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Effect gingivitis on anti-mullierian hormone in females with polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome is a mutual disease affected about 4% to 12% women in propagative age. Anti-mullerian hormone (AMH) is a glycoprotein, a member of the transforming growth factor-b family. It plays a master role in cell growth and differentiation. Polycystic ovary syndrome (PCOS) is a hormonal disorder of women that not only is the leading cause of infertility but also shows a reciprocal link with oral health. Gingivitis was inflammed disorders it has linked with altered vascular reaction, high levels of adhesion molecules and elevated expression of local and systemic inflammatory cytokines and adipokines like tumor necrosis factor-alpha and transforming growth factor-beta. Increased gingival inflammation is related with increased synthesis of steroid hormone. Therefore, The aims of the study to examine the effect gingivitis and PCOS on levels of AMH and study the relation between PCOS and gingivitis in women and if AMH is useful marker in diagnosis of gingivitis and PCOS. A total 84 women of age group between (20 - 28) years were included in this study. Twenty one female who had PCOS and (21) female with periodontal diseases (gingivitis), (21) female who possess both PCOS and gingivitis and (22) healthy females with regular menstrual cycle who presented sequentially and prospectively to the Department of Endocrinology of Al-Rumadi teaching hospital/ Al-Anbar/Iraq. High significant elevation in saliva AMH in PCOS with gingivitis group than in PCOS, gingivitis and control group $p<0.05$. High concentration of AMH in PCOS with gingivitis make suggested all Physicians must refer PCOS patients to oral health clinic providers for a comprehensive oral evaluation and treatment.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women seen in 4-18% of childbearing age (Diamanti-Kandarakis *et al.*, 1999; March *et al.*, 2010). In addition to reproductive derangements, patients with PCOS may develop other metabolic and psychological comorbidities (Rahiminejad *et al.*, 2014). Classically, PCOS is characterized by the entity of menstrual abnormalities (oligomenorrhea or amenorrhea), chronic anovulation or oligoovulation, clinical/biochemical proof of hyperandrogenism (hirsutism, acne, or androgenic alopecia), and ultrasound findings.(Rotterdam 2004) women with this gender-specific form of metabolic syndrome are at higher risk for rise insulin resistance (IR), obesity, dyslipidemia, cardiovascular disease (CVD), and hypertension (Sam and Dunaif 2003; Moran *et al.,* 2010) Moreover, recent researches that shown the higher spread of wreaked glucose tolerance (IGT), type II diabetes mellitus (DM), and lipid profile trouble in women with PCOS (Evangelista and McLaughlin 2009; Wild 2012). Korhonen *et al.,* (2003); Diamanti-Kandarakis *et al.,* (2006) was linked the PCOS with low-grade systemic inflammation as confirmed by rise of multiple biomarkers of inflammation as C-reactive protein, interleukin-18, monocyte chemoattractant protein-1 and white blood count in addition to endothelial dysfunction and elevated oxidative stress.

Anti Mullerian Hormone (AMH), too named Mullerian Inhibiting Substance (MIS), was a homodimeric glycoprotein of biomarker TGFb kindred. It responsible for a master functions in cell growth and differentiation. It have a molecular weight of 140 kDa, it was biggest than LH or FSH in four times (Annemarie *et al.,* 2002). AMH contend a function in sex differentiation meantime embryo growth. Under the effect of the AMH synthesis in Sertoli cells, the Mullerian ducts decadent in male fetuses. This leads into the natural expansion of the male genitals. fetuses of Female not have this hormone, and so growth the internal genital organs in female (Annemarie *et al.,* 2002; Visser *et al.,* 2006).

In women, during the beginning of maturity AMH, like inhibin B, is synthesized by the granulosa cells of the ripeness ovarian follicle, not by the primative follicles and as well not by the antral follicles under immediate regulation of FSH in the end stage of follicular developed (Pankhurst 2017). AMH is the biological organizer of folliculogenesis and of primative follicular tearing. It decreased the ratio of follicle diversion of the primative to the processing phase and control follicle developed by inhibition of FSH-induced diversion of the early to the end stage (Gnoth *et al.,* 2008).

Periodontal diseases are chronic inflammatory procedure that caused tooth loss by simulating tooth-supporting tissues, inclusive the gingiva, alveolar bone, and periodontal ligaments (Genco *et al.,* 2005; Reeves *et al.*, 2006). Increment to the function of bacterial infections, once upon a time studies have demonstrated the association of periodontal diseases and systemic conditions as dyslipidemia, obesity, IR, DM, and CVD.(Kim and Amar 2006; Katz *et al.*, 2012).

Marchetti *et al.,* (2012) defined gingivitis as an inflammatory disorders resulting from pathogenic microbiota existent in the oral biofilm that trigger inflammatory and adaptive immune responses. Gingivitis correlating with high vascular response, higher levels of adhesion molecules (as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1) and raise expression of topical and systemic inflammatory cytokines, inclusive tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and monocyte chemoattractant protein-1, conducting in impaired endothelial function (Dursun *et al.,* 2011; Ozcaka *et al.,* 2012).

Many reported for the first time, Dursun, (Dursun *et al.,* 2011), Ozcaka, (Ozcaka *et al.,* 2013) revealed linked between PD and polycystic ovary syndrome (PCOS), the fundamental reason of an ovulatory infertility, and the major concerted gynecologic and endocrine state between women in reproductive age, affecting between 5 and 10% of the female

inhabitance. However, just limited numbers of studies 16–22 have investigated this linked (Porwal *et al.,* 2014).

Materials and methods

This study involving 84 women; (21) well characterized women with PCOS and (21) with periodontal diseases (gingivitis), (21) with PCOS and gingivitis and (21) systemically and periodontally healthy women with regular menstrual cycle who presented sequentially and prospectively to the Department of Endocrinology of Al-Ramadi teaching hospital/ Al-Anbar/Iraq for duration from February 2017 until march 2018. All subjects were given written informed consent. This study was approved by Ethics committee of college of dentistry, University of Anbar. The diagnosis of PCOS by gynaecologist was based on Rotterdam crireria that necessitating the presence of two out of three of the following criteria: clinical/biochemical hyperandrogenism, oligo-/anovulation, and polycystic ovaries (Yildiz *et al.,* 2010). Exclusion criteria involved any illness except PCOS, gingivitis, medication for the preceding 3 months, hyperprolactinemia, Cushing's disease and androgen-secreting tumours.

Gingivitis women were diagnosed in department of periodontology/ college of dentistry / Al-Anbar University. All of the women in control group had regular periods, no clinical or biochemical hyperandrogenism, no polycystic ovaries on ultrasound, no significant background medical history and none of them were under medications including oral contraceptive pills or over the counter medications. All women with measured HbA1c to checking if any woman have diabetic type 2. Height, weight, and body mass index (BMI) were estimated according to WHO guidelines (World, Health and Organization. 2008).

Periodontal examination was carried out for all study groups under the supervision of periodontitis senior to minimize the observer bias. The severity of periodontal disease was assessed used gingival index (GI) according to Loe and Silness (Loe and Silness 1963).

The saliva collection In brief, the participants were asked to spit directly or drool into sealable polystyrene tube (4 mL) and to provide at least about 3 mL of the saliva. We used unstimulated saliva samples to avoid any assay interference. The "passive drool" technique was used for the collection of saliva instead of the 'salivette' method. Salivary AMH (A79765), LH, and FSH were estimated by using (ELISA technique). Participants were instructed to relax and to swallow all saliva found in their mouths and prior to teeth brushing. Saliva was collected in a screw capped sterilized bottle. Then the saliva sample was centrifuged for about ten minutes at 3000 round per minutes (r.p.m) and the clear supernatants was separated by micropipette and then stored at -20 C^{o} in freezer until the time of biomarkers analysis. The concentration of LH, FSH, and AMH was measured using ELISA technique while HbA1C estimated using spectrophotometer. All measurements were carried out in Laboratory Teaching Unit/Baghdad Teaching Hospital/ Baghdad/Iraq.

Statistical analyses were performed using SPSS statistical software (version 19). The data were expressed as (mean ± SEM) mean± standard error of mean. All Results was compared by (ANOVA) one way analysis of variance and (LSD) the least significant differences were calculated. Correlations between variables were estimated using Pearson's correlation coefficient and simple retrogression analysis. Statistically, the $p<0.05$ value considered as significant.

Results

Estimation of AMH, FSH, LH, and HbA1C in saliva of 85 females (22 control, 21 had PCOS, 21 had gingivitis, and 21 had PCOS and gingivitis). The clinical characteristics of the patient groups and controls showed in table (1). This table revealed significant differences in BMI and gingival index between study groups. Table (2) show highly significant differences in the mean of AMH concentration in PCOS, gingivitis, PCOS+gingivitis

group when comparison with the mean of AMH in control group. Group comparison revealed high significant increase in the mean of AMH in gingivitis and PCOS+gingivitis group than in PCOS group. Furthermore, the mean concentration of AMH was significant higher in PCOS+gingivitis group than those of the gingivitis. The Same table demonstration high significant differences in saliva FSH and LH in PCOS group than in control group p<0.05.

Table 1. Clinical and biochemical data of controls and patients.

	Controls	PCOS	gingivitis	PCOS +gingivitis
Number	22	21	21	21
Age (years)	24.0±0.54	24.1±0.64	24.1±0.55	24.4±0.58
BMI (Kg/m2)	20.9±0.36	22.0±0.38a*	21±0.37b*	22.4±0.33a*, c*
HbA1C %	3.8±0.16	3.74±0.16	3.6±0.17	3.94±0.19
Gingival index	0.14±0.07	0.14±0.08	2.1±0.17 a*,b*	2.5±0.15a*, b*,c*

Results expressed as mean±SEM.

[a]*ANOVA test: PCOS, gingivitis, PCOS+gingivitis vs control group *p<0.05.*

[b]*ANOVA test: gingivitis, PCOS+gingivitis vs PCOS: *P<0.05.*

[c]*ANOVA test: PCOS+gingivitis vs gingivitis: *<0.05.*

In the same table found High Significant increase in saliva FSH and large significant decrease in saliva LH in PCOS group comparison with gingivitis group p<0.05. By using Pearson correlation test found significant negative correlation between AMH and age and between HbA1C and gingival index in females who had PCOS with gingivitis table 3. In same table positive significant correlation found in same group between FSH and LH and also between LH and gingival index.

Table 2. The mean±SEM saliva level among control, PCOS, gingivitis, and PCOS with gingivitis groups.

	Controls	PCOS	gingivitis	PCOS +gingivitis
AMH((ng/ml)	1.3±0.08	3.8±0.12 a*	1.9±0.08 a*, b*	4.6±0.13 a*, b*, c*
FSH(mIU/ml)	8.4±0.95	6.14±0.19 a*	8.8±0.98 b*	7.3±0.34
LH(mIU/ml)	14.4±1.3	17.4±0.87 a*	12.1±1.14 b*	18.81±0.34 a*, c*

[a]*ANOVA test: PCOS, gingivitis, PCOS+gingivitis vs control group *p<0.05.*

[b]*ANOVA test: gingivitis, PCOS+gingivitis vs PCOS: *P<0.05.*

[c]*ANOVA test: PCOS+gingivitis vs gingivitis: *<0.05.*

Discussion

This work was the first in study the effect of saliva AMH concentration on PCOS and gingivitis females in Iraq. All subjected women had polycystic ovaries with and without gingivitis by ultrasound and therefore it might have expected raised in all of the concentration AMH level. Current study reported highly significant increased in the mean of saliva AMH in females that had PCOS with gingivitis than in control, PCOS, and gingivitis groups. This finding are inconsistent with results of Porwal (Porwal *et al.,* 2014), Akcali, (Akcali *et al.,* 2014), Rahiminejad, (Rahiminejad *et al.,* 2015) who reveals a positive relation between PCOS and periodontal disease when measured serum AMH. Consequently, it is tempting to hypothesize that increased risk on developing PD in controls than in patients with PCOS.

PCOS is examined to be a metabolic syndrome related to risk factors such as cardiovascular (Dokras 2016), insulin-dependent diabetes, dyslipidemia and endothelial dysfunction (Hsu 2013) and visceral obesity (Lim *et al.*, 2013). PCOS is likely to be characterised by chronic low-grade inflammation

(Ebejer and Calleja-Agius 2013) and it is responsible for metabolic abnormalities. Lately, Dursun, (Dursun *et al.,* 2011), Ozcaka, (Ozcaka *et al.,* 2012) were reported that certain pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor-α (TNF-α) were higher in PCOS women, compared to systemically healthy individuals. Additionally Ozcaka, (Ozcaka *et al.,* 2013) was reported that prolonged low-grade inflammatory state can be caused by gingivitis, which is a common pathology seen in patients with PCOS.

Table 3. Pearson correlation analysis as dependent variable including other variable in PCOS with gingivitis women group.

Correlation between	r	p
AMH Age	-0.538	0.012
FSH LH	0.443	0.04
HbA1C Gingival index	-0.443	0.04
LH Gingival index	0.522	0.015

Nair (Nair *et al*, 2017) reported that raised spread of periodontal disease was revealed in females with PCOS compared to women without PCOS (Healthy women). Same results were showed by Dursun *et al*, 2014 where PCOS women showed increase periodontal indices (Hsu 2013). It is a well-documented fact that hormonal changes occurring during puberty, pregnancy, and menstrual cycles have an impact over the gingival health of women (Ozcaka *et al.,* 2012). In PCOS women, ovulation and follicle growth often do not occur due to the disrupted levels of follicular stimulating hormone (FSH) and luteinising hormone (LH). Extra LH production leads to the excessive production of androgens (Ebejer *et al.*, 2013; Llim *et al.*, 2013). This excess androgen is responsible for the many features like hirsutism (Dokras 2016), acne and weight gain (Dokras 2016). It is a known fact that the increase in the steroid hormone levels like oestrogen causes gingival inflammation (Hsu 2013).

Table 4. Pearson correlation analysis as dependent variable including other variable in PCOS women group.

Correlation between	r	p
FSH LH	0.704	0.000
HbA1C Gingival index	0.536	0.012

It is difficult to establish worldwide diagnosis of PCOS using solely European or North American guidelines since the clinical presentation of PCOS varies between continents (Molly *et al.,* 2017). Moreover, because of ultrasound features of polycystic ovaries is commonness in healthy women, the embodiment of this sign to the diagnostic criteria of PCOS is still questionable (Kubota 2013.). It is therefore hypothesized that besides PD other risk factors embedded to a misdiagnosed PCOS, such as chronic hyperglycemia, raised systemic levels of proinflammatory cytokines and cardiovascular disorders (Bachanek *et al.,* 2015), and may have contributed significantly in provoking the local and systemic inflammation. Our study demonstrate that significant negative correlation between AMH and age $p<0.05$. This finding in line with Quinn and

coworkers, 2017 that reported decreased AMH with age increased.

Javed (Javed *et al.*, 2016) stated that not surgical treatment of periodontal diseases with adjunct laser therapy is potential linked in inhibit serum proinflamatory cytokines concentration in heart disease patients. Therefore, it is reported that comprehensive PD treatment may participate in the treatment of PCOS women by inhibiting concentration of proinflammatory markers, reactive oxygen species and oxidative stress.

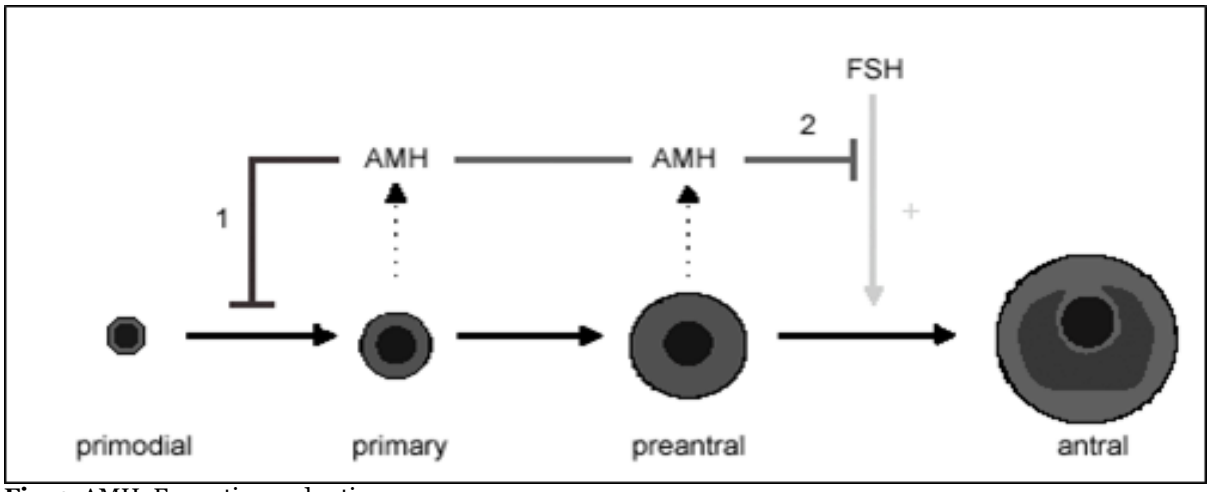

Fig. 1. AMH; Formation and action.

In the future, the evaluation of PD in women with PCOS should determine the effect of non-surgical periodontal therapy in the prediction of severity of PCOS and inflammatory marker.

Conclusion

Gingivitis and high concentration of AMH in PCOS suggests that all physicians should refer patients with PCOS to dental clinics to assess oral health and treatment. AMH should be considered as a vital marker for diagnosis of PCOS and gingivitis women.

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