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Vitamin D, Hormonal profiles and Homa-IR in Polycystic Ovary Syndrome (PCOS) Women who are candidates for In Vitro Fertilization at a Tertiary Hospital in Western Kenya

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a condition that presents with high androgens levels, disturbances in menstrual cycles and polycystic ovaries in shape. Studies report of 30% to 80 % of PCOS gets insulin resistance, hyperinsulinemia and type II diabetes mellitus. It is one of the most common cause of infertility and thus lowering their quality of life. Research is emerging that 70 % of PCOS patients do have Vitamin D deficiency, whether there is a connection between Vitamin D and insulin resistance in PCOS, studies have remained inconsistent and inconclusive. To compare the insulin resistance, hormonal profiles and Vitamin D levels in PCOS women who are candidates for IVF at a tertiary hospital. A case control study carried out between June 2023 and April 2024 at Mediheal Fertility Centre and MTRH in Eldoret. A sample of 60 women. Group 1 of 20 participants with infertile PCOS diagnosed according to Rotterdam criteria and group II of 20 women who are fertile without PCOS and group III of 20 infertile non PCOS who are also candidates for IVF served as controls. Luteinizing hormone, Testosterone, Ant Mullerian hormone, Insulin, fasting blood sugar and Vitamin D levels were carried out using automated Chemiluminescence (ECLIA) methods at MTRH. Data was analyzed using STATA version 15 and Wilcoxon rank-sum test. P values set at <0.05.AMH, Testosterone, LH and Insulin hormone levels were significantly higher as compared to fertile non PCOS and infertile non PCOS p<0.05. However, Vitamin D was significantly lower in infertile PCOS as compared to fertile non PCOS and infertile non PCOS p< 0.05. Vitamin D correlated negatively with LH and Testosterone but positively with AMH and Insulin suggesting that higher levels of LH and Testosterone are linked to lower levels of Vitamin D unlike AMH and Insulin though the correlation was not statistically significant p> 0.05. HOMA-IR, HbA1C and fasting blood sugar were significantly higher in infertile PCOS as compared to fertile non PCOS and infertile non PCOS p< 0.05. Vitamin D correlated negatively with HbA1C but positively with HOMA-IR though not statistically significant p> 0.05. Further research may be warranted to explore additional factors influencing IR and randomized clinical trials to test impact of Vitamin D on metabolic dysregualtions. Healthcare professionals should ensure adequate Vitamin D levels in patients to potentially mitigate insulin resistance.

***Keyword:** PCOS, VITAMIN D, HOMA-IR and HORMONAL PROFILES*

INTRODUCTION

Polycystic ovarian syndrome (PCOS), is considered a common endocrine disease, that affects most women of reproductive age (Farquhar, 2007). The prevalence of Polycystic Ovary

Syndrome (PCOS) worldwide varies from 8% to 21% based on the diagnostic criteria (Neven, A et al., 2018). Bozdag et al., (2016), did a thorough valuation of 24 research describing the prevalence of PCOS. They demonstrated that one of the most prevalent endocrine pathological diseases is PCOS. There have been reports of PCOS in numerous African nations. According to Ugwu GO et al. (2013), PCOS is predicted to impact one in every six infertile Nigerian women. Pembe AB et al. (2009) found that 32% of Tanzanian infertile women had PCOS. According to Odera et al., (2020) at least 131 women who attended the GOPC at Kenyatta National Hospital in 2018, 49 (37%) received the Rotterdam diagnosis, 26 (20%) received the NIH 1990 diagnosis, and 15 (12%) received the AE-PCOS 2006 diagnosis.

PCOS individuals exhibit hyperinsulinemia, which is unrelated to obesity, according to 1987 research by Dunaif et al., (1990) and Legro R. (2011) as well as his friends. According to a 1999 study, 31.5% of the 254 PCOS-affected women who developed type 2 diabetes (DM) had decreased glucose tolerance. IR significantly influences the reproductive difficulties that PCOS-affected women experience (Mishra., 2016). Dunaif and associates (Dunaif et al, 1990) discovered a causal link between high testosterone levels and IR in these individuals. Women with PCOS had Vitamin D levels that are 20 ng/ml lower than those of healthy controls, according to recent studies (Hahn S et al 2006). It has a negative effect on certain people, but it's also linked to IR (Werh E et al 2011). The relationship of Vitamin D and predictors of insulin resistance in women with PCOS is still ambiguous and inconclusive even though correlation studies have demonstrated negative correlation between Vitamin D and BMI and IR (Hans S et al 2006).On the basis that insulin secretion from the beta islets cells of the pancreas is calcium dependent thus Vitamin D studies are justified in different geographical and ethnic background to mirror PCOS manifestation and pathophysiology (Cywes, R. et al., 2023).

MATERIALS AND METHODS

This case-control study was conducted at Mediheal Fertility Center's in the Reproductive, Endocrine and infertility clinic between July 2023 and February 2024. PCOS was diagnosed based on the Rotterdam criteria 2003, classifying participants into four phenotypes; A (full-blown syndrome PCOS: hyperandrogenism; HA + ovulatory dysfunction {OD} + PCO), Phenotype B (HA+OD), Phenotype C (ovulatory PCOS: HA+PCO), and Phenotype D (non-hyperandrogenic PCOS: OD+PCO). Diagnosis was confirmed if two out of three criteria were met.

Sample size was determined using G* Power program 3.1.9.2 software, employing an exact statistical test correlation under a bivariate normal model. To achieve statistical significance, a sample size of 20 participants per group was calculated, later adjusted to 22 per group to account for a 10% drop out rate due to potential pre-analytic errors. Transvaginal ultrasound was performed on PCOS patients to assess ovarian morphology, with criteria including >20 follicles per ovary or ovarian volume >10ml. Serum analysis for vitamin D, LH, AMH, Testosterone hormone and fasting insulin hormone levels were performed using an automated Electro chemilumescent immuno assay method (Roche Diagnostics; Basel, Switzerland e Cobas 311 series) at Moi Teaching and Referral Hospital.

The study was approved by joint MTRH/Moi university IREC (0004610) and permission granted by the National Commission of Science and Technology (NACOSTI/23/26592). All participants provided written informed consent and the study adhered to the principles of Helsinki Declaration. Exclusion criteria included diabetes, hyperprolactinemia, hypothyroidism, androgen-secreting tumors, as well as Cushing syndrome. Data analysis was conducted using STATA version 15. Normality was assessed using the Shapiro-Wilks test. The Wilcoxon rank-sum test was used for comparisons of hormonal values among the groups. Correlation was applied using Pearson moment product of correlation with Vitamin D as dependent factor while other hormone parameters and HOMA-IR as independent variable. Significance was set at $P< 0.05$ and a 95% confidence interval

RESULTS

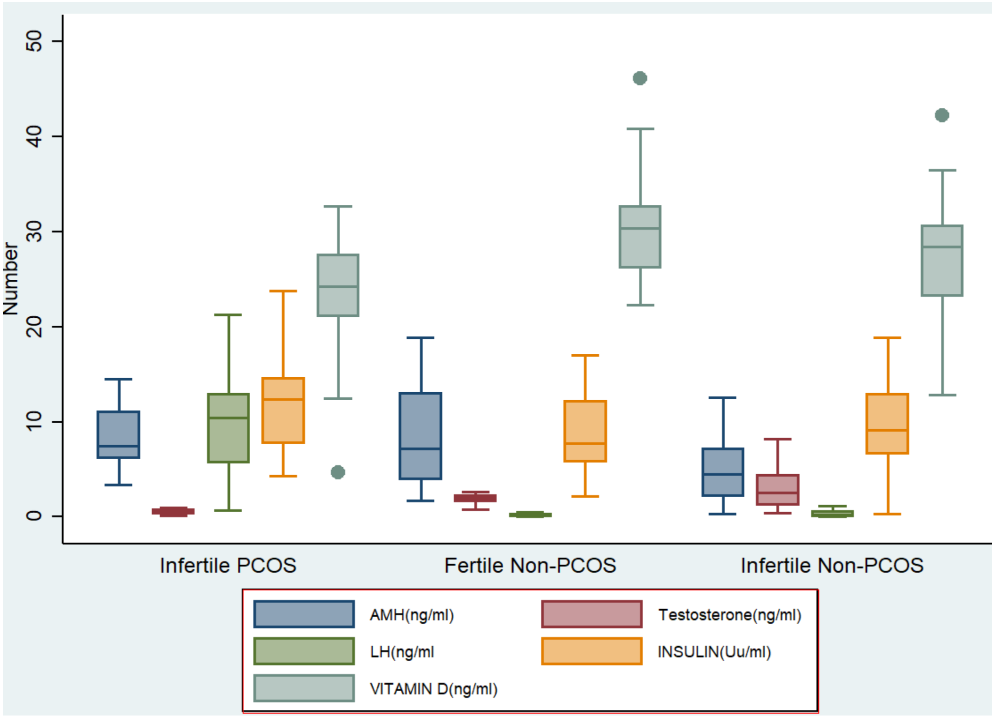

Figure 1: Comparisons of AMH, LH, Testosterone, Insulin and Vitamin D hormone levels among Infertile PCOS, Fertile non PCOS and Infertile non PCOS

The box plot above and the table below shows comparisons of Infertile PCOS group having a higher AMH, Testosterone, LH and insulin values 7.5(6.2, 11.1), 2.56(1.21,4.39), 10.46(5.71, 12.98) and 12.37(7.74, 14.59) compared to that of Fertile Non-PCOS 7.2(4.0, 13.0), 2.0(1.67,2.25), 0.16(0.05, 0.29) and 7.72(5.81, 12.20) and Infertile Non-PCOS 4.5(2.2,7.2), 0.49(0.31,0.77), 0.21(0.08, 0.64) and 9.14(6.64, 12.97). However, Infertile PCOS group had lower vitamin D values 24.3(21.1, 27.6) compared to that of Fertile Non-PCOS 30.3(26.2,32.7) and Infertile Non-PCOS 28.4(23.2, 30.7). The difference in AMH, Testosterone, LH, vitamin D and Insulin hormone levels was statistically significant (all $p<0.05$).

Table 1: Comparisons of AMH, LH, Testosterone, Insulin and Vitamin D among Infertile PCOS, Fertile non PCOS and Infertile non PCOS

Measure	Group			Statistic	p-value
	Infertile PCOS	Fertile NON-PCOS	Infertile NON-PCOS		
AMH	7.5(6.2,11.1)	7.2(4.0,13.0)	4.5(2.2,7.2)	9.391	0.009
Testosterone	2.56(1.21,4.39)	2.0(1.67,2.25)	0.49(0.31,0.77)	31.884	0.001
LH	10.46(5.71,12.98)	0.16(0.05, 0.29)	0.21(0.08,0.64)	38.804	0.001
Insulin	12.37(7.74,14.59)	7.72(5.81,12.20)	9.14(6.64,12.97)	4.199	0.023
Vit D	24.3(21.1, 27.6)	30.3(26.2,32.7)	28.4(23.2, 30.7)	10.909	0.0043

Table 2: Correlation between Vitamin D against AMH, Insulin, LH and testosterone hormones

```
. pwcorr VITAMINDngml AMHngml LHngml Testosteronengml INSULINUuml,sig

             | VITAMI~l  AMHngml   LHngml Testos~l INSULI~l
-------------+---------------------------------------------
VITAMINDngml |   1.0000
             |
             |
     AMHngml |   0.0756   1.0000
             |   0.5657
             |
      LHngml |  -0.1853   0.1472   1.0000
             |   0.1564   0.2619
             |
Testostero~l |  -0.0103  -0.1823  -0.4280   1.0000
             |   0.9378   0.1632   0.0006
             |
 INSULINUuml |   0.0878   0.1493   0.1582  -0.2058   1.0000
             |   0.5049   0.2548   0.2273   0.1147
             |
```

The table above shows a correlation between Vitamin D and AMH and Insulin is positive while that of Vitamin D and LH and testosterone is negative. However, the correlations are not statistically significant (all p>0.05). This therefore implies that lower Vitamin D is associated with higher LH and testosterone.

Below is a box plot on comparisons of HbA1C, Fasting blood sugar and Homeostatic model assessment of Insulin resistant (HOMA-IR) among infertile PCOS, Infertile non PCOS and Fertile non PCOS as markers of glycemic status. The results demonstrates that Infertile PCOS group had a higher HBA1c, FBS and HOMA-IR values 6.2(5.9,6.4), 5.9(5.6,6.4 and 3.2(2.5,3.3) compared to that of Fertile Non-PCOS 5.5(5.4,5.8), 5.2(4.9,5.8) and 2.1(1.8,2.3) and Infertile NON-PCOS 5.6(5.3,5.7), 4.55(4.55,6.05) and 2.3(2.1,3.2) respectively. The difference was statistically significant (p<0.001).

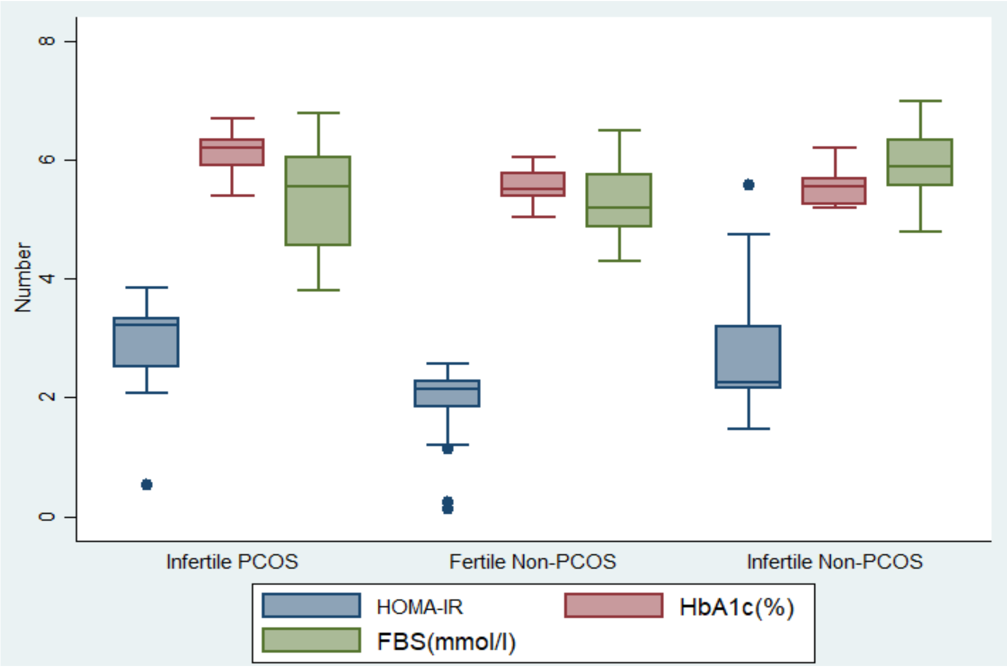

Figure 2: Comparisons of HbA1C, HOMA-IR and Fasting blood sugar among Infertile PCOS, fertile non PCOS and infertile non PCOS

The table below shows a correlation between Vitamin D and HBA1c was negative while that of HOMA-IR was positive. The correlations were not statistically significant ($p>0.05$). This implies that lower Vitamin D levels are associated higher HbA1C thus Diabetes mellitus.

Table 3: Correlations between Vitamin D, HbA1C and HOMA-IR

```
. pwcorr VITAMINDngml HbA1c HOMAIRFBSXINSULIN225,sig
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	VITAMI~l	HbA1c	HOMA~225
VITAMINDngml	1.0000		
HbA1c	-0.0292	1.0000	
	0.8249		
HOMAIRFB~225	0.0154	-0.1037	1.0000
	0.9068	0.4302	

DISCUSSION

The impact of vitamin D on AMH in PCOS individuals with high or even low levels is debatable. Numerous studies reveal inconsistencies and disagreements. In this study it has been established that AMH was 7.5 (6.2, 11.1) in infertile PCOS, while fertile non PCOS had AMH of 7.2 (4.0, 13.0) and the infertile non PCOS group had AMH of 4.5 (2.2, 7.2). This was statistically significant with a $P< 0.009$. In the study of Sharma et al.,2023), it found a positive association between vitamin D and AMH, in contrast to (Chang et al., 2014) who found a negative correlation. The Australian study's concluded that there is no connection between vitamin D and AMH which was later reinforced by Perce et al. (2015) and Bakeer et al. (2018). According to Wang et al. (2018), vitamin D supplementation and AMH are related. According to Seyyed Abootorabi et al.'s (2018) research, vitamin D increases adiponectin levels and lowers fasting blood sugar.

For instance, Soya et al. (2019) discovered a negative association, but Gupta et al. (2015) discovered no correlation. Bahadar et al. (2021) discovered a positive link. In a 2014 study, La Marca et al. established a connection. The present research on the connection between HOMA-IR and AMH is therefore quite inconsistent. Xiang-juan et al. (2021) assert that AMH may contribute to the pathophysiology of PCOS, which is centered on IR, as it is anticipated that AMH would enhance the pathological development of IR.

In a significant multicenter study conducted by Dunaif and colleagues in 1989, AMH was linked to a higher incidence of type 2 diabetes. Reyes et al. (2016) state that it is still unclear if AMH has a role in the pathophysiology of IR in PCOS. As a result, although further research is required to confirm this, AMH may aid in the development of IR in PCOS. In their investigation on the impact of hypoglycemic drugs on AMH, Liping et al. (2020) found that metformin lowers AMH and raises insulin sensitivity.

HOMA-IR and insulin were found to be positively correlated with AMH in a study by Nardo L et al. (2019), and this was also supported by Naeugu M et al. (2012), despite the fact that metformin had no favorable effects on AMH. Researchers are interested in the relationship between AMH and HOMA-IR because it may have substantial therapeutic ramifications, according to Jun et al. (2023).

These erratic and inconclusive results require more investigation. The association of IR and vitamin D levels only exists in old people who are obese and disappears beyond age 60, claim Brassard et al. (2008) when weight is lost. Because the majority of this study seems to be cross-sectional, RCTs are required. According to Zella et al. (2003), 1,25-dihydroxyvitamin D3 given orally to lean diabetic mice reduced the development of insulin-dependent diabetes.

Vitamin D, a fat-soluble vitamin with uses besides bone and calcium metabolism, is present in food. Cell growth regulation, neuromuscular, immunological, and anti-inflammatory functions are additional responsibilities (Bikle et al., 2009).

According to Wild RA et al. (2011), some of these genes regulate blood pressure in addition to lipid and glucose metabolism. According to research by Al-Daqhri et al. (2014), women with increased insulin production is due to VDR gene polymorphisms in the Cdx2, Taq1, Bsm1, Apa1, and Fok1 genes. It has been revealed that a polymorphism in VDR Fok1 protects against the risk of type 2 diabetes, whereas Bsm1 raises that risk.

Additionally, vitamin D insufficiency is less common in those who have the Apa1 polymorphism (Wild et al., 2011). By encouraging the development of insulin receptors, vitamin D improves insulin function and glucose absorption (Pitta et al., 2007). The formation of follicles and the irregular ovulatory cycles linked to PCOS are significantly impacted by low vitamin D-induced calcium and parathyroid abnormalities (Azziz R et al., 2016). Vitamin D controls ovarian steroidogenesis and IGFBP-1 development, according to Ameri P. et al. (2013). Last but not least, because PCOS is such a heterogeneous disorder, it is hard to create standard treatment plans.

When insulin levels rise to levels that are compatible with IR, androgen production dramatically increases. This suggests that there is an immediate consequence of insulin androgen that is dose-dependent. A different study (Glintburg , D, et al., 2016) found that a 2-hour insulin infusion increased testosterone levels as opposed to short-term postprandial hyperinsulinemia. All of this suggests that the generation of androgens requires an ongoing rise in insulin levels. On the other hand, nothing is known about how IR in PCOS-affected women is impacted by hyperandrogenism

According to research by Moghetti, P, et al., (2021), PCOS affect insulin sensitivity since antiandrogen therapy has been shown to decrease androgen production while only modestly increasing insulin sensitivity. Insulin sensitivity increased significantly more in studies using antiandrogens such GnRH analogues for six months (Cicek, N. M. et al., 2003).

CONCLUSION AND RECOMMENDATION

AMH, Testosterone, LH and Insulin hormone levels were significantly higher as compared to fertile non PCOS and infertile non PCOS. However, Vitamin D was significantly lower in infertile PCOS as compared to fertile non PCOS and infertile non PCOS. Vitamin D correlated negatively with LH and Testosterone but positively with AMH and Insulin suggesting that higher levels of LH and Testosterone are linked to lower levels of Vitamin D unlike AMH and Insulin though the correlation was not statistically significant. HOMA-IR, HbA1C and fasting blood sugar were significantly higher in infertile PCOS as compared to fertile non

PCOS and infertile non PCOS. Vitamin D correlated negatively with HbA1C but positively with HOMA-IR though not statistically significant. The study recommends that multi centered randomized clinical trials should be established to test the effects of intervention of Vitamin D on hormonal profiles and glycemic index. The health professionals should ensure adequate Vitamin D levels and even supplement to alleviate metabolic and reproductive ramifications associated with Vitamin D deficiency.

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