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ASSESSMENT OF VITAMIN D LEVELS AND OTHER INFLAMMATORY MARKERS IN OBESE AND NON-OBESE PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT The clinical features of PCOS are often associated with obesity and chronic inflammation; it was aimed to assess vitamin D levels along with other inflammatory markers in PCOS women. A total of 200 subjects were collected from Cytomol Labs and various endocrine clinics. Three milliliter blood samples were obtained and used for estimation of Vitamin D. For cases deficient in vitamin D, hs C-reactive protein (hsCRP), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels were estimated using standard kits. The vitamin D deficiency was observed in 76% of cases with a mean level of 12.7 ± 0.89. Estimation of hsCRP, TNF-α and IL6 in vitamin D deficient cases and also in obese and non-obese groups demonstrated difference suggesting the involvement of vitamin D and other markers in the aetiopathogeneis of PCOS. Hence, treatment with vitamin D, calcium supplementation in addition to metformin could be beneficial in managing PCOS.

KEYWORDS : Inflammation; Vitamin D; Aetiopathogenesis; Interleukin 6

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorders affecting 4% to 12% women of reproductive age worldwide [1] and also a major cause of female infertility. The heterogenous clinical features may change throughout the life span starting from adolescence to postmenopausal age and is characterized by hyperandrogenism, polycystic ovaries and chronic anovulation along with insulin resistance, hyperinsulinemia, abdominal obesity and dyslipidemia as frequent metabolic traits. These predispose the individual to serious long term complications such as type 2 diabetes mellitus, endometrial hyperplasia, hypertension, thyroid dysfunction and cardiovascular disease. The cause of PCOS is unknown, however several genetic, biochemical, immunological and environmental factors are implicated in the aetiopathogenesis of PCOS. The phenotypic manifestation of this disorder is associated with various degrees of gonadotropic and metabolic abnormalities determined by the interaction of multiple genetic and environmental factors [2]. The reproductive function in women is greatly influenced by the Vitamin D levels, involved in ovarian follicular development, glucose homeostasis, inflammation and obesity.

The occurrence of chronic low-grade inflammation in women with PCOS is widely reported to be an important feature of endothelial dysfunction, insulin resistance and atherosclerosis [3,4]. The C-reactive protein (CRP), a marker of inflammation is formed by hepatocytes under the stimulatory control of pro-inflammatory cytokines such as interleukin 6 and TNF-α. Interleukin 6 is produced by mononuclear cells and adipose tissue is an endocrine cytokine that stimulates hepatic C-reactive protein (CRP) synthesis [6,7,8]. It is also produced by adipose tissues is a major predictor of metabolic dysfunction in asymptomatic individuals [9,10] and is found to be a mediator of inflammatory processes [11,12]. Hence, the present study was aimed to assess the vitamin D levels along with circulating concentrations of hs-CRP, TNF-α and IL-6 levels in PCOS women and healthy controls.

Materials and Method

A total of 200 individuals comprising of 100 patients and 100 normal healthy, ultrasound scanned controls were enrolled for the present study. All the subjects were collected from Cytomol Labs, Gandhinagar and various endocrine clinics in and around Hyderabad. The patients were selected based on Rotterdam criteria proposed by ESHRE in 2003 [13]. Detailed information on anthropometric measures was collected through proforma. Information was collected from these individuals included anthropometric measures, history of menstruation and signs of hyperandrogenism like hirsutism, acne, and alopecia. Informed consent and permission was obtained for experimentation with human subjects from the local health authorities. Markers for obesity and abdominal obesity were measured by calculating body mass index (BMI) and Waist to Hip ratio (W/H) respectively. Three milliliter of blood samples were obtained from all the subjects. Serum was separated and stored at -20°C till use for estimation of vitamin D levels using the standard kit (TOSOH, Japan). For all the cases deficient in vitamin D, other inflammatory markers such as hs C-reactive protein (Calbiotech), interleukin 6 (Diaclone SAS) and tumor necrosis factor alpha levels (Diaclone SAS) were estimated by ELISA principle. The values obstained were compiled and expressed as mean $\pm$ SD. For statistical comparisons between the patients and control group, t – test for independent samples was used. A two-tailed p value < 0.05 was considered to be statistically significant.

Results

Data analysis of 200 subjects demonstrated vitamin D deficiency in nearly 76% of cases and 10% in controls. The mean Vitamin D level was 12.7 ± 0.89 and 18.4 ± 1.2 in cases and controls respectively and they differed significantly between the groups. Since vitamin D is associated with inflammation, we further estimated the levels of inflammatory markers (hsCRP, tumor necrosis factor alpha and IL6) in vitamin D deficient cases and controls. The levels also varied significantly between the groups (p=0.0001) [Table 1].

Table 1: The levels of vitamin D and inflammatory markers in cases and controls

	Cases (100)	Controls (100)	p-value
Vitamin D	34.5 ± 1.63	52.0 ± 3.2	0.0001*
	Cases (76)	**Controls (90)**	**p-value**
hsCRP	46.93 ± 0.06	14.9 ± 0.4	0.0001*
TNF alpha	232.3 ± 30.9	126 ± 12.7	0.0001*
IL 6	19.87 ± 4.53	9.9 ± 1.04	0.0001*

Nearly 50% of our cases are overweight or obese and in order to see the influence of these levels in cases, we further categorization of cases into obese (BMI >23 kg/mt^2) and non-obese (BMI ≤23 kg/mt^2) was performed [5]. The levels of vitamin D and inflammatory markers were analyzed in these groups and compared with controls. These levels differed significantly when compared with controls and also when compared among obese and non-obese group (p=0.0001) [Table 2].

Table 2: The levels of vitamin D and inflammatory markers hs-CRP, TNF-α and IL-6 levels within cases and controls

		Mean± SD	p-value
Vitamin D	Controls (90)	52.0 ± 3.2	ref
	Obese cases (40)	26.7 ± 1.3	0.0001
	Non Obese cases (36)	50.1 ± 2.3	0.0015
hsCRP	Controls (90)	14.9 ± 0.4	ref
	Obese cases (40)	9.7 ± 0.03	0.0001
	Non Obese cases (36)	8.1 ± 0.04	0.0001
TNF alpha	Controls (90)	126 ± 12.7	ref
	Obese cases (40)	252.4 ± 35.6	0.0001
	Non Obese cases (36)	159.3 ± 21.3	0.0001
Il6	Controls (90)	9.9 ± 1.04	ref
	Obese cases (40)	23.7 ± 3.85	0.0001
	Non Obese cases (36)	14.5 ± 6.9	0.0001

Vitamin D obese vs. non-obese (p=0.0001); hsCRP obese vs. non-obese (p=0.0001); TNF alpha obese vs. non-obese (p=0.0001); IL6 obese vs. non-obese (p=0.0001)

Discussion

Polycystic ovary syndrome is the most common endocrine disorder among women of fertile age and is often associated with a low-grade chronic inflammation reflected by an increase in circulating levels of these mediators. These markers have been linked to the development of metabolic and ovarian dysfunctions, such as insulin resistance, type 2 diabetes mellitus, cardiovascular disease, as well as hyperandrogenism and anovulation [1]. These symptoms often associated with PCOS and hence the present study was aimed to access the Vitamin D levels in cases and controls and also to analyze the inflammatory status in women deficient in Vitamin D levels in cases and controls.

Our study demonstrated a significant difference of vitamin D in obese cases compared to control indicating the role of vitamin D in the causation of PCOS. Several studies have demonstrated associations of vitamin D with ovarian follicular development, glucose homeostasis, inflammation, obesity, infertility and hirsutism [8, 14, 15]. Obesity is often considered as an inflammatory process that may result in the release of cytokines (TNF-α or IL-6) into circulation and also increases the hs-CRP concentrations, suggesting the existence of low-grade chronic inflammation in this condition [16, 17]. Inadequate vitamin D is associated with obesity and related chronic disease [2]. Since obesity, a chronic inflammatory feature often linked with PCOS, the subjects were further categorized into obese and non-obese groups and the levels were analyzed.

Our study demonstrated a significant decrease in the vitamin D levels that led to increased PTH and is independently associated with anovulatory infertility, increased testosterone leading to the clinical manifestation of PCOS. Deficiency of vitamin D increases parathyroid hormone production and is usually regulated by the serum calcium levels with dietary calcium insufficiency is largely responsible for menstrual abnormalities in PCOS. A recent study in PCOS women demonstrated vitamin D to regulate estrogen biosynthesis through direct regulation or expression of the aromatase gene and by maintaining extracellular calcium homoeostasis [18, 19].

Further analysis of the common inflammatory markers hs-CRP, IL-6 and TNF-α in the studied subjects revealed a significant difference between the groups. Several studies had reported hs-CRP association with PCOS independent of obesity while a few demonstrated that a rise in hs-CRP levels, increases body mass index and the waist circumference in PCOS. The serum TNF-α levels also showed a significant difference similar to the findings of Yang et al in Chinese population [20]. It is further observed that an increased TNFα levels showed an increase in fasting blood sugar, HbA1C, DHEAS, SHBG while the hormones such as AMH, LH and estradiol levels were found to decrease along with vitamin D. The higher serum IL-6 levels in PCOS compared to controls in our studied population were corresponding to the findings of Tarkun et al, 2006 [21]. Raised IL-6 is also shown to a increase the body mass index, fasting blood sugar, HbA1C and high density lipoprotein levels in PCOS women compared to the controls.

When the inflammatory markers were correlated with each other, all the markers correlated positively with disease susceptibility. CRP has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and is also produced by adipose tissue [9, 10]. IL-6 is an endocrine cytokine produced by MNC and adipose tissue that is directly responsible for stimulating hepatic C-reactive protein synthesis [6,7,8]. The low vitamin D levels are often significantly correlated with insulin resistance and chronic inflammation and obesity in women with PCOS. Hence, treatment with vitamin D and calcium supplementation in addition to metformin therapy for PCOS could be beneficial in managing PCOS.

Conflict of interest

None

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