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ASSOCIATION OF THYROID DYSFUNCTION AND OBESITY IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age. Women with PCOS are at a higher risk of developing hypothyroidism. Studies have shown that women with PCOS have a higher prevalence of elevated TSH levels, suggesting an association between these two conditions. The hormonal imbalances associated with PCOS may lead to changes in the thyroid gland function, causing changes in the TSH levels. Early detection of hypothyroidism in women with PCOS is important, as it can lead to improved management and treatment of both conditions.

Aim and Objectives: The present case control study was conducted to evaluate Thyroid profile, Endocrine parameters and BMI in PCOS patients and control subjects and find out the correlations between these parameters.

Methods: In this case-control study, total 209 subjects were included 104 cases and 105 controls aged between 20- 40 years. Cases were diagnosed PCOS women and controls were apparently healthy subjects. The Rotterdam criteria were used to diagnose PCOS in women between the ages of 20 and 40 years who had no other known causes of irregular menstruation. Biochemical parameters such as thyroid stimulating hormone (TSH), Triiodothyronine (T3), Thyroxine (T4), Luteinizing hormone (LH), and Follicle stimulating Hormone (FSH) were estimated by using commercially available ELISA kit and BMI were measured.

Results: The mean of age for both case and control were 25.47±4.40 and 26.49±4.54. Serum LH and TSH levels were found to be significantly increased (4.73±1.504: 4.21±1.5), (3.31±0.89: 2.23±0.80) with a p value 0.0164 and 0.0001 respectively. T3 and T4 values were decreased (1.59±0.04: 2.23 ±0.46), (89.94±8.66: 92.44±9.12) with p value 0.0001 and 0.0431 respectively. BMI (23.73±3.20: 23.83±3.78) with p value 0.8363. FSH (4.040±1.44: 4.68 ±1.59) with p value 0.0026.

Conclusion: Patients with Polycystic Ovary Syndrome (PCOS) have a higher subclinical hypothyroidism prevalence than healthy individuals according to the study. This finding underscores a possible relationship between PCOS and thyroid dysfunction. Further research and clinical evaluation are needed so as to comprehend the underlying mechanisms and implications of such an association.

Keywords: *Polycystic ovary syndrome, Hypothyroidism, Thyroid profile, BMI.*

INTRODUCTION

Thyroid disorders and polycystic ovary syndrome (PCOS) are two of the most common endocrine disorders in the general population. Although hypothyroidism and PCOS have very separate etiopathogenesis, they share many characteristics. Primary hypothyroidism has been associated with an increase in ovarian volume and cystic abnormalities in the ovaries. On the

other hand, it is becoming more and more clear that women with PCOS have higher rates of thyroid issues than women in the general population.**[1]** Various factors involved in PCOS are also present in women with hypothyroidism. Some authors have affirmed that hypothyroidism is a state of insulin resistance (IR), and IR has also been considered to be the principal factor in the genesis of PCOS.**[2]** The most prevalent endocrine illness in women of reproductive age is polycystic ovarian syndrome (PCOS), which affects the neuroendocrine and immune systems and has systemic metabolic symptoms. PCOS is defined by a combination of androgen excess and ovarian dysfunction symptoms. **[3]**Thyroid hormone appears to have an impact on the female reproductive system because both hypothyroidism and PCOS have been linked to altered ovarian function, monthly abnormalities, subfertility, and greater (recurrent) miscarriage rates.**[4]** The pituitary-derived TSH drives both thyroid hormone production and thyroid hormone release into the circulation in a traditional negative feedback loop. This explains why hypothyroidism causes elevated TSH levels when the hypothalamic-pituitary axis is functioning. **[5]** Thyroid hormones are crucial for maintaining reproductive health in addition to metabolism regulation. Thyroid and TSH receptors are both expressed in the ovary, uterus, and in significant amounts after implantation in the feto- maternal unit. Delay in the onset of puberty and anovulatory cycles may result from thyroid hormone deficiency, which may also influence gonadal function and fertility.**[6]**The diagnosis of primary hypothyroidism is confirmed by measuring the levels of Thyroid Stimulating Hormone (TSH) and Thyroxine (T4**).[7]**Hypothyroidism can have a direct effect on these hormones: a deficiency in thyroxine leads to lower levels of FSH and LH in the blood**.[8]**The major actions of Thyroid Hormones are mediated by Thyroid Hormones receptors (TRs). Recently, it was reported that TRs are also present in human ovarian surface epithelium and act on ovarian follicles and shows some slight localization in granulosa cells of ovarian follicles. Hypothyroidism causes an increase in the levels of thyroid releasing hormone (TRH) which in turn stimulates secretion of thyroid stimulating hormone (TSH) and prolactin (PRL) and PRL inhibits the synthesis and secretion of gonadotrophins. Several studies have also confirmed abnormal menstrual patterns in overt hypothyroidism.**[9,10]** PCOS and hypothyroidism both have atypical menstruation, anovulatory cycles, obesity, dyslipidemia, and psychological disorders in common. **[11].** PCOS is thought to have a complex dysfunction with a multiple origin. In 6.3% of PCOS patients, it has also been associated to primary hypothyroidism**.[12]** The pathophysiological route linking these two illnesses is yet unknown. The most obvious link, though, is the elevated BMI and insulin resistance shared by both illnesses.**[13]**

Obesity is linked to an altered mechanism and including an increase in pro-inflammatory markers and insulin resistance. This causes decreased deiodinase-2 activity at the pituitary level, resulting in relative T3 deficit and a rise in TSH levels via unidentified pathways**.[14]**

Reproductive activity is regulated by the hypothalamic-pituitary-ovarian (HPO) axis which secretes hormones necessary for reproduction. HPO is comprised of three main components. Hypothalamus secretes certain hormones, including gonadotropin-releasing hormone (GnRH). Pituitary secretes a variety of hormones, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to GnRH. Ovaries are located in the woman's

pelvis, and secrete estrogen and progesterone **[15].** Major endocrine pathways including hypothalamic-pituitary-thyroid axis (HPTA) and HP-gonadal axis are involved in parallel relationship of PCOS and thyroid dysfunction**.[16]**

Hypothalamic-pitutary-thyroid axis and HPO axis are linked physically. Thyroid receptors in the ovaries regulate female reproductive activities, and estrogen influences the HPT axis. This link identifies subclinical hypothyroidism as a risk factor for PCOS. The high prevalence of hypothyroidism among PCOS patients suggests a strong link as well. Thyroid levels are more usually altered in PCOS patients, and they are more frequently connected with anovulation. Insulin resistance is another trait shared by both disorders. The prevalence of subclinical hypothyroidism in PCOS women increases insulin resistance and hyperandrogenism. **[16,17].**

The study of thyroid function in PCOS-affected women is crucial for both researchers and medical professionals who treat them. We conducted the current study, which sought to assess thyroid function and hormonal profile in individuals with PCOS, in response toother conflicting theories regarding the pattern of thyroid function in PCOS.

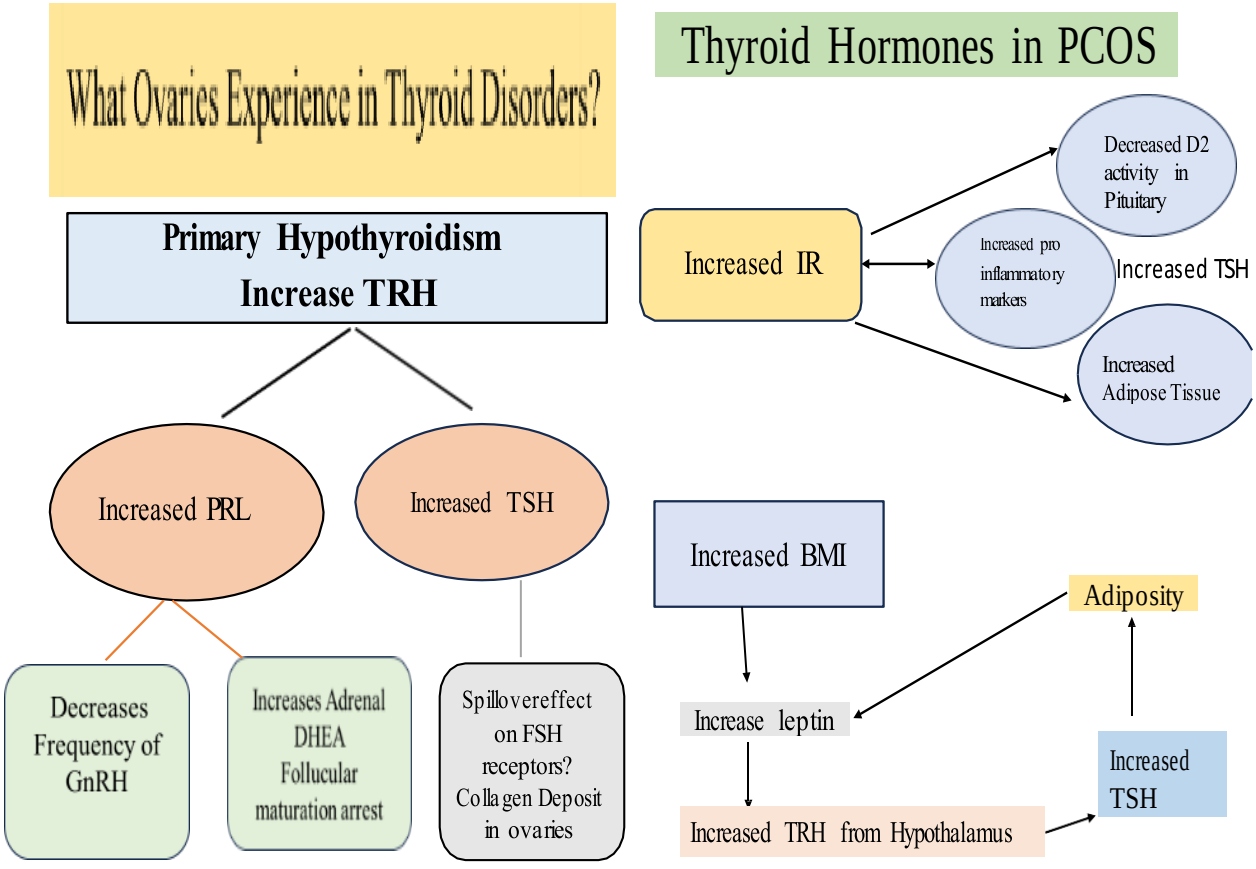


FIGURE:1 Thyroid disorders and PCOS linked together by the symptoms.

PCOS is defined by a combination of androgen excess and ovarian dysfunction symptoms. Hypothalamic-pituitary gonadal (HPG) axis is responsible for the development and regulation of the female reproductive system. Thyroid hormones are crucial for maintaining reproductive health in addition to metabolism regulation.In polycystic ovary syndrome (PCOS) there is disturbance in the HPG axis. Delay in the onset of puberty and anovulatory cycles may result from thyroid hormone deficiency.Major endocrine pathways including hypothalamic-pituitary-thyroid axis (HPTA) and HP-gonadal axis are involved in parallel relationship of PCOS and thyroid dysfunction.It is increasingly realized that thyroid disorders are more common in women with PCOS as compared to the normal population.

METHODS: After obtaining approval from the Institutional Ethical Committee (Integral institute of medical Sciences and Research Lucknow), 210 adult female subjects (105 cases and 105 controls) were selected for the study. Women between the ages of 18 and 40 who did not have any other known causes of irregular menstruation were diagnosed with PCOS based on the Rotterdam criteria were included as cases. The controls were apparently healthy women between the age of 18 to 40 years enrolled from general population without any history of Menstrual irregularities. The anthropometric parameters were measured during patient attending the OPD of Obstetrics and Gynaecology department of the Integral Hospital Lucknow. Sampling was done in collection centre of Integral Hospital by a skilled phlebotomist. Under aseptic condition blood were collected in plane vial to perform endocrine parameters All the endocrine parameters (LH, FSH, TSH, T3, T4) were performed using ELISA kits. The tests were performed in Central Research Laboratory, Department of Biochemistry, IIMS&R, Integral University, Lucknow.

Estimation of serum LH: Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), that is released by the hypothalamus. The LH ELISA kit is a solid phase assay using streptavidin/biotin method. The samples and Anti-LH/AntiBiotin conjugate were added to the wells coated with Streptavidin. LH in the patient's serum forms a sandwich between specific antibodies labeled with biotin and HRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of LH in the samples. The absorbance was read at 450 nm. **[18,19].**

Estimation of Serum FSH: The coated well immunoenzymatic assay for the quantitative measurement of FSH utilizes a polyclonal anti-FSH antibody and an FSH-HRP conjugate. The assay sample and buffer are incubated together with FSH-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader**.[20]**

Estimation of Serum TSH: Thyroid Stimulating Hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland and regulates the synthesis/release of T3 and T4 by thyroid gland. The Thyroid Stimulating Hormone (TSH) ELISA is intended for the quantitative measurement of TSH in human serum. It is a solid phase sandwich ELISA method. The samples, and anti-TSH-HRP/Biotin conjugate is added to the wells coated with Streptavidin. TSH in the sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. The absorbance was read at 450 nm on ELISA Reader. **[21,22]**

Estimation of Serum T3: The T3 ELISA is a competitive immunoassay. Competition occurs between T3 present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-T3 antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of T3 present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of T3 in specimen samples and controls can be directly read. **[23,24]**

Estimation of Serum T4: Competitive Enzyme Immunoassay. The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme- antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.**[25]**

RESULTS

The TSH levels were increased in PCOS women compared to controls and was found to be extremely statically significant with p value 0.0001. While T3(Triiodothyronine): and T4 (Thyroxine) levels were significantly decreased in PCOS women in comparison to healthy controls having p value (0.0001 and 0.0431 respectively). FSH levels showed a slight decrease in cases of PCOS and were statistically significant (0.0026), LH level shows a significant decrease (0.0164) COS patient in comparison to controls. However, the p-value for BMI was found to be 0.6488, suggesting no significant difference between cases and controls shown in **Table 1.**

Table: 1. Baseline characteristics of cases and controls.

Parameters	**Cases (Mean±SD) (n = 101)**	**Controls (Mean±SD) (n=101)**	**p-value**
Age (years)	25.47±4.40	26.49±4.54	0.1065
LH (mIU/ml)	4.73±1.504	4.21±1.5	0.0164
FSH (mIU/ml)	4.040±1.44	4.68 ±1.59	0.0026
TSH (mIU/L)	3.31±0.89	2.23±0.80	0.0001
T3 (nmol/L)	1.59±0.04	2.23 ±0.46	0.0001
T4(ng/dl)	89.94±8.66	92.44±9.12	0.0431
BMI (kg/m^2)	23.73±3.20	23.83±3.78	0.8363

*$p<0.05$ was considered statistically significant

Data were represented as Mean ± SD (Standard Deviation)

BMI: Body mass index, FSH: Follicular stimulating hormone, LH: Luteinizing hormone, T3: Triiodothyronine, T4: Thyroxine

FSH has shown a significant positive correlation with LH among PCOS women ($r= 0.476$, $p<0.01$), shown in **Table2 & Figure 1.**

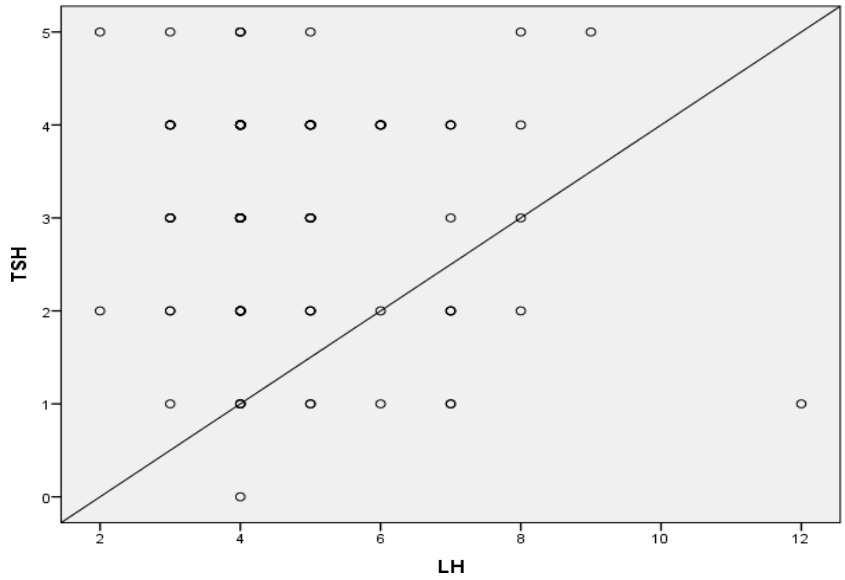
Table 2: Correlation between variables among PCOS women.

Variables	BMI (kg/m^2)	LH (mIU/ml)	FSH (mIU/ml)	TSH (mIU/L)	T3 (nmol/L)	T4 (ng/dl)
BMI(kg/m^2)	1	.085	.082	.108	.099	-.177
LH(mIU/ml)		1	.082	-.080	-.166	.030
FSH(mIU/ml)			1	0.183	0.100	.170
TSH(mIU/L)				1	.053	-.082
T3(nmol/L)					1	-.018
T4 (ng/dl)						1

**. Correlation is significant at the 0.01 level (2-tailed).

BMI: Body mass index, FSH: Follicular stimulating hormone, LH: Luteinizing hormone, T3: Triiodothyronine, T4: Thyroxine.

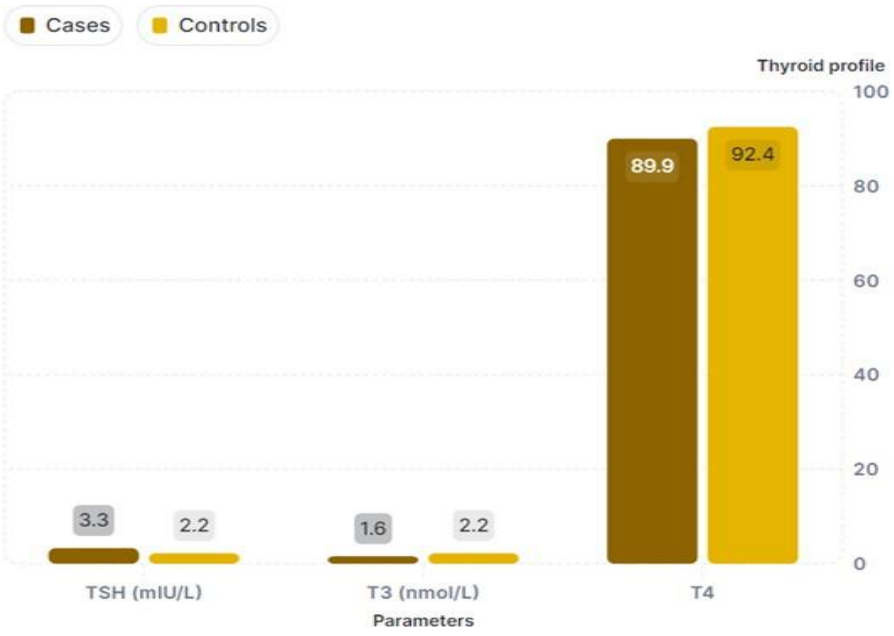
Graph 2: Correlation between TSH and LH levels of PCOS cases.



THYROID DYSFUNCTIONS IN PCOS WOMEN

1. Desforges-Bullet, V., Gallo, C., Lefebvre, C., Pigny, P., Dewailly, D., &Catteau-Jonard, S. (2010). Increased anti-Müllerian hormone and decreased FSH levels in follicular fluid obtained in women with polycystic ovaries at the time of follicle puncture for in vitro fertilization. *Fertility and sterility*, *94*(1), 198-204.

PCOS women shows higher average TSH levels (3.31 mIU/L) compared to the controls (2.23 mIU/L), suggesting potential hypothyroidism among the cases. Conversely, the T3 level is lower in cases (1.59 nmol/L) than in controls (2.23 nmol/L), reinforcing the thyroid dysfunction hypothesis. There's a marginal difference in the average T4 levels. The 'Controls' have a slightly higher average, suggesting better thyroid gland function compared to the 'Cases'.



GRAPH 1: Comparison of Thyroid profile mean values between cases and controls

DISCUSSION:

Prevalence studies almost consistently remark on the frequent co-occurrence of PCOS and HT in women of reproductive age. As a result of the above discussion, it is reasonable to assume that thyroid problems and PCOS are inextricably linked in terms of etiology, pathophysiology, and clinical repercussions. This chapter, on the other hand, provides scientific groundwork for additional research into the relationship between thyroid problems and PCOS. Decreased fertility is associated with Hypothyroidism and PCOS, though it seems that in patients with these two diseases, fertility disorders appear more frequently and are be more pronounced. Antithyroid antibodies are observed in 5-10% of women of reproductive age.Desforges-Bullet, 2010 et al., findings suggest that the granulosa cells from polycystic ovaries continue to produce elevated levels of AMH, possibly because of impaired access of FSH to follicles**.** suggesting a partial insufficiency of endogenous FSH **[26]** It is now clear that the administration of small amount of FSH into the system will induce ovulation and pregnancy in very large proportion of afflicted women **[27]** Indeed, in women in fertile age, hypothyroidism impairs the cycle length and can cause oligomenorrhea, amenorrhea, polymenorrhea, as well as menorrhagia. In fact, TH adjusts the stimulatory effects of FSH on follicular growth and apoptosis suppression. Numerous studies evidence an alteration of the reproductive system with an abnormal thyroid function. Lowertriiodothyronine (T3) and positive TPOAb are associated with a lowerantral follicle count (AFC)woman seeking fertility.**[28]**

Hypothyroid disturbances and elevated TSH levels are common findings in PCOS, which are associated with an adverse metabolic profile. Therefore, women with diagnosed PCOS should be screened for thyroid dysfunction.[29]Sumithra NU et al., Reported that Erythrocyte malondialdehyde (MDA), superoxide dismutase (SOD), serum hsCRP, gonadotrophins, thyroid stimulating hormone, prolactin, glycemic status and lipid profile were estimated. Erythrocyte MDA ($p < 0.001$), SOD ($p = 0.007$) and serum hsCRP ($p < 0.001$) were significantly elevated in PCOS patients than controls**.[30]** We suggest that Thyroid profile are gets altered in Polycystic Ovary Syndrome. The females with PCOS are more prone to get thyroid dysfunction. it is suggested that a routine monitoring of thyroid profile with other parameters needed to be done for the early prognosis. The meta-analysis of 6 studies involving 692 PCOS patients and 540 controls indicates a significant association between polycystic ovary syndrome (PCOS) and an increased risk of subclinical hypothyroidism (SCH).

CONCLUSION:

To conclude, our study provides insight into the probable correlation between Polycystic Ovary Syndrome (PCOS) and subclinical hypothyroidism thus indicating a high occurrence of thyroid disorder among PCOS patients compared to normal individuals. This observation is significant in terms of reproductive health and endocrine diseases. The association of PCOS with subclinical hypothyroidism implies a complicated interaction between the reproductive and thyroid glands. Further investigations are needed to untangle these intricacies, examining genetics, hormones, and environmental factors as may contribute towards both PCOS and subclinical hypothyroidism development as well as progressi

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Ethical Approval: The study was approved by the Institutional Ethics Committee.

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