Research Article

Assessment of oral health status, leptin, and inflammatory markers in serum and saliva of patients with polycystic ovarian syndrome in reference to metabolic syndrome

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Abstract

Background: Polycystic ovarian syndrome was first described in1935 by Stein and Leventhal as classical triad of amenorrhea, obesity and hirsutism

Aims of the study: Assessment of oral health status including gingival inflammation by (GI), periodontal situation by (PI), dental status by (DMFT), and salivary flow rate (SFR) and their relationship with the severity of symptoms, and evidence of metabolic syndrome in PCOS patients and assessing the serum and salivary levels of leptin, CRP, and fibrinogen in PCOS patients and find out the associations with the oral health and metabolic syndrome.

Patients and Methods: Clinical examination had been done for (42) PCOS women and (30), age and BMI, matched control subjects for blood pressure (systolic and diastolic) and anthropometric measurements (BMI, waist to hip, and waist to height ratios). Oral clinical examination including an assessment of gingival inflammation (GI), periodontal situation (PI), dental status (DMFT), and salivary flow (SFR) had also been done. Samples of blood and whole unstimulated (resting) saliva were collected from all participants then investigated for the level of leptin, CRP, and fibrinogen, lipids (HDL and triglyceride), and FBG had also been measured.

Results: Oral health measurements including: GI, PI, and DMFT were significantly higher in PCOS patients, while SFR were less in PCOS compared to controls. Leptin and fibrinogen (in blood and saliva) of the PCOS women were higher than that of controls. Whereas (26.19%) serum and (40.47%) salivary CRP levels >6mg/L, but no one of the controls exhibited high CRP levels. Significant correlation between serum and salivary leptin were resulted in both PCOS and controls, and the same story for CRP and fibrinogen. Elevated levels of the rest variables in both blood and saliva were significantly correlated with MS. GI, PI, and SFR were also significantly correlated with MS, while DMFT was not. GI, PI, and DMFT had significant correlation with BMI in PCOS and controls. Severe gingival inflammation and established destructive periodontitis were significantly correlated with salivary: CRP and leptin, but it were non-significant with salivary fibrinogen and SFR.

Conclusion: Saliva was found to be a useful alternative to serum for the determination of leptin, CRP, and fibrinogen in PCOS. The susceptibility for dental carries, gingivitis, and periodontitis may significantly increase in PCOS that gingivitis and periodontitis are common finding in patients with PCOS, and that was supported by significant decrease in salivary flow rate. Salivary leptin and CRP have become important markers for inflammation related to oral health and in assessing the risk of developing cardiovascular disease.

Key words: Oral health, Metabolic syndrome, Leptin, CRP, Fibrinogen.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorder affecting women in reproductive age; it is a genetic disorder that can be inherited from either parents [(1)]. PCOS can be divided into two main types: insulin-resistant (obese PCOS) and non-insulin-resistant. In 2003 the Rotterdam criteria for the diagnosis of PCOS states 2 of the 3 features were needed to be present to make the diagnosis. These features include: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries [(2)]. It would appear that many women with PCOS fulfill the criteria for the metabolic syndrome in view of a higher reported incidence of hypertension, dyslipidemia, visceral obesity, insulin resistance and hyperinsulinemia [(3)].

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism [(4)]. Most obese individuals are thought to be leptin resistant [(5)]. CRP is (115 kDa) protein synthesized by the liver. Many studies reveal elevated serum CRP levels in women with PCOS. Recent research has shown that fibrin plays a key role in the inflammatory response [(6)]. Oral health is integral to general health. Because of interest in the link between oral and general health clinicians are increasingly using salivary analyses to diagnose systemic disease and to monitor general health. There has also been an increase the association between periodontal inflammation and PCOS [(7)]. In recent times there has been increasing interest in saliva-based analyses, because saliva collection methods are simple and noninvasive. Oral fluid sampling is safe for both the operator and the patient, and has easy and low-cost storage [(8)]. Since the saliva was put forth as a potential diagnostic tool, its use for surveillance of disease and general health has become a highly desirable goal in healthcare and medical research [(9)].

MATERIALS AND METHODS

Forty-two patients having PCOS, based on Rotterdam criteria, and thirty healthy women were matched in age and BMI.

Exclusion criteria: smoking, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, hypertension, and pregnancy.

History with examination were performed which included: age, onset of disease, family history, weight, height, systolic and diastolic blood pressure, collection of blood and saliva, other anthropometric (BMI, WHR, and WHtR), metabolic (leptin, CRP, and fibrinogen), and oral health (DMFT, GI, PI, and SFR) measurements. Venous blood was obtained and the serum was separated and kept in deep freeze for further analysis. To avoid circadian variations, saliva sample was collected between 9 AM and 11 AM into small plastic polyethylene cups. Whole unstimulated saliva for 10 minutes was collected from participants. Then centrifuged and the supernatant is kept at -20ºC until use for further analysis including:

1-Leptin by Sandwich ELISA kit(DRG),catalog no. 2395 (Germany).

2-C-reactive protein(LTA) by qualitative determination kit catalog no. AK00111 (Italy).

3.Fibrinogen (Spinreact) Clauss method by kit catalog no. 1709211 (Spain) .

Statistical Analysis: Statistical tests were achieved by using Microsoft "Excel 2010" statistical package which run under "windows" operating system and a computerized program, the statistical package for social sciences (SPSS). T- student's test was used for comparison with control group.

RESULTS

Table (1) shows comparable mean, standard deviation and median values of clinical characteristic and anthropometric measurements (age, onset of disease, family history, weight, height, systolic and diastolic blood pressure, other anthropometric (BMI, WHR, and WHtR).

Table (2) shows Lipid profiles including (TG, HDL, and TG/HDL) and FBG.

Oral health measurements

Table (3): shows the clinical oral examination of PCOS and controls that include the GI and PI: each group was subdivided into subgroups according to the severity of inflammation in the gingival tissue and periodontal tissue respectively (with the expression of no.%) for each grade of inflammation within the subgroup). DMFT: calculation of the number and percentage of decayed, missed, and filled teeth of each group and mean±SD of salivary flow rate were less in PCOS than that of controls.

Oral health measures, inflammatory biomarkers and leptin

Table (4) shows mean ± SD, and the correlation between the components' number on one hand and the elevated salivary and serum leptin, salivary and serum

CRP, salivary and plasma fibrinogen, GI, PI, DMFT, and SFR in PCOS on the other hand. Salivary and serum leptin had significant linear correlation with all components (P <0.05). Whereas the rest had significant linear correlation only with the (4 and 5 components) (P <0.05) except for DMFT which is non-significant for all components.

Table 1. Clinical characteristics and anthropometric measurements of study subjects

Subject	Control (30)	PCOS (42)
Age (year) Mean ± SD Median	 27.66 ± 5.81 27.5	 27.95 ± 5.67 28.5
Onset of disease (year) Mean ± SD Median	_	 3.93 ± 2.71 3
Family history No. (%)	_	 12 (28.5%)
Weight (kg) Mean ± SD Median	 76.27 ± 17.59 71.35	 78.41 ± 16.25 77.65
Height (cm) Mean ± SD Median	 161.9 ± 5.28 163	 160.9 ± 7.95 161.5
BMI (kg/m²) Mean ± SD Median 18.5 - 24.9 No.(%) 25 - 29.9 No.(%) ≥ 30 No.(%)	 29.38 ± 5.88 28.95 6 (20%) 10 (33.3%) 14 (46.7%)	 30.39 ± 5.96 29.73 8 (19%) 14 (33%) 20 (47.6%)
Waist (cm) Mean ± SD Median	 92.33 ± 11.50 90	 93.02 ± 15.07 91.5
Hip (cm) Mean ± SD Median	115.8 ± 6.04 115	112.4 ± 10.26 110
Waist/hip ratio Mean ± SD Median ≥ 0.8 No.(%)	 0.78 ± 0.06 0.78 13 (43.3%)	 0.82 ± 0.09 0.83 27 (64.3%)
Waist/height ratio Mean ± SD Median ≥ 0.5 No.(%)	 0.55 ± 0.08 0.53 19 (45.3%)	 0.57 ± 0.09 0.57 35 (83.3%)
Systolic BP (mmHg) (Mean ± SD) ≥ 130 No. (%)	 119.76 ± 6.8 3 (10%)	 124.38 ± 6.49 10 (23%)
Diastolic BP (mmHg) Mean ± SD ≥ 85 No. (%)	 78.83 ± 3.37 1 (3.3%)	 81.59 ± 3.90 7 (16.6%)
Mean BP (mmHg) Mean ± SD	 92.47 ± 3.82	 95.85 ± 4.57

The results are expressed as mean±SD, number (%) and median. BMI: body mass index, BP: blood pressure, SD: standard deviation, No.: number, %: percentage.

Relationship of leptin and inflammatory markers levels to endocrine-metabolic parameters

In figure (1,2) the correlations between salivary and serum leptin levels for PCOS and controls were strong linear statistically highly significant (t-test =7.256, P < 0.001) for controls and (t-test =5.938, P < 0.001) for PCOS, While salivary and plasma fibrinogen levels for PCOS and controls had a weak linear correlation statistically non-significant for controls (t-test =0.806, P > 0.05) and positively correlated (linear correlation) statistically significant for PCOS (t-test =5.243, P < 0.05).

In figure (3A)PCOS and controls matched for BMI but salivary leptin levels for PCOS differ widely from those of controls and had strong positive linear correlation with BMI statistically highly significant (t-test = 5.23, P < 0.001) for PCOS and had positive linear correlation statistically significant for controls (t-test = 2.21, P < 0.05). Figure (3B) shows that serum leptin levels for PCOS differ from those of controls and were strongly correlated (positive linear correlation) with BMI statistically highly significant (t-test = 4.58, P < 0.001) for PCOS and had positive linear correlation statistically significant in controls (t-test = 2.33, P > 0.05).

In figure (4A)Salivary leptin levels for PCOS differ from those of controls and had positive linear correlation with W/H Ratio statistically significant in both PCOS and controls (t-test = 2.86, 1.99, P < 0.05) respectively. In figure (4B) serum leptin levels for PCOS differ from those of controls and had strongly positive linear correlation with W/H Ratio statistically highly significant in both PCOS and controls (t-test = 3.47, 3.06, P < 0.001) respectively.

Table 2. Lipid profile and fasting blood glucoseof PCOS patients.

Subject	PCOS (42)	Min – Max
TG (mmol/L) Mean ± SD ≥ 1.78 No. (%)	 1.31 ± 0.62 8 (19%)	 0.7 – 2.95 2.1 – 2.95
HDL (mg/dl) Mean ± SD ≤ 50 No. (%)	54.60 ± 7.38 11 (26.1%)	 32.5 – 67 32.5 – 50
TG/HDL (American units) Mean ± SD > 2 No. (%) ≥ 4 No. (%) ≥ 6 No. (%)	2.39 ± 1.67 11 (26.1%) 3 (7.1%) 3 (7.1%)	1.031 – 6.84 2.21 – 3.76 4.09 – 4.33 6.53 – 6.84
Fasting blood glucose (mmol/L) Mean ± SD ≥ 5.6 No. (%)	4.68 ± 0.82 5 (11.9%)	3.5 – 6.9 5.7 – 6.9

The results are expressed as mean±SD, no. (%), and min-max. Min: minimum, Max: maximum, TG: triglyceride, HDL: high density lipoprotein, SD: standard deviation.

Relationship of oral health measures with BMI

PCOS and controls were matched for BMI but gingival index (GI) scores for PCOS were higher than that of

controls and had strong positive linear correlation with BMI statistically highly significant in PCOS and controls (t-test = 3.081, 3.215, P < 0.001) respectively. (Figure 5A)

Periodontal index (PI) scores for PCOS were higher than that of controls and had strong positive linear correlation with BMI statistically highly significant (t-test = 3.385, P < 0.001) for PCOS and significantly correlated in controls (t-test = 2.807, P < 0.05). (Figure 5B)

Table 3. Oral health measurements of PCOS patients and controls.

		Control (30)	PCOS (42)
GI No. (%)	Normal	8 (26.66%)	-
	Mild	18 (60.13%)	16 (38.09%)
	Moderate	4 (11.72%)	16 (38.09%)
	Severe	-	10 (23.8%)
PI No. (%)	Normal PDT	12 (40%)	-
	Simple Gingivitis	13 (43.33%)	9 (21.42%)
	Beginning Destructive	5 (16.66%)	19 (45.23%)
	Established Destructive	-	14 (33.33%)
	Terminal	-	-
DMFT %		(33.69%)	(39.88%)
DT %		(8.8%)	(16.75%)
MT %		(2.02%)	(4.67%)
FT %		(19.16%)	(18.45%)
SFR (ml/min) (Mean ± SD)		0.394 ± 0.144	0.346 ± 0.079

The results are expressed as no. (%) for the indices, and mean ± standard deviation for the salivary flow rate. GI: gingival index, PI: periodontal index, PDT: periodontal tissue, DMFT: decayed, missed, and filled teeth, DT: decayed tooth, MT: missed tooth, FT: filled tooth, SFR: Salivary flow rate.

DMFT index scores for PCOS had a little difference from those of controls and had strong positive linear correlation with BMI statistically significant t-test for controls and PCOS = 3.869, 2.786, P < 0.05 respectively. (Figure 5C)

DMFT index was shown to have a weak inverse linear correlation with SFR, salivary CRP, salivary fibrinogen, and salivary leptin in PCOS statistically non-significant (t-test = 0.903, 1.83, 0.821, 2.01, P >0.05) respectively. (Figure 6 A, B, C, and D).

Gingival index (GI) had inverse correlation with mean values of salivary flow rate in all grades of inflammation (mild, moderate, and severe) statistically non-significant (P >0.05) (Figure 7A). There was linear correlation between scores of gingival index (degree of inflammation) and mean values of salivary leptin and CRP statistically significant in severe inflammation only (P <0.05). (Figure 7B and C) But it was non-significant with mean values of salivary fibrinogen (P >0.05) in all grades of inflammation (Figure 11D).

Periodontal index (PI) had inverse correlation with mean values of salivary flow rate in all grades of inflammation (simple gingivitis, beginning destructive, established destructive) statistically non-significant (P >0.05) (Figure 8A). There was a linear correlation between grades of periodontal index (degree of inflammation) and mean values of salivary leptin and CRP statistically significant (P <0.05) with the last grade of inflammation only (Figure 8B and C) But it was non-significant with mean values of salivary fibrinogen (P >0.05). (Figure 8D)

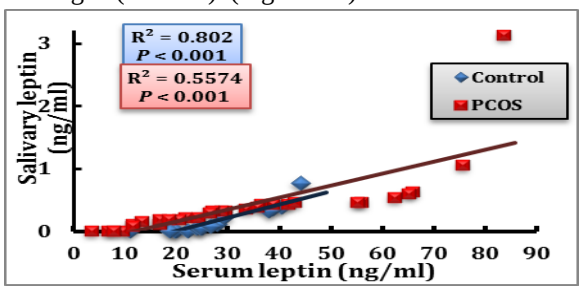

Figure 1. Regression line showing the strength of correlation between serum and salivary leptin concentration in both PCOS and controls.

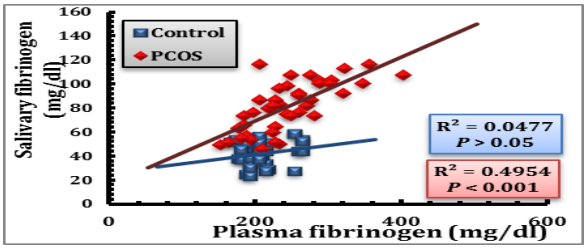

Figure 2. Regression line showing the strength of correlation between salivary and plasma fibrinogen concentration in both PCOS and controls.

DISCUSSION

In this study, examination of salivary leptin and salivary fibrinogen levels, salivary CRP in PCOS, DMFT in PCOS, the association between the oral health status and the metabolic syndrome in PCOS, the association between serum and salivary leptin, CRP, and fibrinogen and the metabolic syndrome in PCOS were done.

In the last ten years, several epidemiological studies have assessed the association between oral infection and systemic diseases that oral infections may confer independent risks for different systemic conditions (e.g. cardiovascular diseases). This study suggests an increased susceptibility for dental caries, gingivitis and periodontitis in women with PCOS compared with

healthy women in agreement with the report by Erhan et al. [7]. The underlying biological mechanisms for the association of obesity with periodontitis are not well-known; however, adipose-tissue-derived cytokines and hormones may play a key role, which in turn may modulate periodontitis [11] Furthermore, significant correlations between the clinical signs of gingivitis and periodontitis with leptin and CRP indicate a possible interaction between hormonal and metabolic phenotype in PCOS. On the other hand the clinical sign of dental caries, gingivitis and periodontitis correlate non-significantly with SFR and salivary fibrinogen. In persons with periodontitis, bacterial pathogens, endotoxins, and inflammatory cytokines may systemically trigger synthesis of acute-phase proteins (CRP), and enhanced lipid metabolism, along with increased serum cholesterol and triglyceride levels, which may contribute to the risk of systemic diseases such as CVD in agreement with Mattila et al.[12]. In women with PCOS, insulin stimulated leptin secretion is limited by the insulin resistance in adipocytes. An important feature of the obesity of PCOS is the accumulation of visceral fat (increased waist to hip ratio), which secretes more leptin in agreement with McConway et al. [13]. Leptin levels (serum and salivary) significantly increased with increasing metabolic syndrome score. Other associations were observed for CRP and fibrinogen levels in which it show significant correlations only in high scores of metabolic syndrome.This evidence in agreement with Feng [14]. The exact mechanism indicates that women with PCOS frequently have insulin resistance, meaning their body does not respond as quickly to insulin. The sluggish response will cause larger and larger amounts of insulin to be required before glucose is taken into the body tissues, and eventually a change in the way the body deals with sugar. Consistently high levels of glucose will be in the blood in agreement with Nicole and RN [15].

In Conclusion saliva was found to be a useful alternative to serum for the determination of leptin, CRP, and fibrinogen in PCOS. The susceptibility for dental carries, gingivitis, and periodontitis may significantly increase in PCOS that gingivitis and periodontitis are common finding in patients with PCOS, and that further supported by significant decrease in salivary flow rate. Salivary leptin and CRP may become important markers for inflammation related to health.

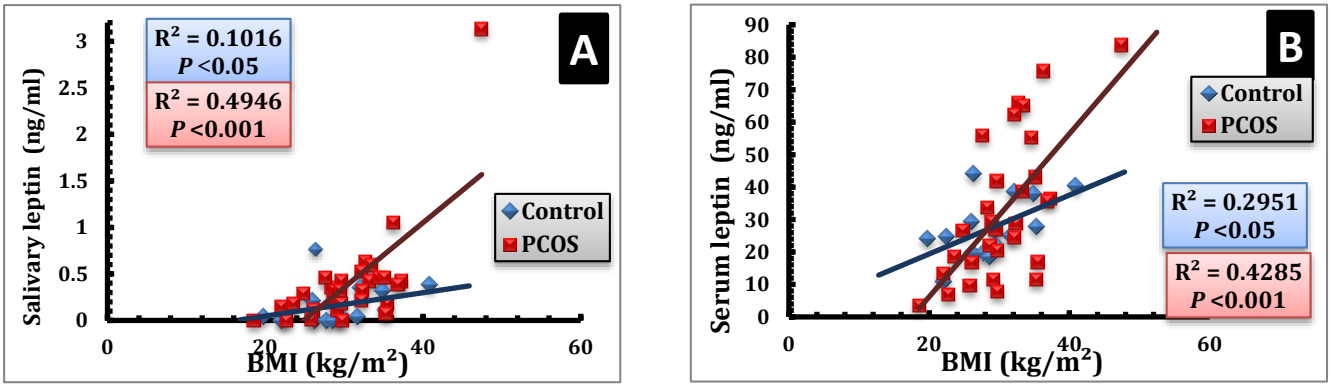

Figure 3. Regression line showing the strength of correlation between BMI and leptin concentration of both PCOS and controls in: A Saliva B Serum.

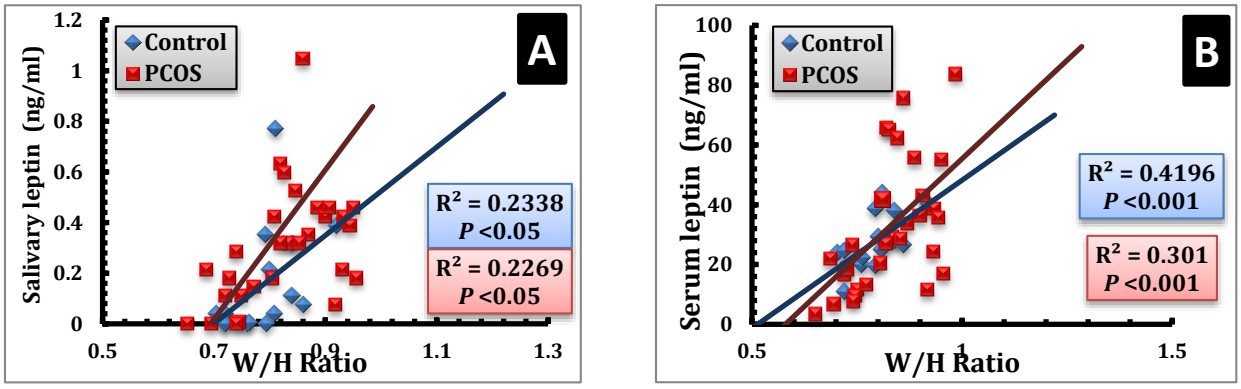

Figure 4. Regression line showing the strength of correlation between W/H Ratio and leptin concentration of both PCOS and controls in A Saliva B Serum.

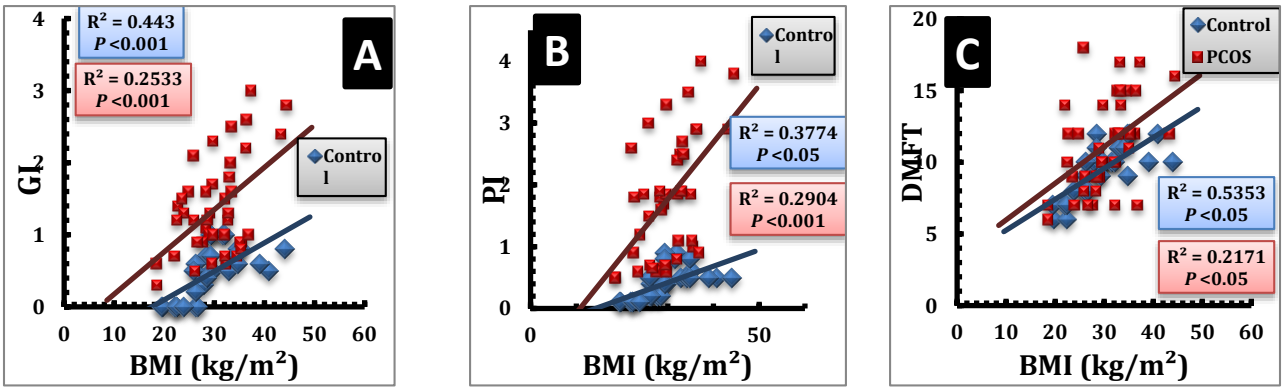

Figure 5. Regression line showing the strength of correlation between body mass index and oral health measures of both PCOS and controls that include A-Gingival index B- Periodontal index C- DMFT index.

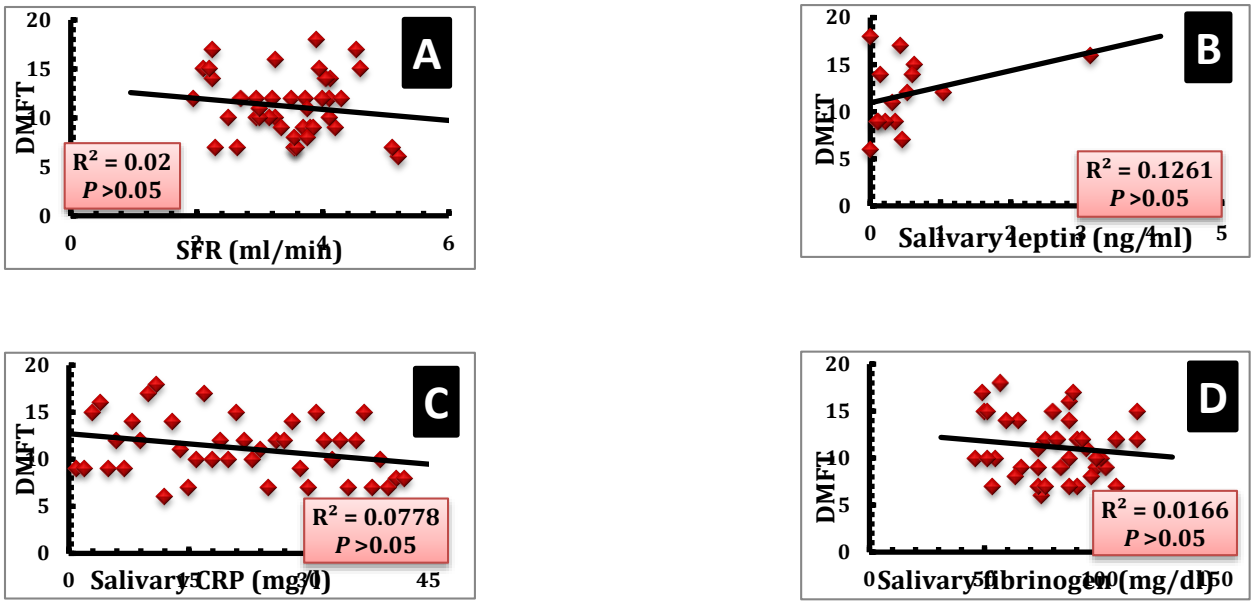

Figure 6. Regression line showing the strength of correlation between DMFT index score and A Salivary flow rate, B Salivary leptin, C Salivary CRP, D Salivary fibrinogen.

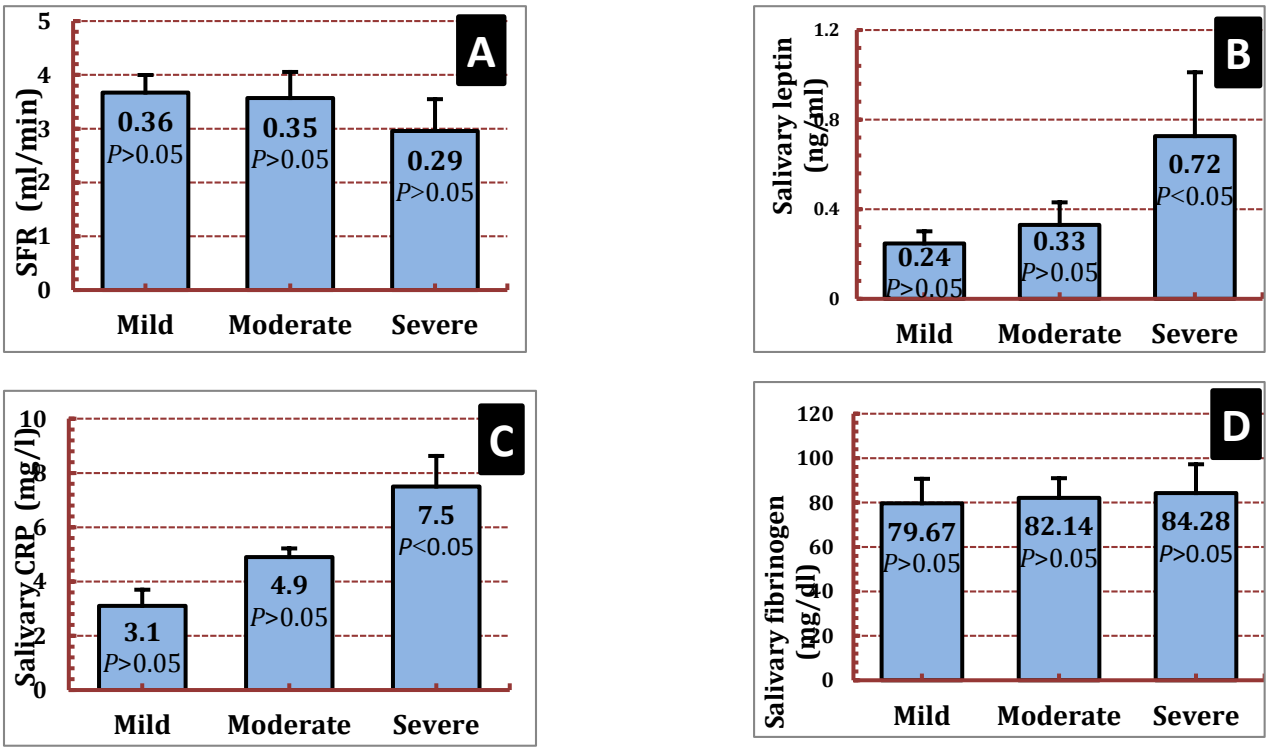

Figure 7. Comparison of the relationship between gingival inflammation grades in PCOS A Salivary flow rate, B Salivary leptin, C Salivary CRP, and D Salivary fibrinogen.

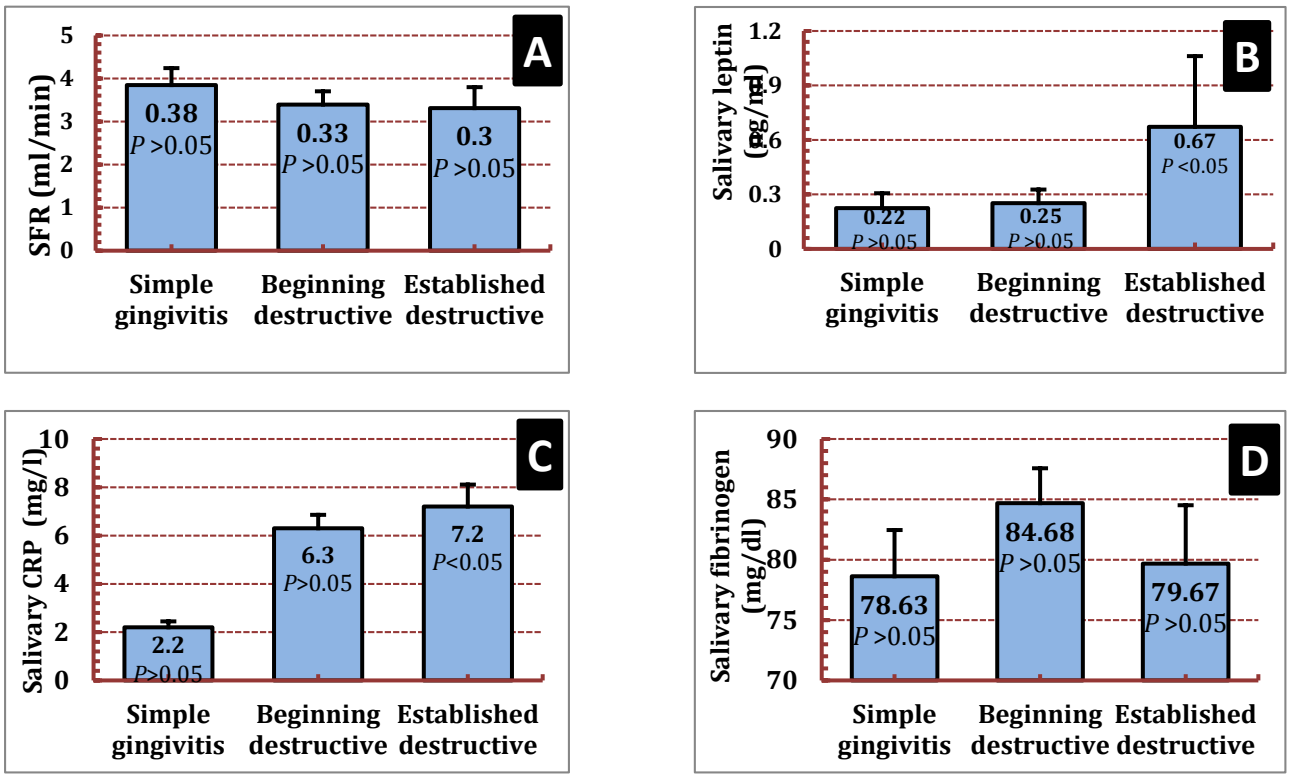

Figure 8. Comparison of the relationship between periodontal tissue inflammation grades in PCOS A SFR, B Salivary leptin, C Salivary CRP, and D Salivary fibrinogen.

Table 4. Oral health measures, inflammatory biomarkers and leptin correlation between the components

	No.	Salivary Leptin ng/ml	Serum Leptin ng/ml	Salivary CRP ≥ 6 (mg/l)	Serum CRP ≥ 6 (mg/l)	Salivary Fibrinogen (mg/dl)	Plasma Fibrinogen (mg/dl)	GI	PI	DMFT	SFR
		(Mean ±SD)		No.		(Mean ±SD)					
1Component	13	0.46 ± 0.08 *	52.33 ± 12.21 *	4 NS	2 NS	103.06 ± 9.21 NS	355.67 NS	1.7 ± 0.7 NS	1.9 ± 0.8 NS	11.3 ± 3 NS	0.36 ± 0.05 NS
2Components	7	0.42 ± 0.11 *	45.85 ± 13.69 *	1 NS	1 NS	94.48 ± 3.98 NS	304.15 346.88 NS	1.5 ± 0.75 NS	1.8 ± 0.99 NS	11.5 ± 3.62 NS	0.326 ± 0.093 NS
3 Components	4	1.04 ±0.31 *	55.74 ± 10.2 *	1 NS	1 NS	116.61 NS	359.17 NS	1.75 ± 0.59 NS	1.9 ± 0.93 NS	12.25 ± 2.06 NS	0.345 ± 0.036 NS
4 Components	3	2.13 ±0.42 *	73. 63 ±36.47 *	2 *	2 *	88.63 *	322 *	2.7 ± 0.14 *	3.56 ± 0.58 *	15 ± 1 NS	0.246 ± 0.05 *
5 Components	1	3.13 *	83.74 *	1 *	1 *	92.45 *	320.06 *	3 *	2.9 *	12 NS	0.195 *

The results are expressed as number and mean±SD. NS: Not significant, *: Significant, CRP: C-reactive protein, GI: Gingival index, PI: Periodontal index, DMFT: Decayed Missed Filled Teeth SFR: Salivary flow rate, no.: number, SD: standard deviation.

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