



Measurement of the Levels of Salivary Lipocalin-2 and C - reactive protein in Women with Polycystic Ovarian Syndrome

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Abstract

The aim of the present study is to investigate salivary lipocalin-2 levels as well as C-reactive protein (CRP) in women with and without polycystic ovary syndrome (PCOS). Lipocalin-2 levels and C-reactive protein were measured in 104 women within the age group of (20 – 29) years were divided into two groups. Group I consisted of 55 diagnosed female patients of PCOS and Group II comprised of 49 female age matched control group with normal menstrual cycles. Highly significant differences were found using t-test in inter group comparison. Levels of lipocalin-2 in PCOS group were decreased significantly compared to the control group ($p < 0.05$), while the salivary level of CRP were increased significantly in the PCOS group ($p < 0.05$). Salivary levels of lipocalin-2 were found to be low whereas CRP were high among women with PCOS.

It was suggested that the measurement of salivary lipocalin-2 and CRP may be specific method for making a diagnosis of women with polycystic ovary syndrome.

Introduction:

Human saliva is a biological fluid with myriad of biological functions important for the maintenance of oral and general health. It is a plasma ultra-filtrate containing proteins either synthesized in situ from blood or in the salivary glands⁽¹⁾.

Saliva contains a large number of proteins that have metabolic, immune response, transporting, and several other cellular functions. Its collection is non-invasive compared to the collection of other body fluid and, hence, has a great potential for use in the diagnosis of systemic and

localized disease^(2,3). Although saliva has not yet become a mainstream sample source for hormone analysis, it has proven to be reliable and in some cases, even superior to other body fluids. Nevertheless, much effort will be required in this approach to receive acceptance over the long-term, especially by clinicians⁽⁴⁾. The polycystic ovary syndrome (PCOS) also known as Stein-Leventhal syndrome is a heterogeneous female endocrine disorder primarily affecting the reproductive system, with substantial collateral negative health effects on reproductive (hyperandrogenism, hirsutism, anovulation, infertility, and menstrual disturbance), metabolic (obesity and diabetes mellitus as well as cardiovascular risk), and psychological features (mood disorders and decreased quality of life)⁽⁵⁻⁷⁾. According to the Rotterdam consensus, the presence of two out of the three following criteria is deemed necessary for diagnosis of PCOS: (i) oligo-ovulation or anovulation, (ii) clinical and/or biochemical hyperandrogenism, and (iii) ultrasound appearance of polycystic ovaries⁽⁸⁾. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa secretory glycoprotein was first identified in activated neutrophils. It is expressed in adipocytes, endothelial cells, macrophages, vascular smooth muscle cells and hepatocytes which induced by many pro- and anti-inflammatory cytokines, factors such as lipopolysaccharide (LPS), tumor necrosis factor- α (TNF- α), IL-1 β , IL-6 or IL-17, and anti-oxidant enzymes reducing free radicals in a variety of cell types^(9,10). Lipocalins are bioactive peptides that belong to a novel adipokines that contributes to obesity and insulin resistance⁽¹¹⁾. C-reactive protein (CRP) is an acute phase protein secreted from the liver, which was stimulated by interleukin-6, originating from the adipose tissue was considered to estimate the low grade chronic inflammation in PCOS⁽¹²⁾. There has been no study on the possible effects of PCOS on lipocalin-2 in saliva. Therefore, the aim of the present study is to evaluate saliva levels of lipocalin-2 in

healthy group and among women with PCOS.

Materials and Methods:

This comparative cross-sectional study included 104 women aged between 20-29 years, which were divided into; Group(I) 55 PCOS patients and Group(II) 49 normal and healthy woman as controls. All individuals were randomly admitted into Kirkuk teaching hospital in Kirkuk city during the period from June, 2017 to January 2018. The detailed case history and informed written consent was taken for each patient. The institutional ethical committee clearance was obtained to conduct the study.

Saliva sampling

Questionnaires which requested details of demographic data and medical history were administered to the subjects, following stimulated saliva was collected from them in the morning between (8:30–9) am in the fasting state by expectoration into polypropylene tubes. Approximately 5 mL of whole saliva was collected and centrifuged for 10 minutes at 1,000 \times rpm at 4°C to remove cell debris. Supernatant was stored in 500 μ L aliquots at –80°C until the biochemical analyses were performed. Lipocalin-2 was measured by enzyme linked immunosorbent assay. C-reactive protein(LTA) by qualitative determination kit catalog no. AK00111 (Italy). The body mass index (BMI)= weight (kg)/height² (m) was measured for participants in this study. Statistical analysis was performed by using SPSS, version 15. Salivary parameters were compared between PCOS group and healthy by using Student's t test.

Results:

The mean age of participants in the study is 23.12 \pm 6.25 years. The results also suggested that 55 patients were in the BMI range of (20 - 25) kg/m² and the remaining 49 individuals were in BMI range of (24-29.9) kg/m². Table (1) shows a significant increased levels of lipocalin-2(p=0.0001) in the PCOS patients (41.4 9 \pm 11.5

ng/mL) as compared to healthy controls (54.45 ± 7.1 ng/mL) as in Fig (1). It shows a non-significant increased levels of CRP ($p=0.0688$) in the PCOS patients (4.67 ± 0.761 mg/dl) as compared to healthy controls (0.67 ± 0.011 mg/dl) as illustration in Fig (2).

Discussion:

This study showed that salivary lipocalin-2 levels were significantly lower in PCOS group compared to healthy individuals. Our findings are in harmony with result of previous findings of Sahin et al and Soner Gok , et al indicate that PCOS has insulin resistance and low-grade chronic inflammation, it is reasonable to presume that lipocalins-2 action is highly expressed in adipocytes, that its expression is regulated by obesity, may contribute to the development of insulin resistance in PCOS^(13,14), through glucocorticoids and TNF- α . Promote insulin resistance induce the expression of lipocalin-2. Considering the fact that lipocalin-2 is clearly involved in the final steps of vascular damage, their decreased concentrations in PCOS women indicate that they might be part of a protective mechanism⁽¹⁵⁾. Studies investigating the relationship between NGAL levels and PCOS found confusing results. Gencer et al.⁽¹⁶⁾ found that lipocalin-2 concentrations were significantly lower in women with PCOS.

Moreover, lipocalin-2 was not associated with cardiovascular risk indicators as defined by echocardiographic examination. Cakal E et al.⁽¹⁷⁾ demonstrated that NGAL levels were higher in PCOS patients and was related with insulin resistance. While Panidis et al. 2010⁽¹⁸⁾, Koiou et al. 2012⁽¹⁹⁾, however, revealed that PCOS per se does not affect LCN2 levels. Circulating C-reactive protein may reach saliva via gingival fluid or the salivary gland⁽²⁰⁾. Salivary levels of hs-CRP were measured as a marker for systemic inflammation and found that salivary hs-CRP levels were higher in females with PCOS compared to systemically healthy controls may be due to the reflection of the chronic low grade inflammation present in the disorder or it may be of a genetic origin since it was found that single nucleotide polymorphism (SNPs) in CRP genes are associated with increased plasma level of CRP^(21, 22).

Conclusions:

This study demonstrations a pointedly decrease in salivary lipocalin-2 and increase in CRP in in women with PCOS these biomarkers may be valuable in diagnosis PCOS. Large sample sizes are needed to minimize the effects of interactions in such diseases, and the relatively small sample size of the present study is a major limitation.

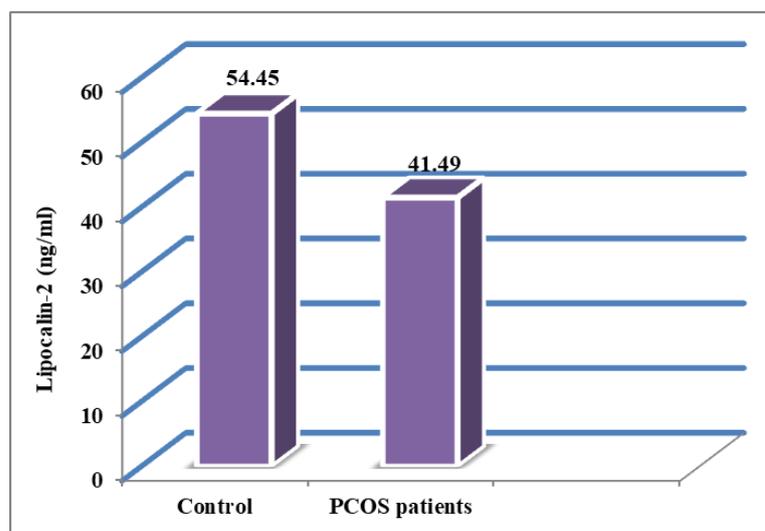


Fig. (1): Salivary lipocalin-2 in controls and PCOS group.

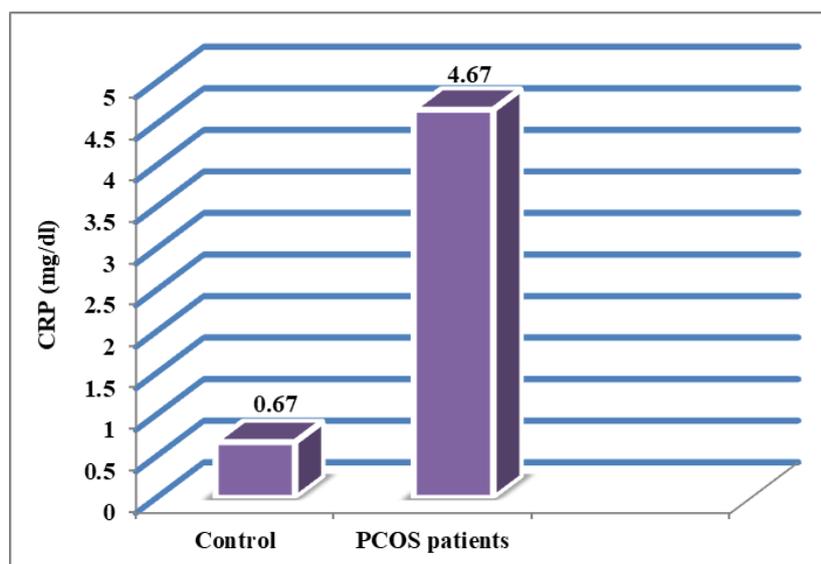


Fig. (2): Salivary CRP in controls and PCOS group.

Table (1): Anthropometric, and biochemical characteristics of the PCOS and control groups.

	PCOS (n= 55)	Control (n= 49)	p
Parameters	55	49	-
Age (years)	23.41 ± 4.8	24.83 ± 4.47	0.62
Body mass index (kg/m ²)	25.2±3.450	24.8±3.435	0.62
CRP(mg/dl)	4.67 ± 0.761	0.67 ± 0.011	<0.001
Lipocalin-2 (ng/ml]	41.4 9 ± 11.5	54.4 5 ± 7.1	<0.001

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