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RESEARCH PAPER

Role of vitamin D and some immunological markers in polycystic ovarian syndrome

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A B S T R A C T:

Polycystic ovarian syndrome (PCOS) is the most widespread endocrine-metabolic disorder in women of reproductive age, which is a very common disorder. The current study aimed to determine the levels of vitamin D and immunological markers like antinuclear antibodies (ANA), anti-double strand DNA (dsDNA) and C. reactive protein (CRP) in the serum of PCOS-affected women. Blood samples were collected from forty polycystic women depending on the Rotterdam criteria. Forty fertile women of similar ages made up the control group. The Blood samples were analyzed to measure the levels of an endocrine hormone and vitamin D by Cobas e411; ANA and ds DNA determined by Enzyme-Linked Immunosorbent Assay (ELISA), And CRP measured by Cobas C111. This study revealed a significant decrease in vitamin D in PCOS patients contrasted to the control group, while CRP, ANA, and dsDNA increased significantly in PCOS patients. Finding low vitamin D and high ANA, ds DNA in PCOS women may have a role in the aetiology of PCOS. Screening for autoantibodies and vitamin D in women with PCOS may provide better insight into its role in PCOS.

KEYWORDS: Autoimmune markers, C. reactive protein, Polycystic ovary syndrome, Vitamin D.
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1. INTRODUCTION:

The heterogeneous disease PCOS affects women who are of fertile age (Bogari, 2020). This complex condition is characterized by inconsistent menstrual periods, a high testosterone level, and tiny cysts on one or both ovaries (Ndefo et al., 2013).

Hyperandrogenemia is a common clinical characteristic of PCOS, (60–80) % of women with PCOS are influenced by this status (Johansson and Stener-Victorin, 2013). It may prevent follicular growth, anovulation, ovarian microcysts, and changes to the menstrual cycle (Lin et al., 2013). As a result of elevated androgen levels, acne, hirsutism, and alopecia can develop (Johansson and Stener-Victorin, 2013). PCOS symptoms usually appear during puberty. However, they may have been programmed as early as the perinatal period (Nisar et al., 2012).

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with this condition. Genetic disorders probably result in PCOS pathology (Bruni and Capozzi, 2022), as well as an autoimmune disease has been suggested in PCOS (Romitti et al., 2018).

The steroid hormone vitamin D is embroiled in the metabolism of calcium and the formation of bones. It may help to prevent cancer, autoimmune disorders, hypertension, diabetes, and obesity, in addition, to inducing spermatogenesis (Menichini et al., 2022, Karim et al., 2021)

Serum vitamin D levels were discovered to be negatively correlated to androgen levels. It is a medication that improves the insulin receptor function, lipid metabolism, menstrual cycle and folliculogenesis of PCOS patients by lowering serum androgen and anti-Müllerian hormone levels and decreasing endometrial thickness. In addition, vitamin D levels were also inversely linked with insulin receptors and body fat mass characteristics (Mu et al., 2021).

There is not enough investigation on the interrelation between PCOS, vitamin D lack, and the immunological parameter.; therefore, this study focused on assessing the interconnection

between PCOS and vitamin D as well as several immunological markers such as ANA, anti-dsDNA, and CRP

2. MATERIALS AND METHODS

The Human Ethics Committee of the College of Science at Salahaddin University in Erbil gave its blessing and authorization to the current investigation (Approval No: 45-36Date: 1/3/2019). Before the data were published, the patients gave their signed, informed consent. The study was carried out from March to September 2019 at Rizgary teaching hospital, Erbil.

2.1 Patients and Control:

In this study, forty PCOS women who marched the 2003 Rotterdam Criteria were assessed, and forty fertile, age-matched control women served as the study's control group. None of the participants had any chronic illnesses or was taking any drugs, vitamins, or minerals. The patients were in the follicular phase.

Blood sampling was performed by withdrawing venous blood from each patient and control group using a disposable syringe (venipuncture technique). The blood was collected in a vaccination tube with gel, allowed to clot at room temperature then centrifuged at 2500 rounds per minute for five minutes to obtain the serum.

2.2 Determination of serum hormones:

Serum levels of thyroid-stimulating hormone (TSH), testosterone, follicle-stimulating hormone (FSH), free androgen index (FAI), and luteinizing hormone (LH) were quantitively estimated by Cobas e411which is dependent on the electrochemiluminescence principle.

2.3 Determination of ANA and dsDNA:

Enzyme-Linked Immunosorbent Assay (ELISA) was used for the quantitive estimation of the serum level of ANA and dsDNA.

2.4 Determination of Vitamin D and C. reactive protein:

Body Mass Index was calculated for patients and control by the formula,

BMI = Weight (kg)/ Height (m)

Weight categories	BMI
Healthy weight	18.5 – 24.9
Overweight	25.0 -29.9
Obese	30.0 – 39.9

2.6 Statistical Analysis

GraphPad Prism Software was used to create the graphics and statistical analyses (version 6.0). Kolmogorov-Smirnov, Shapiro-Wilk, and D'Agostino-Peaarson tests were employed to determine whether or not the data were normally distributed. A t-test is used to compare the values between the patients and the control. If the data were not normally distributed and reported as the median (Range), the Mann-Whitney test was used for all analyses, and P values below 0.05 were deemed statistically significant.

3. RESULTS AND DISCUSSION

The median age of patients was (25.5) year, and the median BMI of patients were 26.2 kg/m2 (overweight).

Figure 1 summarizes the comparison of endocrine hormones between patients and the control group, the median LH concentration of patients was nearly twice of FSH concentration (median 14; P= 0.001; Fig 1A), & median 6.6; P= 0.006; Fig 1B), respectively. This was accompanied by an increase in LH/FSH ratio (median 2.3, P = 0.001; Fig1 C). whereas the median concentration of testosterone was 0.6 (P = 0.001, Fig1D) and the median concentration of free androgen index was 6.7 (P= 0.001; Fig1E). Regarding TSH, there was no significant difference in median concentration between patients and control (median 1.6, 1.8; P = 0.4; Fig1F).

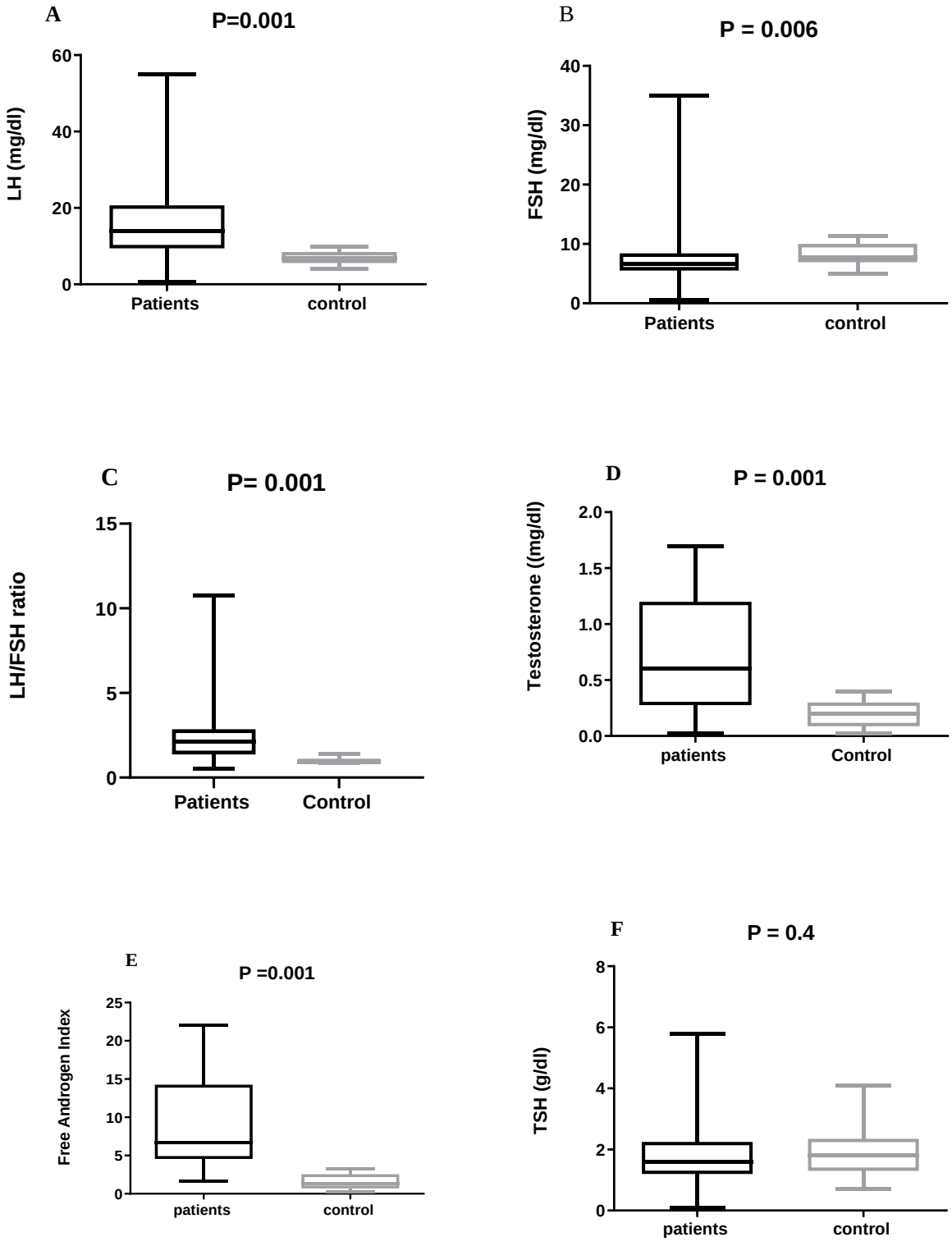

Figure 1: Comparison of endocrine hormones between patients and control group**.**

A. LH; B. FSH; C. LH/FSH ratio; D. Testosterone; E. Free Androgen Index; F. TSH.

In PCOS, the hypothalamic-pituitary-ovarian (HPO) axis has received considerable attention (Burt Solorzano et al., 2012). The most obvious neuroendocrine factor controlling the growth of aberrant ovarian follicles is increased LH secretion with relatively low FSH secretion and increasing theca cell production of androgen, respectively (Patel et al., 2004, Burt Solorzano et al., 2012). In generally healthy women, the ratio of LH to FSH ranges between 1 and 2. This ratio is inverted in polycystic ovary disease patients and may even increase to 2 or 3 (Richard and Ricardo, 2003). Thus with raised LH/FSH ratio in PCOS patients, ovulation does not occur (Johansson and Stener-Victorin, 2013).

The main reasons for LH hypersecretion in PCOS have raised the sensitivity of the pituitary to gonadotropin-releasing hormone (GnRH) and change in GnRH secretion manners (Patel et al., 2004). Additionally, changes in obesity, sex steroid synthesis, and metabolic dysfunction contribute to alterations in LH secretion (Eagleson et al., 2000). Insulin resistance and hyperandrogenemia both contribute to aberrant gonadotropin output, with the androgen of the ovary being the primary source of hyperandrogenemia in PCOS (Tosi et al., 2012). The level of bioavailable androgens increases as a result of compensatory hyperinsulinemia and insulin resistance, which both increase androgen levels in the ovary and decrease SHBG levels in the liver (Sir-Petermann et al., 2009).

In this study, vitamin D had low concentrations in patients and the control, although significant differences existed between the two groups (median 7 and median 20.4 respectively, P = 0.001). Figure 2A.

The Nordic Council of Ministers and the World Health Organization accepted to define adequacy at 30-70 ng/mL; however, deficiency reported as under 10 ng/mL, from 10-30 ng/mL is considered to be insufficient.

Women with and without PCOS frequently have vitamin D deficiencies, which may be related to metabolic and endocrine complications (He et al., 2015).

The current results agree with studies which suggest that vitamin D levels in PCOS individuals are low (He et al., 2015, Thomson et al., 2012). Another investigation found that vitamin D levels were constant in both polycystic women and control (Kim et al., 2014).

The metabolism of glucose may be aided by vitamin D by promoting insulin production, and secretion and enhancing insulin receptor expression or lowering pro-inflammatory cytokines that may lead to insulin resistance (Mu et al., 2021). Previous research has shown that a Lack of vitamin D is believed to increase the incidence of T2DM, impaired glucose tolerance, and decreased insulin sensitivity (Pittas et al., 2006, Mattila et al., 2007).

In the current study, we chose CRP as an indicator of inflammation and well-known indicators of autoimmunity (ANA and anti-dsDNA), which are employed clinically to diagnose autoimmunologic disorders. The median concentration of ANA raised significantly in patients compared with the control group (median 9.2 and median 5.9 respectively, P = 0.002; Figure 2B). Furthermore, dsDNA increased dramatically in patients (P= 0.01; Figure 2C). Finally, compared to the control group, CRP significantly increased in the patient group (median 5.8 and 2.2 respectively, P = 0.003; Figure 2D). The normal range of CRP is less than 5 mg/L. According to a prior study, ANA and smooth muscle antibodies were the most often found in women with PCOS (Reimand et al., 2001). Another study discovered that polycystic women have higher serological autoimmune markers like anti-histone and anti-dsDNA Ab (Hefler-Frischmuth et al., 2010). TNF- and IL-6 serum levels in PCOS women have been found to be higher than normal (Gao et al., 2016, Abraham Gnanadass et al., 2021). Additionally, a number of publications have observed a connection between PCOS and certain autoimmune diseases like autoimmune thyroiditis (Kowalczyk et al., 2017), Graves' disease (Nisar et al., 2012), chronic lymphocytic thyroiditis (Ganie et al., 2010), and diabetes mellitus (Busiah et al., 2017).

According to studies, people with PCOS had increased CRP levels, which can serve as an early indicator of cardiovascular risk (Hu et al., 2011, Boulman et al., 2004). In contrast, a study

showed that PCOS is not related to increased rates of CRP (Sánchez-Ferrer et al., 2019). Most polycystic women are obese and have insulin resistance (Macut et al., 2017, Ezzat et al., 2021). The CRP is a marker of acute inflammation, and it is produced by liver cells controllable by IL-6 and TNF-α, which are produced by adipocytes. As a result, obese individuals with more and larger adipocytes have higher initial serum CRP levels (Sproston and Ashworth, 2018).

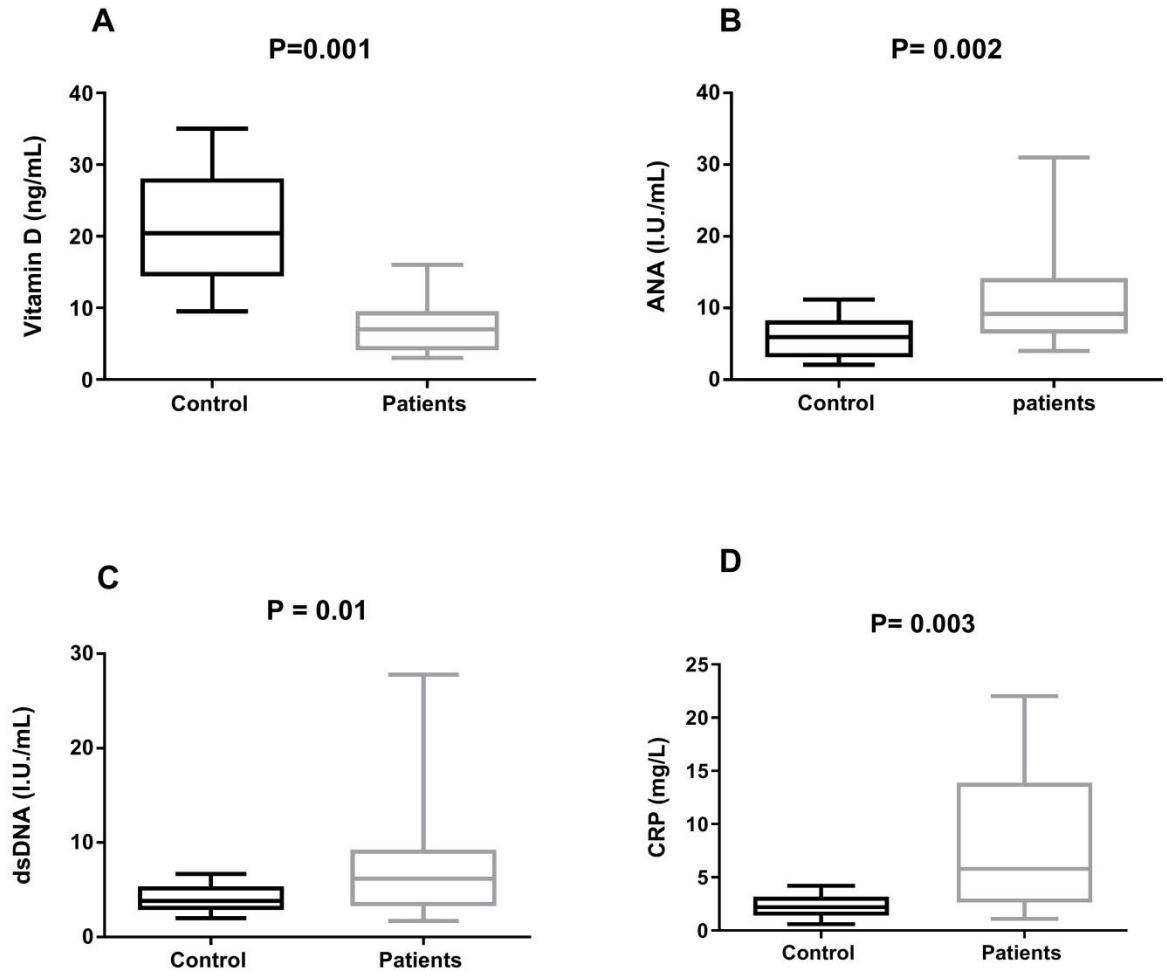

Figure 2: Comparison of Vitamin D and immunological parameters between patients and control group.

A- Vitamin D3; B. ANA; C.dsDNA; D. CRP

3.3 CONCLUSION

Finding low vitamin D in PCOS women may have a role in the pathogenesis of PCOS but is insufficient to be a PCOS diagnostic tool. To fully understand the vitamin D rule in infertility, more research is still required. High ANA, dsDNA, and CRP levels may point to an underlying autoimmune etiology in the pathophysiology of PCOS.

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