Role of Vitamin B_{12} and Vitamin D with Their Clinical Relevance in Normal and Polycystic Ovarian Syndrome (PCOS)

Thesis

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By

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DECLARATION BY THE CANDIDATE

I, **Shahid Khan**, do hereby declare that the work presented in this thesis entitled **"Role of Vitamin B12 and Vitamin D with their Clinical Relevance in Normal and Polycystic Ovarian Syndrome(PCOS)''** submitted to the Maharishi University of Information Technology, Lucknow in partial fulfilment for the award of Ph. D degree in Biochemistry is an authentic record of original research work accomplished by me under the supervision of **Prof. Akhand Pratap Singh** and Co-Spervison of **Dr. Mohd. Ashraf Ganie**

I further declare that the work done in the thesis has not been submitted for the award of any other degree to the best of my knowledge and belief.

Date: 08/08/2023
Place: Lucknow

Shahid Khan

SUPERVISOR'S CERTIFICATE

This is certified that the thesis entitled, **"Role of Vitamin B12 and its Clinical Relevance in normal and Polycystic Ovarian Syndrome(PCOS)"** is an original piece of research work carried out by **Mr. Shahid Khan** for the award of **Doctor of Philosophy (Ph.D.)** degree in Biochemistry under my guidance and supervision. I further certify that the work has not been submitted either partly or fully to any other University or Institution for the award of any degree.

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ABSTRACT

In the progenitive age range, PCOS is the most prevalent endocrine disorder and the main contributor towards infertility. Due to a lack of research on the burden of PCOS, its relevance is still incomprehensible at this point in time. Although a portion of the mechanism involved in the development of PCOS has been identified, the precise aetiology and pathophysiology are still not fully understood. The most significant reasons for PCOS are impaired ovarian steroidogenesis, insulin resistance (IR), neuroendocrine abnormalities, obesity, and elevated cortisol metabolism-related adrenal hyperandrogenism. However, the underlying cause of PCOS is still unknown. Insulin and androgens have both been identified as critical contributors to its development. As a result, treating PCOS entails reducing hyperandrogenism and hyperinsulinemia. Additionally connected to insulin resistance, obesity, and elevated homocysteine in PCOS patients are low serum vitamin B12 concentrations. Moreover, insulin resistance is strongly connected with vitamin D insufficiency, which is frequently detected in PCOS-afflicted women. However, the Indian Scenario has not undergone a thorough investigation of this. In this thesis, we hypothesize that variations in vitamin B12 and vitamin D levels either alone or in combination with one another cause PCOS.

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Chapter 1

INTRODUCTION

The main factor in infertility is polycystic ovarian syndrome (PCOS), a common endocrinological condition in the progenitive age range. If left untreated, PCOS puts women at risk of developing issues such as infertility, metabolic illnesses such as type 2 diabetes mellitus, hypertension, and other cardiovascular and cerebrovascular disorders (1). Such challenges are detrimental to the patient's quality of life, as well as to their social and emotional health. Long-term monitoring of 786 PCOS patients revealed an increased risk of endometrial cancer (2,3). Worldwide, PCOS prevalence ranges from 2.2% to 26%(4). The frequency of PCOS varies among Asian nations, from as low as 2.4% in China(4) to 19.5% in Iran (5). The prevalence of PCOS among teenagers varies regionally, according to a recent metanalysis (2019) by Naz et al(6). Different diagnostic criteria, such as those used by the National Health Institute (NIH) in 1990, Rotterdams (2003), and the Androgen Excess Society (AES) in 2006, have resulted in varying prevalence. The reported prevalence with Rotterdam criteria was 11.04%, 3.39% with NIH criteria, and 8.03% with AES(7). Any person between the ages of 10 and 19 is considered an adolescent according to the World Health Organization (WHO). The adolescent age range frequently experiences PCOS because of the modern lifestyle. Adolescence may mark the end of PCOS's clinical manifestation, although diagnosing the condition can be difficult because some of its

symptoms overlap with those of the transition from puberty to adulthood (8). Early adolescent individuals are not thought to meet the hyperandrogenism and oligoanvolution criteria proposed by AES (2006)(9). The small sample sizes of the studies that are currently available have not caught the attention of decision-makers. Due to a lack of understanding among school personnel, healthcare workers, and beneficiaries, as well as prevalent cultural taboos, this issue goes untreated, ignoring the long-term impact on women's physical and emotional health.

This syndrome is frequently linked to enlarged and dysfunctional ovaries, high levels of androgen, insulin resistance, etc. (10). One in ten women are thought to experience PCOS before menopause and struggle with its implications. The precise aetiology and pathophysiology of PCOS are not completely understood (11,12), despite the fact that the high luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio and increased frequency of gonadotropin-releasing hormone (GnRH) are known to be its underlying causes. Evidence points to the involvement of numerous internal and external factors, including as genetics, epigenetics, hyperandrogenism (HA), insulin resistance (IR), and environmental factors. The risk of other consequences such cardiovascular diseases(12,13), type 2 diabetes mellitus (12,13), metabolic syndrome (13), depression, and anxiety(14) is also something that should be mentioned. The most important step in managing this illness is to decrease at least 5% of body weight, so every woman with PCOS is advised to follow a regular exercise regimen and a diet low in fat and sugar. Additionally, due to their preexisting beliefs, reduced prices, etc., complementary and alternative medicine practises can occasionally be preferred to conventional therapies. Oral contraceptives, antiandrogens, insulin sensitizers, and

ovulation inducers are frequently prescribed by doctors (11). The Food and Drug Administration (FDA) has not yet approved any drugs, especially for PCOS, and all of the pharmaceuticals described are used outside of their intended medical uses (15) . Drug repurposing techniques could lead to the identification of novel drugs in addition to the critical need for advancement in the research and development of new drug molecules and new drug discovery (16) .There is considerable interest in using a number of medications that the FDA previously licenced for uses other than PCOS as treatment options for the management of PCOS.These treatments range from 3-hydroxy-3-methyl-3-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors like simvastatin and atorvastatin to 3-hydroxy-3-methyl-3-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors like pioglitazone, empagliflozin, sitagliptin, and liraglutide, as well as mucolytic drugs like N-acetyl cysteine(17). The current registration of clinical trials serves as an example of the pharmaceutical industry's lack of interest in PCOS: at the end of August 2017, only 28 commercial studies on PCOS had been registered at ClinicalTrials.gov, whereas 4,632 studies had been registered for diabetes mellitus, despite the fact that both conditions have comparable global prevalence (18,19). As a result, PCOS may now be regarded as an orphan condition affecting adolescent and adult women with the highest prevalence from the standpoint of pharmaceutical treatment. Given that PCOS is a rising problem that unfortunately has many unfavourable side effects and that the present treatments and drugs are not always successful, it is crucial to carefully research its pathophysiology and identify novel pharmacological targets. Repositioning techniques could do this while saving money and time.

It has been proposed that nutrition-related signalling pathways are key regulators of ovarian follicle advancement and ovulation rates. Vitamin and mineral supplements definitely help PCOS symptoms by promoting the production of healthy oocytes (20). Additionally improved are hyperinsulinemia, hyperandrogenism, elevated body mass index, and cardiovascular problems. Additionally, the psychological problems linked to PCOS have improved (21). (22) (23).

Androgens and insulin are two important elements in the pathophysiology of PCOS, even though the exact reason is currently unknown. Significant roles for vitamin B12 in homocysteine (Hcy) control. In the pathophysiology of PCOS, Insulin Resistance (IR) and high Hcy levels have been found to positively correlate (24) (25). Kaya et al. showed that low serum insulin B12 concentrations in PCOS-affected women were associated with IR, obesity, and elevated Hcy levels. Three months of supplementation with folic acid and vitamin B-12 was effective in reducing elevated serum Hcy levels, especially in women without IR. Metformin inhibited the binding intrinsic factor-B12 complex and its receptor, lowering serum levels of folic acid and vitamin B12.The Hcy levels were further elevated by metformin. However, vitamin B12 supplements had no impact on levels of androgen or lipids in the pathophysiology of PCOS (26). These results suggested that the Hcy-increasing effects of metformin therapy could be reduced by supplementing with B-group vitamins and folic acid.

Furthermore, research has demonstrated that difficulties linked to PCOS pathogenesis can result from vitamin D3 deficits. D3 measurement stops the inflammatory process

that leads to PCOS. Additionally, vitamin D3 therapy is essential for folliculogenesis because it lowers increased levels of the anti-mullerian hormone (27). Intriguingly, Jafari-Sfidvajani et al.showed that vitamin D treatment in PCOS did not alter the androgen profile when paired with a low-calorie diet, but it did enhance menstruation frequency (28). A cross-sectional study reported that lower vitamin D was linked with IR as a result of the pathophysiology of PCOS (29). Inflammatory development in the pathophysiology of PCOS is inhibited by vitamin D3.Vi ta m i n E Vitamin E (400 IU) and omega-3 fatty acid (1,000 mg) co-supplementation in women with PCOS for 12 weeks provided significant improvement in IR and androgen levels(30).

Therefore, supplementation with particular nutrients and complementary therapies may be useful in improving health outcomes in women with PCOS by modulating key pathways hypothesised to produce PCOS (e.g., insulin signalling, IR, lipid metabolism, etc.).This would change the symptomatology and severity of PCOS. According to this concept, PCOS is either caused by variations in vitamin B12 and vitamin D levels alone or when they occur together.

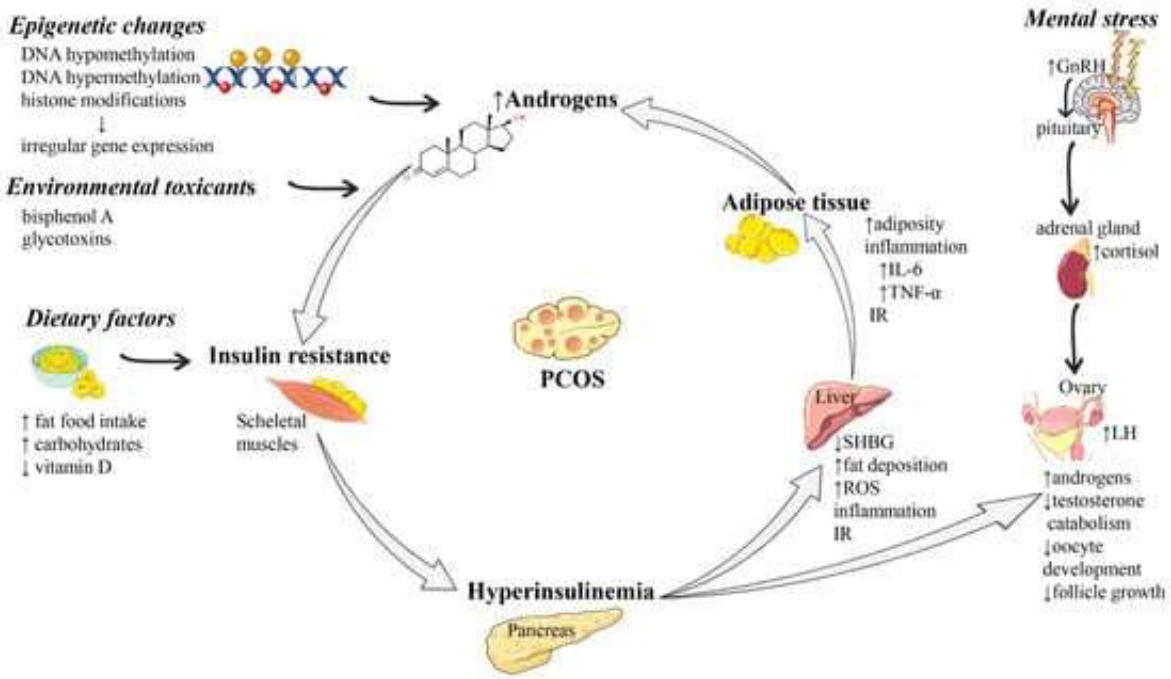

Figure 01:- Summarized scheme regarding the pathophysiology of PCOS. Abbreviations and symbols: ↑ (increased), ↓ (decreased), DNA (deoxyribonucleic acid), GnRH (Gonadotropin-releasing hormone), IL-6 (interleukin 6), IR (insulin resistance), LH (luteinizing hormone), PCOS (polycystic ovary syndrome), SHBG (sex hormone-binding globulin), TNF-α (tumor necrosis alpha) Image Source:- (17)

Chapter 2

Review of Literature

Polycystic ovarian syndrome (PCOS) also known as Stein-Leventhal syndrome, is a frequent endocrinopathy that affects 3-7% of women of reproductive age (31). It is unknown if PCOS-afflicted women are more susceptible to cardiovascular disease than comparably aged controls (32). All available evidence suggests that PCOS has the inherent conditions that lead to an increased incidence of factors predisposing to cardiovascular diseases, even though epidemiological studies have not demonstrated an increased incidence of death from cardiovascular events in women with PCOS(33) (34). Hyperinsulinemia affects more than 30% of lean and 75% of obese PCOS women(35).

Insulin resistance, dyslipidaemia, impaired glucose tolerance, type 2 diabetes mellitus, and elevated systolic blood pressure are more prevalent in obese young women with PCOS, which suggests that women with PCOS are at an increased risk of CVD (36) (37) (38). Cross-sectional studies examining prevalence imply that women with PCOS are at greater risk for premature development of diabetes mellitus (2). Studies on high blood pressure and dyslipidemia in PCOS-affected women have shown inconsistent findings thus far(39). Sampson et al. (1996) found no variations between 24-hour ambulatory blood pressure readings taken during the day and at night(40). However, Holte et al. (1996) found greater mean and systolic ambulatory arterial blood pressure readings during the day(41). In PCOS-afflicted women of normal weight, Orio et al.,2005 reported a diastolic filling and a substantial rise in left ventricular mass index (42). On the other hand, Zimmermann et al.

(1992) contend that women with PCOS do not have elevated arterial pressure or left ventricular mass despite having severe hyperinsulinemia and insulin resistance(43). Independent of body weight, women with PCOS appear to have greater triglyceride (TG) levels and lower high-density lipoprotein (HDL) cholesterol levels(44). Most studies—but not all—report this profile. When obese women with PCOS had comparatively elevated HDL-C levels, Legro et al., (2001) found that elevations in low-density lipoprotein cholesterol (LDL-C) were the primary lipid abnormality(45). According to Talbott et al. (1998), young women with PCOS have LDL-C values that are significantly higher than age-matched controls, but pre- to peri-menopausal women with PCOS (aged 40 years) have LDL-C and total cholesterol levels that are comparable to those of age-matched controls(46). In addition, several researchers have found that women with PCOS have significantly higher serum Hcy concentrations than controls (47) (48), while others have found no correlation between PCOS and Hcy (49). When atherosclerotic markers such the intima-media thickness of the femoral and carotid arteries are assessed, women with PCOS appear to have a higher risk of Coronary Vascular Disease(50). Furthermore, aortic and coronary artery calcification have recently been found to be more common in middle-aged women with PCOS when compared to controls (51) (52). Although it's uncertain whether PCOS is a standalone risk factor for circulatory death, recent research suggests that women with PCOS cluster risk variables for early morbidity and mortality(44).

Recognizing insulin resistance as a key element in the development of PCOS has led to the use of insulin sensitizers in its therapy (53). The most well researched insulin sensitizer for PCOS is metformin, an oral antihyperglycemic medication initially developed to treat type 2 diabetes mellitus. According to research by Carlsen et al.,1997 metformin increases total

serum Hcy levels in non-diabetic male patients with coronary heart disease(54). When metformin is added to insulin therapy for type 2 diabetes mellitus, it lowers levels of folic acid and vitamin B12 and causes a modest increase in Hcy levels within 12 weeks (55). Vrbikova et al's study on PCOS patients showed that metformin had a homocysteine (Hcy) - increasing impact (Vrbikova et al., 2002)(56). Further, a study showed that individuals with PCOS who received metformin plus rosiglitazone for 3 months experienced a considerable rise in plasma Hcy concentrations (24). However, elevated total plasma homocysteine (Hcy) is a recognised risk factor for early cardiovascular disease (CVD) and stroke risk in populations that are otherwise healthy (57). High plasma levels of Hcy have been linked to vascular damage and changes in the clotting process, according to experimental studies (58). An increase in Hcy levels causes 10% of the general population's risk of coronary artery disease (59). According to their findings, plasma Hcy levels that rise by 5 mol/l raises the risk of CVD by 1.6–1.8 times. Plasma Hcy levels have also been linked to blood pressure (60), and elevated Hcy levels have been linked to higher insulin levels (61) (62) as well. According to research by Vrbikova et al., 2002, metformin use in PCOS-afflicted women can cause an increase in homocysteine levels, a risk factor for atherosclerosis(63). As a result, it is possible to draw the conclusion that women with PCOS who use insulin sensitizers may have an increase in Hcy levels (24).

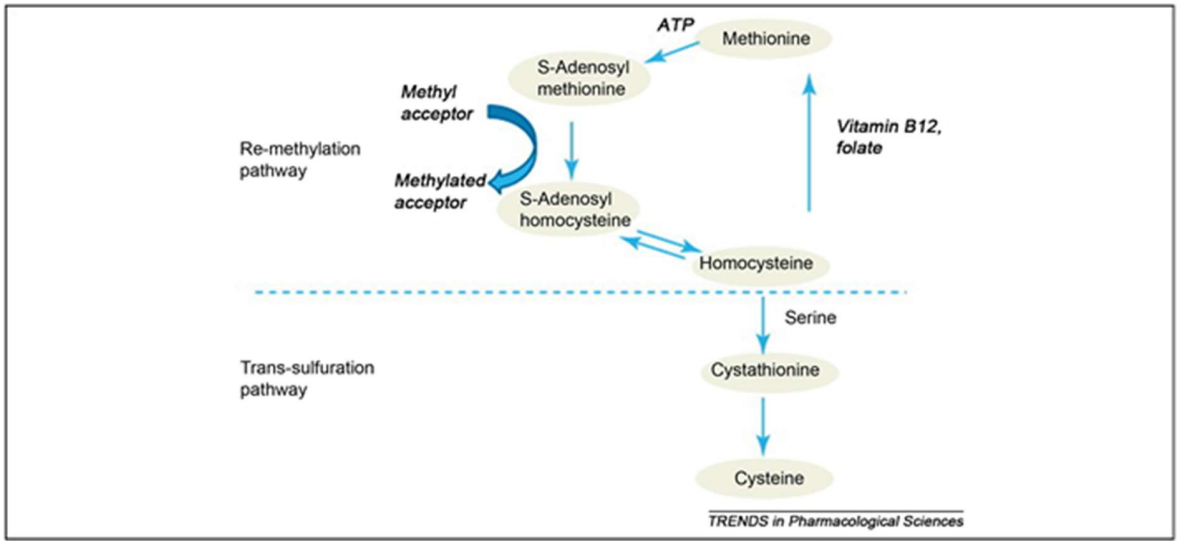

Figure 02:- Homocysteine (Hsy), a product of the production of methionine, has been identified as a key characteristic of PCOS. It is a cysteine and a-amino acid homologue that is not a protein. This figure depicts its metabolic pathway, which includes either remethylation to methionine or by transsulfuration to cystathionine. While the second process needs pyridoxal-5'-phosphate, the first requires folate and vitamin B12. S-adenosylmethionine (SAM), an inhibitor of cystathionine -synthase and a moderator of methylenetetrahydrofolate reductase (MTHFR), increases the synthesis of both routes (CBS). Any defective function, such as inadequate transsulfuration brought on by a CBS mutation, a vitamin B6 deficit, or even a remethylation blockade, might disrupt the metabolic pathways and cause an aberrant buildup of plasma Hcy (64).

However, studies in patients with metabolic syndrome, folate and vitamin B_{12} supplementation showed reduced insulin resistance and endothelial dysfunction while decreasing Hcy levels(65). Taking folic acid and B-group vitamins can help PCOS patients with short-term metformin therapies lower their serum levels of Hcy, according to a 2005 PCOS study by Kilicdag et al. After 12 weeks, metformin medication caused a 26.5% increase in Hcy levels, while the groups that received B-group vitamins and folic acid plus metformin experienced 21.17 and 8.33% declines in Hcy levels, respectively. These results

suggested that the Hcy-increasing effects of metformin therapy could be mitigated by the administration of folic acid and B-group vitamins, particularly B-group vitamins (26). Kaya and his group back in 2010 first assessed whether vitamin B12, folate, and Hsy concentrations in PCOS patients were lower or greater than in healthy, age and body mass index-matched controls. They then looked into the correlations between these nutrients, insulin resistance and obesity in patients with PCOS. In PCOS patients with insulin resistance as compared to those without it, Hsy levels and the homeostasis model evaluation score were greater, but vitamin B12 levels were lower. Obese PCOS individuals had significantly lower serum vitamin B12 concentrations than obese control women ($P < 0.05$). In PCOS patients, serum vitamin B12 concentrations were independently influenced by fasting insulin, insulin resistance, and Hsy levels. They hence concluded that lower serum vitamin B12 concentrations were linked to insulin resistance, obesity, and increased homocysteine in PCOS(66) . Gourgari et al., 2014 describe for the first time in a case study of an adolescent girl with PCOS that vitamin B12 deficiency is associated with metformin use. The oral cyanocobalamin treatment was started at a dose of 1000 g per day. A second evaluation after one month of therapy revealed a normal B12. Her psychological symptoms were said to have improved by her family after the stabilisation of her serum vitamin B12 levels. After the patient's vitamin B12 dosage was stopped, she returned five months later. Her serum B12 level had dropped but was still within normal limits. Thus, this study concluded that universal screening for vitamin B12 is necessary among adolescent females with PCOS(67). Take note that this medication may cause a rise in plasma Hcy levels and interfere with the routes for vitamin B12 and folic acid. Strong evidence supports the use of metformin in the regulation of cycle and ovulation induction among PCOS patients who seek conception

(68). Increased levels of Hcy have been linked to spontaneous miscarriages, intrauterine growth retardation, pre-eclampsia, and intrauterine foetal death in addition to neural tube abnormalities and other congenital problems (69). To prevent neural tube defects, many authors recommend the administration of folic acid and vitamin B12 (70) (71) (72) (73) This is because vitamin B_{12} promotes the uptake of folic acid by cells, so even though folic acid concentrations are normal, a vitamin B_{12} deficiency may lead to neural tube defects (74) (71)(Smithells et al., 1983; Czeizel and Dudal, 1992; Eskes, 1998; Leeda et al., 1998; Brouwer, 2000). At the Fatemehzahra Fertility Hospital in Babol, Iran, an interventional trial was planned between 2014 and 2015 with 18 PCOS patients aged between 18 and 35 years. All patients had a 6-month course of metformin therapy (500 mg twice daily). Before and after receiving metformin, the levels of serum Hcy, vitamin B12, and folic acid in the participants were measured. After taking metformin for six months, patients' mean vitamin B12 levels significantly decreased ($P = 0.002$). However, serum folic acid levels did not differ significantly from one group to the next. After therapy, mean Hcy levels rose, although this change was not statistically significant. Body mass index (BMI) and insulin sensitivity were used to divide patients into four subgroups. Results were generally similar among the subgroups, with the exception that Hcy levels in the overweight/obese group (BMI > 25 kg/m2) following therapy showed a substantial rise ($P = 0.01$). These results suggest that metformin raises serum Hcy levels in PCOS patients, particularly in females with BMIs greater than 25 kg/m2. The evident decrease in vitamin B12 levels could be the mechanism underlying this impact (75). In addition to the work mentioned above, Hasan and his team most recently studied individuals with PCOS who attended a specialised endocrinology clinic of a tertiary hospital in Mymensingh, Bangladesh, from July 2021 to June 2022. A total of

50 PCOS patients who had just received a diagnosis and 52 PCOS patients who had been on metformin for at least six months were evaluated in this study. The chemiluminescent microparticle immunoassay principle was then used to determine the serum vitamin B12 content. Except for the metformin group's lower haemoglobin levels and increased plateletcrit, the clinical and laboratory parameters of new drug-naive and metformin-treated PCOS participants were similar. In comparison to drug-free patients, those who received metformin had reduced serum levels of vitamin B12 (385.5 pg/mL [interquartile range, or IQR, 298.7-535.2] vs. 272.0 pg/mL [IQR 217.0-395.7]; P 0.001). B12 deficiency and borderline deficiency were more prevalent in the metformin group (15.4% vs. 6% and 42.3% vs. 18%, respectively; P = 0.003). This study found that metformin-using PCOS patients had lower serum B12 levels than those who had just been given the diagnosis. However, they came to the conclusion that more comprehensive data were required before routine periodic testing for B12 levels in PCOS patients receiving metformin (76).

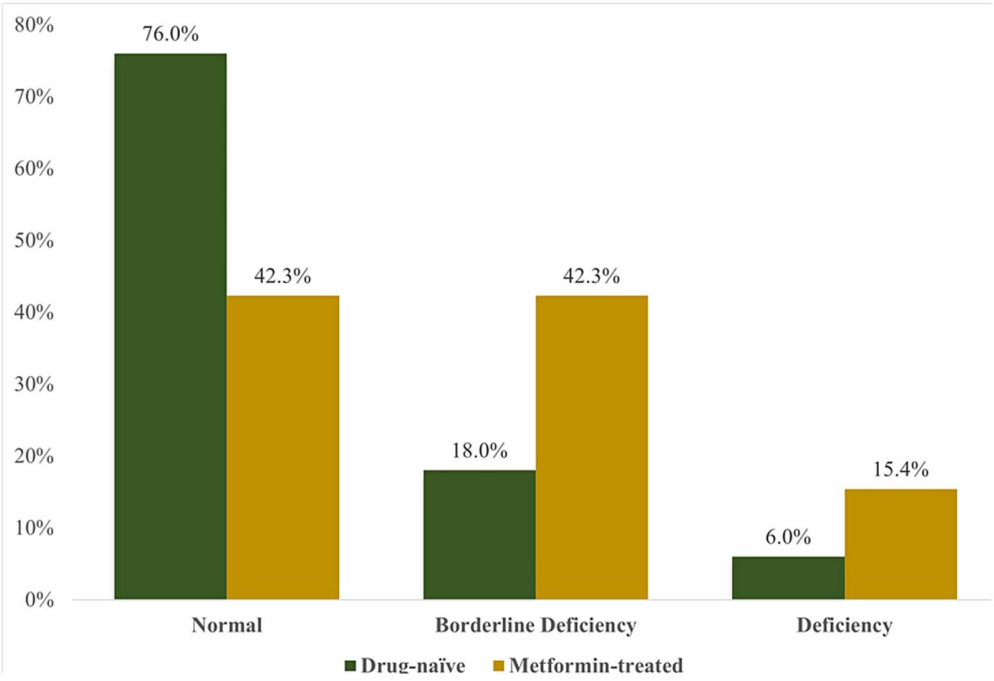

Figure 03:- Vitamin B12 readings in Patients with PCOS on metformin versus those not consuming any drugs (data taken from Hasan et al.,2022)(76)

Similar to the above cited papers a review by our team by using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement and the Cochrane handbook for Systematic Reviews of Interventions was initiated (77). Studies that met the following criteria were added to our list:

(1) It should include people with type 2 diabetes or polycystic ovary syndrome (PCOS) who met strict diagnostic criteria and hadn't taken any B group vitamins before joining the study.

(2) Patients should be randomly randomised to receive metformin, a placebo, or another blood sugar-lowering medication for randomised controlled trials (RCTs). Valid statistical

methods should be used to compare the groups. For observational studies, a group given metformin should be compared to another group given a placebo or other diabetes drugs.

(3) The main result should be a change in the amount of vitamin B12 in the blood, or serum.

(4) All studies should report enough information to do a meta-analysis or enough information to estimate it. These factors are linked to changes in vitamin B12 levels. Studies that had no data, were published more than once, or were written in a language other than English were not included. All papers published up to October 2013 were looked for in PubMed, Embase, and the Cochrane central registry of controlled trials. Subject headings were put together with keywords and their synonyms. For example, search terms like "B12" were combined with "metformin," "Glucovance," "dimethylbiguanid," "vitamin B12," and "cobalamin." References in some articles and reviews that were already out there were also looked up by hand. Two researchers did their own searches of the literature, and any differences were worked out in group discussions. Through direct author contact, we looked for more studies and information that was missing from reports that had already been published.

The validity of the eligible RCTs was evaluated according to the Cochrane Collaboration guidance which includes the following criteria:

(1) random sequence generation,

(2) allocation concealment,

(3) blinding of participants and staff,

(4) blinding of outcome assessment,

(5) incomplete outcome data,

(6) selective reporting, and (7) other bias.

An answer of "Yes" denoted a low likelihood of bias, an answer of "No" suggested a high risk of bias, and an answer of "Unclear" showed that there was either insufficient information or uncertainty on the likelihood of prejudice. We also used the Newcastle-Ottawa Scale (NOS) to judge quality of the observational studies that were included. We looked at the titles and abstracts to find clinical trials and observational studies. Studies that satisfied the requirements for inclusion were discovered in articles with their complete texts. The title, authors' names, publication year, research design, participant characteristics, and information regarding the conclusion were all gathered from each article. A meta-analysis was conducted to examine the connection between metformin use and variations in vitamin B levels. The amount of vitamin B12 and how it changed was measured in pmol/L. Using the chi-square test and I2 statistics, we looked at how different the studies were. P=0.1 and I2=.50% were both signs of heterogeneity. For heterogeneity that wasn't important, a fixed effects model was chosen, and for heterogeneity, a random effects model was chosen. In each study, the mean difference (MD) was found. The MDs were added together, and Review Manager was used to figure out the pooled MDs and their 95% confidence intervals (CIs) (RevMan, version 5.2). Separating the studies by comparators, follow-up time, and background treatment made it possible to do subgroup analyses. z-statistics were used to measure the overall effect of tests.

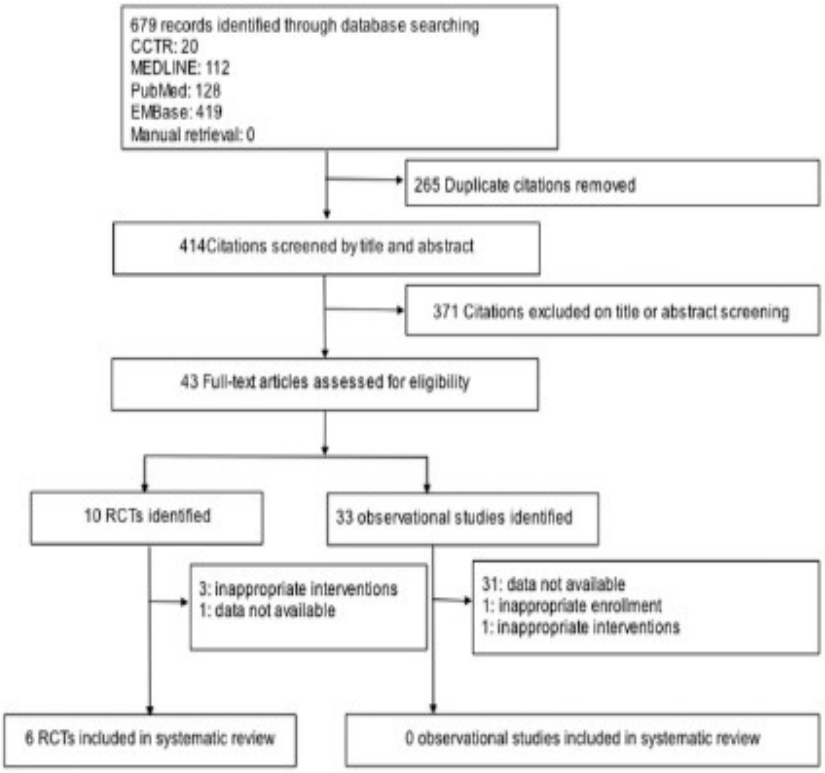

Figure 04:- The study's flowchart (77)

Overall, we found that patients with diabetes and PCOS who took metformin had statistically significant drops in their vitamin B12 levels. The 33 observational studies demonstrated that using metformin was associated with a significant decline in vitamin B12 levels, despite the fact that none of these studies matched the criteria for inclusion. A meta-analysis of RCTs revealed that individuals with hyperglycemia and PCOS who received metformin had reduced levels of vitamin B12, which is consistent with the current observational studies. According to a subgroup analysis, a larger dose of metformin may cause vitamin B12 levels to drop even more. Subgroup analysis showed that a higher dose of metformin could lower vitamin B12 levels more. Because both PCOS and T2DM are caused by insulin resistance, we included RCTs of patients with both conditions in our meta-analysis. Subgroup analysis

showed that the effects of metformin on vitamin B12 in people with T2DM or PCOS were almost the same. The clinical importance of biochemical changes in vitamin B12 levels in the blood is still up for debate. Some past studies have shown that a normal-range decrease in serum B_{12} concentration caused by metformin could be clinically important. Metformin may also speed up cognitive impairment and the spread of diabetic peripheral neuropathy in a way that depends on vitamin B_{12}. The decrease in serum B12 levels within the normal range was therefore advised not to be disregarded.

Although metformin has been associated to an increase in Hsy-concentration in PCOS patients, resulting in Vitamin B12 deficiency, another component that has recently been observed is a drop in Vitamin D and an increase in depressed symptoms within the same group of patients. Twenty women of reproductive age with PCOS, insulin resistance, low vitamin D, and depression participated in the MINDD (Metformin, vitamin D, and Depression) pilot trial. For 12 weeks, subjects were given a 1:1 random assignment to either metformin or vitamin D. To measure sadness, anxiety, and quality of life, standardised, validated questionnaires were employed. Measures of depression severity, serum 25-hydroxyvitamin D levels, and HOMA-IR (Homeostasis Model Assessment of Insulin Resistance), which were derived from fasting insulin and glucose, were retrieved at baseline, six weeks, and at the end of the procedure. The idea was that treating women with PCOS and depression for metabolic issues, notably insulin resistance or vitamin D deficiency, could lessen their symptoms of depression (78). Although their preliminary data showed that there was change in BDI-II score (depression symptoms) in PCOS patients who were given metformin showed decrease in Vitamin D levels and signs of depression. However, this study was terminated because of patient recruitment challenges back in 2021.

Vitamin D influences ovarian follicular growth and luteinization in human granulosa cells via modulating anti-müllerian hormone (AMH) signalling, follicle-stimulating hormone sensitivity, and progesterone production (79) . The presence of a specific vitamin D receptor (VDR) in pancreatic beta-cells and skeletal muscle, the expression of the enzyme 1-α-hydroxylase, which can catalyse the conversion of 25-hydroxyvitamin D [25(OH)D] to 1,25-dihydroxyvitamin D, and the presence of a vitamin D response element in the human insulin gene promoter are all examples of potential vitamin D influences on glucose homeostasis (80) . The prevalence of vitamin D deficiency in women with PCOS is about 67-85 per cent, with serum concentrations of 25(OH)D <20 ng/ml (81) . Although there is no discernible difference in 25(OH)D levels between PCOS and normal control women, it has been discovered that metabolic syndrome is strongly connected with a high incidence of vitamin D insufficiency, which could have a substantial influence on public health (82). Infertility, hyperandrogenism, obesity, insulin resistance, monthly abnormalities, and an elevated risk of cardiovascular diseases are just a few of the PCOS symptoms that may worsen with low levels of 25(OH)D.The inconsistent results from different individual studies and from a recent meta-analysis report of a systematic review make it difficult to draw a firm conclusion about the causal relationship between vitamin D and metabolic disturbances in PCOS, despite the fact that many observational studies have suggested a possible role for vitamin D (83).

In vitamin D-deficient women with PCOS, vitamin D administration can decrease the excessively increased serum AMH levels and enhance the serum anti-inflammatory soluble receptor for advanced glycation end-products (79). Menstrual regularity and ovulation in particular may benefit from vitamin D and calcium supplements in addition to metformin therapy in women with PCOS (84). However, Garg et al demonstrated that supplementing

with vitamin D for six months at a dose of 4,000 IU/day did not significantly improve insulin kinetics or cardiovascular risk factors in PCOS-affected women using metformin. The results of earlier observational studies and clinical trials on the effects of vitamin D supplementation in reducing symptoms in women with PCOS remain unclear due to the small sample size and the relatively short duration of follow up (84) (85). As a result, more research with high-quality randomised controlled trials is necessary to determine how vitamin D supplementation affects PCOS therapy.

Insulin resistance has been reported to be highly linked with low 25(OH)D levels in PCOS women (80). As a result, genes related to vitamin D metabolism have been proposed as potential genetic markers for PCOS risk. Several VDR gene polymorphisms, including Cdx2, Taq1, Bsm1, Apa1, and Fok1, have been documented to have an impact on insulin production and sensitivity in PCOS women (86). Results of a study conducted in Hyderabad, India, to look into the connection between four VDR polymorphisms (Cdx2, Fok1, Apa1, and Taq1) and PCOS in Indian women were published by Dasgupta et al.,2015 (87). They identified a significant variation in the genotype and allele frequency distributions of the Cdx2 polymorphism between PCOS and control women. When comparing controls to patients, a significantly greater frequency of the heterozygous GA genotype and the A allele of Cdx2 polymorphism was found ($p<0.001$), showing a protective effect for this single nucleotide polymorphism (SNP) against the PCOS phenotype. The carriers of the GA genotype and the A allele continue to give protection against PCOS after the factors of age and body mass index have been adjusted. However, no other noteworthy correlations between PCOS and the other three VDR polymorphisms (Fok1, Apa1, and Taq1) were found. Their further analysis of the connections among VDR genotypes and a few of the specific clinical and biochemical

characteristics associated with PCOS revealed a significant association between the Cdx2 genotypes and testosterone levels and a significant association between the Fok1 polymorphism and the presence of infertility. Additionally, the two haplotypes ACCA and ACTA, each made up of four polymorphisms, were discovered to be substantially related with PCOS. Studies have connected vitamin D deficiency and SNPs in the gene that codes for the vitamin D receptor (VDR) to an increased chance of developing polycystic ovarian syndrome, according to a similar study conducted on a population from Pakistan (PCOS).The effect of vitamin D status and genotypes for 24 SNPs in four genes involved in the vitamin D pathway (VDR, DBP, CYP27B1, and CYP24A1) on PCOS was investigated in a case-control research. To determine the phenotypic and genotypic risk factors for PCOS and to look for interactions between the genotype and vitamin D status, statistical studies were carried out. Lower age, higher BMI, lower waist-hip ratio, vitamin D deficiency (serum 25-hydroxyvitamin D concentration 10 ng/mL), lack of outdoor exercise, greater fasting glucose, and a history of PCOS in at least one first-degree relative were all independently linked to PCOS. No main effect or interaction between the genotype of any SNP under investigation and the risk of PCOS was found to be statistically significant. In this study, a significant and unwavering correlation between vitamin D insufficiency and the incidence of PCOS, which is unaffected by genetic variation in the vitamin D route (88). However, the VDR Cdx2 polymorphism and the Apa1 polymorphism were linked to decreased insulin resistance and lower testosterone levels, respectively, in a cohort of Austrian women with PCOS (89). Others, however, could not discover any appreciable variations in the frequencies of the VDR gene polymorphisms between women with PCOS and healthy controls (89). In a cohort of Taiwanese Asian PCOS women, Lin et al. (2012) found that the VDR 1a promoter

polymorphisms were not related to the risk of PCOS but were related to serum 25(OH)D levels (90). In addition, Lin and the team's 2015 investigation revealed that participants with the heterozygous 1521CG/1012GA haplotype of the VDR 1a promoter polymorphisms in both PCOS and control women had significantly lower serum 25(OH)D levels (91). However, the effect of metformin therapy on blood 25(OH)D levels was limited to PCOS patients with homozygous 1521G/1012A haplotype (92). Despite the evidence suggesting that VDR gene variations affect PCOS characteristics, it is still challenging to demonstrate a definite relationship between VDR polymorphisms and the onset of PCOS (93).

Intriguingly, it has been demonstrated that low VD levels may exacerbate PCOS symptoms, leading to a reported inverse association between serum VD levels and PCOS-related metabolic and hormonal abnormalities (94) (95) (96). Few research examined the connection between PCOS characteristics and VD deficiencies. According to the Rotterdam criteria, PCOS women were divided into three diagnostic phenotypes for analysis by Davis et al. There are three groups of women: group 1, who have polycystic ovaries and ovulatory dysfunction; group 2, who have both excess androgen and ovulatory dysfunction; and group 3, who have both excess androgen and polycystic ovaries. This study suggests that PCOS subjects with androgen excess may have a greater prevalence of VD insufficiency (97). On VD-deficient (serum contents 20 ng/mL) women with phenotypic B-PCOS in accordance with the Rotterdam criteria, Maktabi et al. conducted a placebo-controlled experiment. VD supplementation significantly reduced fasting plasma glucose, insulin, the HOMA-IR score, and boosted quantitative insulin sensitivity following the 12-week intervention (98).On VD-deficient (serum contents 20 ng/mL) women with phenotypic B-PCOS in accordance with the Rotterdam criteria, Maktabi et al. conducted a placebo-controlled experiment.VD

supplementation significantly reduced fasting plasma glucose, insulin, the HOMA-IR score, and boosted quantitative insulin sensitivity following the 12-week intervention (98) . Figure 05 plots the primary pathways that play a significant part in the indirect and direct activity of VD on female fertility.Therefore, each of these studies point to possible therapeutic benefits of VD supplementation in treating PCOS-related sequelae and hormonal milieu in women who are VD deficient.

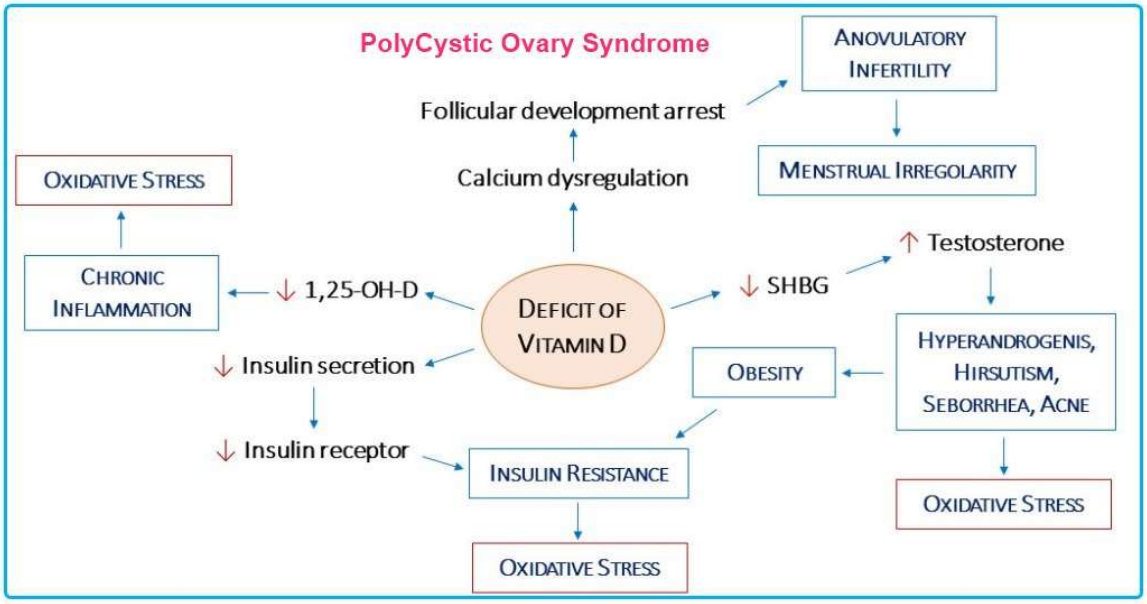

Figure 05:- Relationship between vitamin D deficiency and hormonal changes, insulin resistance, metabolic syndrome, and infertility in PCOS (99)

A thorough search of electronic databases was conducted up to January 2013 for observational studies and clinical trials in women with PCOS with outcome measures related to vitamin D levels. Based on published research, Krul-Poel ., 2013 performed a univariate and multivariate regression analysis of the weighted means to obtain an understanding of the relationship between vitamin D, BMI, and IR. 29 trials that were eligible were found to have

inconsistent findings. There has only been one carefully planned randomised controlled experiment performed so far. Vitamin D was found to be an important and reliable predictor of insulin resistance (IR) in both PCOS and control women by univariate regression analysis of the weighted averages. The importance vanished in PCOS women after BMI was taken into account. According to this research, there is a negative correlation between vitamin D level and PCOS-related metabolic abnormalities. The studie's variability makes it challenging to reach a firm conclusion. Well-designed placebo-controlled randomised clinical trials are still needed to establish the causal link between vitamin D level and metabolic issues in PCOS (83) .

Further, a few studies on the impact of vitamin D supplementation on PCOS patients metabolic condition. The goal of the study by Maktabi et al.,2017 was to determine how vitamin D supplementation affected PCOS patients' metabolic condition. 70 women between the ages of 18 and 40 who met the Rotterdam criteria for phenotypic B-PCOS and were vitamin D deficient (serum concentrations 20 ng/ml) participated in this randomised double-blind, placebo-controlled experiment. Every two weeks for 12 weeks, participants were randomly divided into 2 groups and given either 50 000 IU of vitamin D (n=35) or a placebo (n=35). Biomarkers for oxidative stress, inflammation, and metabolic processes were measured before and after a 12-week intervention. After the 12-week intervention, vitamin D supplementation significantly improved quantitative insulin sensitivity check index (+0) and decreased fasting plasma glucose (FPG) (3.17.3 vs. +0.56.3 mg/dl, p=0.02), insulin (1.43.6 vs. +2.67.0 IU/ml, p=0.004), and homeostasis model of assessment-estimated insulin resistance (0.30.8 vs. +0.61.6, p In addition, supplementing with vitamin D significantly decreased plasma levels of malondialdehyde (MDA) and high-sensitivity C-reactive protein

(hs-CRP) when compared to placebo (p=0.009 and p=0.01, respectively). Overall, glucose homeostasis indices, hs-CRP, and MDA were improved in vitamin D-deficient women with phenotypic B-PCOS who received vitamin D supplements for 12 weeks (98).

Serum 25OHD levels were measured in patients seeking specialised reproductive endocrinology care for PCOS-related symptoms. A validated 9-item Patient Health Questionnaire (PHQ) was used to assess depressive symptoms (score 0-4, Not depressed; 5-9 Mild; 10-14 Moderate; 15-19 Moderately severe and 20-27 as Severe depressive symptoms). The relationship between serum 25OHD (continuous as well as dichotomized at 20ng/ml vs. higher) and depression (PHQ score>4) and with moderate-severe depression (PHQ score>10) was established after controlling for age, overweight body habitus, family history of depression, and symptoms of acne, hirsutism, and oligomenorrhea.For 51 individuals who met PCOS criteria, serum 25OHD and PHQ scores were available. 20/51 (39%) of the respondents matched the PHQ criteria for depression (PHQ>4), and 9/51 (17%) of the respondents fulfilled the criteria for moderate-to-severe depression (PHQ> = 10). Serum 25OHD levels and PHQ ratings showed an inverse relationship (r -0.24, P=0.08). Patients who met the criteria for depression were nearly 4 times more likely to have serum 25OHD levels below 20ng/ml (PHQ>4, OR 3.47, 95% CI 0.78-16.19, P=0.056). According to adjusted analyses, serum 25OHD<20 was a reliable predictor of PHQ score (AR2 0.19, 4.24); women with 25OHD<20ng/ml were 18 times more likely to score >10 and 6 times more likely to score >4 (OR 6.51, 95% CI 1.03-324.91, P=0.023). Hence it could be concluded from this study that in women with PCOS, a lack of vitamin D has been found to increase the incidence of depressive symptoms (100).

Too far, no such combined study has been carried out for the Indian population to support the usefulness of Vitamin D and Vitamin B12 taken together. So, in order to comprehend the crucial role played by both of these essential vitamins in PCOS, we present this study.

Chapter 3

Hypothesis

We henceforth hypothesize that either changes in the level of Vitamin B12 and Vitamin D may alone or synergistically leads to PCOS.

So, the following are the primary objectives of this study:-

I. To study the role of Vitamin B12 and Vitamin D in PCOS Patients.

II. To correlate the clinical relevance of Vitamin B12 and Vitamin D in normal and PCOS subjects.

OBJECTIVE NO I :-To study the role of Vitamin B12 & Vitamin D in PCOS Patients

Introduction:- The multidimensional disorder known as polycystic ovarian syndrome (PCOS) is linked to a number of co-morbid conditions, such as obesity, metabolic syndrome (MS), insulin resistance (IR), aberrant glucose tolerance (AGT), non-alcoholic fatty liver disease (NAFLD), psychiatric disorders, increased cardiovascular disease (CVD), and cancer risk, among others (32) (101) (102) (103) (12) in addition to several reproductive and cosmetic dysfunctions. In the West, the illness is known to affect 5–10% of women who are of reproductive age, but it is more prevalent in India, where early data indicate a prevalence as high as 22.5%. Since the exact cause of the disorder is unknown, two primary pathogenic

mechanisms—hyperandrogenism and IR—are thought to be responsible. Both of these may cause unique clinical phenotypes and ovarian morphological abnormalities on ultrasonography in PCOS patients (104) (105) (106). According to a meta-analysis, IR and compensatory hyperinsulinaemia are present in 30–40% of women with PCOS, and only 10% of these women will go on to acquire type 2 diabetes (107). Additionally, PCOS women were more likely than healthy women to present with dyslipidaemia, which includes lower levels of high-density lipoprotein cholesterol (HDL-C) and higher levels of triglycerides (TGs) and low-density lipoprotein cholesterol (LDL-C)(36).A lack of vitamin B12 (cobalamin), which is essential for health, may have negative haematological, neuropsychiatric, and cardiovascular effects (108). The literature is replete with information about B12 deficiency in metformin-treated patients with type 2 diabetic mellitus (T2DM), however there is little information about PCOS(108) (23). Similar to Vitamin B12 it is well known that vitamin D controls the metabolism of calcium and phosphorus as well as skeletal growth and development. However, vitamin D is also linked to a number of other conditions, such as cancer, immunological disorders (109), T2DM (110), cardiovascular disease (111), infectious infections and cancer (112). The link between vitamin D and metabolic variables in PCOS women as well as the variation in vitamin D levels between PCOS and healthy women have both been the subject of discussion in recent years. According to several research, women with PCOS had lower levels of serum 25(OH)D than healthy women, and patients with PCOS who were vitamin D deficient also had higher levels of hyperinsulinemia, dyslipidemia, and metabolic risk factors (113) (29). However, other researchers have discovered diverse findings (114).Given the above, we conduct this study to examine vitamin B12 and vitamin D levels in PCOS patients who have been diagnosed. Although both of these

vitamin deficiencies are linked to PCOS and metabolic diseases, nothing is known about how prevalent they are among Indian women who have PCOS. Consequently, the purpose of this cross-sectional study is to investigate vitamin B12 and vitamin D levels in PCOS-affected women, as well as the relationship between risk factors for metabolic disease and their deficiencies.

Materials and Methods:- From December 2014 to January 2018, individuals were recruited for this cross-sectional study in northern India specifically New Delhi. The study was carried out in conformity with the principles outlined in the Helsinki Declaration of 1975, and it received the approval of the Ethics Committees of AIIMS (IEC Number). Before each participant was enrolled, they all provided written informed consent (Consent Form Attached).

Subjects:- The study was explained to all consecutive women (18–40 years old) who visited the endocrinology and gynaecology outpatient clinics at the All India Institute of Medical Sciences (AIIMS), New Delhi, complaining of excessive hair growth, irregular menstrual cycles, and other PCOS symptoms. Women who agreed to participate in the study and met the Rotterdam 2003 criteria for the diagnosis of PCOS had to complete an informed consent form. The women were recruited in blocks of age (18-20, 21-25, 26-30, 31-35, 36-40 years) and BMI categories(20, 20-25, >25 kg/m2) in order to exclude the confounding effects of age and body weight on inflammatory markers and other metabolic parameters. A standardised protocol was followed at the centre for the study's instruments, data collection techniques, SOPs, investigator training, lab evaluation, etc.

Clinical Assessment:- All women were questioned at the participating centre about their menstrual cyclicity (age of menarche, duration, and number of cycles per year),

characteristics of hyperandrogenism (duration and extent of unwanted hair growth, acne vulgaris, and androgenic alopecia), body weight, fertility problems, history of drug use, etc.) according to the pre-designed uniform questionnaire (Consent Form along with the Questionnaire). Oligomenorrhea was defined as a cycle interval of >35 days or <8 cycles per year and amenorrhea as cessation of cycles for more than 6 months. Qualified and certified dieticians conducted a thorough diet assessment utilising a questionnaire on food frequency (FFQ) and a 72-hour dietary recall to quantify different dietary elements using specially created diet software (Diet Cal, Profound Tech solutions, New Delhi). Women who refused to participate, took medications known to affect glucose tolerance, insulin sensitivity, or inflammatory markers (glucocorticoids, insulin sensitizers, anti-epileptics, NSAIDs, etc.), were pregnant, or had a history of Cushing's syndrome, non-classical congenital hyperplasia, diabetes, or androgen-secreting tumours at the time of enrollment were also excluded from the study. Any prior history of illness, trauma, surgery, or substantial stress like exams, bereavement, psychiatric disorder, etc. that was known to cause an inflammatory response was also excluded (for at least 2 weeks). Using standard calibrated instruments (SECA 213, Hamburg, Germany), body weight, height, and waist circumference were measured. This was followed by a thorough systemic examination, which included the measurement of blood pressure (Omron HEM7120). The final value for these parameters was determined using the mean of three observations. Acanthosis nigricans, acne vulgaris, and androgenic alopecia were graded by a single observer, as well as the revised Ferriman-Gallwey (mFG) rating (8 or above out of a total of 36 from nine body locations chosen as significant) for quantifying hirsutism.

Laboratory Evaluation:- All of the individuals underwent overnight fasting (9–11 hours) before blood was drawn during the follicular phase (2–7 days) of a natural menstrual cycle. Each subject had five millilitres of blood taken from a peripheral vein for the study. 3.0 ml of blood was collected in plain gel tube (yellow top) and two ml in EDTA tube (Lavender top). Collected Blood was centrifuged in plain and EDTA tubes at 2500 g for 10 min at 4 °C. From the appropriate tubes, serum and plasma were removed, and aliquots were produced for various experiments. The divided samples were kept at -80°C pending further examination. A single sonologist at the location used a 7.5 mHz probe (AlokaSSD-500, Tokyo, Japan) to perform trans-abdominal ultrasonography in the follicular phase to measure ovarian volume, count the number of ovarian follicles, and evaluate thecal hyper echogenicity using a standard operating procedure (SOP).

Controls:- As part of the outreach health awareness-cum-screening programmes run by the institute, women who appeared to be in good health and whose age and BMI matched were selected from community clusters. Similar clinical and laboratory testing was performed on these ladies as it was on the cases.

Assays:- On fully automated biochemical analyzers, biochemical parameters including plasma glucose, lipids, uric acid, calcium, phosphorus, liver function, and kidney function were assessed using standard commercially available kits in accordance with manufacturer's instructions (Hitachi 920, Japan). Using Cobas e411 and an electrochemiluminescence immunoassay (ECLIA), samples for hormonal parameters (serum total T4, TSH, LH, FSH, PRL, cortisol, 17OHP, total testosterone, and insulin) were analysed from both locations (Roche Diagnostics Limited, USA). The coefficients of variation between and within tests were 7%. TNF-, IL-1, IL-6, IL-10, hs-CRP, resistin, and adiponectin serum inflammatory

marker levels were measured by ELISA using commercially available kits and in accordance with the supplier's methodology (Diaclone, France and Calbiotech, CA, USA). The coefficients of variation for the tests within and between samples followed the manufacturer's guidelines. The departmental laboratory at AIIMS New Delhi tested for both hormonal and inflammatory markers.

Sample Size Calculation:- Software called G*Power was used to determine the sample size (version 3.1.9.2). A minimum of 50 patients per group were needed when taking into account type one error (α) as 0.05, study power as 90%, and effect size as 0.3. In order to control the 2:1 ratio of cases to controls, we therefore intended to enrol a minimum of 62 cases and 124 controls in each group to account for quasi and incomplete data.

Statistical Analysis:- The statistical analysis was performed using the Statistical Package for Social Sciences-22 programme (SPSS Inc., Chicago, IL, USA). Data has been presented as mean standard deviation and, when appropriate, log-transformed. The Kolmogorov-Smirnov test was used to evaluate normalcy. And over two groups were compared using one-way ANOVA, and two groups were compared using the Mann-Whitney U-test. Parameters with $p \leq 0.05$ were considered statistically significant.

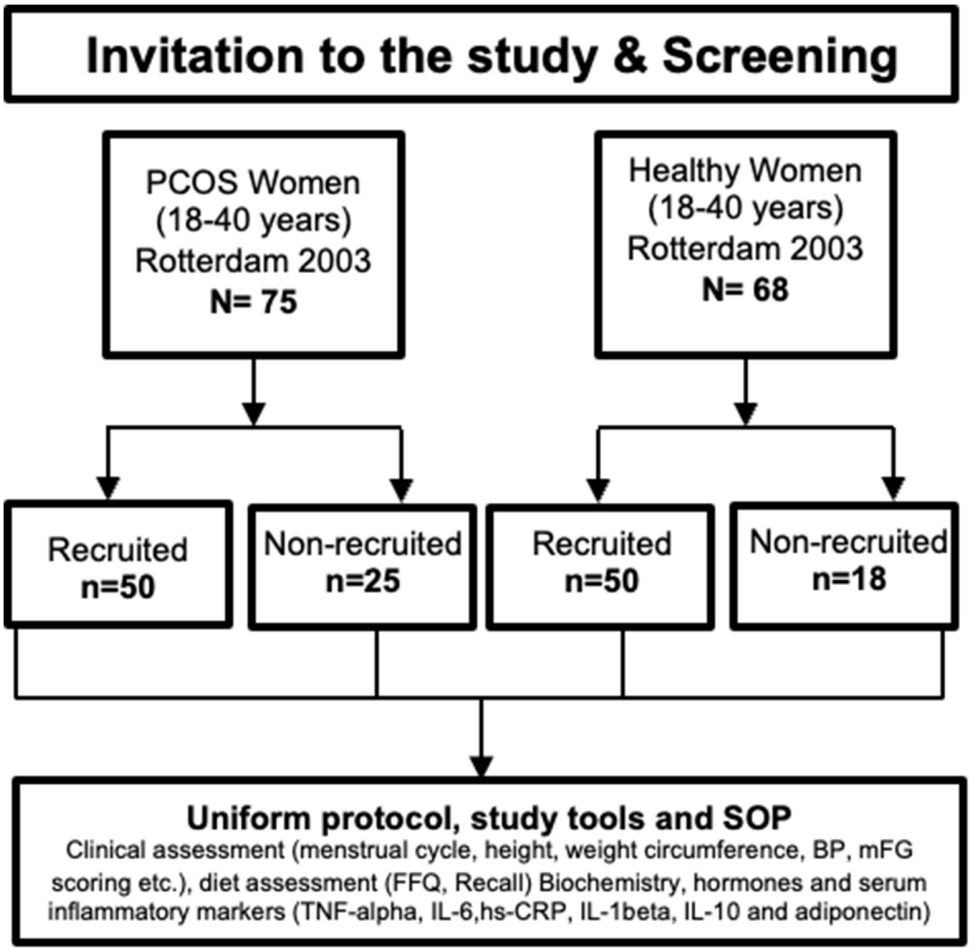

Figure 06:- Program evaluation and review technique (PERT) Chart describing the flow of subjects throughout this study.

Rule of Vitamin B12 an vitD With their Clinical relevance in Normal and Polycystic Ovarian Syndrome (PCOS) .

DATE…… **SUBJECT ID……**

INSTRUTOS: This questionnaire contains ten section and will administered by qualified person to the women consent

Section 1 : Background information

Name	……. ……. ……. Family Middle First
Sex: Hight: Weight:	DOB: ☐☐ ☐☐ ☐☐☐☐ DD/MM/YYYY Age …… Years …… Month …… Days
School/collage / university	……
Departmental Address	……
Class / section	
Residential address	
Contact number (At least one Relative)	Mob: Landline- 1. 1. 2. 2.
Email ID	
Type of Family	1☐ Nuclear 2☐ Joint 3☐ Extended 4☐ Any other (Please Specify)
Total family income (per month) (INR)	1☐ <25,000 2☐ 25,001-50,000 3☐ 50,00-075,000 4☐ 75,000-1,00,000 5☐ >1,00,000
Has consent been taken?	1 ☐ Yes 2☐ No
24 Hour Dietary Recall Status	1 ☐ Day 1 2☐ Day 2 3☐ Day 3
Semi Quantitative food Frequency Questionnaire Status	1☐ Complete 2☐ Incomplete

Interviewee……..Sig……..Date……..

Section 2: Menstrul History and Diagnosis for Polycystic Ovary Syndrome

A	Do you have ANY unwanted (dark, coarse) hair Anywhere on the body in the male pattern?	1 ☐ Yes 2 ☐ No If No, to A.4.	
A.1.	If yes, kindly select the areas and the density they Have?	(Tick on the figure overleaf) Please score with the help of a picture	
A.2.	Without any hair removal technique, please rate on a scale of 0 (no hair growth) to 4 (excessive hair growth),the amount of hair that would grow on your body parts? [Distribution of Hirsutism Score (ferryman Gallwey)]	☐ Upper lip	
		☐ Chin	
		☐ Chest	
		☐ Upper abdomen	
		☐ Lower abdomen	
		☐ Back	
		☐ Sacrum	
		☐ Thighs	
		☐ Arms	
		Total	----------/36
A.3.	Do you employ hair removal techniques (shaving, laser, depilatory creams, electrolysis, tweezing, waxing) to remove hair from any of the body parts?	.....	
A.4.	Do you have any loss of scalp hair?	1 ☐ Yes 2 ☐ No	
A.5.	Have you noticed that you have dandruff (white flakes on your scalp) ?	1 ☐ Yes 2 ☐ No	
B.	At what age did you have your first period ?	☐☐ Years	
B.1.	Menstrual cyclicity-Are your cycles regular ?	1 ☐ Yes 2 ☐ No If No, Go to B.3.	
B.2.	If regular, what is the inter-menstrual interval? (Time from the first day of one period to the first day of the next period)	☐☐ Days	
B.3.	If regular, how many menstrual cycles did you have in the last one-year?	☐☐	
B.4.	How many days is your longest cycle period?(Days Between two consecutive cycle from day 1)	☐☐ Days	
B.5.	How many days is your shortest cycle period? (days between two consecutive cycle from day 1)	☐☐ Days	
B.6.	What is the duration fof your periods (Bleeding) on an average ? AND Amount of bleeding	☐☐ Days ☐☐ Number of pads / Days	
C.	Did you/ do you have acne (PIMPLES) anywhere One the body?	1 ☐ Yes 2 ☐ No	

C.1.	If yes, how severe are/were they?	1 ☐ Mild 2 ☐ Moderate			
C.2.	Have you noticed that you have oily skin ?	1 ☐ Yes 2 ☐ No			
C.3.	Have you noticed dark patches on your skin, skin tags, or tiny excess flaps of skin (neck, armpits, etc.)?	1 ☐ Yes 2 ☐ No			
D.	H/O Hypothyroidism	1 ☐ Yes 2 ☐ No			
E.	Does anyone in your family (1st/2nd degree relative) have any of these ?		**Family History**	Yes	No
		A	PCOS		
		B	Severe acne		
		C	Menstrual disturbances		
		D	Abnormal hair growth(Hirsutism)		
		E	Diabetes mellitus(High blood sugar)		
		F	Hypertension (High blood pressure)		
		G	Infertility(female)		
		H	Gout		
		I	Early coronary artery disease/heart attacks		
		J	Breast cancer		
		K	Uterine cancer		
		L	Obesity, overweight		
		M	Hypothyroidism		
		N	NAFLD		
		O	Any other(please specify)		
F.	Are you taking hormonal therapy for a reason other than birth control ?		**Hormonal therapy for**	yes	No
		A	PCOS		
		B	Acne		
		C	Menstrual pain		
		D	Heavy/ irregular bleeding		
		E	Any other(please specify)		
F.1.	Do you take any medications / (steroids, anti-epileptices, oral contraceptives, Anti-androgens, any other)	1 ☐ Yes 2 ☐ No			
F.2.	If yes,what is the NAME of the medicine /				
G.	Did you experience weight gain ?	1 ☐ Yes 2 ☐ No			
G.1.	If yes, how many kilograms ?	☐☐ KG			

Section 3: Dietary intake pattern

1	Food habits	1 ☐ vegetarian 2 ☐ non-vegetarian 3 ☐ ovo-vegetarian			
2	How many meals do you consume in a day? (multiple tick)	1 ☐ Breakfast 2 ☐ mid-morning 3 ☐ lunch 4 ☐ tea time 5 ☐ dinner 6 ☐ bed time 17 ☐ other (please specify).....			
3	Do you eat or drink anything else in between two in meals?	1 ☐ Yes 2 ☐ No			
4	If yes, how many in between meals do you consume?	1 ☐ 1 meal 2 ☐ 2 meals			
5	What are food items generally consumed in between the main meals?		Food items	Yes	No
		1	Biscuit (sweet/salty)		
		2	Tea /coffiee		
		3	Fried snacks (samosa, bread pakora, French fries)		
		4	Chinese/momos(stemmed/fried/tandoori)		
		5	Dosa/ idle/ bada		
		6	Burger/ sandwich		
		7	Desserts (pastry, sweets)		
		17	other(please specify)		
6	Do you skip main meals?	1 ☐ Yes 2 ☐ No			
7	If yes, which main meal do you skip?		**Main meal**	Yes	No
		1	Breakfast		
		2	Mid-morning		
		3	Lunch		
		4	Tea time		
		5	Dinner		
		6	Bed time		
		17	other(please specify)		
8	What is the main reason for skipping main meals?			Yes	No
		1	Lack of appetite		
		2	Lack of time		
		3	Not appealing		
		4	Trying to lose weight		

		17	Other (specify)		
9	Do you get your tiffin/lunch from home or eat out/from the canteen?	1 ☐ Bring from home 2 ☐ Eat out/ canteen			
10	What do you eat if don't get lunch from home?			YES	NO
		1	Pizza/pasta		
		2	Chole buature		
		3	Fried snacks (samosa, bread pakora, French fries)		
		4	Chinese/momos(stemmed/fried/tandoori)		
		5	Dosa/ idle/ bada		
		6	Burger/ sandwich		
		7	Desserts (pastry, sweets)		
		17	other(please specify)		
11	In usual routine, do you eat out?	1 ☐ Yes 2 ☐ No			
12	If yes, what do you mostly eat?			YES	NO
		1	Pizza/pasta		
		2	Chole buature		
		3	Fried snacks (samosa, bread pakora, French fries)		
		4	Chinese/momos(stemmed/fried/tandoori)		
		5	Dosa/ idle/ bada		
		6	Burger/ sandwich		
		7	Desserts (pastry, sweets)		
		17	other(please specify)		
13	Are you influenced by the discounts offered by various received online (Facebook, group on, mail) or the pamphlets circulated by various eating joints?	1 ☐ Yes 2 ☐ No			

NOTE: Annexure 1 (optional)

Section 4: Clinical Examination

1.	Systolic Blood pressure (mm Hg)		
2.	Diastolic Blood pressure (mm Hg)		
3.	Pulse / minute		
4.	Acne (Grade 1, 2 , 3)		
5.	Androgenic Alopecia (Grade 1, 2 , 3 , 4)		
6.	Acanthosis nigricans		
7.	Chest		
8.	CVS		
9.	Abdomen		
10.	CNS		
11.	Secondary sexual characteristics		
12.	Miscellaneous		

Section 5: Biochemical assessment

1. Routine

1.	Hb	TLC	DLC	
2.	Platelet	PBF		
3.	Bil (mg/dl)	OT	PT	ALP
4.	T. Pr (g/dl)	Alb	Urea (mg /dl)	Cr
5.	Na (mmol/l)	K	Ca (mg /dl)	P
6.	TG (mg /dl)	CHOL	HDL	LDL
7.	GTT (mg/dl)	BGF	1 hr	2 hr

2. Hormones

1	T4	TSH	LH(IU/ML)	FSH (IU/ML)	Prolactin
2	T TESTO (µg/ml)	DHEAS(µg/ml)	17-OHP(µg/ml)	Cortisol M	Cortisol E
3	ODST (µg/ml)				
4	Insulin(µg/ml)	0hr	1 hr	2 hr	
5	TNF-Alpha				
6	IL-6				
7	Body Composition				

Section 6: Perception about self, health status and lifestyle

1	What do perceive your health as:			Yes	No	
		1	Excellent			
		2	GOOD			
		3	Fair			
		4	Poor			
2	What do you feel about your present weight?			Yes	No	
		1	Would like to lose			
		2	Would like to gain			
		3	Satisfied			
3	What do you perceive your lifestyle as?			Yes	No	
		1	Active			
		2	Moderately active			
		3	Sedentary			
4	Do you suffer from any of the diseases?			Yes	No	
		1	Tuberculosis			
		2	Diabetes			
		3	High blood pressure			
		4	Thyroid			
		5	Bone or joint related problem			
		17	Other (please specify)			
5	Do you get any leisure time?	1 ☐ Yes		2 ☐ No		
6	If yes how do you spend your leisure time?			Yes	No	
		1	Rest			
		2	Watch T.V.			

		3	Sit/chat with family member/ Friends			
		4	Listen to music			
		5	Talk on phone			
		6	Work on computer/laptop			
		17	Other (please specify)			
7.	Do you feel the need to work out?	1 ☐ Yes		2 ☐ No		
8.	If yes, do you plan your daily schedule so that you can accommodate a session for workout?	1 ☐ Yes		2 ☐ No		
9.	Do you undertake any physical activity?	1 ☐ Yes		2 ☐ No		
10.	If yes, which of the following activities so you undertake?			Yes	No	
		1	Walking			
		2	Jogging			
		3	Yoga			
		4	Aerobic exercises, Pilates, power yoga			
		5	Sports (please specify)			
		6	Gym			
		7	Swimming			
		8	Other (please specify)			
		17	Other (please specify)			
11.	What is the number of hours you spend in school/collage?					
12.	What is the distance of your school/collage from home?					
13.	On which floor is your class located?	0		yes	No	
		1	Ground floor			
		2	First floor			
		3	Second floor			
		17	Other (please specify)			
14.	Do you climb stairs or use lift to go to your class?					
15.	On which floor is your house located?			yes	No	
		1	Ground floor			
		2	First floor			
		3	Second floor			
		17	Other (please specify)			

16.	Do you climb stairs or use lift to go to your home?		
17.	What is the distance of the most accessed market form home?		
18.	What is the usual mode of travelling use by you		
19.	Do you carry weight and walk?		
20.	Do you have a television in your bedroom?	1 ☐ Yes 2 ☐ No	
21.	How many hours on an average (per day) do you spend sitting in front of a screen (laptop/television)?	Weekday-.....hours Weekend .....hours	
22.	How many hours do you sleep at night?		
23.	Do you smoke?	1 ☐ Yes 2 ☐ No	
24.	If yes, please specify the number of cigarettes and the frequency with which you smoke?		

		Yes	No
1	On food		
2	Bus		
3	Metro		
4	Auto		
5	Car		
17	Other (please specify)		

Weight	never	Daily	Weekly	Occasionally
<1 kg				
1-2 kg				
3-4 kg				
>5 kg				

Annexure 1 : 24 hour dietary recall (2 weekdays + weekend)

S. NO	Type	Monthly (lit)	Number of family members Who are sharing the meal	Per person Consumption / Month
1	Sunflower oil			
2	Soybean oil			
3	Mustard oil			
4	Coconut oil			
5	Groundnut oil			
6	Olive oil			
7	Corn oil			
8	Rice bran oil			
9	Rapeseed oil (canola oil)			
10	Blend of oils			
11	Hydrogenated oil			
12	Palm oil			
13	Butter			
14	Ghee			
15	Cream			
16	Dalda/vanaspati			
17	Other (please specify)			

Day 1 (Weekday)

Time	Menu	Quantity (household Measures)	Ingredients	Amount of ingredients(g)	
				Household measures	Grams

Day 2 (Weekday)

Time	Menu	Quantity (household Measures)	Ingredients	Amount of Ingredients (g)	
				Household measures	Grams

Day 1 (Weekend)

Time	Menu	Quantity (household Measures)	Ingredients	Amount of Ingredients (g)	
				Household measures	Grams

OBJECTIVE NO II:- To correlate the clinical relevance of Vitamin B_{12} and Vitamin D in normal and PCOS subjects.

A total of **N=75** women who met the criteria for a PCOS diagnosis in Rotterdam 2003 were screened; of these, **n=50** women were recruited and **n=25** women were not recruited due to a lack of permission. Another **N=68** women of similar age and BMI who appeared healthy were examined and invited to take part as controls; 50 of them signed up, while the remaining 18 were unable to do so due to a lack of consent. Figure 06.

Tables 1-2 demonstrate group-wise assessments of their inflammatory, hormonal, biochemical, and clinical marker profiles.

Comparison between Women with PCOS and Healthy Controls

The overall respective mean age of PCOS subjects (n = 50) and controls (n = 50) was 26.06 ± 4.13 vs. 26.55 ± 5.02 years while as their mean BMI was 24.81 ± 3.55 vs. 23.97 ± 3.92 kg/m2. The mean number of menstrual cycles per year (8.10 ± 2.83 vs. 11.86 ± 2.88) was significantly lower while as mFG scores (11.57 ± 4.36 vs. 5.88 ± 1.77), serum LH (7.58 ± 3.57 vs. 6.42 ± 2.37 IU/ml) and serum total testosterone (0.37 ± 0.27 vs. 0.17 ± 0.13 ng/ml) levels were significantly higher among women with PCOS as compared to healthy women from the center. Fasting plasma insulin (12.57 ± 7.27 vs. 8.59 ± 6.26 mIU/ml was higher among PCOS women than healthy controls. Pro-inflammatory markers (TNF-α, IL-6, IL-1β and hs-CRP) were significantly higher ($p \leq 0.05$) and anti-inflammatory markers (IL-10 and adiponectin) significantly lower among women with PCOS than their healthy counterparts. Other parameters like waist circumference, blood pressure, uric acid and serum phosphorous did not differ significantly among the groups. The Follicle Stimulating hormone (FSH), Prolactin levels were significantly lower in subjects with PCOS as compared to the higher levels of Testosterone, Cortisol and C-reactive proteins in them. The Vit B_{12} level was however found to be comparatively low in PCOS subjects than Vit D level.

Comparison of Diets between Women with PCOS and Control Cases

The overall respective mean age of PCOS subjects (n = 50) and controls (n = 50) was 26.06 ± 4.13 vs. 26.55 ± 5.02 years while as their mean BMI was 24.81 ± 3.55 vs. 23.97 ± 3.92 kg/m2 as compared to the differences in most of the clinical parameters (mean number of menstrual cycles per year, mFG score, BP), biochemical (mean plasma glucose, HOMA-IR, urea, uric acid When compared to vegetarian PCOS women, non-vegetarian PCOS women had higher levels of biochemical markers like blood creatinine, serum triglycerides, and LDL cholesterol (p 0.05). It's interesting to note that PCOS women had greater serum total testosterone levels. According to the diet intake enlisted below it was observed that the consumption of fatty acids (saturated, mono-unsaturated, poly-unsaturated) were more in the cases as compared to the controls Table 1 and 3.

Parameters	Women with PCOS (n=50) Mean ± SD	Healthy women (n=50) Mean ± SD	p-value
Age (years)	26.06±4.12	26.55±5.05	0.10
No. of menstrual cycles/year	8.10±2.83	11.86±2.88	<0.01
Ferriman–Gallwey score (mFG)	11.57±4.37	5.88±1.77	<0.01
BMI (Kg/m^2)	24.81±3.53	23.97±3.90	0.11
Serum LH (IU/ml)	7.58±3.57	6.42±2.37	<0.01
Serum FSH (IU/ml)	6.19±2.07	7.07±2.19	<0.01
Serum total testosterone (ng/ml)	0.52±0.27	0.27±0.13	<0.01
Serum 25OHD (ng/ml)	11.45±8.19	15.78±8.02	0.05
Blood glucose- fasting (mg/dl)	87.14±10.61	84.96±9.36	0.12
Fasting plasma insulin-(mIU/ml)	12.57±7.27	8.59±6.26	<0.01
HOMA-IR	2.67±1.58	1.73±1.25	<0.01
QUICKI	0.35±0.06	0.37±0.04	0.02
FGIR	9.05±4.76	15.21±10.57	<0.01
Serum TNF-α (pg/ml)	40.97±31.40	23.65±18.60	<0.01
Serum IL-6 (pg/ml)	30.62±12.09	6.65±5.35	<0.01
Serum IL-1β (pg/ml)	10.96±6.60	8.10±5.30	<0.01
Serum hs-CRP (ng/ml)	4.21±1.56	3.96±1.52	<0.01
Serum resistin (ng/ml)	8.88±4.36	5.88±3.46	<0.01
Serum adiponectin (ng/ml)	4.52±2.85	6.95±4.88	<0.01
Serum IL-10(pg/ml)	6.57±2.53	9.92±5.66	<0.01

Values are presented as mean ± standard deviation. P values were calculated using independent sample t-test. A p value of ≤ 0.05 was considered as significant.

Table 1:- Comparison between healthy controls and PCOS patients based on menstrual cycle, age, and several biochemical characteristics

Diet Parameters		
	Control Diet (mean ± SD)	**Cases diet ((mean ± SD)**
BMI	23.28863875	27.3022546
Protein (PROTCNT) [gms]	37.34137947	38.0131009
Total Fat (FATCE) [gms]	26.99811653	28.74365
Total Dietary Fibre (FIBTG) [gms]	26.68477946	27.2219803
Carbohydrate (CHOAVLDF) [gms]	200.4516924	202.574551
Energy in Kcal (ENERC) [Kcal]	1218.283416	1244.5332
Thiamine B1 (THIA) [mgs]	0.903449459	0.91708528
Riboflavin B2 (RIBF) [mgs]	0.528705407	0.5530432
Total Ascorbic Acid (VIT C) [mgs]	55.27985448	56.7291296
Calcium (CA) [mgs]	360.7349257	402.152664
Iron (FE) [mgs]	10.06881812	10.3124972
Magnesium (MG) [mgs]	292.1445709	305.194402
Phosphorous (P) [mgs]	824.8458485	837.591818
Potassium (K) [mgs]	1665.778376	1711.83948
Zinc (ZN) [mgs]	6.707678453	6.70976034
Total Available Carbohydrate [gms]	165.3380035	165.058382
Total Saturated Fatty Acids TSFA (FASAT) [mgs]	8552.452948	9347.60818
Total Mono-unsaturated Fatty Acids TMUFA (FSMS) [mgs]	6146.244275	6664.34639
Total Polyunsaturated Fatty Acids TPUFA (FAPU) [mgs]	6350.351422	6472.11648

Table 2:- Comparison of the nutrient intake amongst the Control and PCOS Cases

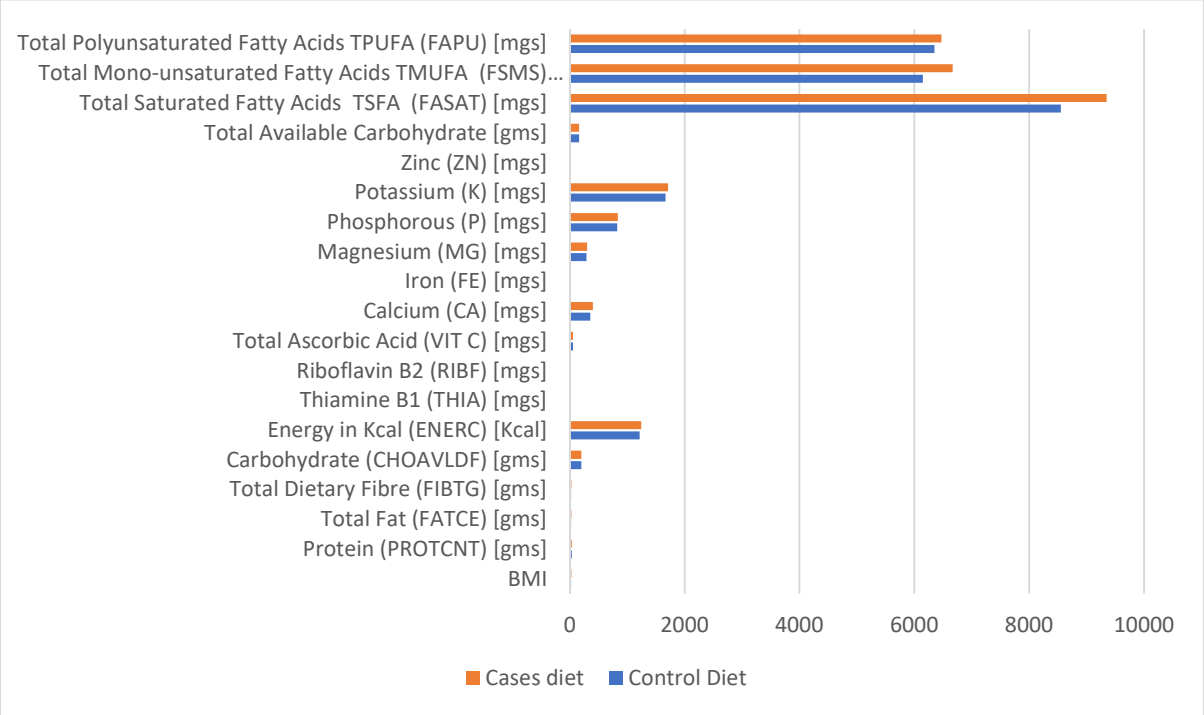

Figure 7:- Showing comparison of nutrient levels among women with PCOS and healthy controls

Between the two groups of women, the majority of the clinical, biochemical, and hormonal characteristics were equivalent. There were a few exceptions, including greater levels of mFG score, serum creatinine, TG, LDL cholesterol, and total testosterone in subgroups of women with PCOS. Mean fasting plasma glucose and insulin levels were greater in healthy women. In comparison to the group of healthy women, pro-inflammatory markers were higher and anti-inflammatory markers were lower in PCOS subgroups. However, once more, only in the cases of serum hs-CRP and IL-6 did this reach statistical significance. In a comparison of the macronutrient consumption of the two groups, women with PCOS reported consuming more calories per day 1895.40 ± 208.11 vs. 1857.47 ± 385.59 Kcal, $p \leq 0.05$) with higher daily fat (46.10 ± 9.46 vs. 42.05 ± 16.93 g) and protein intake (54.19 ± 11.03 vs. 52.02 ± 16.62 g) and a lower carbohydrate intake (304.93 ± 44.16 vs. 313.83 ± 72.87 g; $p \leq 0.05$) than their control counterparts.

Chapter 4

Results and Discussion

We compared the inflammatory biomarker profiles of Indian women with PCOS in the current study after the PCOS patients were followed up after consuming vitamin B12 and vitamin D dosages for 3 and 6 months, respectively along with their diet patterns. The major results demonstrated that PCOS-affected women had higher levels of pro-inflammatory markers than healthy controls in the various groups who were age-and BMI-matched (TNF-, IL-6, IL-1, resistin, and hs-CRP).

The lack of information on certain micronutrients, such as vitamin B12, vitamin D, and omega-3 fatty acids, the measurement of visceral fat mass, and the consideration of lifestyle differences between the two populations, such as pollution and stress levels, among others, mean that the study's findings can only be used to draw limited inferences. The community is habitually divergent, so while it would have been helpful to enrol both vegetarian and non-vegetarian women from the centre, this was not practical. However, this is the first study with a respectable sample size, matched control groups for age and BMI, and reporting the effect of diet on inflammatory markers among women with PCOS. Low-grade chronic inflammation also referred to as inflammaging is incriminated in the aetiopathogenesis of many chronic illnesses, notably metabolic syndrome (115), obesity (116) T2DM (117), CAD (118), neurodegenerative diseases (119), reproductive dysfunctions including PCOS (120). Data on diet-induced inflammation among Asians as a whole, and Indian women with PCOS in particular, are scarce. As a result, we conducted this study to assess how food affected cytokines in North Indian women with PCOS.

According to previously published data, PCOS women had fewer menstrual cycles annually, more severe hirsutism, and higher serum total testosterone, fasting plasma glucose, and insulin

levels, as compared to healthy controls(102,121) (122). Women with PCOS had lower serum 25OHD concentrations while having greater serum alkaline phosphatase levels. This may be due to their higher body fat percentage, particularly their earlier reported visceral adiposity (123) (124). Except for serum LDL cholesterol, triglycerides, and creatinine, we found that the majority of the clinical, biochemical, and hormonal indicators in the PCOS women from the centre were higher. This is explained by consuming more protein and fat on a daily basis. It is difficult to explain the surprising finding that healthy and PCOS-positive women had greater mFG scores and serum total testosterone than women of similar age and BMI. This result, however, is in line with our earlier results and is explained by ethnic differences. Although the prior findings assessing the impact of androgens on inflammation is conflicting and requires additional investigation, the finding of greater androgen levels among women with PCOS may be one of the reasons of superior inflammatory marker profile.

The observation of a stronger pro-inflammatory state in women with PCOS compared to healthy controls is similar to other research revealing higher hs-CRP levels in women with PCOS compared to healthy controls (120) (125). Although Escobar-Morreale et al systematic's review and meta-analysis found no differences in the serum levels of IL-6 and TNF- between women with PCOS and controls, this meta-analysis had some drawbacks (126). They included 10 studies with a decent number of individuals in the present analysis, a generally homogenous population, and a limited number of PCOS subjects (n = 523) and controls (n = 330) with backgrounds from various ethnicities.

It's interesting to note that women in the PCOS subgroups had lower levels of the anti-inflammatory protein adiponectin and considerably higher levels of serum hs-CRP and resistin. These results appear to be at odds with the majority of earlier studies demonstrating lower inflammatory marker levels among non-PCOS patients, despite the fact that there are no data examining the effects of various diets among women with PCOS internationally (127) (128)

(126). In a multi-ethnic study, Nettleton et al. found a negative relationship between serum levels of hs-CRP and IL-6 with a diet rich in whole grains, fruits, and green leafy vegetables(127). A conventional Mediterranean diet is quite helpful in reducing inflammation, according to randomised trials. Koloverou et al. found that rigorous adherence to the Mediterranean diet lowers the risk of acquiring type 2 DM by 23% in a recent meta-analysis of 136,846 participants (129) (130). To the best of our knowledge, this is the first study examining the effect of food and dietary supplements for Vitamin B12 and Vitamin D on inflammatory markers in PCOS and healthy women, even if a small number of reports among non-PCOS individuals support our observations. As our ladies were age and BMI matched, it is unlikely that this increased inflammatory response among women (both healthy and PCOS) following a high-calorie diet is attributable to a higher fat mass or higher age. Different dietary compositions (micronutrient or macronutrient) or diet preparation techniques may be to blame for this.

Indian vegetarian diets tend to be high in carbohydrates and poor in omega-3 fatty acids, which may also explain why pro-inflammatory markers are elevated more than in Mediterranean diets(131). A higher total fat intake was linked to a lower risk of total mortality, cardiovascular illnesses, and stroke, according to a recent cohort study by Dehghan et al. that involved people from varied ethnic backgrounds and was done across eighteen nations(132). In accordance with this study, we found that participants recorded a larger percentage of energy intake from carbohydrates and a lower percentage from fats when compared to non-vegetarian subjects, which may help to explain why they were in a pro-inflammatory state.

Despite having lower BMIs, Asian Indians have an increased prevalence of metabolic syndrome, obesity, T2DM, CAD, etc. (105) (133) (134) . Since the specific cause of these illnesses is uncertain, food has been blamed as one of the major contributors to their increased prevalence. There is a dearth of information on diet-induced inflammation in Asians in general

particularly in Indian women with PCOS . Our findings go against previous research and typical advice offered to patients, even while they are not immediately applicable to clinical practise and might not be able to generalize until well-designed, longer-term studies are conducted to replicate the findings.

Ovarian follicle growth and ovulation rates are mostly regulated by signalling pathways connected to nutrition. Vitamin B12 and Vitamin D supplements definitely help PCOS symptoms by promoting the production of healthy oocytes. Additionally improved are hyperinsulinemia, hyperandrogenism, elevated body mass index, and cardiovascular problems. Additionally, the psychological problems linked to PCOS have improved. The link between vitamin B_{12}, vitamin D, and PCOS patients with in Indian community has never been studied before. The unique result of the current investigation was the correlation between PCOS and decreased serum vitamin B12 and vitamin D concentrations. In PCOS women, higher Hcy concentrations were linked to decreased serum vitamin D and B12 levels. Patients with PCOS had considerably reduced serum levels of vitamin B12. Consumption of protein is also correlated with plasma vitamin B_{12} concentrations. Being a steroid hormone, vitamin D has the power to prevent a variety of illnesses, including cancer, autoimmune disorders, hypertension, diabetes, and obesity. It also plays a crucial role in calcium metabolism and bone construction. Vitamin D status is linked to PCOS patients' ability to reproduce, metabolic changes, and mental health, according to recent reports. Vitamin D deficiency is also a typical PCOS consequence. A study by Yang et al. showed that vitamin D supplementation might lower serum levels of androgen and anti-Müllerian hormone (AMH), as well as endometrial thickness, which improved PCOS patients' menstrual cycles and folliculogenesis (135). IR and lipid metabolism benefit from vitamin D supplementation. Additionally, it has been suggested that vitamin D benefits PCOS sufferers' mental health. To effectively treat and stop the

advancement of PCOS, it is crucial to comprehend how vitamin D status and PCOS patient symptoms are related.

In conclusion, this study found that women with PCOS had greater levels of inflammatory markers than healthy controls, and that Vitamin B12 and vitamin D supplements had a positive effect on this profile.

Tests	Control	Cases	3 Month	6 Month
LH	5.1885938	7.88763158	7.55654545	7.53135135
FSH	7.4406875	6.10608696	6.01928571	5.92864865
Testo	0.171	0.37908772	0.40976786	0.39997297
Prolactin	19.210313	0.37908772	17.2709091	16.3078378
T4	8.4296875	8.6733913	8.54267857	8.42
TSH	2.4459375	3.01478261	2.72285714	3.23972973
Cortisol (M)	11.899063	13.0036111	12.4828571	12.44
VIT-D	14.78125	14.1786111	21.0785714	17.8540541
DHEAS	260.09375	250.334907	270.716964	259.558649
BG	85.40625	86.4649123	85.0714286	84.2432432
INS	12.78125	15.4861468	12.97	11.8775676
BIL	0.625	0.47636364	0.44464286	0.49459459
SGOT	27	22.8859649	23.2678571	21.2702703
SGPT	28.78125	23.2807018	20.2321429	21.5675676
ALP	211.84375	206.460177	193.196429	191.081081
Total Protein	7.2875	7.9122807	7.30714286	7.39459459
Albumin	4.190625	4.69447368	4.75535714	4.82972973
Urea	25.40625	20.7719298	20.6071429	20.8918919
Cretinine	0.7375	0.63596491	0.64285714	0.64054054
Cholesterol	177.8125	160.885965	165.071429	162.162162
Total Glucose	110.625	112.132743	105.208929	109.297297
HDL	40.28125	42.745614	43.5178571	41.8918919
LDL	85.75	92.754386	94.9107143	92.9166667
U ACID	4.65625	4.67168142	4.61071429	4.62432432
Cal	9	9.09823009	9.13214286	9.15135135
Hemoglobin	11.5625	12.6636364	12.7946429	12.4621622
Platelet Count	208.71875	276.663636	271.767857	257.891892
Total Leukocyte Count	7.838125	7.85227273	7.80892857	7.55675676

IL-6 (pg/ml)	5.490625	45.5924146	45.3432828	43.394587
hs-CRP (mg/L)	3.2728125	4.69108911	4.285	4.42105263
TNF-α (pg/ml)	41.632188	77.7822772	75.4135714	78.8785212
MCP-1 (pg/ml)	126.74188	145.621372	122.360661	151.336371
Homocysteine (umol/L)	42.399375	41.1414851	41.4096429	35.8786486
B12 (pg/mL)	101.71875	64.4071287	55.9973214	69.6772973
MMA (pg/ml)	150.54344	332.89396	288.649821	359.518649

Table 3:- Demonstrating the differences in biochemical markers between PCOS patients and healthy women after intake of Vitamin B12 and vitamin D supplements

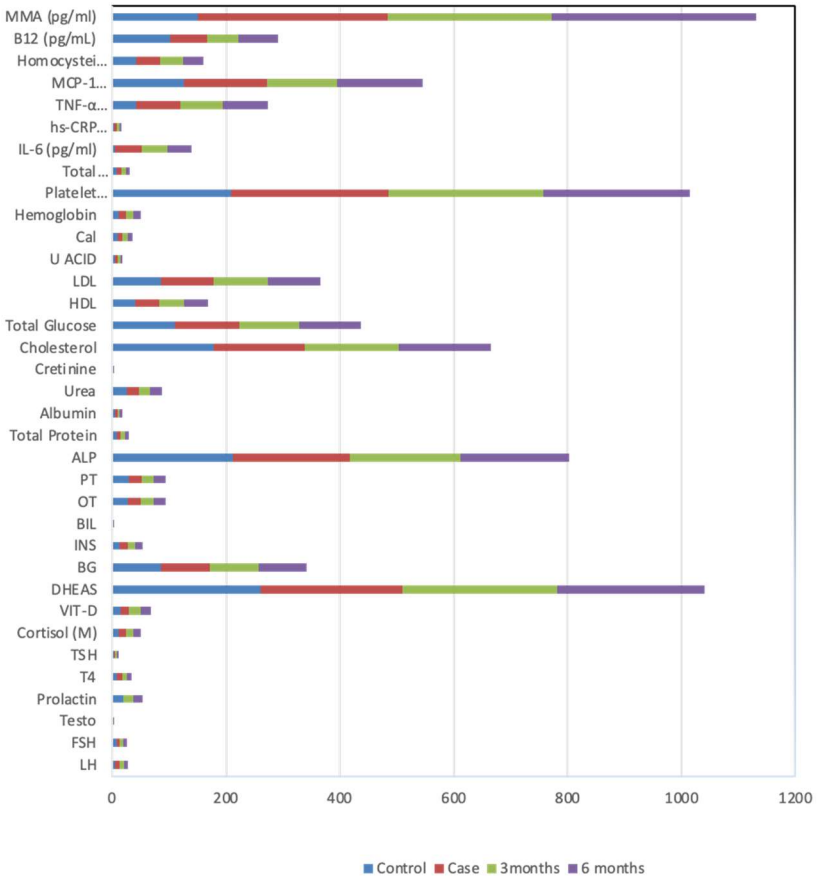

Figure 8:- A bar-diagram representation of the biochemical markers of PCOS patients and healthy women after taking vitamin B12 and vitamin D supplementation is shown.

As such, PCOS pathogenesis is complicated and currently poorly understood. The most significant processes include impaired ovarian steroidogenesis, insulin resistance (IR),

neuroendocrine abnormalities, and enhanced cortisol metabolism-related adrenal hyperandrogenism. Androgens and insulin are two important elements in the pathophysiology of PCOS, even if the exact reason is currently unknown. As a result, treating PCOS entails reducing hyperandrogenism and hyperinsulinemia. In order to keep insulin and androgen receptors functioning properly, nutrients work as cofactors. Myoinositol and vitamin D deficiency can result in issues linked to PCOS aetiology. As a result, nutritional supplements may help those with PCOS who have problems with immature oocytes, IR, hyperandrogenism, and oxidative stress.

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