a state of a low grade chronic inflammation[25].Furthermore, PCOS is one of the endocrine disorder characterized by hormonal imbalance and among these, steroid hormones [26] which have been mostly linked with periodontal pathogenesis [27]. It was found that the gingival tissue is one of the targets for steroid hormones which can exacerbate gingivitis during period of hormonal flactuation[28]. A previous study reported an increase in the level of estrogen hormone among women with PCOS[26], the gingival tissue respond to increased level of estrogen hormone by undergoing vasodilatation and increased capillary permeability also there is increased migration of fluid and white blood cells out of blood vessels. Cyclic increase in the production of sex steroid hormones often alter the biology of gingival tissue and vasculature, and recognition by effector cells of the local immune system [29,30].

The current study revealed that salivary free testosterone level was higher in the study group than that in the control group with statistically highly significant difference (p<0.01). This result was also reported by previous study[31]. The highly significant increase in salivary free testosterone among women with PCOS could be explained by the fact that androgen excess is the central defect in PCOS women and hyperandrogenism is the most common characteristic feature of this syndrome, the level of serum free testosterone is increased in women with PCOS [5] due to decrease sex hormone binding globulin (SHBG) production [7]. It was reported that there is a correlation between serum free testosterone and salivary free testosterone [32],so, the increase in the level of free testosterone in serum could explain the increase in salivary free testosterone level among women with PCOS. In this study, it was found that salivary free testosterone is considered as the most specific and sensitive biochemical marker that used as a diagnostic tool for women with PCOS, this is supported by the result of Receiver Operative Characteristics Curve (ROC) that was used to assess the specificity and sensitivity of the use of salivary free testosterone as a biomarker for PCOS. In medicine, the ROC analysis had been extensively used in the evaluation of diagnostic tests [33]. It was found that the area under the curve for free salivary testosterone was (1.00) and (100%) sensitivity and (100%) specificity with cut off point of (30.86) indicating very good predictive capacity for the use of salivary free testosterone as a biomarker for the diagnosis of PCOS.

In current study, the correlations between salivary free testosterone with oral variables (PlI, GI) were found to be statistically not significant (p>0.05). The result of this study was inconsistent with previous study[11] which found that GI, PlI were significantly correlated with increased salivary free

testosterone. On the other hand, another study[34]found that the level of salivary free testosterone decreased in gingivitis patients. It was reported that testosterone has an anti-inflammatory effect on periodotium[35,36]. It has an inhibitory effects in the cyclooxygenasepathway of arachidonic acid metabolism in the gingiva by inhibiting prostaglandin secretion[37],and stimulates bone cell proliferation and differentiation and therefore has a positive effect on bone metabolism [38].

One must keep in mind the contrast in the result among studies could be attributed to differences in diagnostic criteria employed,sample size, differences in biochemical procedures, even differences in statistical methods employed and difference in the way of saliva collecting, type of saliva collected (stimulated or unstimulated),in addition to differences in inclusion criteria used for selection of women, in this study, only women with a normal weight were included in the study while in previous studies, there was no restriction in the criteria used for selection of women with PCOS.

From the results of this study, it was concluded that women with PCOS had high gingival inflammation, thus, an organized, comprehensive oral health preventive and educational programs in addition to the intense oral hygiene programs are essential to improve the gingivalhealth condition of women with polycystic ovary syndrome.Frthermore, salivary levels of free testosterone were found to be high among women with polycystic ovary syndrome.

An interesting result in this study that salivary free testosterone can be used as a marker for diagnosis of women with polycystic ovary syndrome.

REFERENCES

1. Sirmans S, Kristen pate A. Epidemiology, Diagnosis and Management of polycysticovary syndrome. J Clin epidemiology 2014;(6): 1-13.
2. Tang T, Glanville J, Orsi N, Balen AH. The use of metformine for women with PCOS undergoing IVF treatment. Hum Reprod 2006;21(6):1416-1425.
3. Goodarzi M O, Dumesic D A, Chazenbalk G, Azziz R. Polycystic ovary syndrome: Etiology, pathogenesis and diagnosis. Nature Reviews Endocrinology 2011; 7: 219–231.
4. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M. Years lived with disability (YLDs) for1160 sequelae of289diseases and anjuries. EMJ 2012;380(9859):2163-86.
5. Hacker Neville F:Hacker and Moore's essentials of Obstetrics and Gynecology:5thed. China: Elsevier;2012.
6. Azziz R, Carmina E, Dewailly D. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. J ClinEndocrinolMetab 2006; 11:4237–4247.
7. Escober-Morreale HF, Calvo RM, Villuendas G, Sanchoj, San Millan JL. Association of polymorphism in interleukine-6 receptorcomplexwithobesity and hyperandrogenenism. ObesRes 2001;11:987-996.
8. Fauser BC, Tarlatzis BC, Rebar RW. Consensus on Women's Health Aspects of Polycystic Ovary Syndrome (PCOS): The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. FertilSteril2012;97:28-38.
9. Akcalı A, Bostanci N, Özçaka O, Öztürk-Ceyhan B, Gümüş P, Buduneli N, Georgios N. Association between Polycystic Ovary Syndrome, Oral Microbiota and Systemic AntibodyResponse. JPeriodontol2014;24(5):78-88.
10. Erhan D, Guliz D , Nese C, Ayla H, Murat O, Erdem K, Tolga F, TozumA, Kamer K, Alev A, Bulent O . Periodontal disease in polycystic ovary syndrome. TDJ 2009; 20:666-671.
11. Abdulla HI, Helmi Z, kadhem ZK. Effects of Estradiol, Progesterone and Testosterone on Oral Health Status and Gender Identification of Fetus in Women During Pregnancy.IDJ 2014;6(24)34-45.
12. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis–a review. J ClinPeriodontol 2000; 27:453–465
13. Socransky S, Haffajee A, Smith C, Duff G. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. J ClinPeriodontol 2000; 27:810–818.
14. Zhang L, Xiao H, Wong D. Salivary Biomarker for Clinical Applications. Molecular Diagnosis and Therapy 2009;13(4):245-259.
15. Aps JK, Martens LC. The physiology of saliva and transfer of drugs into saliva. Forensic SciInt 2005; 150: 119-31.
16. Tremblay M, Loucif Y, Methot J, Brisson D, Gaudet D. Salivary PH as a Marker of PlasmaA diponectin Concentration in Women. 2012; ADJ 4:4.
17. Rotterdam ESHRE/ASRM –Sponsored PCOS Consensus Work- shop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks relted to polycystic ovary syndrome. FertilSteril 2004;81(1): 19-25.
18. Navazesh M, Kumar S. Measuring salivary flow Challenges and opportunities. JADA 2008; 139(5): 35-40.
19. Karen K.: Measurement of free Testosterone in normal women and women with androgen deficiency: Comparison of methods J Clin Endocrin Metabol 2004; 89 (2): 525 – 533.
20. Silness J, Löe H. Correlation between oral hygiene and periodontalcondition. ActaOdontolScand 1964; 22: 121-135.
21. Loe H, Silness J. Peiodontal disease in pregnancy I. ActaOdentalScand 1963; 21:533-551.
22. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod, 2010;25(2):544-51.
23. Abbas MJ, AL-Hashemy E H, Abdul Hassan OJ. Elevation Salivary C-reactive protein (CRP) Levels in Iraqi Women with Polycystic ovary syndrome (PCOS) and oral diseases. MDJ 2014;11(1):136-138.
24. Loos BG, Craandijk J, Hoek FJ, Paulein ME, Wertheim-van Dillen PM, van der Velden U. Elevation of Systemic Markers Related to Cardiovascular Diseases in the Peripheral Blood of Periodontitis Patients. J Periodontol 2000;71:1528–34.
25. Kelly CJ, Speirs A, Gould GW. Altered vascular function in young women with polycystic ovary syndrome. J ClinEndocrinolMetab 2001;87(2):742-746

26. Laven JS, Imani B, Eijkemans MJ, Fauser BC.New approach to polycystic ovary syndrome and other forms of anovulatory infertility. Obstet Gynecol Surv 2002;57(11):75567.
27. Apoorva SM, Suchetha A. Effect of sex hormones on the periodontium. Ind J Dent Sci2010 ; 2 (5): 36-40.
28. Mariotti A. Sex steroid hormones and cell dynamics in the periodontium. Crit Rev Oral Biol Med 1994;5:27-53.
29. Scannapieco FA. Periodontal inflammation from gingivitis to systemic disease. CompendContEduc Dent 2004; 25(7): 16-24.
30. Marcuschamer E, Hawley CE, Speckman I, MaríaDíaz Romero R, Molina JN. A lifetime of normal hormonal events and their impact on periodontal health. PerinatolReprod Hum 2009; 23(2): 53-64.
31. Sharquie K.E., Al-Bayatti A.A., Al-Ajeel A.I.,. Al-Bahar A.J, Al-NuaimyA.A.. Free testosterone, luteinizing hormone/follicle stimulating hormone ratio and pelvic sonography in relation to skin manifestations in patients with polycystic ovary syndrome Saudi Med J 2007; 28(7): 1039–1043.
32. Youssef O, El AttySA ,Sharaf El Din HM. , Kamal M , Youssef G, Al-Inany H . Reliability of salivary testosterone measurements in diagnosis of Polycystic Ovarian Syndrome. Middle East Fertility Society Journal 2010; 15: 183–187.
33. Pepe Margaret S. The statistical evaluation of medical tests for classification and prediction. AMJ 2003;9(4):81-87.
34. Al-AniRH ,AlKazzaz FF. Salivary and seral sex steroid hormones variation among group of peoples with gingivitis. IDJ 2012;24(4):24-35.
35. Parkar M, Tabona P, Newman H, Olsen I. IL-6 expression by oral fibroblasts is regulated by androgen. Cytokine 1998;10:613-619.
36. Gornstein RA, LappCA, Bustos-Valdes SM, Zamorano P. Androgen modulateintrleukine -6 production by gingival fibroblast in vitro. J Periodontal 1999;70:604-609.
37. ElAttar TM, Lin HS, Tira DE. Testosterone inhibits prostaglandin formation by human gingival connective tissue: relationship to 14C-arachidonic acid metabolism. Prostaglandins Leukot Med 1982;9:25-34.
38. Kasperk CH, Wakley G, Hierl T, Ziegler R. Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. J Bone Miner Res 1997;12:464-471.

الخلاصة

خلفية الموضوع :متلازمة تكيس المبايض هي واحدة من اكثر اضطرابات الغدد الصماءشيوعا"التي تؤثر على النساء في عمر الانجاب. تتميز متلازمة تكيس المبايض بعدم الاباضة اواضطراب الاباضة وزيادة الهرمون الدكري.زيادة الهرمون الدكري هو الاضطراب الاساسي لمتلازمة تكيس المبايض . ان متلازمة تكيس المبايض هي اضطراب معقد تؤثر على الصحة العامة بالاضافه الى صحة الفم.ان اهداف هذا البحث كانت لتقييم حدوث امراض اللثة لمجموعة من النساء المصابات بمتلازمة تكيس المبايض ولتقييم مستوىالهرمون الذكري الحر في اللعاب غير المحفز وعلاقتها بصحة اللثة.

المواد وطرائق العمل:شملت هده الدراسة اثنان وستون من النساء تراوحت اعمارهن بين (20-25) سنة وباوزان طبيعية تراوحت بين (18.5-24.9) وتم تقسيم النساء الى مجموعتين : واحد وثلاثون امراة من المصابات بمتلازمة تكيس المبايض في مستشفى بابل التعليمي /وحدة العقم(مجموعة الدراسة), و واحد وثلاثون امراة تشتمل على نفس المواصفات وبدورة شهرية منتظمة وليس لديهم اي علامات او اعراض على ارتفاع الاندروجين اوالاصابة بمتلازمة تكيس المبايض تحت الامواج فوق الصوتية (المجموعة الضابطة).تم جمع اللعاب غير المحفزفي ظروف موحدةتم قياس الهرمون الذكري الحر في اللعاب غير المحفز.تم استخدام مقياس الصفيحة الجرثومية وفقا لتصنيف Silness and Löe(1964) اما التهاب اللثة فقد تم قياسها وفقا لمقياس Löe and Silness (1963).

النتائج:اظهرت النتائج ان متوسط قيمة الصفيحة الجرثومية كان اعلى لدى المجموعة الضابطة(1.18±0.03)من المجموعة تحت الدراسة(1.00±0.08) مع وجود فرق معنوي عالي بين المجموعتين. بينما القيمة المتوسطة لالتهاب اللثة كانت اعلى لدى مجموعة الدراسة(1.52 ±0.06)منه لدى المجموعة الضابطة (1.31 ±0.05) مع وجود فرق معنوي عالي. وجد ان مستويات الهرمون الذكري كانت اعلى لدى النساء المصابات بتكيس المبايض(44.12±1.37)من مستوياتها لدى النساء في المجموعة الضابطة (18.50 ±0.87) مع وجود فرق معنوي عالي. اظهرت نتائج هذه الدراسة بانه لايوجد فرق معنوي بخصوص ارتباطات الهرمون الذكري مع متغيرات الفم (الصفيحة الجرثومية, التهاب اللثة).لقد اظهرت نتائج هذه الدراسة بان اختبار منحني خصائص المستقبل الفاعل الذي يستعمل لتقييم الخصوصية والحساسية لاستعمال الهرمون الدكري اللعابي كمؤشر لتشخيص متلازمة تكيس المبايض وجد بان المنطقه تحت المنحني بالنسبه للهرمون الدكري كانت (1) مع (100٪)حساسية و(100٪)خصوصية.مشيرا" الى امكانية اعتباره كمؤشر جيد لتشخيص متلازمة تكيس المبايض.

الاستنتاجات:لقد تم الاستنتاج بان التهاب اللثة كان عاليا" لدى النساء المصابات بمتلازمة تكيس المبايض. وكانت مستويات الهرمون الذكري الحرعالية في لعاب النساء المصابات بمتلازمة تكيس المبايض مما يقترح امكانية قياسه في اللعاب كمؤشر جيد لتشخيص متلازمة تكيس المبايض.