ASSOCIATION OF ANTI-MULLERIAN HORMONE (AMH) WITH POLYCYSTIC OVARIAN SYNDROME (PCOS) IN PATIENTS WITH, AND WITHOUT HYPOTHYROIDISM

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A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Philosophy (physiology)

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To Dr. Syedna Mohammad Burhanuddin &

Abde Syedna, Huzefa Zahid Lotia

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is the leading cause of female infertility due to anovulation. Another condition that has a significant impact on ovarian health is hypothyroidism. Both these conditions are widely prevalent. Hypothyroidism, as well as PCOS bring about a similar effect on the ovary in terms of polycystic appearance, which is why it is important to rule out hypothyroidism while planning workup of PCOS. The anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting hormone (MIH) is produced by granulosa cells of the pre-antral and small antral follicles in women until menopause. Production of AMH regulates folliculogenesis by inhibiting recruitment of follicles from the resting pool, thus promoting growth of a single dominant follicle. AMH can help predict the functional ovarian reserve since it is a product of the granulosa cells, which envelop each egg and provide them energy. An AMH level of >3.8ng/mL is being considered to help in the diagnosis of PCOS. However, hypothyroidism can alter the levels of AMH, and so it is important to ascertain whether serum AMH levels are reliable in PCOS patients with hypothyroidism. The objective of this study was to measure and compare the levels of AMH in women of reproductive age that have been diagnosed with PCOS (by Rotterdam Criteria, 2003) alone, and those that have been diagnosed with PCOS as well as hypothyroidism (TSH>4.2 mIU/L). In this case-control study, carried out at the National Medical Centre (NMC) hospital in Karachi, subjects were divided into two groups. Cases, consisting of 42 subjects; these were subjects diagnosed with both conditions, PCOS as well as hypothyroidism. Controls; 42 subjects diagnosed with PCOS alone. Serum TSH, Fasting Insulin, AMH, and BMI were measured in both groups. Age, and duration of infertility was recorded in both groups. Statistical analysis was done using SPSS software version 26. The results showed a statistically insignificant difference between AMH levels (4.39 ± 2.05 in cases vs. 4.19 ± 1.86 in controls) in both groups. Fasting insulin (16.17 ± 8.91 in case vs. 14.26 ± 6 controls) , BMI (28.14 ± 5.12 in cases vs. 29.39 ± 6.09 in controls), and duration of infertility (4.62 ± 3.87 in cases vs. 3.86 ± 3.04 in controls) was similar in both groups. The results of this study support use of AMH as a diagnostic marker in PCOS, even in patients suffering from hypothyroidism.

Keywords: Polycystic ovary syndrome (PCOS), hypothyroidism, & anti-mullerian hormone (AMH)

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LIST OF ABBREVIATIONS

AES	Androgen Excess Society
AMH	Anti-mullerian hormone
BMI	Body Mass Index
FI	Fasting Insulin
PCOS	Polycystic Ovary Syndrome
SHBG	Sex Hormone Binding Globulin
TPO	Thyroid Peroxidase antibodies
TSH	Thyroid Stimulating Hormone

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND:

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder affecting women of reproductive age. The prevalence of PCOS worldwide is reported to be as high as 21.27% of women worldwide (Deswal, R. et al., 2020), with other studies suggesting that it may affect up to 10% of women of reproductive age if the Rotterdam and AES criteria is used (Bozdag, G. et al., 2016). In comparison to Caucasians, the prevalence of PCOS is significantly higher in the south Asian population. Two studies conducted in various regions of the Indian Punjab suggested prevalence of PCOS to be 4% in their rural, and 6% in their urban population (Sharma, P. et al., 2022 & Deswal, R. et al., 2019). Studies in Pakistan suggest a higher prevalence of PCOS (~50%), in accordance with the Rotterdam criteria 2003 (Akram, M. et al., 2015). Similarly, (Baqai, Z. et al., 2010) reported a 40% prevalence of PCOS among infertile women seeking medical advice in Karachi, Pakistan. However, the exact prevalence may vary depending on the population studied and the diagnostic criteria used. An article by (Zhao, Y., & Qiao, J., 2013) has extensively explored prevalence along with phenotypical differences noted in different ethnic groups in various studies on PCOS prevalence around the globe.

A range of symptoms helps in characterization of polycystic ovarian syndrome. These symptoms include, but are not limited to, irregular menstrual cycles, hirsutism, and acne. PCOS is more likely associated with metabolic disturbances such as insulin resistance, dyslipidemia, and obesity (Teede HJ. et al; 2018).

In the current study, the Rotterdam criteria (2003) was used in order to identify PCOS patients. The Rotterdam criteria was developed during a meeting of experts in Rotterdam, Netherlands, in 2003. This criteria is used to identify polycystic ovary Syndrome (PCOS).

The criteria demands that diagnosis of polycystic ovarian syndrome can be made if a woman has at least two of the following three features: this includes the presence of an irregular menstrual cycle or anovulation which is absence of a menstrual cycle for a period of 6 months; the next criterion is presence of clinical and/or biochemical signs of hyperandrogenism. Lastly, Polycystic ovarian morphology detected by an ultrasound examination (12 or more follicles measuring 2-9 mm in diameter, or ovarian volume >10 mL) is required for diagnosis in accordance with the Rotterdam criterion of 2003.

It is of interest to note that some other conditions can also mimic the symptoms of PCOS, these include, but are not limited to, thyroid disorders and congenital adrenal hyperplasia. Such conditions should be ruled out therefore, before a diagnosis of PCOS can be made.

A more recent criteria, proposed by the AES (Androgen excess society) in 2006 is based on the Rotterdam criteria developed by The European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003. AES criteria has the following features: this includes the presence of an irregular menstrual cycle or anovulation which is absence of a menstrual cycle; the next criterion is presence of clinical and/or biochemical signs of hyperandrogenism. Polycystic ovarian morphology, and lastly, exclusion of related disorders

There are distinct differences in prevalence of PCOS in various ethnicities across the globe (Zhao, Y., & Qiao, J., 2013). Prevalence was noted to be 4.8 percent and 8 percent in white and black women in south- eastern United States (Azziz R, et al., 2004); 6.8 percent in the white women population of Greece; 6.5% in white women in Spain (Asuncion M. et al., 2000); 6.3% in south asian in Sri Lanka (Kumarapeli, V. et al., 2008), and 5% in Thai women (Vutyavanich T. et al., 2007). Contradictory to the findings of Zhao et al. (2013), it seems that prevalence is similar across above regions because of similar estimates. However, the studies quoted above, while widely accepted for estimation of prevalence, are outdated.

New studies studying prevalence are the need of the time as the disease burden of PCOS has increased far more in the recent years (Sendur, S. N., & Yildiz, B. O., 2021)

Several studies have documented the differences in PCOS symptoms with respect to ethnicity, age, insulin resistance, and BMI. There are many differences in phenotypical presentation of PCOS among various ethnicities as well (Fauser, B. C. et al. 2012).

Phenotypically, the severity of various PCOS symptoms demonstrates high variability. Caucasians have more severe hirsutism compared (east) Asians (Huang Z., & Yong E. 2016). There is a very high prevalence of hirsutism among women of Middle East and Mediterranean origin (Glintborg D. et al., 2010) (Kauffman RP. Et al., 2006). Hispanic women had higher fasting insulin levels and higher HOMA-IR scores than that of nonHispanic White women (Kazemi, M. et al., 2022). Interestingly, even though Japan has lower rates of obesity and hirsutism, the Japanese still have comparable rates of androgen excess and insulin resistance to the US and Italy (Zhao, Y., & Qiao, J., 2013). Black women had increased levels of fasting insulin, pertaining to more severe insulin resistance compared to white women (Engmann, L. et al., 2017).

The anti-Mullerian hormone (AMH), alternatively called Mullerian-inhibiting hormone (MIH) is a product of granulosa cells of the pre-antral and small antral follicles in women until menopause (Pellatt et al., 2007). Regulation of folliculogenesis is done by production of AMH as AMH inhibits recruitment of follicles from the resting pool, thus promoting growth of a single dominant follicles (Kollmann et al., 2015). Since it is a product of the granulosa cells, AMH provides a reliable estimate of the functional ovarian reserve. AMH is being considered as a biomarker to help in the diagnosis of PCOS. (Depmann M et al., 2016)

While the serum Anti-Mullerian hormone serves as a valuable tool to assess functional ovarian reserve, its use as a biomarker for the diagnosis of PCOS remains debatable(Scheffer, 2017). Partly because, PCOS patients, if diagnosed using only the ESHRE/ASRM Rotterdam Criteria (2003) could be suffering from a thyroid dysfunction which could in turn affect the AMH levels. A study has suggested a new criteria to diagnose PCOS using AMH. They substituted PCOM measurement on ultrasound with measurements of AMH levels. An AMH level above **3.8ng/mL** was considered as positive for PCOS (Sahmay, S. et al., 2014). The study has pointed out that since PCOM on ultrasound is unreliable due to: Differences in measurement of number of follicles subject to technician skill; Limited visibility of small antral follicles; Difficulty in

assessing virgin patients; AMH is a very useful and reliable measure of follicle excess, as seen in PCOS.

Investigation relating to the association between AMH and PCOS has been extensive in the recent years. PCOS patients had significantly higher levels of AMH than women without PCOS. AMH levels correlated positively with the number of ovarian follicles and the severity of the syndrome (Pigny P. et al; 2006). Subsequent studies, exploring the relation of elevated AMH levels have also shown that AMH levels are elevated in women with PCOS, regardless of age, BMI, or menstrual status (Li HW. et al., 2011).

Relationship of patient age with polycystic ovary syndrome (PCOS) has been found to have a significant association with anti-Mullerian hormone (AMH) levels. The relationship between age and AMH levels in PCOS patients gives a better understanding of the impact of aging on ovarian function. Logic suggests that with declining ovarian function due to aging alone, the AMH levels should have a declining trend. That being said I have further discussed the studies that have proven that this is the case in the paragraphs that follow.

AMH levels were significantly higher in younger patients compared to older patients with PCOS. This finding suggests that age plays a role in the decline of ovarian reserve and follicular activity in PCOS. Similarly, another study demonstrated a negative correlation between age and AMH levels in women with PCOS, indicating that as age increases, AMH levels tend to decline (Laven J. S. et al., 2004). AMH levels are higher in the PCOS group compared to age matched controls, this finding is in agreement with both of these studies.

With advancing age, there is a decrease in the number of primordial follicles. Thus, with advancing age, there should also be a decrease in the level of AMH. The decrease in primordial follicle pool over time (even in patients with PCOS) is responsible for the age related decline of AMH levels. This decline is still slower if compared to age matched and BMI matched controls without PCOS.

Insulin resistance and obesity are metabolic disturbances that may exacerbate the decline in AMH levels with age, especially in patients suffering from PCOS. These metabolic factors can negatively affect ovarian function and accelerate the depletion of ovarian

reserve. Insulin resistance, especially, has been shown to contribute to the progression of ovarian dysfunction in PCOS, leading to diminished AMH production (Nardo, L. G., 2009). (Refer to figure 1.1)

It should be noted that while age is associated with a decline in AMH levels in PCOS patients, this decline may occur at a slower rate compared to women without PCOS. PCOS is characterized by increased AMH production due to the presence of excessive small antral follicles in the ovaries. This hyperandrogenic state in common in PCOS may further delay age-related decline in AMH levels, resulting in relatively higher AMH levels in older PCOS patients compared to women without PCOS (Laven J. S. et al., 2004). Body Mass Index (BMI) is calculated by dividing an individual's weight in kilograms by the square of their height in meters. A high BMI is a frequently observed sign in PCOS patients, with a significant percentage falling into the overweight or obese BMI categories (Zhao H. et al., 2023).

Metabolic disturbances, including insulin resistance and dyslipidemia are attributed to coexistence of elevated BMI and PCOS together (Barber T. M. et al., 2015). Therefore, understanding the influence of BMI on AMH levels can shed light on the potential effects of excess body weight on ovarian function in PCOS.

Decreased AMH levels have been associated with a raised BMI, which indicates an association of excess body weight on follicle activity (Hirschberg A. L., 2009). Insulin resistance may be a factor that is involved, as it is seen to be higher in overweight and obese individuals (Sahmay, S. et al., 2013). Since Insulin resistance can lead to higher androgen levels, which, in turn stimulates the ovaries to produce more AMH (Homburg, R. et al.,2013). In fact, hormones secreted by adipose tissue also tend to affect ovarian function, potentially contributing to elevated AMH levels (Legro, R. S. et al., 2013). A significant difference has been observed when patients with BMI less than 24.9 were compared with those with a BMI between 25-29.9, and 30 and above respectively (Piouka A. et al., 2009). It is apparent that BMI is negatively correlated with BMI level. However, review articles have documented studies that have also shown a statistically insignificant difference in AMH levels between obese and non-obese women (Kloos, J., Coyne, K., &

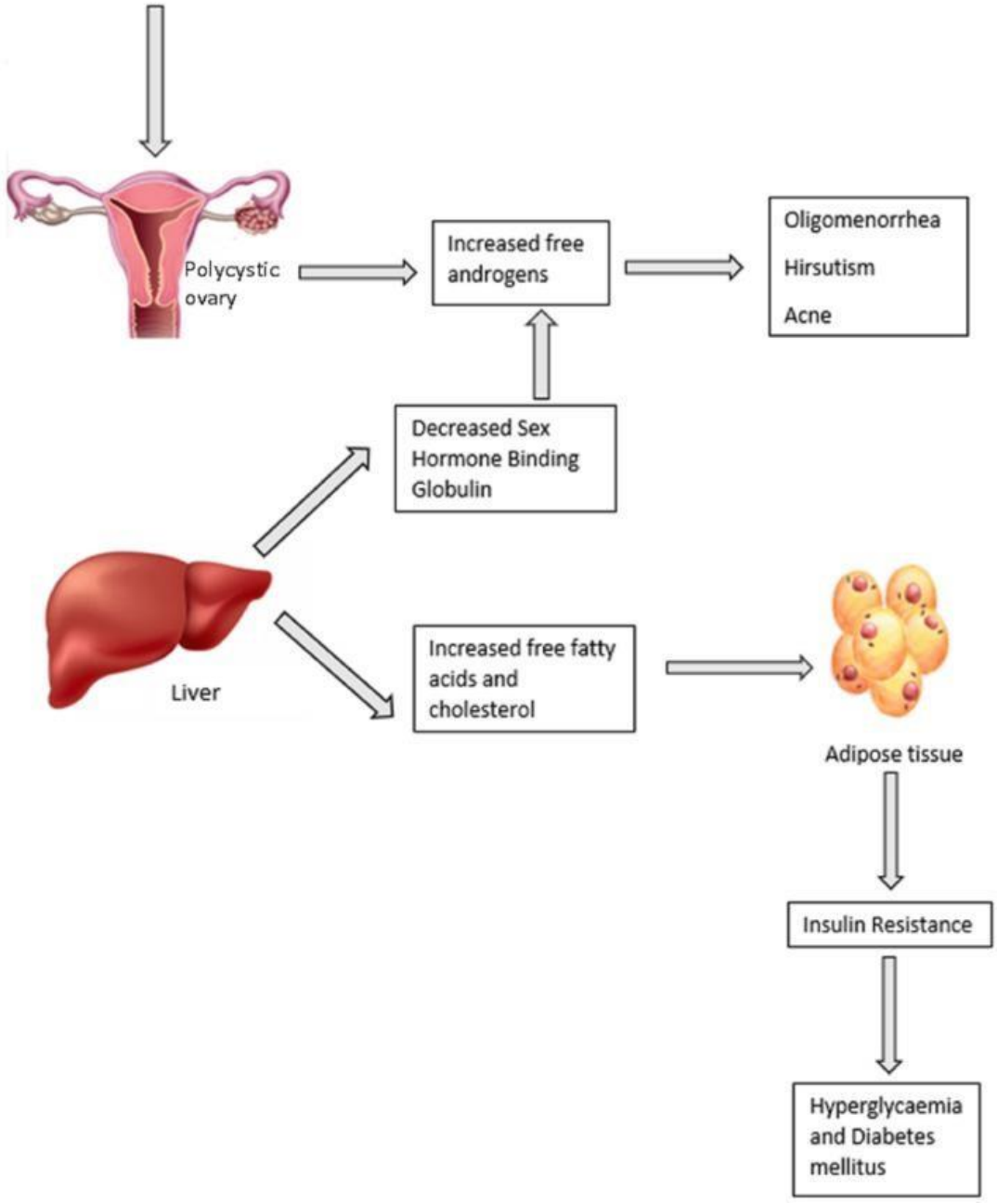

Figure 1.1: Mechanism of insulin resistance and other clinical features of PCOS (Peddemul A. et al., 2022)

Weinerman, R., 2022)). In these studies, AMH levels are not conclusively correlated with BMI, changing the previous perspective of BMI affecting AMH levels.

The hormonal imbalance observed in polycystic ovary syndrome (PCOS), characterized by elevated luteinizing hormone (LH) levels; and a higher leutinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio, has been associated with alterations in ovarian function and the regulation of anti-Mullerian hormone (AMH) production.

It has been seen that LH independently is a predictor of high AMH levels in PCOS patients; LH can result in up to a four hundred percent increase in AMH levels (Silva M. et al., 2021). FSH is not correlated with AMH as AMH is secreted in the LH dependent stage of the cycle thus there is no effect of altered AMH levels on FSH (Zhao H et al., 2023). AMH in turn, however, plays a paracrine role in order to inhibit FSH-induced aromatase activity in granulosa cells that aids in the development of a dominant follicle (Pellatt, L. et al. 2011) (Crespo, R. P. et al., 2018). Higher AMH levels in PCOS makes follicles resistant to to FSH action, causing inhibition of follicular maturation and ovulation; inhibition of aromatase expression; consequently, resulting in hyperandrogenism. (Azziz et al., 2016).
(Refer to figure 1.2)

LH to FSH ratio and AMH levels in a cohort of PCOS patients revealed a positive correlation between the LH to FSH ratio and serum AMH levels, indicating that a higher LH to FSH ratio is associated with increased AMH production (Piltonen et al., 2005). This can be explained by the fact that elevated LH levels will result in an altered LH to FSH ratio observed in PCOS, which will in turn contribute to the overproduction of AMH by the ovaries.

The underlying mechanisms that affect the relationship between the LH to FSH ratio and AMH levels in PCOS are multifactorial. LH stimulates androgen production in the ovaries, which in turn leads to excess androgen levels in PCOS patients (Silva M. et al., 2021. Testosterone, an androgen, has been shown to upregulate AMH production in ovarian granulosa cells (Dumont, A. et al., 2015). Thus, an elevated LH level and higher LH to FSH ratio in PCOS promotes androgen excess, and results in higher levels of AMH in PCOS patients.

Disturbances in regulation of insulin signaling pathways which are seen in PCOS can also contribute to the LH to FSH ratio disturbance, and in turn have its effect on AMH levels. Insulin resistance, which happens to be a hallmark of polycystic ovary syndrome, can stimulate ovarian androgen production and disrupt the normal feedback mechanism between LH and FSH. The disruption that results further promotes the LH to FSH ratio imbalance, which, in turn, contributes to the excessive production of AMH (Piltonen et al., 2015)

The relationship that exists between LH to FSH ratio and AMH levels in PCOS may vary among individuals due to the heterogeneity of the syndrome. Obesity, genetic predisposition, and environmental influences are factors that can further modulate this relationship. However, the overall trend suggests that a higher LH to FSH ratio is associated with elevated serum AMH levels in PCOS patients, indicating an impact on ovarian reserve and follicle activity.

Insulin resistance (IR) is also correlated with levels of AMH in PCOS patients. It has been shown that higher levels of AMH in the circulation alter the function of the pancreatic cells; result in hyperinsulinemia (as release of AMH by the granulosa cells through uptake of androgens) can lead to hyperinsulinemia. (Sahmay, S et al., 2013). What this implies is that the higher levels of circulating AMH in PCOS tend to cause a state of hyperinsulinemia, and in turn increase the risk of type II diabetes in such patients in later years. However, another study's findings suggest that higher AMH levels tend to decrease the function of the Islet cells, implying that AMH is directly causal in the development of type II diabetes in PCOS patients.

A cohort study explored the increased AMH levels of pregnant women with PCOS, and its link with etiology of PCOS (when the fetus reached adulthood). The study showed that pregnant women with PCOS had higher levels of AMH compared to pregnant controls without PCOS. They used an animal (mice) model showing that when the fetus was exposed to high levels of AMH in the womb, the female fetus was more likely to develop PCOS as an adult. This was attributed to the neuroendocrine disturbances caused by the elevated levels of AMH which in turn played havoc on the estrus cycle (Tata, B. et al., 2019).

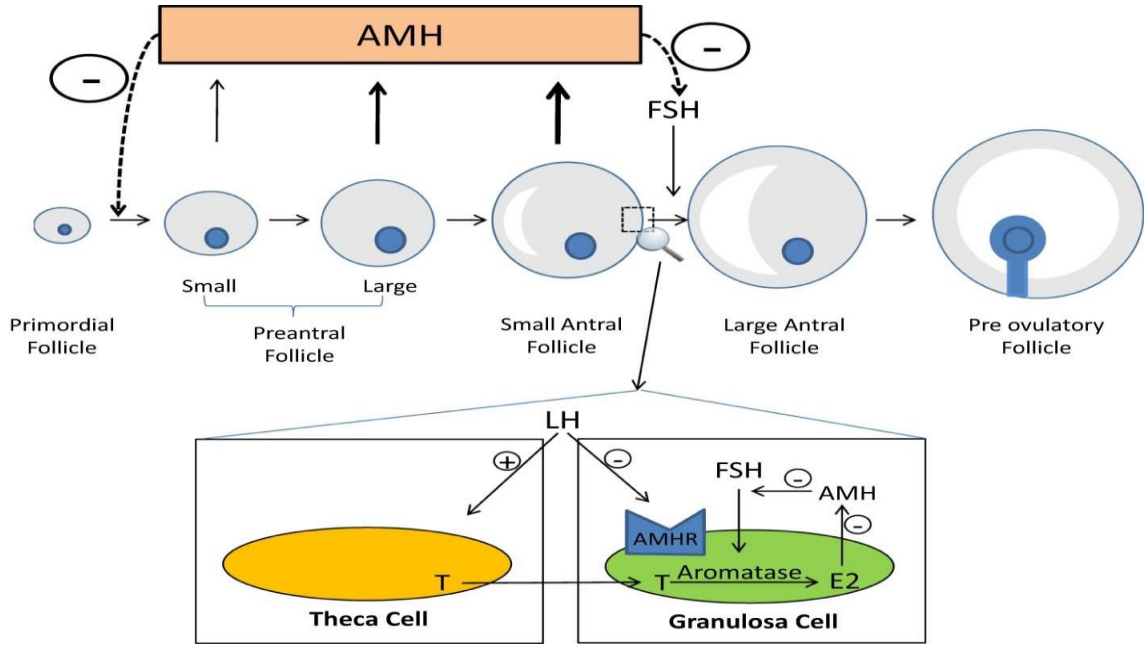

Figure 1.2: **Role of AMH in folliculogenesis.**
Adapted from Azziz et al., (2016) (Crespo, R. P. et al., 2018)

PCOS has a strong genetic component, with twin studies estimating a 72 percent variance risk in the development of the disease (Vink J. M et al., 2006). Genetic factors play a crucial role in the pathogenesis of PCOS, it has been shown through twin studies that the inheritance in PCOS is X-linked polygenic. It is not autosomal dominant or monogenic as previously theorized. Genetic factors influence clinical features and underlying molecular mechanisms in PCOS (Afzal T. et al., 2021).
(Refer to figure 1.3)

Various genetic variants associated with PCOS, including those involved in hormone regulation, insulin signaling pathways, and folliculogenesis have been identifies (Dapas, M., & Dunaif, A., 2022). These genetic variants can in turn effect the the expression and function of AMH and may contribute to the raised serum AMH levels that are widely seen in in PCOS patients.

Specific genetic variants in women with PCOS has been widely researched. An association exists between AMH levels and polymorphisms in genes such as FSHR, AMHR2, and INSR; these are known to play vital roles in ovarian function and follicular development (Khan, M. J. et al., 2019)). These findings suggest that genetic variations in these genes may contribute to the dysregulation of AMH production and secretion in PCOS patients.

Another study examined the genetic influence on AMH levels in women with and without PCOS. The researchers identified several genetic loci associated with AMH levels, including variants near the AMHR2 and AMH genes (Gorsic L. K. et al., 2019). These loci showed stronger associations in women with PCOS, compared to normal matched controls, indicating a potential link between genetic predisposition to PCOS and AMH gene regulation.

A genome-wide association study (GWAS), has revealed genetic variants in the THADA gene that were associated with both PCOS susceptibility and elevated AMH levels. THADA is involved in ovarian development and has been implicated in PCOS pathogenesis (Hayes et al., 2015) (Shi, Y. et al., 2012)). This link of PCOS with THADA further strengthens the genetic etiology of PCOS (Zeber-Lubecka, N. et al., 2021)

An association between AMH and PCOS, and how this relationship is impacted by

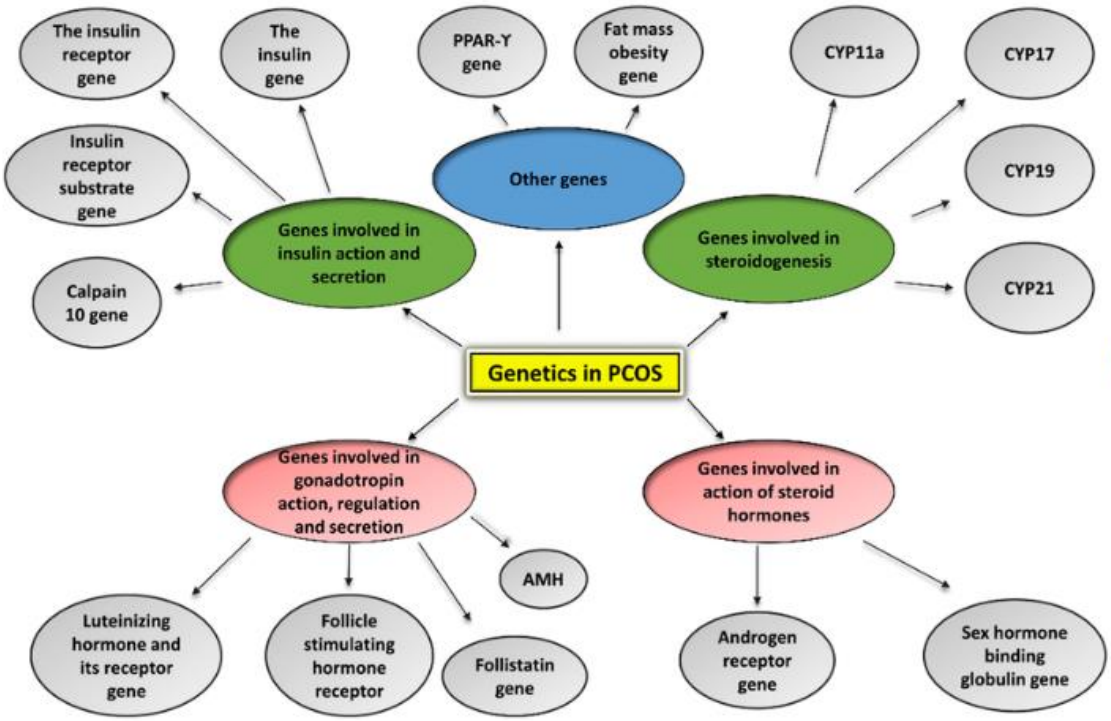

Figure 1.3: **Summary of genes involved in PCOS (Khan, M. J. et al., 2019)**

other comorbidities such as hypothyroidism is currently a much researched niche. Hypothyroidism is a common condition in PCOS patients, affecting up to 25% of women with PCOS (Unuane D. et al; 2017). Both PCOS and hypothyroidism are known to affect reproductive function, and it is thus important to understand the potential impact of these conditions on each other.

Hypothyroidism is characterized by neuromuscular symptoms such as; Lethargy in performing regular mundane tasks such as filling of water, fatigue while performing basic medium level exercised, excessive sleepiness that disrupts the normal day to day life, weakness that is unexplained and makes it difficult to perform every day tasks, slow reflexes that make it difficult to perform functions required for day to day life.

Weight gain is common in hypothyroidism. It can have a primary or secondary cause. It is difficult to determine the exact metabolic disorder resulting in weight gain Hypothyroidism can have a primary cause or a secondary cause. A primary cause is a condition that directly impacts the thyroid and causes it to create low levels of thyroid hormones. A secondary cause is something that causes the pituitary gland to fail, which means it can't send thyroid stimulating hormone (TSH) to the thyroid to balance out the thyroid hormones.

Primary causes of hypothyroidism are much more common. The most common of these primary causes is an autoimmune condition called Hashimoto's disease. Also called Hashimoto's thyroiditis or chronic lymphocytic thyroiditis, this condition is hereditary (passed down through a family). In Hashimoto's disease, the body's immune system attacks and damages the thyroid. This prevents the thyroid from making and releasing enough thyroid hormone. (Utiger et al., 2021)

FSH to LH ratio is altered by the hormone prolactin. Prolactin causes an increase in the level of dehydroepiandrosterone. These fluctuating hormone levels increase ovarian volume, inhibit ovulation, and lead to cysts in the ovary(de Medeiros et al., 2018)

FSH to LH ratio is altered by the hormone prolactin. Prolactin causes an increase in the level of dehydroepiandrosterone. These fluctuating hormone levels increase ovarian volume, inhibit ovulation, and lead to cysts in the ovary(de Medeiros et al., 2018)

Due to the similarities that exist between symptoms of hypothyroidism and PCOS It is important to perform workup of both conditions for a more accurate diagnosis. The diagnostic criterion for hypothyroidism remains the measurement of the serum thyroid stimulating hormone (TSH); A serum TSH level of greater than 4.2 mIU/L is considered to be diagnostic of hypothyroidism; additionally, serum free thyroxine (Free T4) is measured if the level of serum TSH is greater than 4.2 mIU/L. A free T4 index (FTI) is a commendable alternative to the value of the free hormone level. The product sum of T3 resin uptake and total T4 levels is the FTI. Free T4 levels, it must be remembered, however, are unreliable in the event of pregnancy and severe illness(Garber et al. 2012).

Regarding pathophysiology of hypothyroidism, it occurs when the thyroid gland, is unable to produce enough thyroid hormones to meet the needs of an individual. The thyroid gland produces two hormones, namely, thyroxine (T4) and triiodothyronine (T3). These hormones play a crucial role in regulating metabolism and controlling the rate at which the body uses energy. A decrease in the production of T4 and T3, leading to a decrease in the metabolic rate and a variety of symptoms occurs in hypothyroidism. This can be due to a number of reasons, including autoimmune disease, iodine deficiency, and radiation exposure. (de Medeiros et al., 2018.)

Lowering the circulating thyroid hormones has an effect on fertility; thus hypothyroidism has been found to have a potential association with serum AMH levels. This relationship between AMH levels and hypothyroidism has been explored numerous studies. Correlation of AMH levels with TSH levels in women with infertility was examined by one such study; the study found that TSH and AMH were negatively correlated (Kuroda K. et al. 2015). Levels of AMH in subclinical hypothyroidism were evaluated by another study and it was found that significantly lower AMH levels compared to euthyroid women exist in subclinical hypothyroid women (Kucukler, F. et al 2019). The same study also found significantly reduced AMH levels in overtly hypothyroid women compared to euthyroid controls (Kucukler, F. et al 2019)

The exact reasons for the association between hypothyroidism and decreased AMH levels are not yet fully understood. However, it has been suggested that hypothyroidism may disrupt the normal development and function of ovarian follicles, leading to a decline in

AMH production. Thyroid hormones are known to play a crucial role in follicular development, oocyte maturation, and ovulation (Janssen et al., 2004). When thyroid hormone levels are inadequate due to hypothyroidism, it may negatively impact the quantity and quality of ovarian follicles, consequently affecting AMH production (Osuka, S. et al. 2018)

Treatment of hypothyroidism with thyroid hormone replacement therapy can lead to the restoration of AMH levels. A study demonstrated that after levothyroxine treatment in hypothyroid women, AMH levels significantly increased (Kuroda, M. et al., 2018). This suggests that correcting thyroid hormone imbalances through appropriate treatment may help restore AMH levels and improve ovarian function in women with subclinical as well as overt hypothyroidism.

Association between hypothyroidism and serum AMH levels has many clinical implications for reproductive health.. AMH levels are widely used in assessing ovarian reserve, and also help in predicting response to the assisted reproductive techniques. These then help in guiding fertility treatment plans. Thus, identifying link between hypothyroidism and AMH levels can aid in optimizing fertility management plans for women with hypothyroidism.

Association between age and serum anti-Müllerian hormone (AMH) levels in patients with hypothyroidism is widely investigated. It is known that due to diminished ovarian function with age, AMH levels also decrease. However, since hypothyroidism alone also lowers AMH levels, then patients with hypothyroidism should have even lesser AMH levels compared to women without thyroid disease. A study examined the relationship between age and AMH levels in women with hypothyroidism. The results showed a negative correlation between age and serum AMH levels, indicating a decline in ovarian reserve with advancing age in hypothyroid patients (Hasegawa, Y. et al., 2021)). This finding suggests that age-related decline in fertility may be more pronounced in women with hypothyroidism due to the combined effects of aging and impaired thyroid function.

Another study conducted further investigated the impact of age on AMH levels in hypothyroid women. The research showed that AMH levels negatively correlate with

TSH levels as well as age. Thus indicating that increasing age as well as hypothyroidism result in decreased levels of AMH (Kuroda, K. et al., 2015)

These studies highlight the importance of considering age as a factor influencing AMH levels in patients with hypothyroidism. The decline in AMH levels with age in hypothyroid patients are an important area of study as more and more women are opting to have children at an advanced age. These studies have shown that women with both hypothyroidism and advanced age may have a diminished ovarian reserve, which may impact their chances of conceiving naturally and may need assisted reproduction treatments.

Several studies have explored the association between body mass index ((BMI) and serum anti-Müllerian hormone (AMH) levels in patients with hypothyroidism. Association between BMI and AMH levels in women with hypothyroidism was evaluated by a study. They found that a higher BMI was associated with lower serum AMH levels in hypothyroid patients, suggesting a negative impact of increased body weight on ovarian reserve (Adamska, A. et al. 2021).

Negative correlation between BMI and AMH levels suggests that higher body weight, particularly in the form of obesity, may contribute to diminished ovarian reserve in hypothyroid individuals. They also suggest that fertility treatments in hypothyroid women should focus on the importance of a healthy BMI for better outcomes.

Genetic variations that relate to thyroid function in women with subclinical hypothyroidism and overt hypothyroidism has been documented in many studies; the results of one such study revealed that specific genetic variants, including those in the TSHR and DUOX2 genes, were associated with altered thyroid function (Sun, F. et al. 2018). These findings suggest that genetic factors related to thyroid function may contribute to variations in AMH levels even in the subclinical hypothyroidism population. These studies highlight the potential influence of genetic predisposition of of hypothyroidism on serum AMH levels, indicating a complex interplay between genetic factors, thyroid function, and ovarian reserve. Genetic variants involved in thyroid hormone synthesis and metabolism may contribute to variations in AMH levels in individuals with hypothyroidism.

PCOS and Hypothyroidism share a number of symptoms; including menstrual irregularities and infertility. It has been shown that there is a high prevalence of hypothyroidism in women with PCOS, and that the two conditions may be linked via complex derangement of hormone levels as well as metabolic factors.
(Refer to figure 1.4)

The link between hypothyroidism and PCOS may be due to a number of factors, including hormonal imbalances and metabolic disturbances. Thyroid hormones play an important role in the regulation of the menstrual cycle; follicular development; and ovulation (Carmina E., et al., 2005). And thus, any disruption in thyroid function, such as that seen in hypothyroidism, can lead to menstrual irregularities and impaired fertility. Thyroid hormones tend to interact with sex hormones, estrogen and progesterone, which are involved in the pathogenesis of PCOS (Azziz R et al., 2004). Disturbances in the delicate balance of these hormones contribute to the development or the exacerbation of PCOS symptoms in individuals with hypothyroidism.

Insulin resistance, which is a hallmark of PCOS, has been implicated in the development of hypothyroidism as well (Diamanti-Kandarakis E., & Dunaif A., 2012). Hypothyroidism has been shown to influence insulin resistance and glucose metabolism, potentially exacerbating insulin resistance in women with PCOS (Brenta, G., 2011). Interaction between thyroid function, insulin resistance, and PCOS may further contribute to the shared clinical features and metabolic abnormalities observed in individuals that suffer from both conditions.

Impact of hypothyroidism on AMH levels in PCOS remains unclear. Hypothyroidism has been shown to have a negative impact on AMH levels, and PCOS patients have a higher AMH level compared to controls that are age and BMI matched (Kuroda K. et al. 2015) (Kucukler, F. et al 2019) (Sahmay S. Et al. 2014), so the effect of hypothyroidism on AMH levels in females with PCOS still warrants research if AMH is being considered as a diagnostic and prognostic marker in PCOS

AMH levels in patients with PCOS as well as Hashimoto's thyroiditis were found to be lower than AMH levels of patients with PCOS alone (Serin A. N. Et al., 2021).this recent study pointed out that limited studies have evaluated AMH levels in patients with both conditions, PCOS as well as hypothyroidism.

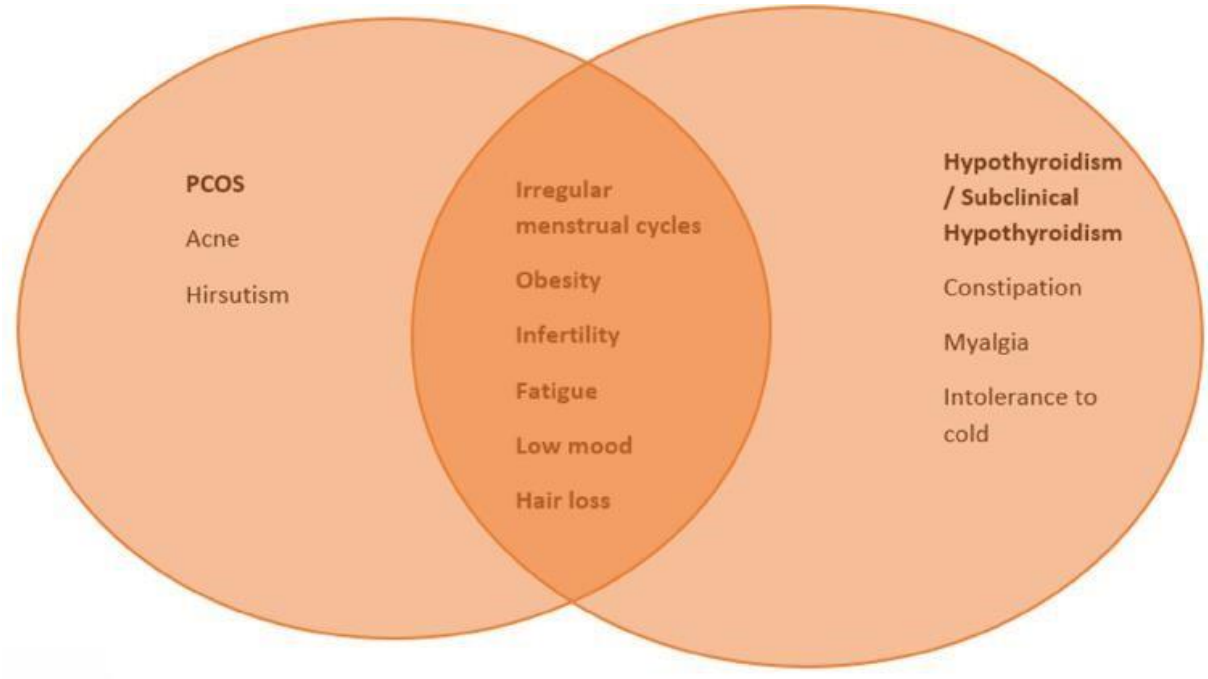

Figure 1.4: Overlapping symptoms of PCOS and hypothyroidism. (Peddemul, A. et al., 2022)

Another study in 2016 found that there was no significant difference in AMH levels between PCOS patients with and without hypothyroidism (Pergialiotis. Et al; 2016). These conflicting findings highlight the need for further research to fully understand the impact of hypothyroidism on the association between AMH and PCOS.

Elevated levels of AMH are a key diagnostic feature of PCOS, and may have prognostic value in the assessment of fertility potential. However, the potential impact of hypothyroidism on the association between AMH and PCOS remains unclear, and further research is needed to fully understand this relationship. In addition, the potential influence of other comorbidities such as obesity and insulin resistance should also be taken into account when investigating the association between AMH and PCOS.

1.2 RESEARCH GAP/ RATIONALE OF STUDY

The aim of this study is to compare the levels of serum AMH in women with PCOS as well as hypothyroidism, and in women with PCOS alone, as serum AMH may prove to be a potential marker for the diagnosis of PCOS.

1.2.1 Theoretical gap:

It has been well established by previous studies that high serum AMH levels are associated with PCOS. However, larger scale research is still required to explore the fluctuations in levels of AMH should the patient of PCOS be suffering from an additional condition. Since there are ethnic variations in symptoms and signs of PCOS, studies need to be conducted in all regions to establish the best tools needed for PCOS diagnosis in a particular region. This study aims to show that females with PCOS and hypothyroidism tend to have differed AMH levels compared to PCOS patients without hypothyroidism in the Pakistani population. The findings of this study will highlight whether the use of current diagnostic levels of AMH are reliable for PCOS diagnosis when a patient has hypothyroidism.

1.2.2 Contextual Gap/ Analysis:

There is limited literature on serum AMH in PCOS patients with hypothyroidism in Pakistan, which shows a severe lack of study in this field. Therefore, is important to explore serum AMH which can serve as a diagnostic tool that may help with the treatment, and better outcome of PCOS patients in Pakistan. The discovery of these gaps reveal the necessity and significance of further research into the link of serum AMH with PCOS and hypothyroidism in the unique setting of Pakistan. This will help researchers and healthcare professionals fight to lessen the burden of this disease.

1.2.3 Methodological gap/analysis: NA

1.3 PROBLEM STATEMENT:

The most common cause of female infertility worldwide is PCOS (Deswal et al., 2020). The condition is widely prevalent in Pakistan as well (Akram et al., 2015). PCOS is difficult to diagnose especially since many of its symptoms may be seen in other conditions (Agapova et al., 2014); and there are ethnic variations in symptoms as well (Bozdac et al. 2016). Serum AMH can serve as a useful tool for diagnosis, but AMH levels may be lower in patients with hypothyroidism making it an unreliable biomarker (Kuroda et al., 2015). Given that PCOS is important to diagnose correctly since it affects quality of life and predisposes a woman to cancer, it is pertinent to establish how the available diagnostic tools can be used effectively to diagnose this condition. This study aims to demonstrate the differences in AMH levels in PCOS patients in Pakistan when they have a high serum TSH level. This information may prove helpful in the consideration of AMH as a biomarker for PCOS by highlighting the changes in AMH levels that occur due to lower free thyroxine levels. It is hoped that the study will add to the findings of previous studies and contribute to the hunt for finding the perfect tools required to diagnose PCOS in Pakistani population accurately.

1.4 RESEARCH QUESTION/ HYPOTHESIS OF STUDY

Research question: Is hypothyroidism in PCOS patients associated with lower levels of serum AMH compared to patients that do not have hypothyroidism?

Ha**:** hypothyroidism in PCOS is associated with lower levels of serum AMH, compared to controls that have PCOS, but not hypothyroidism

1.5 OBJECTIVES:

- To measure serum AMH levels in patients of hypothyroidism and PCOS
- To measure serum AMH levels in patients with PCOS only
- To compare the levels of AMH in both these groups

1.6 SIGNIFICANCE OF STUDY:

PCOS remains a highly prevalent, difficult to diagnose condition. When PCOS presents with an additional related disorder such as hypothyroidism, accurately diagnosing PCOS becomes difficult. With limited resources available in developing countries such as Pakistan, it is the need of the time to have economical and effective diagnostic and therapeutic tools to manage the condition. This study will help to identify the degree of change in serum AMH levels in patients with PCOS that have hypothyroidism. This in turn can contribute to further polishing the criteria currently in use for the diagnosis of PCOS in Pakistan.

CHAPTER 2

LITERATURE REVIEW

Deswal, R. et al. (2020) have shown that the accurate prevalence of PCOS is yet to be established as there is still debate going on regarding the exact criteria for the diagnosis. The study proposed a mean prevalence of 21.27% after analyzing 27 surveys conducted recently in different regions.

Bozdag et al. (2016) have conducted research based on prevalence of PCOS based on three different diagnostic criteria. They assessed prevalence of PCOS on criteria defined by the NIH, Rotterdam, and AES. The results show differences in prevalence of PCOS when the above three criterias are used. They have shown that when NIH criteria was used in the population studied, the prevalence of PCOS was 6 percent. When the Rotterdam criteria was used, the prevalence in their study population was 10 percent, and when the Androgen Excess Society (AES) criteria was used, the prevalence was also 10 percent. They demonstrated that prevalence is also dependent upon the diagnostic criteria used.

Sharma P. et al. (2022) have conducted prevalence studies in the Indian Punjab and have shown that menstrual irregularities prevalence varied in the urban and rural population. The most prevalent menstrual problem they found was dysmenorrhea (60%). They inferred that BMI, age of menarche, fitness and mental health were significant predictors of menstrual irregularity. They also found that awareness of PCOS was very low, approximately 3 percent in the urban are rural population.

Deswal, R. et al. (2019) studied PCOS prevalence in the rural and urban regions of Haryana, India. They measured prevalence of each phenotypical feature; hyperandrogensim, oligomennorhea/amenorrhea, and PCOS on ultrasound. They found significant differences in prevalence of PCOS in the urban and rural regions. While the

prevalence in the urban region was 71 percent, they found the prevalence in the rural region to be much lower, at 29 percent.

Akram, M. et. (2015) estimated the prevalence of PCOS in Pakistani population to be around 52 percent. Their study conducted in Lahore focused on the hormonal variations and their interactions in a cohort study.

Baqai Z. et al. (2010) carried out a study in the Pakistani metropolitan city of Karachi. They measured prevalence of PCOS in women that presented to the outpatient department for fertility concerns. Out of the 1210 patients assessed, 40 percent were diagnosed with PCOS using the Rotterdam criteria (2003) as a diagnostic tool.

Sidra, S. et al. (2019) in their study evaluated clinical manifestations and health risk of PCOS patients in Pakistan. They showed the percentage prevalence of the various Rotterdam criterion, as well as obesity among the PCOS patients of Lahore and Islamabad. They concluded that obesity (80%) was highly prevalent in PCOS patients. The also noted that quality of life of patients with PCOS was highly affected.

Zhao, Y., & Qiao, J. (2013) in their review highlighted the ethnic variations in prevalence as well as phenotypical presentation of PCOS. They concluded that ethnicity specific guidelines should exist in order to diagnose and treat PCOS

Lizneva, D. et al. (2016), demonstrated in their study that there are geographic variations of PCOS prevalence worldwide. The study estimated PCOS prevalence is to be between 2% and 26%. The differences in diagnostic criteria, prevalence of influential risk factors, sample heterogeneity, socioeconomic level, medical care access, health and education/awareness, were responsible for the geographic differences in the prevalence rate.

Lizneva et al. (2016) and Azziz R et al. (2006) have done valuable work in attempting to discover the pathophysiology of the disease. There is suspected involvement of PCOS genes in modulation of gonadotropin and neuroendocrine action, insulin action, and ovarian androgen biosynthesis. This points towards the potential evolutionary advantages and evolutionary path changes in PCOS as reviewed by (Azziz R et.al, 2006).

Teede H. J. Et al. (2018) in their study showed that since PCOS is characterized by a range of symptoms; irregularity of cycle, hirsutism, acne; and is characterized by a range of metabolic disturbances such as insulin resistance, and dyslipidemia. Thus there is a need for a more extensive assessment and management criteria for PCOS.

Azziz, R. et al. (2006) reviewed all available data linked to PCOS and presented the revised Rotterdam criteria for PCOS diagnosis to the AES. They concluded that PCOS diagnosis should also include exclusion of other disorders that the patient might be suffering from. Thus it was concluded that presence of 2 of three features namely; hyperandrogensim, oligo-ovulation/anovulation, and polycystic ovaries were not a reliable criteria, and that exclusion of other related disorders was pertinent.

Goldzieher et al. (1963) in their study mentioned the additional features of PCOS, these include hirsutism, obesity, acne, glucose intolerance, hyperinsulinemia, insulin resistance, diabetes mellitus type II, Luteinizing hormone (LH) hypersecretion, infertility, metabolic disturbances, and dyslipidemia. Cardiovascular events, uterine cancer, and psychological disorders including depression are long-term complications of PCOS.

Fauser, B. C. et al. (2012) documented in the third PCOS consensus worshop that there are differences in PCOS symptomology with respect to ethnicity, age, insulin resistance and BMI. Their aim was to identify gaps in knowledge and their work focused on adolescence, acne and hirsutism, contraceptives, menstrual disorders, quality of life, ethnicity, pregnancy complications, long-term metabolic and cardiovascular health, and also risk of cancer.

Huang Z., & Young E. (2016) in their study reported that phenotypically, the severity of various PCOS symptoms shows a high variability. They showed that differences in phenotypic presentation between Caucasians and east Asians. Caucasians tend to have more severe hirsutism in comparison to East Asians. While PCO morphology is more prevalent in east Asians compared to Caucasians. Their study has thus suggested that the hirsutism cutoff value for east Asians should be lower than Caucasians in the diagnosis of PCOS.

Glintborg D. et al. (2010) in their study evaluated ethnic differences in the Rotterdam criteria. They compared Caucasians, middle eastern women and Asians. They concluded

that Caucasian women had worse cardiovascular profile compared to Middle Eastern women, and they noted that their insulin sensitivity was higher. The prevalence of the individual Rotterdam criteria showed significant differences especially between the Caucasians and middle eastern women.

Kazemi, M. et al (2022) in their review explored differences in presentation of PCOS women of Hispanic and white ethnicities. They found that Hispanic women were more hirsute and had higher degree of insulin resistance compared to white women.

Zhao, Y. & Qiao, J. (2013) in their review gave evidence of ethnic variation in the expression of PCOS phenotypes. They showed that east Asian women were less hirsute and had lower BMI scores, however they were at high risk of developing metabolic syndrome. South Asians had higher BMI scores with central obesity being more prevalent in PCOS women, and they also had more insulin resistance and risk of diabetes mellitus. The study found that black and Hispanic women had highest BMI, and were also more prone to metabolic syndrome. Hirsutism was more prevalent in middle eastern women. Thus making it apparent that while assessing patients for PCOS a more detailed ethnicity based criteria is warranted.

Engmann et al. (2017) in their study found that Hispanic women differed in the clinical presentation of PCOS. Hispanic women had higher degrees of metabolic aberrations and hyperandrogenism compared to non-Hispanic women, thus further demonstrating the ethnic variations in the presentation of PCOS. Their study also showed that black women had increased levels of fasting insulin which pertains to more severe insulin resistance compared to white females.

Tata, B. et al. (2019) in their cohort study demonstrated that pregnant women with PCOS had higher levels of AMH compared to pregnant controls without PCOS. They then used a mice model to show that when mice foetuses were exposed to higher AMH levels in the womb, the female mice then grew up and started to exhibit PCOS symptoms in the reproductive years. This study demonstrated a likelihood of neuroendocrine disturbances that were caused by raised AMH levels in the womb, as an etiology of PCOS.

Vink, J. M. et al. (2006) showed in that PCOS has a strong genetic component. Their

twin study estimated a 72 percent variance risk in the development of PCOS. This study indicated that PCOS has a strong heritability

.

Afzal T. et al. (2021) in their study pointed out that while genetic factors may play a crucial role in the pathogenesis of PCOS, inheritance in PCOS is neither autosomal dominant or monogenic as previously theorized. Inheritance in PCOS is X-linked polygenic.

Dapas, M., & Dunaif, A. (2022) have studied the genetic basis of PCOS in their review. Their take away is that it is a heritable and complex trait in which roughly 20 common susceptibility variants from genome-wide association studies have been identified. Less common variants in DENND1A, AMH, and AMHR2 have also been identified by nextgeneration sequencing. They also showed that epigenetic features also have an effect on the phenotypical features seen in PCOS.

Khan, M. J. et al (2019) highlighted that PCOS is a multifactorial disease and can be caused by a number of genetic abnormalities. They have inferred that all genes/mutations that affect ovaries whither directly or indirectly are associated with the syndrome. They have summarized the various genes involved in the development of PCOS and have then discussed all of these genes in extensive detail.

Gorsic L. K. et al. (2019) have examined the genetic influence on AMH levels in women with and without PCOS. They identified several genetic loci associated with AMH levels, including variants near the AMHR2 and AMH genes. Since these gene loci showed a stronger association in women with PCOS, it shows that there is a potential link between genetic predisposition to PCOS and AMH gene regulation.

Hayes et al. (2015), Zeber-Lubecka, N. (2021), & Shi, Y. (2012) have focused on GWAS and revealed genetic variants in the THADA gene that is linked to PCOS susceptibility as well as elevation in the level of AMH. THADA is involved in ovarian development and has been implicated in PCOS pathogenesis. This link of PCOS and THADA strengthens the theorized notion that etiology of PCOS in genetic based.

McAllister et al. (2014) in their study showed that DENND 1A isoform overexpression resulted in a PCOS theca phenotype. The study pointed out that cause of PCOS and

selection of PCOS genes pointed towards a direct genetic association in PCOS. They also showed that some subjects did not demonstrate symptoms during their reproductive years. Confirmation of PCOS is therefore based on radiographic, biochemical, and physical evaluations along with the medical history of the patients.

Pellatt et al. (2007) discussed the anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting hormone (MIH). As given in the study, it is produced by granulosa cells of the pre-antral and small antral follicles in women until menopause.

Kollmann et al. (2015) have written in their study that regulation of folliculogenesis is done by production of AMH, as AMH inhibits recruitment of follicles from the resting pool, thus promoting growth of a single dominant follicle

Depmann, M. et al. (2016) showed that fluctuations in the AMH levels cause fluctuations in the antral follicle count which in turn could present as a phenotypical presentation of polycystic ovaries on ultrasound. Thus pointing that AMH could be used as a marker to diagnose PCOS.

Scheffer, J. B. (2017) has argued that AMH levels are difficult to measure and require sound technique. Also, AMH levels are not a reliable marker for condition such as PCOS due to its fluctuation in other related disorders.

Pigny P. et al. (2006) showed that AMH levels correlated positively with the number of ovarian follicles and the severity of PCOS. The higher the levels of AMH the higher the severity of PCOS. Thus inferring that patients with higher levels of AMH needed to be assessed and treated meticulously.

Li, H. W. et al. (2011) showed that AMH levels are elevated in PCOS regardless of age and BMI, pointing out that AMH could serve as an important marker in the identification of PCOS

Nardo, L. G., (2009) concluded from their study that AMH levels are affected by insulin resistance and androgens in women with and without PCOS. However since insulin resistance and hyperandrogensim are key features of PCOS there is ambiguity. This relationship is independent of age. However, an indirect causal effect that results due to

increasing age cannot be ruled out. Excessive granulosa cell activity seems to be responsibe in the abnormal follicular development seen in PCOS

Zhao, H. et al. (2023) in their study on Chinese women found that AMH levels had a negative correlation with BMI; that is, the higher the BMI, the lower the level of AMH. However PCOS women with obesity still showed higher levels of AMH compared to matched controls without PCOS. Their study also showed that higher HOMA-IR scores were linked to higher levels of AMH. And that

Barber, T. M. et al. (2015) in their article discussed that increased BMI was linked to development of PCOS in genetically predisposed women. The article discussed that metabolic disturbances that result due to weight gain were responsible for the exacerbation of symptoms and poor fertility outcomes. They recommended dietary and lifestyle changes in obese women with PCOS.

Hirchberg A. L. et al (2009) also found that increased BMI is linked with decreased levels of AMH, and discussed impact of increased weight on fertility outcomes in women with PCOS.

Sahmay S. et al. (2013) compared AMH levels in the various phenotypes of PCOS. They found that AMH levels were highest when all three features were present and were lowest in the group that did not have polycystic ovarian morphology on ultrasound.

Homburg, R. et al (2013) indicated changes in AMH levels in PCOS, PCOM and normal females. They inferred that insulin resistance can lead to higher androgen levels which in turn can lead to elevated levels of AMH

Legro. R. S. et al (2013) added that even adipose tissues have a role in secreting hormones that alter ovarian function, thereby resulting in alterations in the levels of AMH

Kloos, J., Coyne, K., & Weinerman, R., (2022) in their review documented the various studies exploring the effect of BMI on obese women with PCOS. They showed many studies with satitistically insignificant correlation of AMH with BMI. It should be noted however, that most studies that showed insignificant results had more participants falling into the overweight but not obese category. And the comparison made was between

overweight and normal BMI subjects. Thus it can be concluded that only obese females with PCOS that have a BMI of >35 exhibit statistically significant differences in AMH levels when compared to overweight/normal BMI women with PCOS. AMH levels were found to correlate negatively with AMH.

Silva M. et al. (2021) in their review reported novel AMH actions across the HPG axis. They highlight the effect and involvement of AMH in the reproductive system. Thus highlighting how AMH can be used for diagnosis and assessment of reproductive disorders. They have pointed out that AMH levels were predominantly affected by LH levels, and not FSH levels. LH can raise AMH levels by 400 percent. Additionally, the mechanisms that underlie the relationship between the LH to FSH ratio and AMH levels in PCOS are multifactorial. LH is known to stimulate androgen production in the ovaries, leading to excess androgen levels in PCOS patients

Dumont, A. et al. (2015) in their review discussed that androgens have been shown to upregulate AMH production in ovarian granulosa cells.

Zhao, H. et al (2023) in their review discussed that FSH is not correlated with AMH as AMH is secreted in the LH dependent stage of the cycle, thus there is no effect of FSH levels on AMH.

Pellatt, L. et al. (2011) and Crespo, R. P. et al. (2018) documented that AMH plays a paracrine role in order to inhibit FSH-induced aromatase activity in granulosa cells that aids in the development of the dominant follicle. Thereby contributing to PCOS symptomology.

Azziz, R. et al. (2016) have shown in their study that higher AMH levels in PCOS makes follicles resistant to FSH action, causing inhibition of follicular maturation and ovulation; inhibition of aromatase expression; and consequently, results in hyperandrogenism.

Piltonen, T. et al. (2005) showed that in a cohort of PCOS patients, LH to FSH ratio and AMH levels revealed a positive correlation. Indicating that a higher LH to FSH ratio is associated with increased AMH production.
Asanidze et al. (2018) in their study, studied patients of PCOS with and without insulin resistance; they attempted to find a correlation of AMH with hormonal and ovarian

morphological characteristics in both groups of PCOS patients. The study found that there is a correlation between ovarian morphological and hormonal characteristics and AMH. Positive correlation between volume of the ovaries and AMH was shown. PCOS patients with insulin resistance had a positive correlation between AMH and the volume of ovary, and antral follicular count. A negative correlation between serum AMH and SHBG (Serum hormone binding globulin) was also shown. PCOS patients without insulin resistance also showed a positive correlation between AMH and the volume of ovary, as well as positive correlation between AMH and AFC, and HOMA-IR. Increased levels of AMH was seen in all PCOS patients. The study showed no significant difference in AMH levels in patients of PCOS with, and without insulin resistance.

Sahmay, S. et al. (2013) in their study measured AMH levels in the various PCOS phenotypes. They inferred that Insulin resistance (IR) is also correlated with levels of AMH in PCOS patients. Higher levels of AMH in the circulation alter the function of the pancreatic cells; result in hyperinsulinemia (as release of AMH by the granulosa cells through uptake of androgens) can lead to hyperinsulinemia.

Abbara et al. (2019) in their study ventured to access the diagnostic role of anti-Mullerian hormone (AMH) in PCOS. The study found that serum AMH could be useful as a biomarker to diagnose PCOS related menstrual disturbances. AMH levels of >60 pmol/L were linked to more severe menstrual disturbances in comparison to those with an AMH < 15 pmol/L. Women with the higher AMH had increased number of features of PCOS as well. This showed that AMH could be used for diagnosis. their work further showed that AMH was better at discriminating women with menstrual disturbance from those with regular menstrual cycles than antral follicular count (AFC). The study notes that if both the markers, AMH and AFC are used in combination, then the discrimination is increased compared to when either of them is being measured alone. The study further added that serum AMH was higher in women who presented with all the symptoms of the PCOS triad (menstrual disturbance, hyperandrogenism, polycystic ovarian morphology) in comparison to those women who presented with none of these triad symptoms. The odds of menstrual disturbance were increased by 10.7 times in women with bilateral polycystic morphology ovaries in comparison to those with normal ovaries.

Body mass index was found to be a stronger predictor of free androgen index (FAI) than either anti-Mullerian hormone (AMH) or antral follicular count (AFC)

Sivanandy, M. S., & Ha, S. K. (2023) in their review assessed studies that evaluated AMH levels in PCOS patients in order to determine the effectiveness of AMH levels in PCOS diagnosis. Their review evaluated AMH alone and AMH as a replacement for each criteria present in the Rotterdam criteria. They concluded that an AMH level of greater than 4.1ng/mL could be used to replace one criterion of the Rotterdam criteria to reach a conclusive diagnosis of PCOS. They found that the AMH cutoff for replacement of the hyperandrogenism criteria needs to be higher in order to increase specificity and sensitivity of AMH levels as diagnostic tools in PCOS

Unuane D. et al. (2017) in their work on endocrine disorders and female infertility, showed an association between AMH and PCOS, and how this relationship is impacted by other comorbidities such as hypothyroidism. Hypothyroidism is a common condition in PCOS patients, affecting up to 25% of women with PCOS

Haugen et al. (2016) studied hypothyroidism prevalence. The prevalence ranges from 0.33.7% according to the definition used and the population studied. According to this study, the main cause of hypothyroidism worldwide is still iodine deficiency. The study states that, in iodine-sufficient regions, primary hypothyroidism can be attributed to congenital or acquired causes.

Utiger et al. (2021) have concisely discussed hypothyroidism etiology. The study discusses that thyroid gland disorders result in hypothyroidism termed as primary hypothyroidism. Non development of the thyroid gland, its abnormal development, or inherited defects in the synthesis of thyroid hormone are the conditions responsible for congenital primary hypothyroidism. They also discussed that acquired primary hypothyroidism etiology is linked with chronic autoimmune thyroiditis (inflammation of the thyroid). It has two forms; Hashimoto thyroiditis, characterized by presence of goiter (enlargement of the thyroid). The other division of autoimmune thyroiditis is atrophic thyroiditis, in this type the thyroid gland is reduced in size.

De Medeiros, S.F. et al (2018) in their review suggested that due to differences in PCOS patients with and without subclinical hypothyroidism, the diagnosis of PCOS should exclude patients with subclinical and overt hypothyroidism.they have also discussed in detail the pathophysiology of thyroid disorders in their review.

Mechanick et al. (2011) discussed management of central hypothyroidism, which is yet another form of hypothyroidism. This condition is characterized by deficiency of the anterior pituitary gland hormone thyrotropin, also known as TSH (thyroid stimulating hormone) that controls the thyroid. Etiology is pituitary disease resulting in deficiency of TSH, or the lack of hypothalamic hormone thyrotropin-releasing hormone which in turn maintains thyrotropin secretion. The treatment modality according to this study remains L-thyroxine.

Garber et al. (2012) conducted a study to establish the guidelines for management of hypothyroidism, their findings were based on 52 evidence-based recommendations and sub-recommendations. The study concluded that serum thyrotropin (TSH) is the most reliable screening tool for primary thyroid dysfunction. A value of >4.2mIU/Lis considered as indicative of hypothyroidism. The standard treatment is replacement, according to the findings of this study remains treatment with L-thyroxine. The study recommends that subclinical hypothyroidism treatment when the serum thyrotropin is <10mIU/L needs to be done according to the needs of the individual patient.

Carmina E., et al. (2005) in their study discussed that thyroid hormones play an important role in the regulation of the menstrual cycle; follicular development; and ovulation. They took the various phenotypes of PCOS, namely classic, ovulatory and idiopathic hyperandrogensim and compared the BMI and insulin resistance among these groups. They found that BMI and insulin resistance was highest in the classic PCOS phenotype.

Wu et al. (2021) in their study have explored the relationship between ovarian reserve and hypothyroidism. However, this study failed to find any link between the two.

Janssen et al., (2004) in their study on chinese women demonstrated increased presentation of thyroid autoimmunity in patients with PCOS. Their study showed that higher thyroid antibody levels, larger, more hypoechogenic thyroids were observed in

females with PCOS when compared to controls. Showing that PCOS patients tend to have higher chances of developing more severe thyroid disease

Garelli et al. (2013) in their study showed that thyroid peroxidase (TPO) antibodies were seed in 27% of the PCOS patients in comparison to 8% in normal matched controls.

Raj et al. (2021) in their study demonstrated significant association of SCH (subclinical hypothyroidism) in women with PCOS in comparison to their normal matched controls. The results of this study bring to light the clinical implication of maintaining vigilance for signs and symptoms of SCH and creating awareness among women with PCOS in order to enhance their reproductive and clinical pregnancy outcomes.

Sinha et al. (2013) conducted a cross sectional study in eastern India. This study revealed statistically significant higher prevalence of raised anti-TPO antibody levels, the levels of which rise in autoimmune thyroiditis. In the study, mean TSH level was higher in PCOS patients compared to healthy matched controls. Rise in goiter presence among PCOS patients was also observed. Thyroid Ultrasonography revealed that a significantly higher percentage of PCOS patients had hypoechoic ultrasonography pattern which is relevant for the diagnosis of autoimmune thyroiditis.

Kucukler, F. et al. (2019) evaluated levels of AMH in subclinical hypothyroidism and found significantly lower AMH levels compared to euthyroid women. The same study reported significantly reduced AMH levels in overtly hypothyroid women compared to euthyroid controls. They also showed that hypothyroidism has a negative impact on AMH levels, and PCOS patients have a higher AMH level compared to controls that are age and BMI matched.

Kuroda et al. (2015) in their study explored the relationship of serum thyroid-stimulating hormone with anti-Müllerian hormone (AMH) in infertile women of reproductive age. The study found that thyroid-stimulating hormone (TSH) levels was negatively correlated with AMH levels in infertile patients, but not in normal fertile women. They found that decrease in circulating thyroid hormones does effect fertility and thus serum TSH has been found to have a negative correlation with serum AMH levels. They also mentioned that that after levothyroxine treatment in hypothyroid women, AMH levels significantly increased

Osuka, S. et al. (2018) discussed that when thyroid hormone levels are inadequate due to hypothyroidism, it may negatively impact the quantity and quality of ovarian follicles, consequently affecting AMH production.

Hasegawa, Y. et al. (2021) in their study examined the relationship between age and AMH levels in women with hypothyroidism. The results showed a negative correlation between age and serum AMH levels, indicating a decline in ovarian reserve with advancing age in hypothyroid patients.

Adamska, A. et al. (2021) in their study examined the relationship between BMI and AMH levels in women with hypothyroidism. The findings indicated that higher BMI was associated with lower serum AMH levels in hypothyroid patients, suggesting a negative impact of increased body weight on ovarian reserve.

Sun, F. et al. (2018) studied specific genetic variations related to thyroid function in women with subclinical hypothyroidism and overt hypothyroidism. The results revealed that specific genetic variants, including those in the TSHR and DUOX2 genes, were associated with altered thyroid function. These findings suggest that genetic factors related to thyroid function may contribute to variations in AMH levels even in the subclinical hypothyroidism population.

Azziz, R. et al. (2004) in their study on androgen excess in women discussed that thyroid hormones tend to interact with sex hormones, estrogen and progesterone, which are involved in the pathogenesis of PCOS

Diamanti-Kandarakis E., & Dunaif A., (2012) in their review on insulin resistance in PCOS discussed that insulin resistance, which is a hallmark of PCOS, has been implicated in the development of hypothyroidism as well.

Brenta, G., (2011) in their study on evaluation of hypothyroidism and its link in the development insulin resistance showed that hypothyroidism can influence insulin resistance and glucose metabolism, potentially exacerbating insulin resistance in women with PCOS.

Serin A. N. Et al. (2021) in their study showed that AMH levels in patients with PCOS as well as Hashimoto’s thyroiditis were lower than AMH levels of patients with PCOS alone.

Al-Jaff (2018) concluded in her study that hypothyroidism is accompanied low levels of anti-Mullerian hormone. Hypothroid + PCOS group showed low AMH levels compared with PCOS group (7.034 ± 3.282 vs 11.871 ± 0.495), her study showed that antiMullerian hormone does not present a reliable picture of the condition of the ovaries in polycystic ovarian syndrome (PCOS) in cases with thyroid abnormalities. Her study, like many other studies mentioned that suffering from PCOS for longer period may cause Goiter in the instances where the luteinizing hormone (LH) level is high.

Operational Definitions

1. Polycystic ovarian syndrome (PCOS): diagnosed according to the Rotterdam criteria which states that presence of two out of three of the following is diagnostic of PCOS, namely, Oligo/anovulation, Hyperandrogenism (clinically, hirsutism or less commonly male pattern alopecia), and polycystic ovaries on ultrasound (Azziz et al., 2006).

2. Hypothyroidism: is diagnosed based on TSH (thyroid stimulating hormone) levels greater than 4.2mIU/L (Garber et al., 2012)

3. Anti-Mullerian hormone: A typical level for a fertile woman is between 1.0–3.8 ng/ml; under 1.0 ng/ml is considered low and indicative of a diminished ovarian reserve (Shebl et al., 2011). A level above 3.8ng/mL is indicative of PCOS (Sahmay, S. et al., 2014)

4. Ovarian cancer: National Cancer Institute (NIH) defines it as cancer that forms in tissues of the ovary. Most ovarian cancers are either ovarian epithelial cancers (cancer that begins in the cells on the surface of the ovary) or malignant germ cell tumors (cancer that begins in egg cells) (Bethesda, 2007)

5. Diabetes: it is recommended that An HbA1c of 6.5% is the cut point for diagnosing diabetes (Florkowski, 2013).

6. Endometriosis: World Health Organization defines it as a disease characterized by the presence of tissue resembling endometrium (the lining of the uterus) outside the uterus. (Kreuger et al., 2017)

7. Thyroid dysfunction: Thyroid disease is a general term for conditions that prevent the thyroid gland from making the right amount of hormones (Rugge et al., 2014).

8. Smoking: National Health Interview Survey defines smoking status as a recoded variable based on several questions about cigarette smoking. It includes the categories of current smoker, former smoker, never smoked, and smoking status unknown. (Adams, 2022)

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN:

Case-control study

3.2 SUBJECTS:

84 females of reproductive age (between 15-49 years)

3.3 SETTING:

National Medical Centre (NMC), a tertiary care hospital in Karachi, Pakistan

3.4 INCLUSION CRITERIA:

- Case group; females with PCOS as well as hypothyroidism (n=42)
- Female gender
- Between 18-49 years of age
- Diagnosed with PCOS (according to Rotterdam criteria,2003)
- Diagnosed with Hypothyroidism (TSH>4.2mIU/L)

- Control group; females with PCOS only (n=42)
- Female gender
- Between 15-49 years of age

- Diagnosed with PCOS (according to Rotterdam criteria)
- Hypothyroidism excluded by (TSH <4.2mIU/L)

3.5 EXCLUSION CRITERIA

- Ovarian tumor
- Endometriosis
- Ovarian surgery
- Disorders of liver
- Disorders of kidney
- Diabetes
- Undergoing treatment for cancer
- Treated thyroid dysfunction
- Smoking

3.6 DURATION OF STUDY

Total period of study: 6 months

3.7 SAMPLE SIZE ESTIMATION:

Sample Size for Unmatched Case-Control Study

For:

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	1
Hypothetical proportion of controls with exposure	20

Hypothetical proportion of cases with exposure:	52
Least extreme Odds Ratio to be detected:	4.33

	Kelsey	Fleiss	Fleiss with CC
Sample Size – Cases	36	35	41
Sample Size – Controls	36	35	41
Total sample size:	72	70	82

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-

15

Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19

CC = continuity correction

Results are rounded up to the nearest integer.

Print from the browser menu or select, copy, and paste to other programs.

3.8 SAMPLING TECHNIQUE:

Convenience sampling

3.9 HUMAN SUBJECTS AND CONSENT:

84 human subjects (42 case and 42 controls); informed written consent was taken from each participant. Forms in English and Urdu attached as annexure ‘C’

3.10 MATERIALS USED:

- Subject evaluation form; attached as annexure ‘D’
- ELISA kits
- The following lab equipment were used for the completion of the current study:

1. Ultra Low Temperature freezer
2. Eppendorf tubes
3. Centrifuge machine
4. Micropipette
5. Incubator
6. ELISA reader

Refer to figures: 3.1-3.5

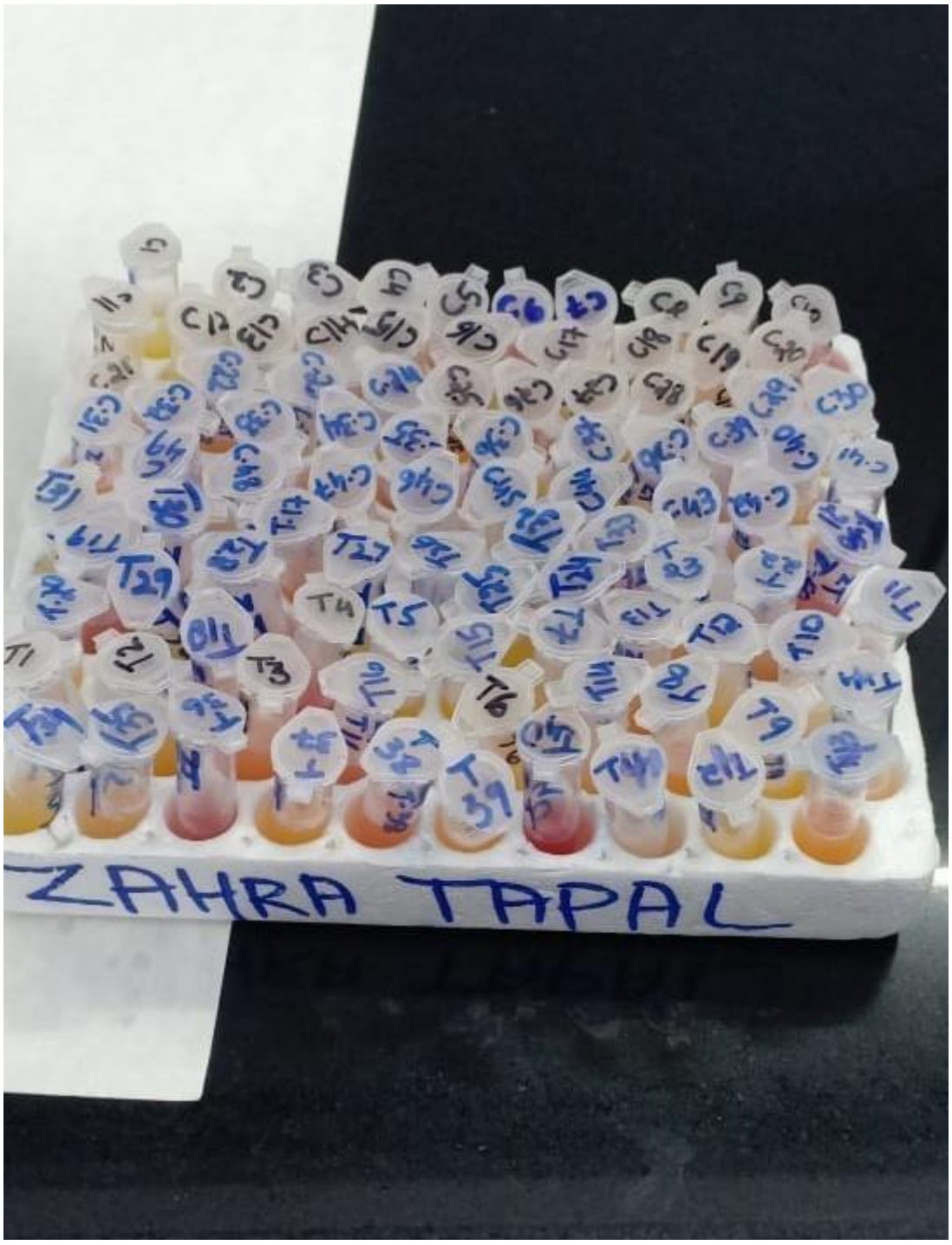

Figure 3.1: Eppendorf tubes with samples

Figure 3.2: centrifuge machine

Figure 3.3: incubator

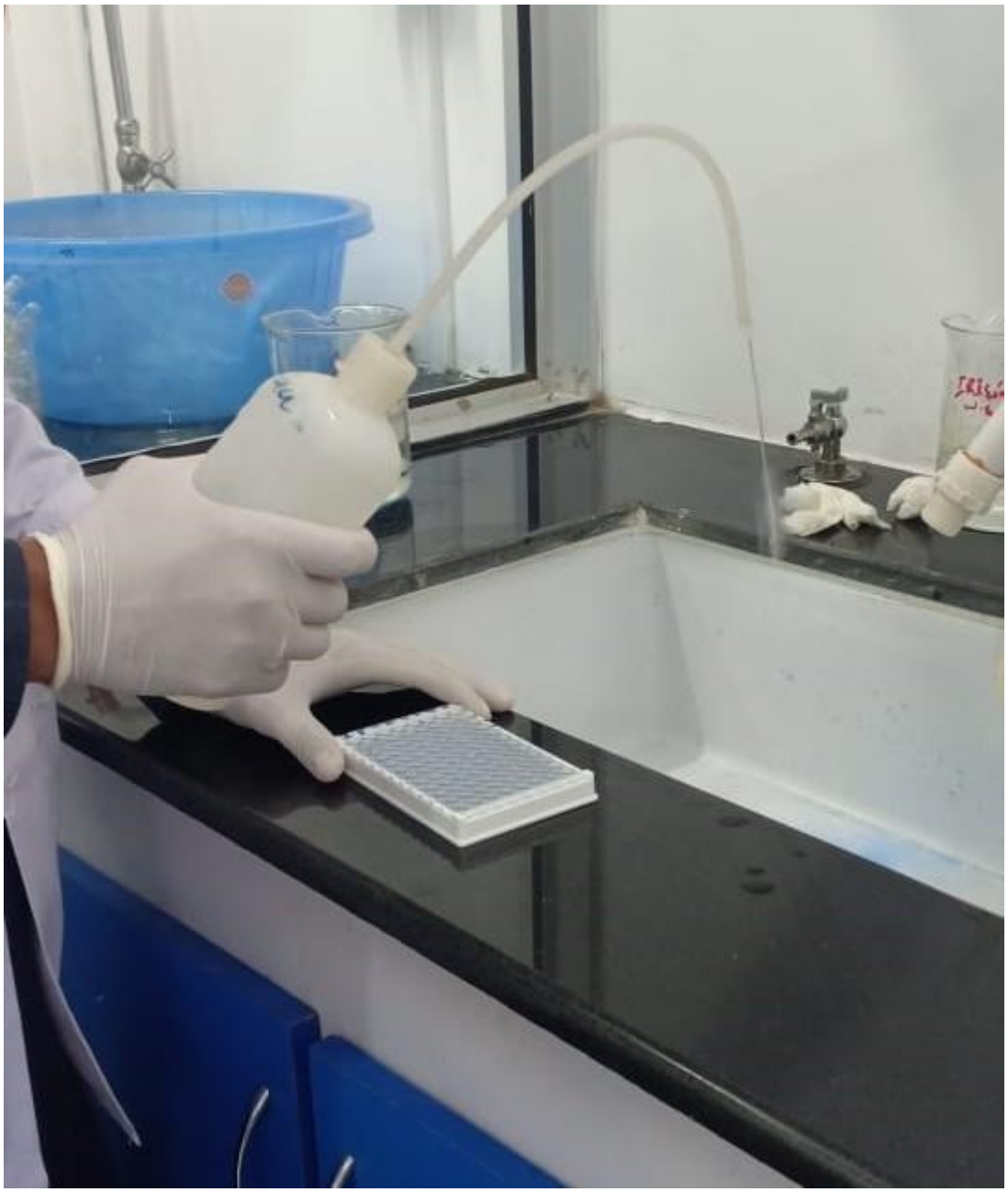

Figure 3.4: Washing of well plate

Figure 3.5: well plate before addition of stop solution

3.11 PARAMETERS OF STUDY

General parameters include:

- Age
- Weight
- Height
- BMI
- Marital status
- Duration of infertility (if married)
- Temperature at the time of presentation
- Pulse rate
- Blood pressure

PCO parameters, these include data for the following:

- Regularity of cycle
- Hirsutism
- Polycystic ovaries on ultrasound
- FSH
- LH
- FSH:LH

Study parameters include the following:

- Serum AMH
- Serum fasting insulin
- Serum TSH

3.12 PROTOCOL / PROCEDURE OF STUDY After ethical approval from institute, and consent from participants, 84 participants at the national medical center hospital (NMC) were selected and divided into two groups. The selection of participants was made on basis of subject evaluation form; which was filled out by the researcher in the presence of the participant. The first group containing the case subjects were

identified. These were the participants that met the inclusion criteria for the case subjects; PCOS as well as hypothyroidism.
The second group containing the control subjects was identified. These subjects met the inclusion criteria for control group; PCOS only.

Each participant was asked to give a blood sample. The blood sample was collected by venipuncture at the national medical center by their trained laboratory staff following all SOPs. The blood whole sample was transported to the Multidisciplinary Research Lab (MDRL). The whole blood was allowed to clot by leaving it undisturbed at room temperature for 30 minutes. The sample was then centrifuged at 2,000 x g for 10 minutes. Following centrifugation, serum was transferred to a clean eppendorf tube using a pipette. The serum was then stored in a -20 degree freezer.

Once sample collection was complete, the samples were ready to be evaluated. The samples were then thawed. And the following kits were used for measurement:

- Human Insulin ELISA kit
- Human Mullerian Inhibiting Substance/Anti-Mullerian hormone, MIS/AMH BT-LAB kit
- Fish Thyroid Stimulating Hormone, TSH ELISA Kit

3.12.1 HUMAN INSULIN ELISA KIT:

Principle of the test

The Calbiotech Inc., Insulin ELISA is based on solid phase sandwich ELISA method. The samples and conjugate reagent (anti-Insulin biotin & HRP) were added to the wells coated with Streptavidin. Insulin in the serum of the patient binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate got washed off, through a washing step. Upon addition of the substrate, the intensity of color was proportional to the concentration of Insulin in the samples. A standard curve was then prepared by relating the color intensity to the concentration of Insulin.

Components of the kit (96 Tests)

1. Microwells coated with Streptavidin 12x8x1
2. Insulin Standards: 6 vials (ready to use) 0.5 ml
3. Insulin Conjugate Reagent: 1 bottle (ready to use) 12ml
4. TMB Substrate: 1 bottle (ready to use) 12 ml
5. Stop Solution: 1 bottle (ready to use) 12 ml 6. 20X Wash concentrate: 1 bottle 25ml

Stability

The kit was stored at 2 - 8C and the microwells were sealed in a dry bag with desiccants.
The reagents were not exposed to heat, sun, or strong light.

Specimen collection and handling

Prior to assay, the frozen sera were completely thawed and mixed well, the grossly lipemic specimens were avoided.

Reagent preparation

1X wash buffer was prepared by adding the contents of the bottle to 475 ml of distilled water and was then stored at room temperature.

Assay procedure

1. Prior to assay, reagents were allowed to stand at room temperature and were gently mixed before use
2. The desired number of coated strips were places into the holder
3. 25 µl of Insulin standards, control and the sera of patients were pipetted into appropriate wells.
4. 100µl of Insulin Conjugate Reagent was added to all wells and was then incubated for 60 minutes at room temperature (20-25C).

5. Liquid was removed from all wells, and these wells were then washed three times with 300 µl of 1X wash buffer, followed by blotting on absorbent paper towels.
6. 100 µl of TMB substrate was added into all wells, followed by further incubation for 15 minutes at room temperature.
7. Finally 50µl of stop solution was added to all wells followed by a gentle shake of the plate to mix the solution.
8. Absorbance was read on ELISA Reader at 450 nm within 15 minutes after adding the stop solution. Standard Curve was used to calculate the readings.

Note: The empty well plate was read using the ELISA reader to check absorbance (pretest absorbance). After the experiment, the post-test absorbance was read and the final absorbance was calculated by subtracting post-test absorbance from pre-test absorbance.

Before the standard curve was plotted, the absorbance of the blank was subtracted from all values so that the concentration at 0 absorbance read as 0 concentration.

3.12.2 HUMAN MULLERIAN INHIBITING SUBSTANCE/ANTI-MULLERIAN HORMONE, MIS/AMH ELISA KIT

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been precoated with Human MIS antibody. MIS present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human MIS Antibody is added and binds to MIS in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated MIS antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human MIS. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Components

- Standard solution: (24ng/ml) 0.5ml x1
- Pre-coated ELISA plate: 12 * 8 well strips x1

- Standard diluent: 3ml x1
- Streptavidin-HRP: 6ml x1
- Stop solution: 6ml x1
- Substrate solution A: 6ml x1
- Substrate solution B: 6ml x1
- Wash buffer Concentrate (25x): 20ml x1
- Biotinylated Human MIS antibody: 1ml x1
- User instruction: 1
- Plate sealer: 2 pics

Stability

The kit was stored at 2 - 8 C and the microwells were sealed in a dry bag with desiccants. The reagents were not exposed to heat, sun, or strong light

Standard curve range: 0.05 – 15ng/ml **Sensitivity**: 0.01ng/ml

Specimen collection and handling

Prior to assay, the frozen sera were completely thawed and mixed well, the grossly lipemic specimens were avoided.

Reagent preparation

- All reagents were brought to room temperature before use.
- Standard Reconstitute was made with 120ul of standard (16ng/ml) with 120ul of Standard/Sample diluent to generate an 8ng/mL standard stock solution. The standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Dublicate standard points were prepared by serially diluting the standard stock solution 1: 2 with diluent to produce 4ng/mL, 2ng/ml, 1ng/mL, and 0.5ng/mL solutions. Standard/Sample diluent alone was added as the blank (0ng/mL).
- 20ml of 25x concentrated wash buffer was diluted with double distilled water to prepare 500 ml of wash buffer. If crystals formed in the concentrate, it was warmed in a 40°C water bath and mixed gently until the crystals completely dissolved.

Assay procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. The number of strips required for the assay were determined. The strips were inserted in the frames for use.
3. A blank well was set without any solution.
4. 50ul standard was added to the standard well.
5. 40ul sample was added to sample well, then 10ul anti-MIS antibody was added to the sample well, then 50ul streptavirdin-HRP was added to sample wells (not the blank control well) and it was mixed and covered with sealant. It was incubated for 60 minutes at 37C.
6. After incubation, the sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels..
7. 50ul substrate solution A was added to each well and then 50ul substrate solution B was added to each well. It was then mixed well. Plate covered with a new sealer was incubated for 10 minutes at 37°C in the dark.
8. 50ul stop solution was added to each well, the blue color changed to yellow immediately.
9. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 15 minutes after adding the stop solution.

Note: The empty well plate was read using the ELISA reader to check absorbance (pretest absorbance). After the experiment, the post-test absorbance was read and the final absorbance was calculated by subtracting post-test absorbance from pre-test absorbance. Before the standard curve was plotted, the absorbance of the blank was subtracted from all values so that the concentration at 0 absorbance read as 0 concentration.

3.12.3 FISH THYROID STIMULATING HORMONE, TSH ELISA KIT

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). Add samples to the precoated plate. Then add biotinylated antigen. The antigens in the samples compete with the biotinylated antigen to bind to the capture antibody and incubate. Unbound antigen is washed away during a washing step. An avidin-HRP is then added and then incubate. Unbound avidin-HRP is washed away during a washing step. TMB Substrate is then added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of TSH in the sample. The concentration of TSH in the sample is then determined by comparing the O.D. of the samples to the standard curve.

Components:

- Pre-coated plate (12 * 8 well strips x 1)
- Fish TSH Standard, lyophilized (2 vials)
- Standard/Sample diluent (6ml ×1 vial)
- Biotinylated antigen, lyophilized (1 vial)
- Avidin-HRP concentrate (100ul × 1 vial)
- Biotinylated antigen diluent (6ml x1)
- Avidin HRP diluent (5.9ml × 1 vials)
- Substrate solution A (6ml x1)
- Substrate solution B (6ml x1)
- Stop solution (6ml x1)
- Wash buffer ((25x) 20ml × 1 vial)
- Plate sealer (2 pics)

Stability

The kit was stored at 2 - 8 C and the microwells were sealed in a dry bag with desiccants. The reagents were not exposed to heat, sun, or strong light

Standard curve range: 0.3 - 90uIU/ml
Sensitivity: 0.13μIU/ml

Specimen collection and handling

Prior to assay, the frozen sera were completely thawed and mixed well, the grossly lipemic specimens were avoided.

Reagent preparation

- All reagents were brought to room temperature before use.
- Standard Reconstitute was made with one vial of standard with 150ul of Standard/Sample diluent to generate a 96μIU/mL standard stock solution. The standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Dublicate standard points were prepared by serially diluting the standard stock solution 1: 2 with diluent to produce 48μIU/mL, 24μIU/mL, 12μIU/mL, 6μIU/mL and 3μIU/mL solutions. Standard/Sample diluent alone was added as the blank (0μIU/mL).
- Biotinylated Antigen vial was briefly centrifuged then 1ml Biotinylated antigen diluent was added and mixed well. All this solution was pipetted back into the Biotinylated antigen diluent vial to mix well and generate a 6ml stock solution. This was allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- Low- speed centrifugation was performed on the avidin-HRP Concentrates solution and then all avidin-HRP was pipetted into the Avidin HRP Diluent vial. It was mixed well to generate a 6ml stock solution. This was allowed to sit for 10 minutes with gentle agitation prior to making dilutions.
- 20ml of concentrated wash buffer was diluted with 480ml double distilled water to prepare 500 ml of wash buffer. If crystals formed in the concentrate, it was warmed in a 40°C water bath and mixed gently until the crystals completely dissolved.

Assay procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. The number of strips required for the assay were determine. The strips were inserted in the frames for use.
3. A blank well was set without any solution.
4. 50ul negative control was added to each of the negative control wells and 50ul positive control was added to each of the positive control wells. 40ul sample diluent was added and then 10ul sample was added to the sample well, and it was mixed.
5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels.
6. 50ul HRP was added to each well (except blank well). It was covered with a plate sealer, and incubated for 30 minutes at 37°C.
7. The sealer was removed and washed as described above.
8. 50ul substrate solution A was added to each well and then 50ul substrate solution B was added to each well. It was then mixed well. Plate covered with a new sealer was incubated for 10 minutes at 37°C in the dark.
9. 50ul stop solution was added to each well, the blue color changed to yellow immediately.
10. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 15 minutes after adding the stop solution.

A standard curve was used to calculate concentrations.
Note: The empty well plate was read using the ELISA reader to check absorbance (pretest absorbance). After the experiment, the post-test absorbance was read and the final absorbance was calculated by subtracting post-test absorbance from pre-test absorbance. Before the standard curve was plotted, the absorbance of the blank was subtracted from all values so that the concentration at 0 absorbance read as 0 concentration

3.13 FLOW CHART/ ALGORITHM OF STUDY

84 participants selected at the National Medical Centre answered a questionnaire containing evaluation and history

Divided in 2 groups: Case and control

Both groups gave blood sample for measurement of AMH, Fasting insulin, and TSH

Test Kits used to measure the serum level of AMH, fasting insulin, and TSH

Entry of data into the SPSS software for analysis

Analysis of all parameters in case and control group done on SPSS software

Presentation of results

3.14 STATISTICAL ANALYSIS

The current study is a case control study based on a sample of 84 female patients, which has been equally divided into two groups (42 subjects in each group). Most of the statistical analysis is carried out comparatively among the case and control group. The statistical data analysis has been carried out descriptively as well as inferentially. The descriptive statistical analysis comprised on the frequency distributions, appropriate graphical presentation of the data, computing measures of central tendency and dispersion, finding out the crosstabs with appropriate percentages. Then inferential

statistics techniques of hypothesis testing are applied in order to test the mean differences among two or more than two groups, and to explore the bivariate linear relationships among the variables. The hypothesis testing methods used for the current study are two independent samples t-test, Mann-Whitney U test, Kruskal Wallis test, Correlation analysis and its significance along with regression analysis.

CHAPTER 4

RESULTS

A total of 84 patients were enrolled in this study. All 84 subjects completed the study. Females between the ages of 18-40 years were included in the study if they met the inclusion criteria for either case or control groups. Informed written consent was taken from each subject.

In order to ascertain differences in the AMH levels between cases and controls. The subjects were divided into two groups. Cases were subjects that were suffering from polycystic ovary syndrome (PCOS) as well as hypothyroidism. Controls were those that were suffering from PCOS alone. The diagnosis of PCOS was made using the Rotterdam criteria, 2003. The diagnosis of overt hypothyroidism was based on a TSH level higher than 4.2mIU/L.

The descriptive analysis of the data is based on frequency distribution of the demographic and other categorical variables along with their graphical representation by pie chart. However, descriptive statistics including mean, minimum, maximum and standard deviation are calculated for the quantitative variables along with the bar charts for average quantity. The frequency distributions and descriptive statistics are calculated separately for the control and case groups. Furthermore, the variables AMH, TSH and Fasting Insulin are also compared on the basis of age and BMI.

4.1 GENERAL PARAMETERS

These include data for:

- Age

- Weight
- Height
- BMI
- Marital status
- Duration of infertility (if married)
- Temperature at the time of presentation
- Pulse rate
- Blood pressure

4.1.1 AGE

It can be seen from the table 4.1 and figure 4.1, that 61.9% (n = 52) of the patients have ages between 18–29 years, whereas 38.1% (n = 32) of the patients have the ages between 30 – 40 years. Furthermore, it can be seen from the table 4.1 and figure 4.2 that in the control groups, 61.9% (n = 26) of the patients have ages between 18–29 years, whereas the 38.1% (n = 16) of the patients are aged between 30–40 years. Similarly, in the case groups, 61.9% (n = 26) of the patients are aged between 18–29 years, whereas 38.1% (n = 16) of patients are aged between 30–40 years. So, majority of the patients in both case and control groups are age matched, and younger.

It can be seen from the table 4.2 and figure 4.2 that age of the patients varies from a minimum of 18 years to a maximum of 40 years in the control group, whereas the age of patients varies from a minimum of 20 years to a maximum of 38 years in the case group. So, age range is wider for the control group as compared to the case group. The mean age for control group is 28.55 years with average variation of 5.72 years, whereas the mean age for the case group is 28.19 years with average variation of 4.70 years. So, average age is somewhat same for the both groups but case group have less variation than control group.

4.1.2 WEIGHT

It can be seen from the table 4.3 and figure 4.3 that weight varies from a minimum of 40 kg to a maximum of 104 kg in the control group, whereas the weight varies from a minimum of 40 kg to a maximum of 96 kg in the case group. So, weight range is wider for the control group as compared to the case group. The mean weight for control group is 71.21 kg with average variation of 14.97 kg, whereas the mean weight for the case

Table 4.1: Frequency Distribution of Age

Age	Overall	Control	Case
18 - 29 Years	52 (61.9%)	26 (61.9%)	26 (61.9%)
30 - 40 Years	32 (38.1%)	16 (38.1%)	16 (38.1%)
Total	84 (100%)	42 (100%)	42 (100%)

Table 4.2: Descriptive Statistics of Age among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Age	Control	42	18	40	28.55	5.72
	Case	42	20	38	28.19	4.70

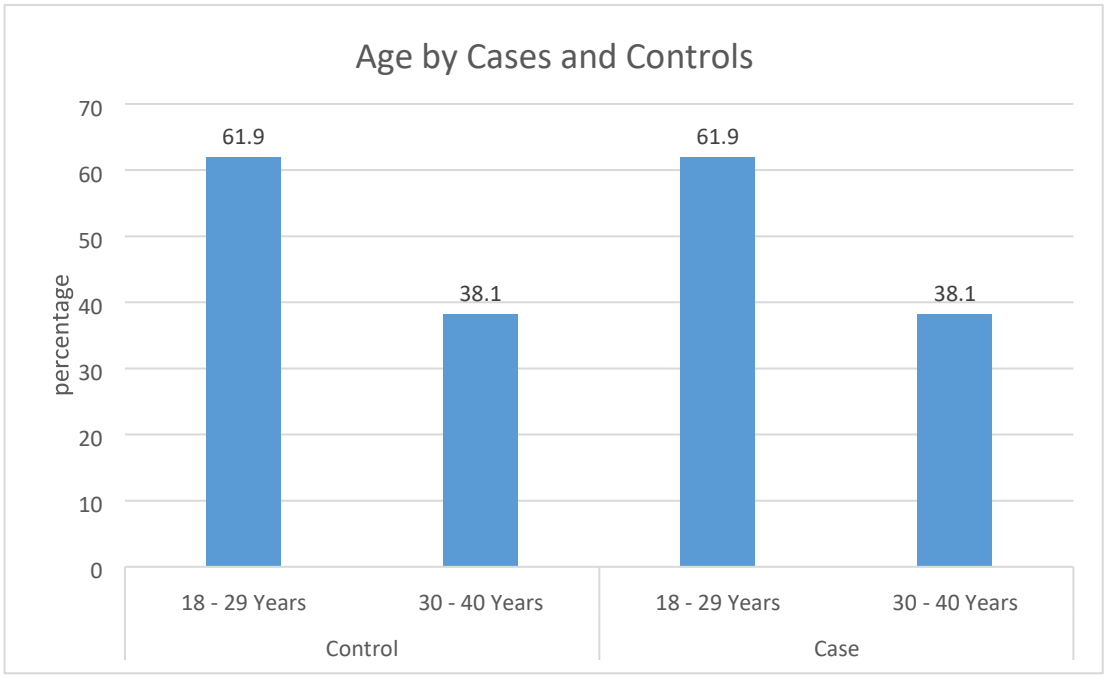

Figure 4.1: Bar Chart of Age for Control and Case

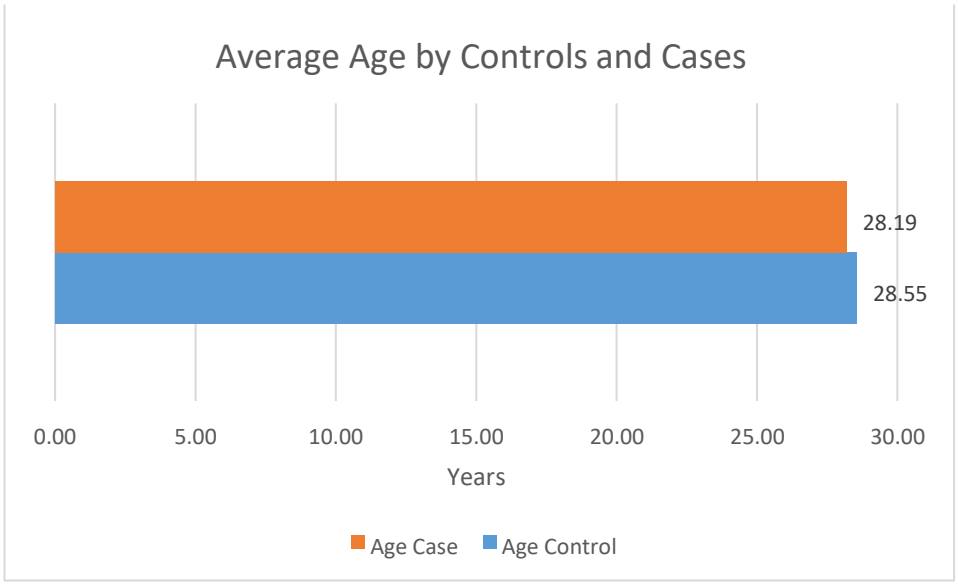

Figure 4.2: Bar Chart of Average Age for Control and Case

group is 68.0 kg with average variation of 12.21 kg. So, average weight is quite higher for the control group as compared to the case group

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4.1.3 HEIGHT

It can be seen from the table 4.4 and figure 4.4 that height varies from a minimum of 144 cm to a maximum of 168 cm in the control group, whereas the height varies from a minimum of 145 cm to a maximum of 168 cm in the case group. So, height range is approximately same for the control group and case group. The mean height for control group is 155.71 cm with average variation of 5.00 cm, whereas the mean height for the case group is 155.52 cm with average variation of 5.05 cm. So, average height is quite similar for the control group and case group.

4.1.4 BODY MASS INDEX (BMI)

It can be seen from the table 4.5 and figure 4.5, that 27.4% (n = 23) of the patients have BMI less than 25, 28.6% (n = 24) of the patients have BMI between 25 and 29.99 and 44.0% (n = 37) of the patients have BMI of 30 or above. Furthermore, it can be seen from the table 4.6 and figure 4.12 that in the control groups, 33.3% (n = 14) of the patients have BMI less than 25, 14.3% (n = 6) of the patients have BMI between 25 and 29.99 and 52.4% (n = 22) of the patients have BMI of 30 or above. Similarly, in the case groups, 21.4% (n = 9) of the patients have BMI less than 25, 42.9% (n = 18) of the patients have BMI between 25 and 29.99 and 35.7% (n = 15) of the patients have BMI of 30 or above. So, majority of the patients have BMI of 30 or above in the control groups, whereas the majority of the patients in the case group have BMI between 25 and 29.99.

It can be seen from the table 4.6 and figure 4.6 that BMI varies from a minimum of 16.02 to a maximum of 43.85 in the control group, whereas the BMI varies from a

Table 4.3: Descriptive Statistics of Weight among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Weight	Control	42	40	104	71.21	14.97
	Case	42	40	96	68.00	12.21

Table 4.4: Descriptive Statistics of Height among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Height	Control	42	144	168	155.71	5.00
	Case	42	145	168	155.52	5.05

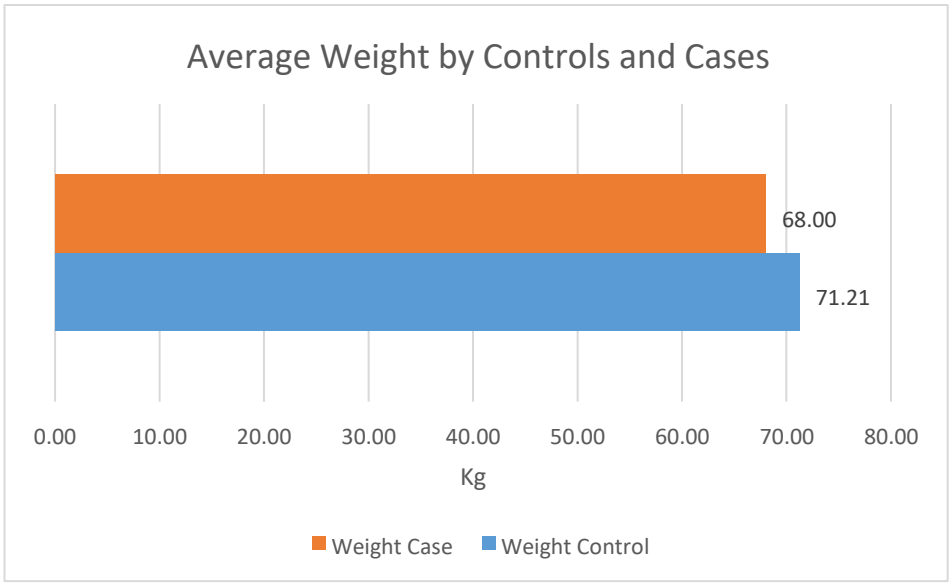

Figure 4.3: Bar Chart of Average Weight for Control and Case

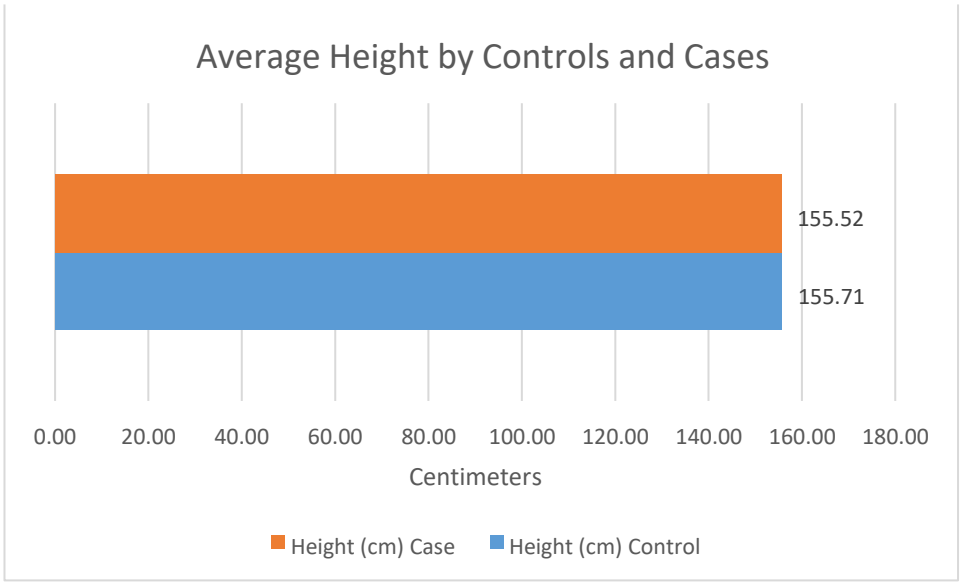

Figure 4.4: Bar Chart of Average Height for Control and Case

Table 4.5: Frequency Distribution of BMI

BMI	Overall	Control	Case
Less than 25	23 (27.4%)	14 (33.3%)	9 (21.4%)
25 - 29.99	24 (28.6%)	6 (14.3%)	18 (42.9%)
30 or above	37 (44.0%)	22 (52.4%)	15 (35.7%)
Total	42 (100%)	42 (100%)	42 (100%)

Table 4.6: Descriptive Statistics of BMI among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
BMI	Control	42	16.02	43.85	29.39	6.09
	Case	42	17.31	43.28	28.14	5.12

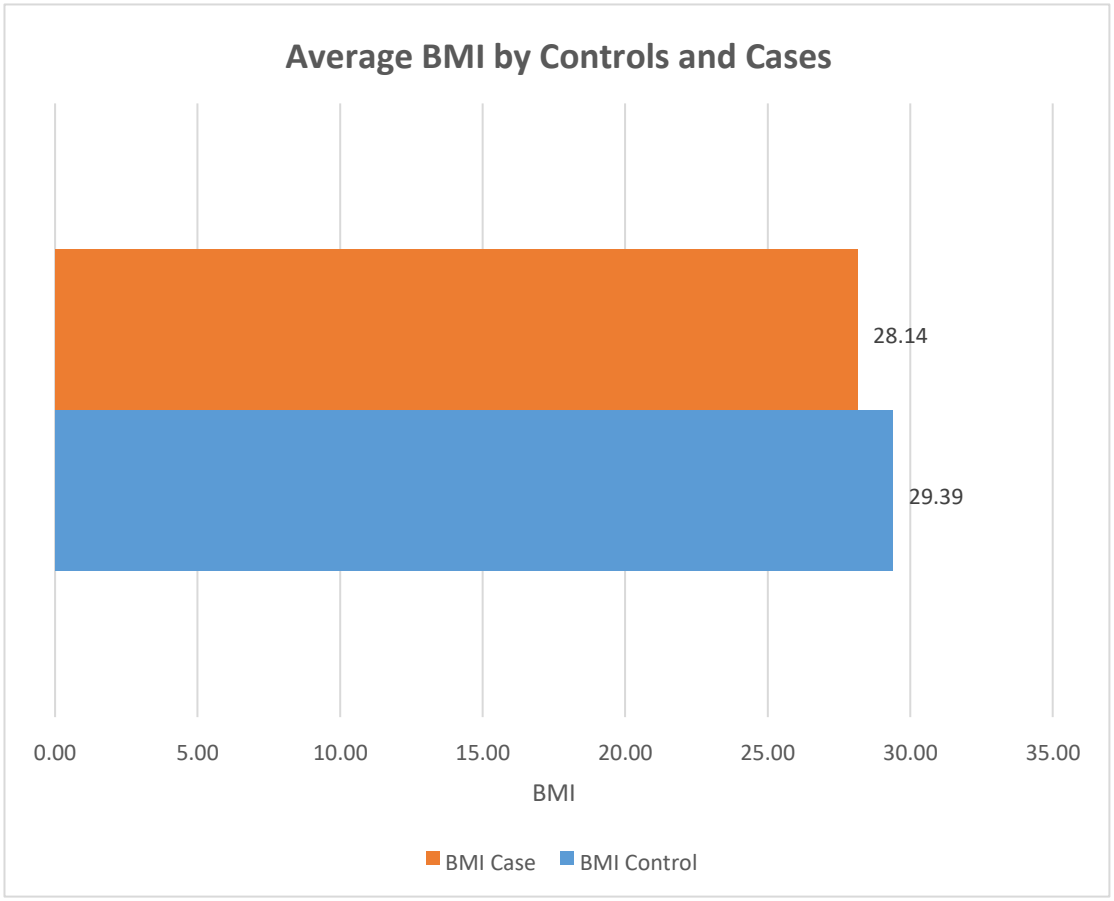

Figure 4.5: Bar Chart of Average BMI for Control and Case

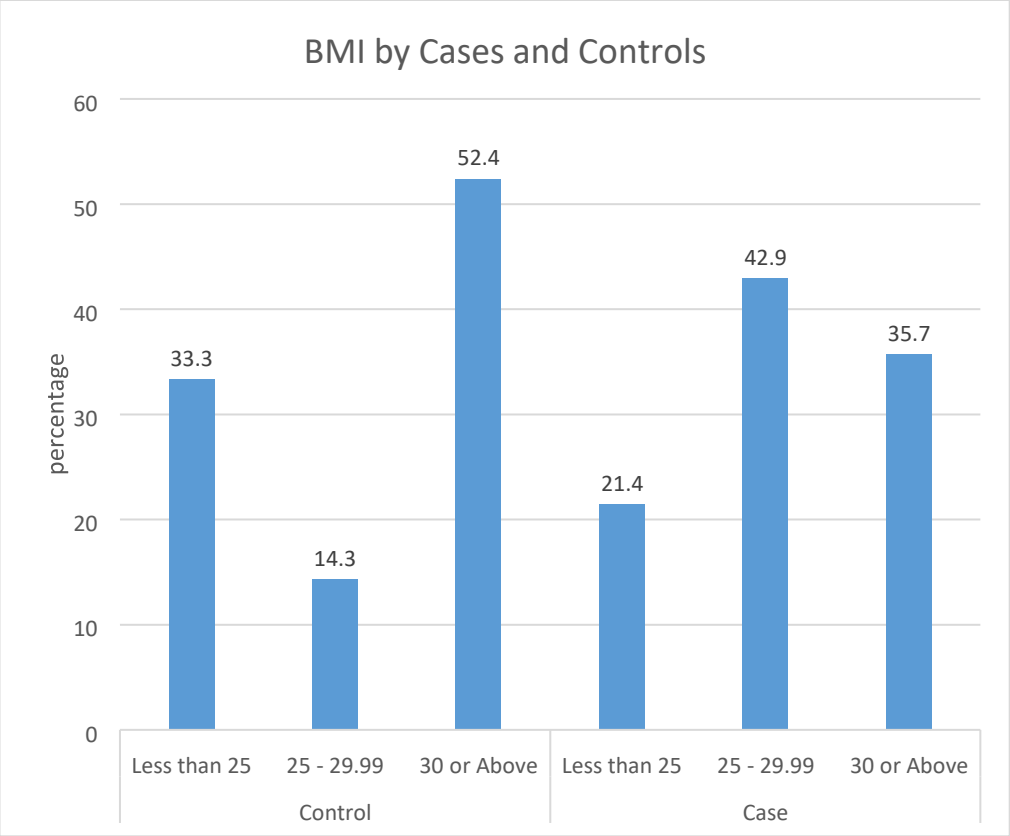

Figure 4.6: Bar Chart of BMI for Control and Case

minimum of 17.31 to a maximum of 43.28 in the case group. So, BMI range is little wider for the control group as compared to the case group. The mean BMI for control group is 29.39 with average variation of 6.09, whereas the mean BMI for the case group is 28.14 with average variation of 5.12. So, the average BMI is little higher for the control group as compared to the case group.

4.1.5 MARITAL STATUS

It can be seen from table 4.7 and figure 4.7 that, 4.8% (n = 4) of the patients were unmarried, whereas the 95.2% (n = 80) patients were married. Furthermore, it can be seen from the table 4.2 and figure 4.4 that in the control groups, 2.4% (n = 1) of the patients were unmarried, whereas the 97.6% (n = 41) of the patients were married. Similarly, in the case groups, 7.1% (n = 3) of the patients were unmarried, whereas the 92.9% (n = 39) of the patients were married. So, majority of the patients in both groups were married. It can be seen from the table 4.8 and figure 4.8 that marriage duration varies from a minimum of 1 year to a maximum of 21 years in the control group, whereas the marriage duration varies from a minimum of 1 year to a maximum of 17 years in the case group. So, marriage duration range is wider for the control group as compared to the case group. The mean marriage duration for control group is 6.24 years with average variation of 5.28 years, whereas the mean marriage duration for the case group is 5.27 years with average variation of 4.37 years. So, average marriage duration is little higher for the control group as compared to the case group.

4.1.6 DURATION OF INFERTILITY

It can be seen from the table 4.9 and figure 4.9 that years of infertility varies from a minimum of 1 year to a maximum of 14 years in the control group, whereas the years of infertility varies from a minimum of 1 year to a maximum of 17 years in the case group. So, years of infertility range is wider for the control group as compared to the case group.The mean years of infertility for control group is 3.86 years with average variation

Table 4.7: Frequency Distribution of Marital Status

Marital Status	Overall	Control	Case
Unmarried	4 (4.8%)	1 (2.4%)	3 (7.1%)
Married	80 (95.2%)	41 (97.6%)	39 (92.9%)
Total	84 (100%)	42 (100%)	42 (100%)

Table 4.8: Descriptive Statistics of Marriage Duration in Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Marriage Duration	Control	41	1	21	6.24	5.28
	Case	39	1	17	5.27	4.37

Table 4.9: Descriptive Statistics of Years of Infertility in Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Years of Infertility	Control	41	1	14	3.86	3.04
	Case	39	1	17	4.62	3.87

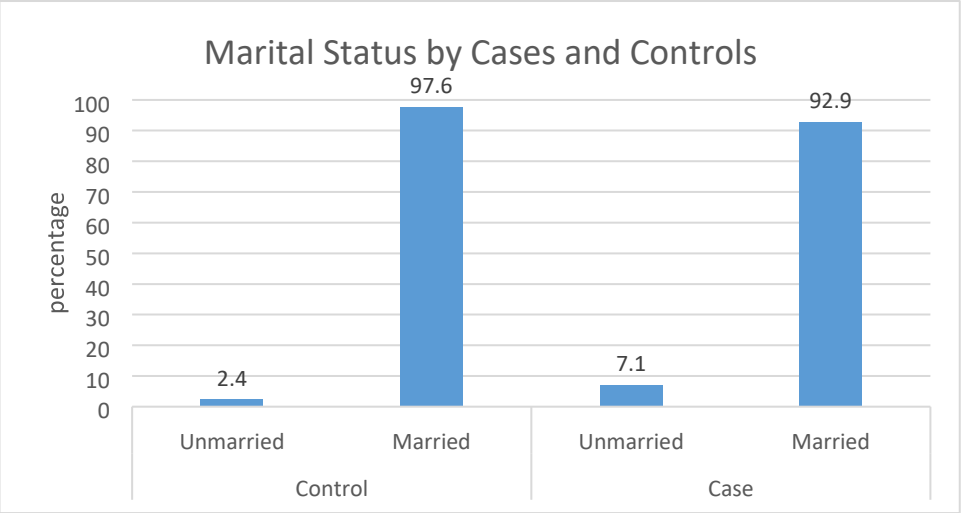

Figure 4.7: Bar Chart of Marital Status for Control and Case

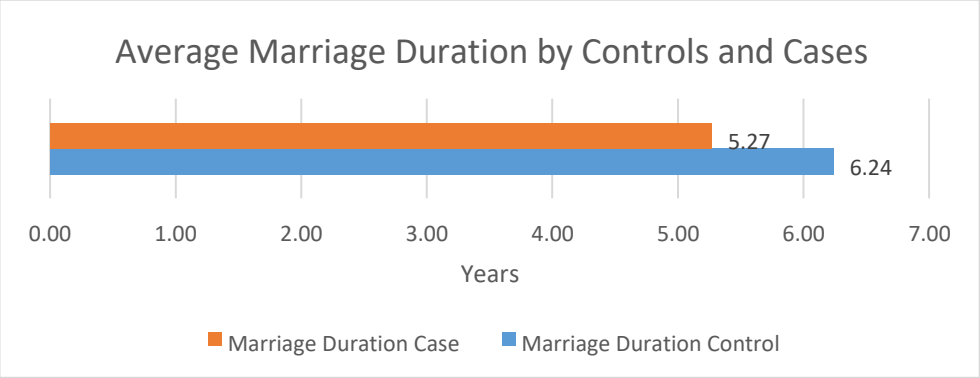

Figure 4.8: Bar Chart of Average Marriage Duration for Control and Case

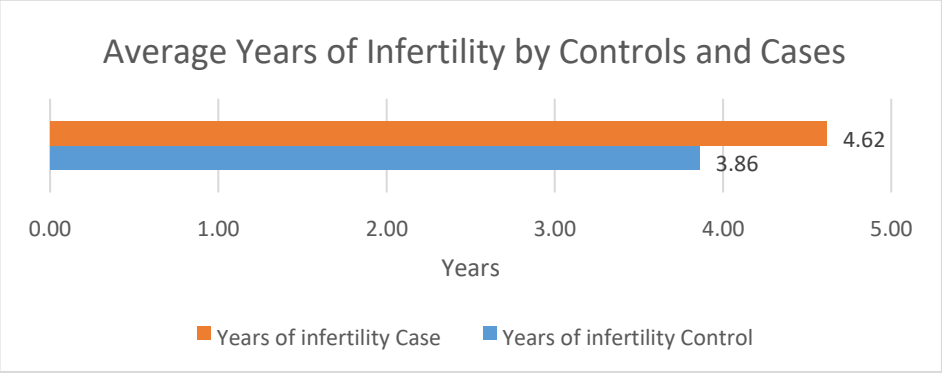

Figure 4.9: Bar Chart of Average Years of Infertility for Control and Case

of 3.04 years, whereas the mean years of infertility for the case group is 4.62 years with average variation of 3.87 years. So, average years of infertility is higher for the case group as compared to the control group.

4.1.7 TEMPERATURE

It can be seen from the table 4.10 and figure 4.10 that temperature varies from a minimum of 97°F to a maximum of 99.2°F in the control group, whereas the temperature varies from a minimum of 97°F to a maximum of 98.4°F in the case group. So, temperature is a little higher for the control group as compared to the case group. The mean temperature for control group is 97.57°F with average variation of 0.49°F, whereas the mean temperature for the case group is also 97.57°F with average variation of 0.36°F. So, the average temperature is exactly same for the control group and case group, however, temperature varies more in the control group.

4.1.8 PULSE

It can be seen from the table 4.11 and figure 4.11 that pulse rate varies from a minimum of 74 to a maximum of 134 in the control group, whereas the pulse rate varies from a minimum of 56 to a maximum of 117 in the case group. So, pulse rate is much higher for the control group as compared to the case group. The mean pulse rate for control group is 101.50 with average variation of 12.99, whereas the mean pulse rate for the case group is 93.19 with average variation of 12.36. So, the average pulse rate is quite higher for the control group as compared to the case group.

4.1.9 BLOOD PRESSURE

It can be seen from the table 4.12 and figure 4.12 that systolic blood pressure varies from a minimum of 100 to a maximum of 170 in the control group, whereas the systolic blood pressure varies from a minimum of 100 to a maximum of 180 in the case group. So, systolic blood pressure is a little higher for the control group as compared to the case group. The mean systolic blood pressure for control group is 130.24 with average variation of 13.70, whereas the mean systolic blood pressure for the case group is 124.29

Table 4.10: Descriptive Statistics of Temperature in Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Temperature	Control	42	97	99.2	97.57	0.49
	Case	42	97	98.4	97.57	0.36

Table 4.11: Descriptive Statistics of Pulse Rate among the Control & Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Pulse	Control	42	74	134	101.50	12.99
	Case	42	56	117	93.19	12.36

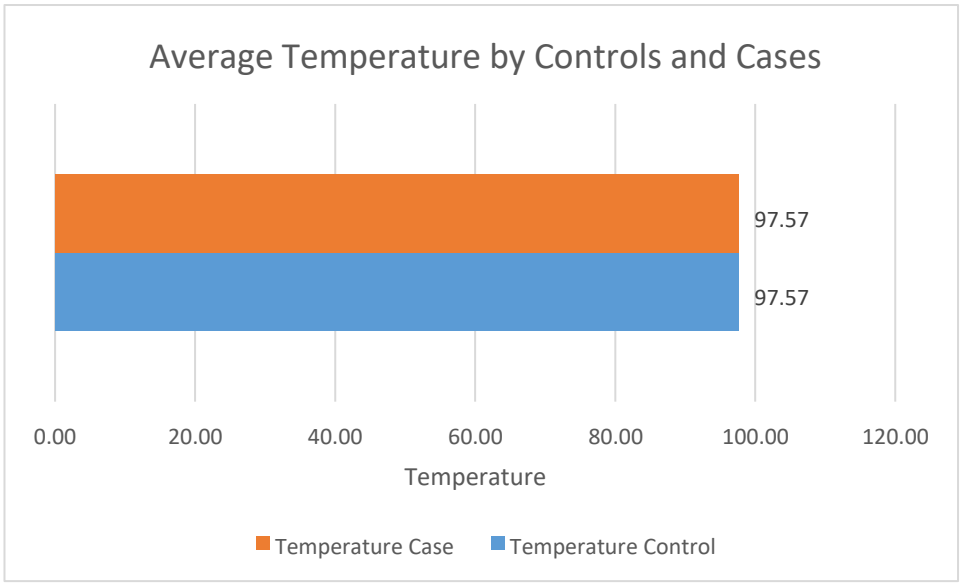

Figure 4.10: Bar Chart of Average Temperature for Control and Case

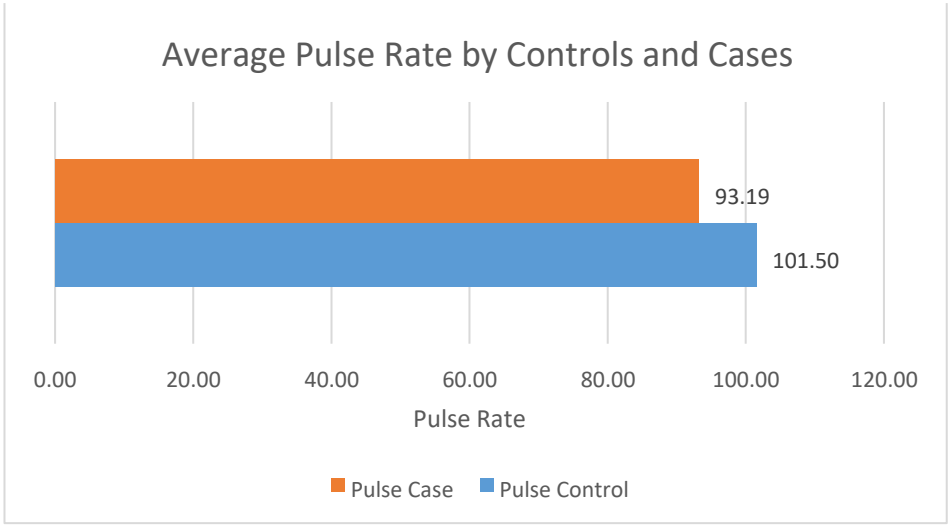

Figure 4.11: Bar Chart of Average Pulse Rate for Control and Case

Table 4.12: Descriptive Statistics of Systolic BP among the Control & Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Systolic BP	Control	42	100	170	130.24	13.70
	Case	42	100	180	124.29	14.34

Table 4.13: Descriptive Statistics of Diastolic BP among the Control & Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Diastolic BP	Control	42	60	100	82.86	8.05
	Case	42	70	100	82.14	7.17

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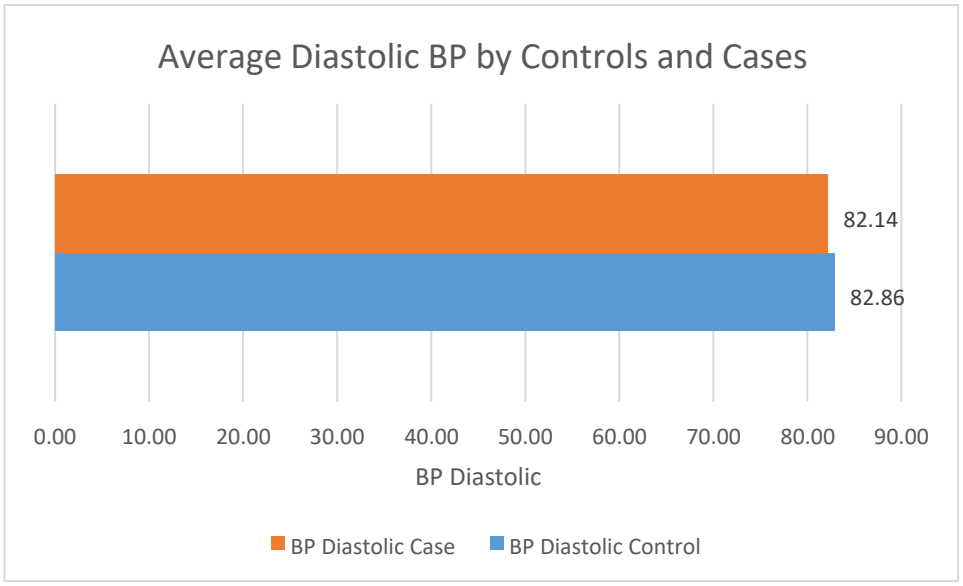

Figure 4.12: Bar Chart of Average Diastolic BP for Control and Case

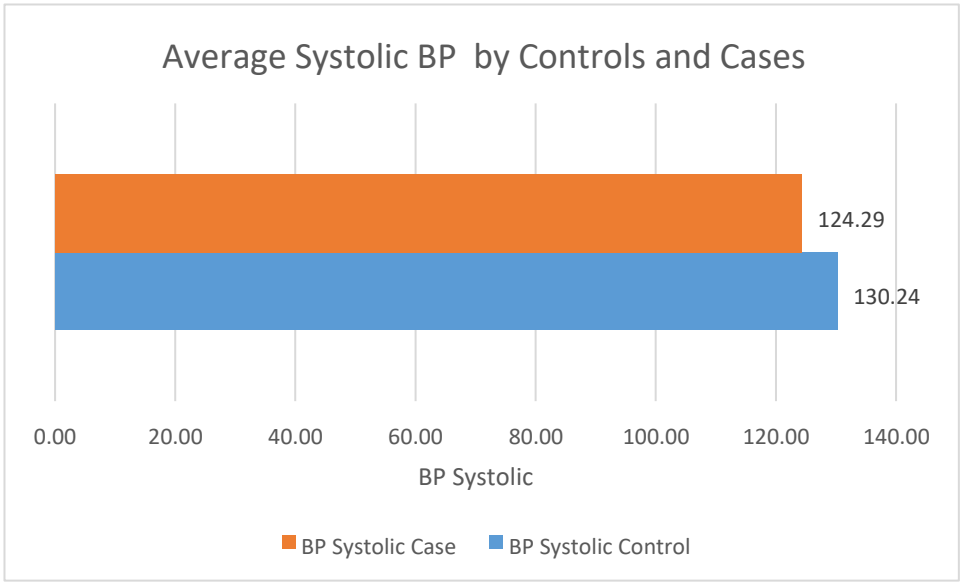

Figure 4.13: Bar Chart of Average Systolic BP for Control and Case

with average variation of 14.34. So, the average systolic blood pressure is higher for the control group as compared to case group.

It can be seen from the table 4.13 and figure 4.13 that diastolic blood pressure varies from a minimum of 60 to a maximum of 100 in the control group, whereas the diastolic blood pressure varies from a minimum of 70 to a maximum of 100 in the case group. So, diastolic blood pressure is a little higher for the case group as compared to the control group. The mean diastolic blood pressure for control group is 82.86 with average variation of 8.05, whereas the mean diastolic blood pressure for the case group is 82.14 with average variation of 7.17. So, the average diastolic blood pressure is little higher for the control group as compared to case group

4.2 PCOS PARAMETERS

These include data for the following:
- Regularity of cycle
- Hirsutism
- Polycystic ovaries on ultrasound
- FSH
- LH
- FSH:LH

4.2.1 REGULARITY OF CYCLE

It can be seen from the table 4.14 and figure 4.14, that 72.6% (n = 61) of the patients have an irregular cycle, whereas the 27.4% (n = 23) of the patients have regular cycle. Furthermore, it can be seen from the table 4.3 and figure 4.6 that in the control groups, 61.9% (n = 26) of the patients have irregular cycle, whereas the 38.1% (n = 16) of the patients have regular cycle. Similarly, in the case groups, 83.3% (n = 35) of the patients have irregular cycle, whereas the 16.7% (n = 7) of the patients have regular cycle. Compared to the control group, the case group had higher likelihood of irregular cycles

4.2.2 HIRSUTISM

Table 4.14: Frequency Distribution of Regularity of Cycle

Regularity of Cycle	Overall	Control	Case
Irregular	61 (72.6%)	26 (61.9%)	35 (83.3%)
Regular	23 (27.4%)	16 (38.1%)	7 (16.7%)
Total	84 (100%)	42 (100%)	42 (100%)

Table 4.15: Frequency Distribution of Hirsutism

Hirsutism	Overall	Control	Case
NO	10 (11.9%)	5 (11.9%)	5 (11.9%)
YES	74 (88.1%)	37 (88.1%)	37 (88.1%)
Total	84 (100%)	42 (100%)	42 (100%)

Table 4.16: Frequency Distribution of PCO on Ultrasound

PCO on Ultrasound	Overall	Control	Case
NO	2 (2.4%)	0 (0.0%)	2 (4.8%)
YES	82 (97.6%)	42 (100.0%)	40 (95.1%)
Total	84 (100%)	42 (100%)	42 (100%)

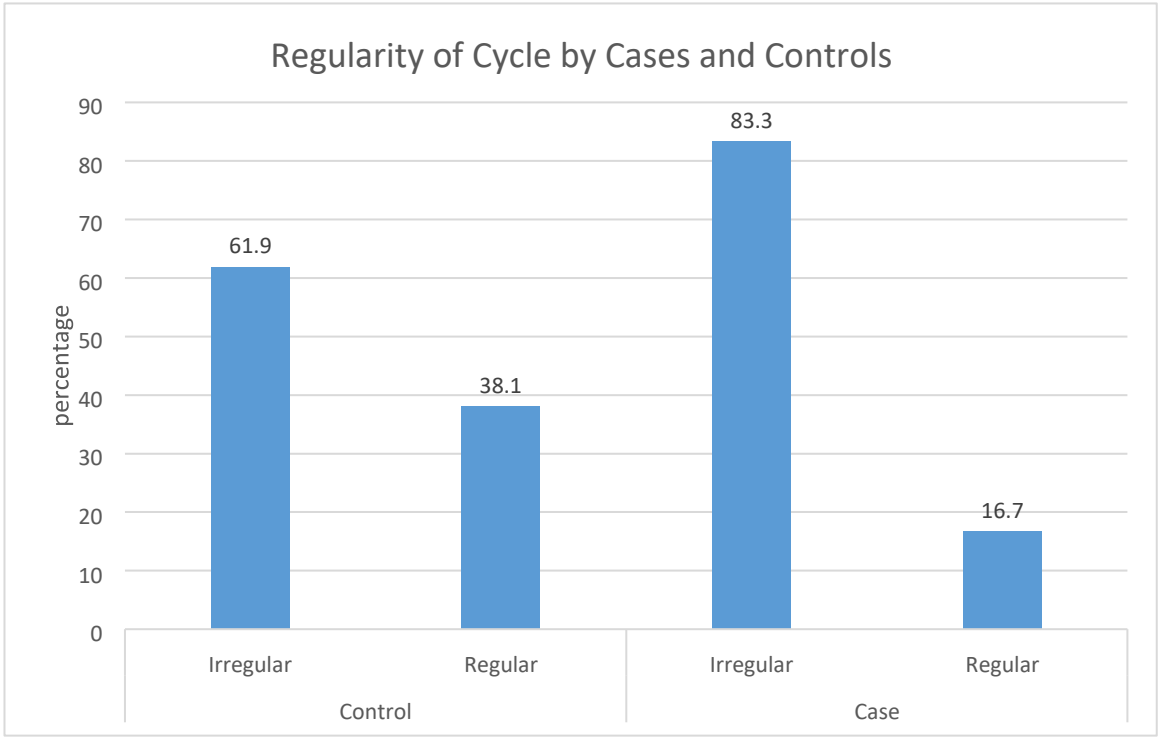

Figure 4.14: Bar Chart of Regularity of Cycle for Control and Case

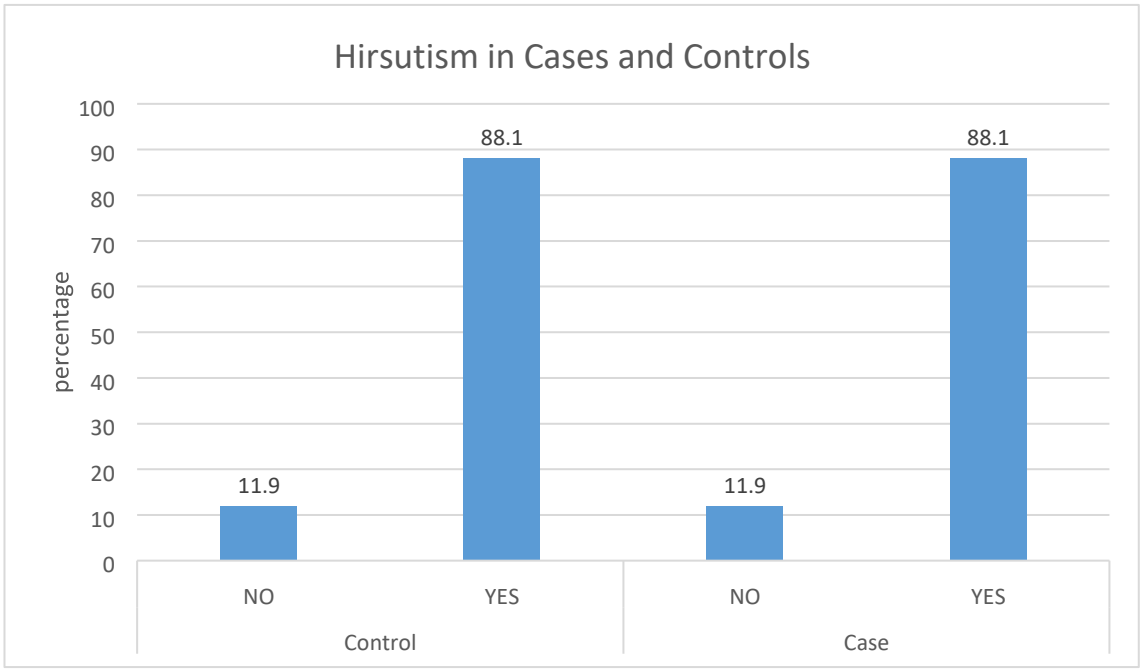

Figure 4.15: Bar Chart of Hirsutism for Control and Case

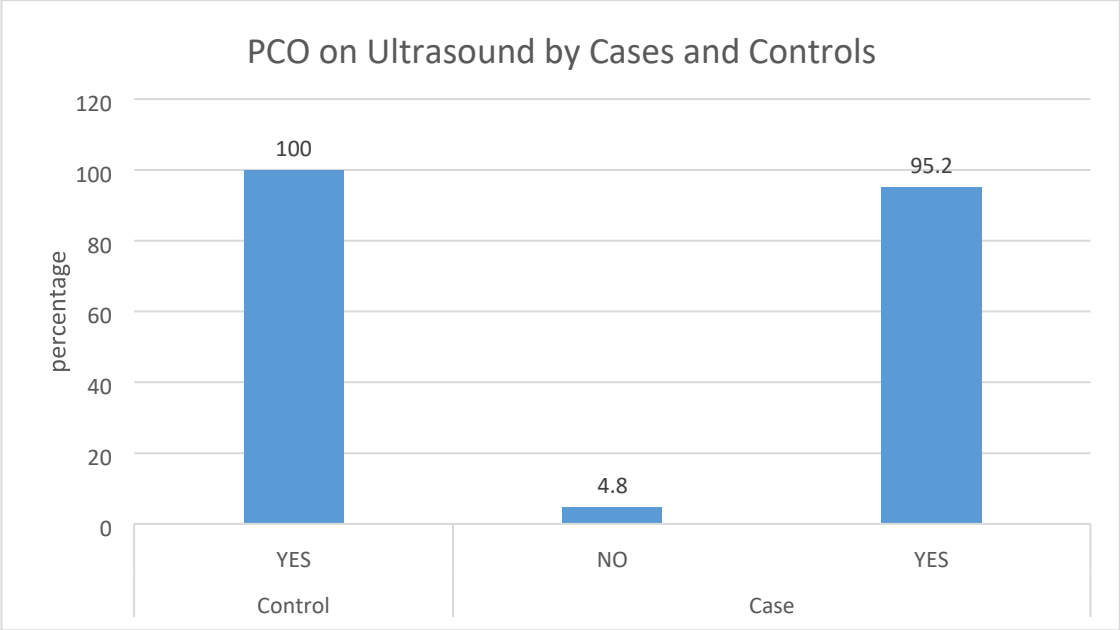

Figure 4.16: Bar Chart of PCO on Ultrasound for Control and Case

It can be seen from the table 4.15 (previous page) and figure 4.15, that 11.9% (n = 10) of the patients do not have hirsutism, whereas the 88.1% (n = 74) of the patients have hirsutism. Furthermore, it can be seen from table 4.4 and figure 4.8 that in the control groups,

11.9% (n = 5) of the patients do not have hirsutism, whereas 88.1% (n = 37) of the patients do have hirsutism. Similarly, in the case group, 11.9% (n = 5) of the patients do not have hirsutism, whereas 88.1% (n = 37) of the patients have hirsutism. Thus, a majority of the patients are hirsute in both groups. Also, it can be seen that hirsutism was comparable in both groups**.**

4.2.3 POLYCYSTIC OVARY ON ULTRASOUND

It can be seen from table 4.16 (previous page) and figure 4.16 that, 2.4% (n = 2) of the patients do not have PCO on ultrasound, whereas the 97.6% (n = 82) of the patients have PCO on ultrasound. Furthermore, it can be seen from the table 4.5 and figure 4.10 that in the control groups, all (n= 42) of the patients have PCO on ultrasound. Similarly, in the case groups, 4.8% (n = 2) of the patients do not have the PCO on ultrasound, whereas the 95.1% (n = 40) of the patients have PCO on ultrasound. So, majority of the patients have PCO on the ultrasound in both groups.

4.2.4 FSH

It can be seen from the table 4.17 and figure 4.17 that FSH varies from a minimum of 3.54 to a maximum of 9.0 in the control group, whereas the FSH varies from a minimum of 0.30 to a maximum of 15.77 in the case group. So, FSH has a wider range for the case group as compared to the control group. The mean FSH for control group is 6.78 with average variation of 1.67, whereas the mean FSH for the case group is 5.67 with average variation of 3.99. So, the average FSH is higher for the control group as compared to case group.

4.2.5 LH

It can be seen from the table 4.18 and figure 4.18 that LH varies from a minimum of 3.1 to a maximum of 10.3 in the control group, whereas the LH varies from a minimum of

Table 4.17: Descriptive Statistics of FSH among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
FSH	Control	8	3.54	9.00	6.78	1.67
	Case	12	0.30	15.77	5.67	3.99

Table 4.18: Descriptive Statistics of LH among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
LH	Control	5	3.1	10.3	6.43	3.33
	Case	10	3.7	19.9	9.26	5.02

Table 4.19: Descriptive Statistics of FSH/LH among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
FSH/LH	Control	5	0.75	2.03	1.35	0.55
	Case	9	0.17	2.92	0.87	0.84

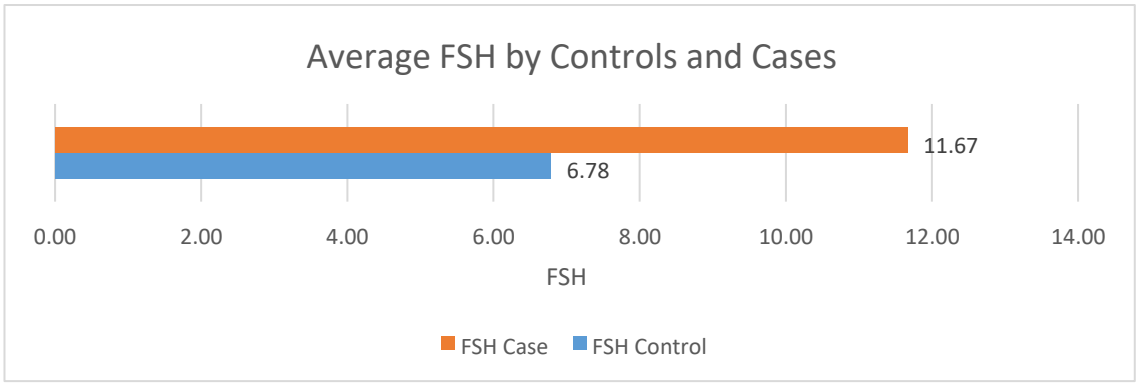

Figure 4.17: Bar Chart of Average FSH for Control and Case

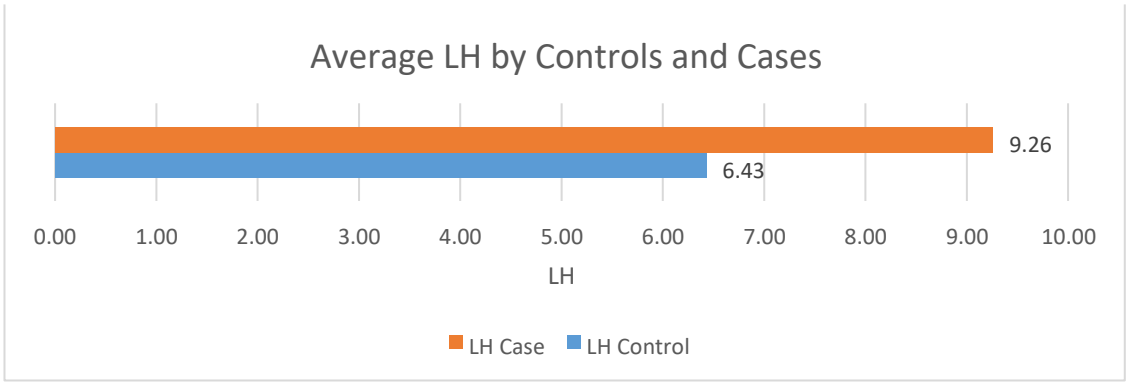

Figure 4.18: Bar Chart of Average LH for Control and Case

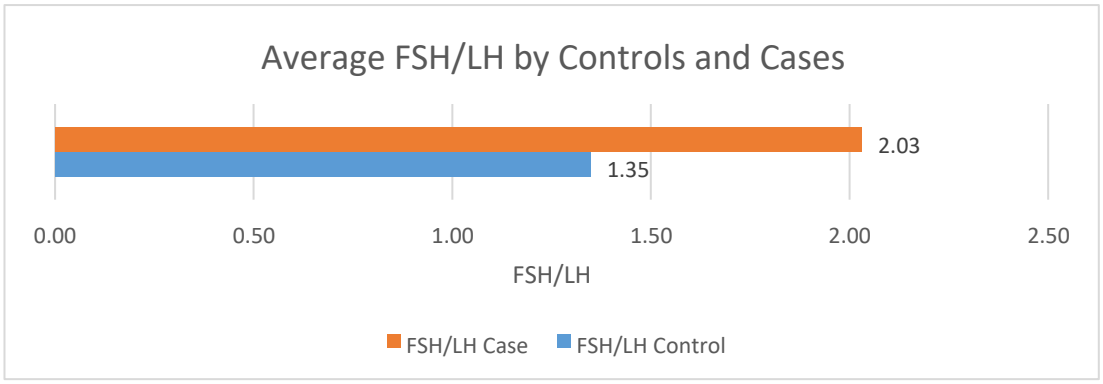

Figure 4.19: Bar Chart of Average FSH / LH for Control and Case

3.7 to a maximum of 19.9 in the case group. So, LH appears to be higher for the case group as compared to the control group. The mean LH for control group is 6.43 with average variation of 3.33, whereas the mean LH for the case group is 9.26 with average variation of 5.02. So, the average LH is higher for the case group as compared to control group.

4.2.6 FSH: LH RATIO

It can be seen from the table 4.19 and figure 4.19 that FSH / LH ratio varies from a minimum of 0.75 to a maximum of 2.03 in the control group, whereas the FSH / LH ratio varies from a minimum of 0.17 to a maximum of 2.92 in the case group. So, FSH / LH ratio has a wider range for the case group as compared to the control group. The mean FSH / LH ratio for control group is 1.35 with average variation of 0.55, whereas the mean FSH / LH ratio for the case group is 0.87 with average variation of 0.84. So, the average FSH / LH ratio is higher for the control group as compared to the case group.

4.3 FASTING INSULIN

It can be seen from the table 4.20 and figure 4.20 that fasting insulin varies from a minimum of 4.0 to a maximum of 37.0 in the control group, whereas the fasting insulin varies from a minimum of 7.9 to a maximum of 57.6 in the case group. So, fasting insulin is much higher for the case group as compared to the control group. The mean fasting insulin for control group is 14.26 with average variation of 6.00, whereas the mean fasting insulin for the case group is 16.17 with average variation of 8.91. So, the average fasting insulin is higher for the case group as compared to the control group.

4.3.1 FASTING INSULIN (FI) BY AGE

It can be seen from the table 4.21 and figure 4.21 that in the control group, mean fasting insulin for 18-29 years group is 15.47 with average variation of 6.87, whereas the mean fasting insulin for the 30-40 years group is 12.30 with average variation of 3.63. So, the,

Table 4.20: Descriptive Statistics of Fasting Insulin in Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
Fasting Insulin	Control	42	4.0	37.0	14.26	6.00
	Case	42	7.9	57.6	16.17	8.91

Table 4.21: Descriptive Statistics of FI by Age in Control and Case Group

Type	Age	N	Mean	Std. Deviation
Control	18 - 29 Years	26	15.47	6.87
	30 - 40 Years	16	12.30	3.63
	Total	42	14.26	6.00
Case	18 - 29 Years	26	16.87	10.19
	30 - 40 Years	16	15.02	6.46
	Total	42	16.17	8.91

average fasting insulin is higher for the 18-29 years group. Similarly, in the case group, mean fasting insulin for 18-29 years group is 16.87 with average variation of 10.19, whereas, the mean fasting insulin for the 30-40 years group is 15.02 with average variation of 6.46. So, the average fasting insulin is higher for the 18-29 years group. So, for both control and case group, average fasting insulin is higher for 18-29 years group.

4.3.2 FASTING INSULIN (FI) BY BMI

It can be seen from the table 4.22 and figure 4.22 that in the control group, mean fasting insulin for group with Less than 25 BMI is 14.02 with average variation of 5.31, the mean fasting insulin for the group with BMI between 25-29.99 is 12.70 with average variation of 3.18 and the mean fasting insulin for the group with BMI of 30 or above is 14.85 with an average variation of 7.02. So, the average fasting insulin is higher for the group with BMI of 30 or above. Similarly, in the case group, mean fasting insulin for group with Less than 25 BMI is 12.43 with average variation of 6.44, the mean fasting insulin for the group with BMI between 25-29.99 is 13.76 with average variation of 4.68 and the mean fasting insulin for the group with BMI of 30 or above is 21.30 with average variation of 11.74. So, the average fasting insulin is higher for the group with BMI of 30 or above. So, for both control and case group, mean fasting insulin is higher for the group with BMI of 30 or above So, the average TSH is much higher for the case group compared to control group.

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4.4 TSH

The only parameter measured in order to determine hypothyroidism in this study was serum TSH. In order to determine whether the subject was to be classified in the Case or Control group, a cut off value of was set. Subjects with serum TSH higher than 4.2mIU/L were put in the Case group.It can be seen from the table 4.23 and figure 4.23 that TSH varies from a minimum of 0.4 to a maximum of 4.19 in the control group, whereas the TSH varies from a minimum of 4.20 to a maximum of 100 in the case group. So, TSH range is much higher for the case group as compared to the control

Table 4.22: Descriptive Statistics of FI by BMI in Control and Case Group

Type	BMI	N	Mean	Std. Deviation
Control	Less than 25	14	14.02	5.31
	25 - 29.99	6	12.70	3.18
	30 or above	22	14.85	7.02
	Total	42	14.26	6.00
Case	Less than 25	9	12.43	6.44
	25 - 29.99	18	13.76	4.68
	30 or above	15	21.30	11.74
	Total	42	16.17	8.91

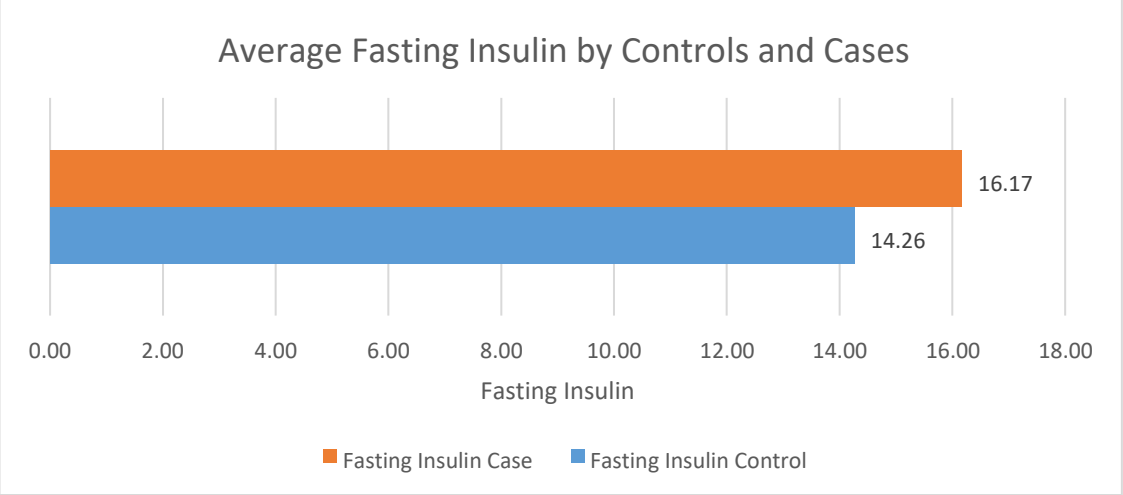

Figure 4.20: Bar Chart of Average Fasting Insulin for Control and Case

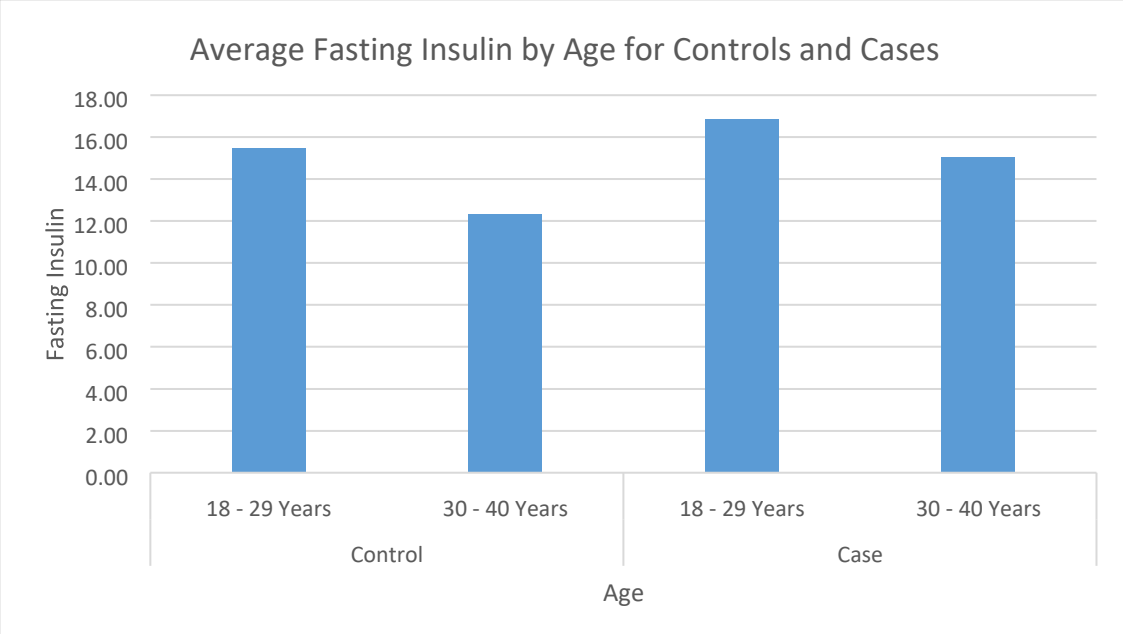

Figure 4.21: Bar Chart of Average Fasting Insulin by Age for Control and Case

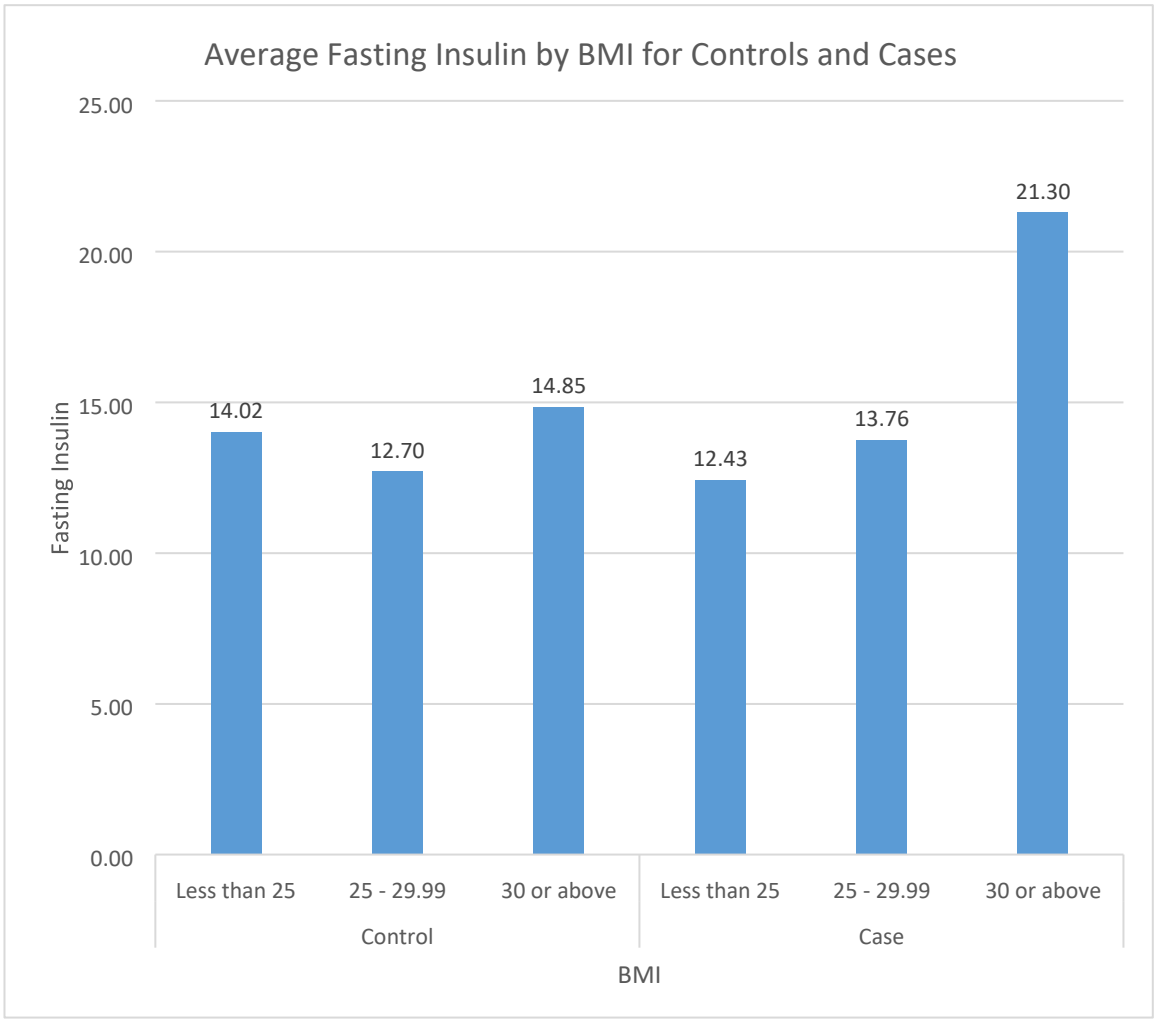

Figure 4.22: Bar Chart of Average Fasting Insulin by BMI for Control and Case

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Table 4.23: Descriptive Statistics of TSH among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
TSH	Control	42	0.4	4.19	2.49	0.97
	Case	42	4.20	100	9.65	15.38

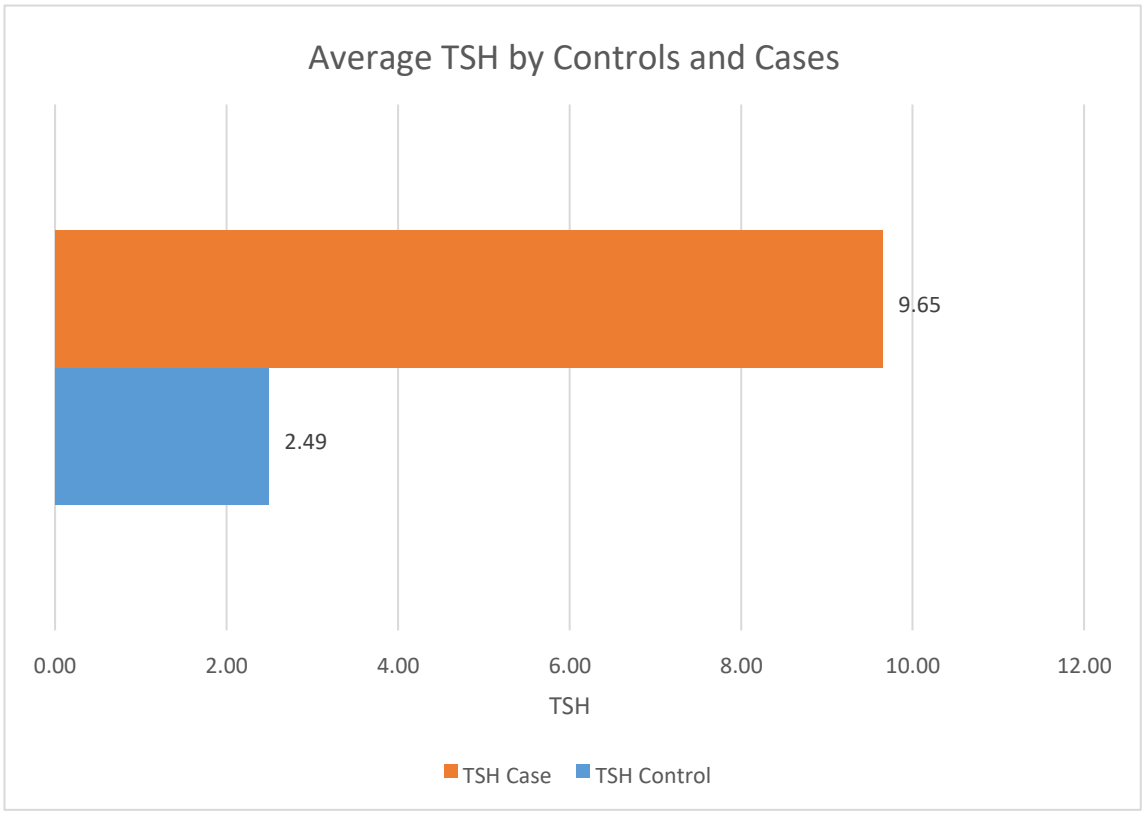

Figure 4.23: Bar Chart of Average TSH for Control and Case

group. The mean TSH for control group is 2.49 with average variation of 0.97, whereas the mean TSH for the case group is 9.65 with average variation of 15.38.

4.5 AMH

It can be seen from the table 4.24 and figure 4.24 that AMH varies from a minimum of 2.41 to a maximum of 11.68 in the control group, whereas the AMH varies from a minimum of 2.56 to a maximum of 13.68 in the case group. So, AMH is higher for the case group as compared to the control group. The mean AMH for control group is 4.34 with average variation of 1.86, whereas the mean AMH for the case group is 4.49 with average variation of 2.05. So, the average AMH is little higher for the case group as compared to the control group.

4.5.1 AMH WITH AGE

It can be seen from the table 4.25 and figure 4.25 that in the control group, mean AMH for 18-29 years group is 4.43 with average variation of 2.31, whereas the mean AMH for the 30-40 years group is 3.81 with average variation of 0.55. So, the average AMH is higher for the 18-29 years group. Similarly, in the case group, mean AMH for 18-29 years group is 4.30 with average variation of 1.98, whereas the mean AMH for the 30-40 years group is 4.55 with average variation of 2.21. So, the average AMH is higher for the 3040 years group. So, for control group, average AMH is higher for 18-29 years group and for case group, average AMH is higher for 30-40 years group.

4.5.2 AMH WITH BMI

It can be seen from the table 4.26 and figure 4.26 that in the control group, mean AMH for group with Less than 25 BMI is 4.36 with average variation of 2.18, the mean AMH for the group with BMI between 25-29.99 is 3.87 with average variation of 0.32 and the mean AMH for the group with BMI of 30 or above is 4.17 with average variation of 1.93. So, the average AMH is higher for the group with BMI of less than 25. Similarly, in the case group, mean AMH for group with Less than 25 BMI is 3.81 with average variation

Table 4.24: Descriptive Statistics of AMH among the Control and Case Group

	Type	N	Minimum	Maximum	Mean	Std. Deviation
AMH	Control	42	2.41	11.68	4.34	1.86
	Case	42	2.56	13.68	4.19	2.05

Table 4.25: Descriptive Statistics of AMH by Age in Control and Case Group

Type	Age	N	Mean	Std. Deviation
Control	18 - 29 Years	26	4.43	2.31
	30 - 40 Years	16	3.81	0.55
	Total	42	4.34	1.86
Case	18 - 29 Years	26	4.30	1.98
	30 - 40 Years	16	4.55	2.21
	Total	42	4.49	2.05

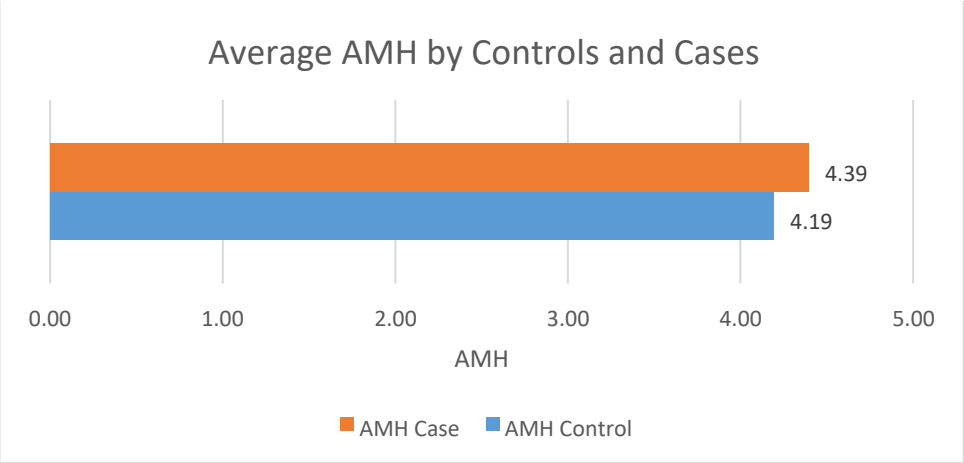

Figure 4.24: Bar Chart of Average AMH for Control and Case

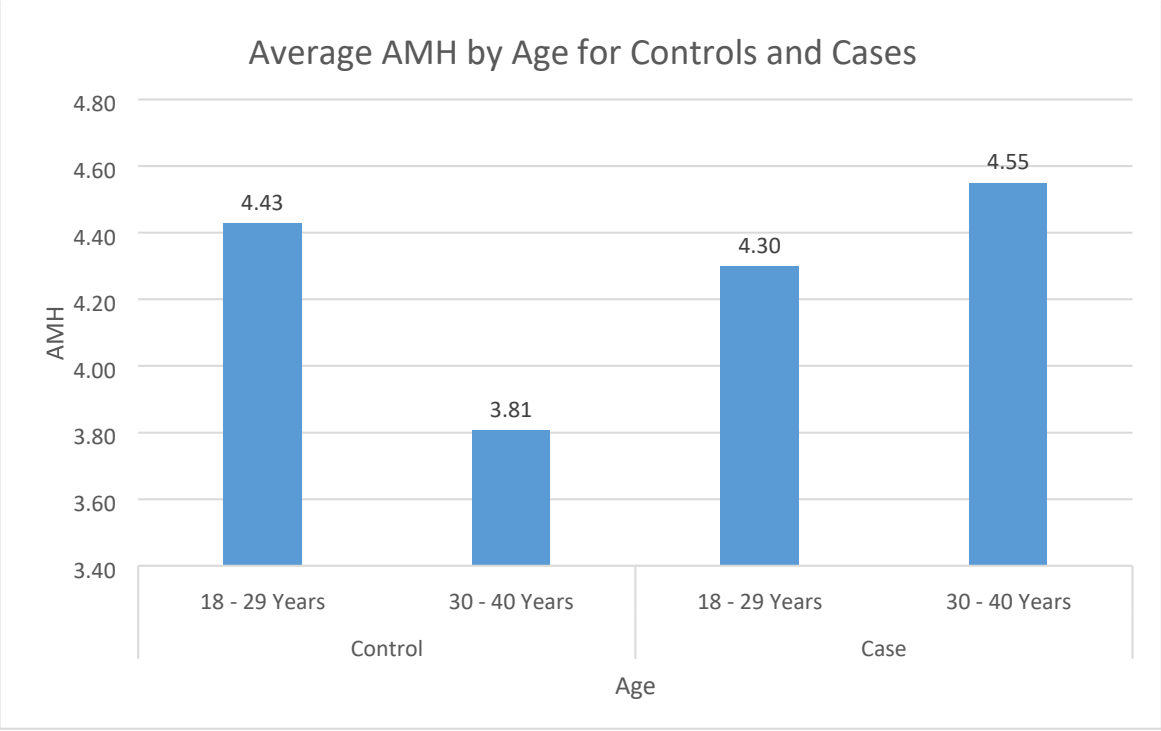

Figure 4.25: Bar Chart of Average AMH by Age for Control and Case

Table 4.26: Descriptive Statistics of AMH by BMI in Control and Case Group

Type	BMI	N	Mean	Std. Deviation
Control	Less than 25	14	4.36	2.18
	25 - 29.99	6	3.87	0.32
	30 or above	22	4.17	1.93
	Total	42	4.34	1.86
Case	Less than 25	9	3.81	0.34
	25 - 29.99	18	4.86	2.78
	30 or above	15	4.18	1.51
	Total	42	4.49	2.05

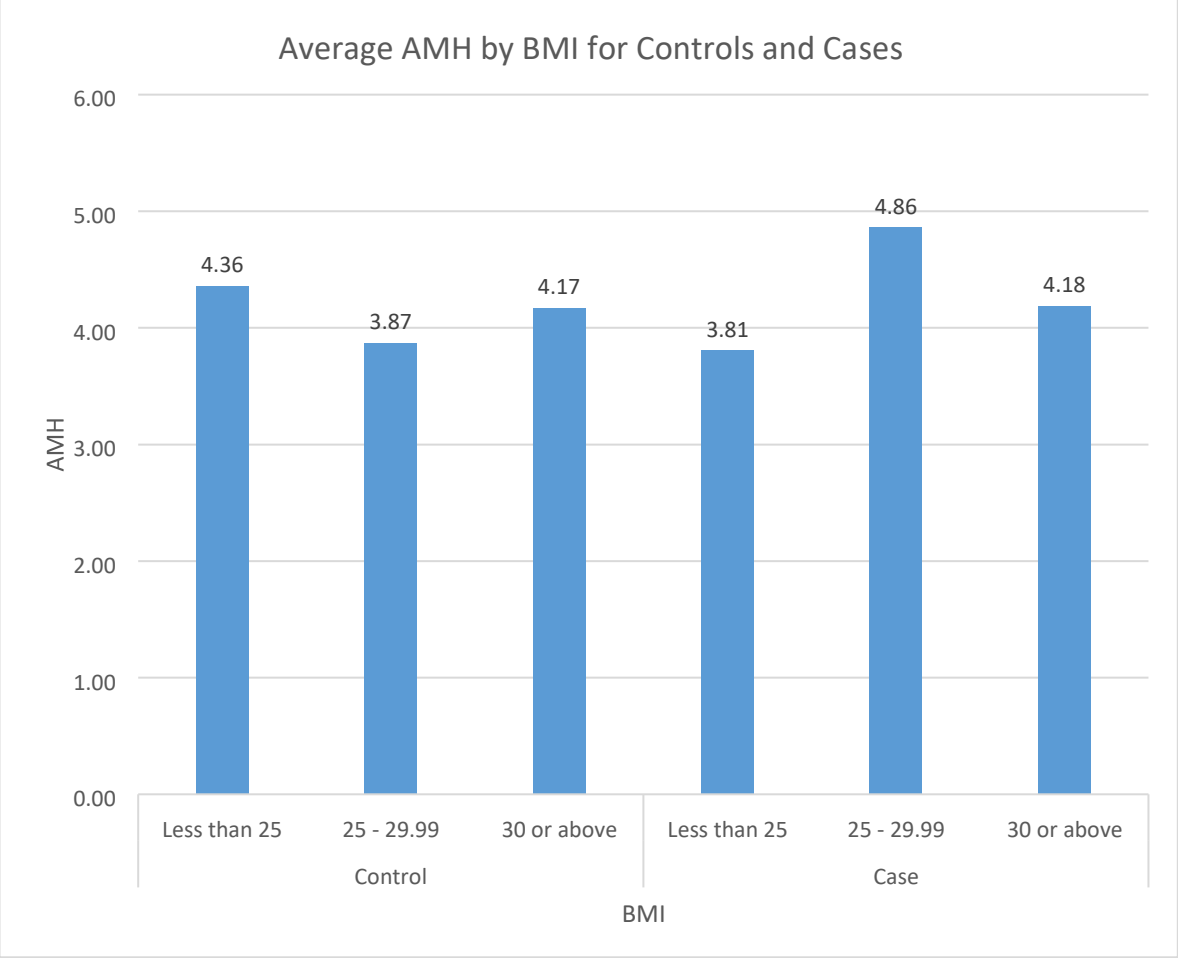

Figure 4.26: Bar Chart of Average AMH by BMI for Control and Case

of 0.34, the mean AMH for the group with BMI between 25-29.99 is 4.86 with average variation of 2.78 and the mean AMH for the group with BMI of 30 or above is 4.18 with average variation of 1.51. So, the average AMH is higher for the group with BMI between 25-29.99. So, for control group, mean AMH is higher for the group with BMI of less than 25, whereas for the case group, average AMH is higher for the group with BMI between 25-29.99.

4.6 HYPOTHESIS TESTING

To test the hypotheses about the significant difference in the mean among the case and control group, two independent samples t-test and Mann-Whitney U test were used. We need certain assumptions to be fulfilled in order to apply certain hypothesis tests and one of the most important assumptions is normality. If the population distribution is normal, we can apply hypothesis test for any sample size but when the population distribution is not normal, we can still consider the sampling distribution as approximately normal, if the sample size is large. The central limit theorem states that for larger sample size (30 or more), the sampling distribution of mean and difference between the two-sample means is approximately normal. So, we applied the two independent samples t-test where sample size is large (more than 30) for both groups and Mann-Whitney U test, where sample size is small (less than 30) for both groups. Similarly, for comparing averages of more than two groups with small sample sizes (less than 30), we used the Kruskal Wallis test. A standard of 5% level of significance is set for all the hypothesis tests

It can be seen from the table 4.27 that results of hypotheses testing for the mean difference among the case and control group for several variables including age, marriage duration, year of infertility, weight, height, BMI, pulse rate, temperature, Diastolic and systolic BP, FSH, LH, TSH, fasting insulin, AMH and FSH/LH ratio are given. Since none of the pvalue is less than 0.05, so we fail to reject the null hypothesis and can say that there is not sufficient evidence to conclude that there is a significant difference among the case and control groups for all these variables. So, we can say that there is difference in the means of control and case group but that difference is not statistically significant at 5% level.

Table 4.27: Two Samples Hypothesis Test among the Control and Case Group

Hypothesis test for Equality of Means			
Variable	**Control**	**Case**	**Sig. (2-tailed)**
Age	28.55 ± 5.72	28.19 ± 4.7	0.755
Marriage Duration	6.24 ± 5.28	5.27 ± 4.37	0.376
Years of infertility	3.86 ± 3.04	4.62 ± 3.87	0.333
Weight	71.21 ± 14.97	68 ± 12.21	0.284
Height	155.71 ± 5	155.52 ± 5.05	0.863
BMI	29.39 ± 6.09	28.14 ± 5.12	0.314
Pulse	101.5 ± 12.99	93.19 ± 12.36	0.004
Temperature	97.57 ± 0.49	97.57 ± 0.36	0.980
BP Diastolic	82.86 ± 8.05	82.14 ± 7.17	0.669
BP Systolic	130.24 ± 13.7	124.29 ± 14.34	0.055
FSH	6.78 ± 1.67	11.67 ± 21.87	**0.157**
LH	6.43 ± 3.33	9.26 ± 5.02	**0.371**
FSH/LH	1.35 ± 0.55	2.03 ± 3.68	**0.190**
TSH	2.49±0.97	9.65 ± 15.38	**0.003**
Fasting Insulin	14.26 ± 6	16.17 ± 8.91	0.255
AMH	4.19 ± 1.86	4.39 ± 2.05	0.636

4.7 HYPOTHESIS TEST: AMH, FASTING INSULIN, AND TSH BY AGE

Table 4.28 shows the results of the significant difference hypothesis test among the case and control groups for AMH and fasting insulin by age groups separately. It can be seen from the above table that p-values for the AMH and fasting insulin are greater than 0.05, so we fail to reject the null hypothesis and conclude that there is no significant difference in AMH and fasting insulin among the case and control groups for each age group.

Table 4.29 shows results of the significant difference hypothesis test among the two age groups for AMH and fasting insulin by case and control groups separately. It can be seen from the table that p-values for the AMH and fasting insulin are greater than 0.05, so we conclude that there is no significant intra group difference in the AMH and fasting insulin among cases, as well as controls.

4.8 HYPOTHESIS TEST: AMH, FASTING INSULIN, AND TSH BY BMI

Table 4.30 shows results of the significant differences among the case and control groups for AMH, fasting insulin and TSH by BMI groups separately. It can be seen from the table that p-values for the AMH and fasting insulin for two groups of BMI are greater than 0.05, so we fail to reject the null hypothesis and conclude that there is no significant difference in AMH among the case and control group for all groups of BMI and no significant difference in fasting insulin among the case and control groups for two groups of BMI. However, the p-values for the fasting insulin for the BMI group “Less than 25” and TSH are less than 0.05, which indicated that we reject the null hypothesis and conclude that mean fasting insulin is significantly different among the case and control groups for the BMI group “Less than 25” and mean TSH is significantly different among the case and control group for all three BMI categories.

Table 4.31 shows results of the significant differences among the three BMI groups for AMH, fasting insulin and TSH by case and control groups separately. It can be seen from the above table that p-values for the AMH, fasting insulin (control group) and TSH are greater than 0.05, so we fail to reject the null hypothesis and conclude that there is no significant difference in the AMH, fasting insulin (control group) and TSH among the

Table 4.28: Two Samples Hypothesis Test: Age Groups among Controls and Cases

Variables	Age	Control	Case	p-value
AMH	18 - 29 Years	2.43 ± 2.31	2.30 ± 1.98	0.297
	30 - 40 Years	1.81 ± 0.55	2.55 ± 2.21	0.323
Fasting Insulin	18 - 29 Years	15.47 ± 6.87	16.87 ± 10.19	1.000
	30 - 40 Years	12.30 ± 3.63	15.02 ± 6.46	0.423

Table 4.29: Two Samples Hypothesis Test: Controls & Cases among the Age Groups

Variables	Type	18 - 29 Years	30 - 40 Years	p-value
AMH	Control	2.43 ± 2.31	1.81 ± 0.55	0.959
	Case	2.30 ± 1.98	2.55 ± 2.21	0.876
Fasting Insulin	Control	15.47 ± 6.87	12.30 ± 3.63	0.423
	Case	16.87 ± 10.19	15.02 ± 6.46	0.657

Table 4.30: Two Samples Hypothesis Test: BMI Groups among Controls & Cases

Variable	BMI	Control	Case	p-value
AMH	Less than 25	2.36 ± 2.18	1.81 ± 0.34	0.926
	25 - 29.99	1.87 ± 0.32	2.86 ± 2.78	0.581
	30 or above	2.17 ± 1.93	2.18 ± 1.51	0.262
Fasting Insulin	Less than 25	14.02 ± 5.31	12.43 ± 6.44	0.026
	25 - 29.99	12.70 ± 3.18	13.76 ± 4.68	0.923
	30 or above	14.85 ± 7.02	21.30 ± 11.74	0.201
TSH	Less than 25	2.64 ± 0.97	16.72 ± 31.34	0.000
	25 - 29.99	2.20 ± 1.16	8.35 ± 7.93	0.000
	30 or above	2.49 ± 0.94	6.97 ± 3.65	0.000

Table 4.31: Kruskal Walis Test for Control & Case Group among the BMI Groups

Variable	Type	Less than 25	25 - 29.99	30 or above	p-value
AMH	Control	2.36 ± 2.18	1.87 ± 0.32	2.17 ± 1.93	0.592
	Case	1.81 ± 0.34	2.86 ± 2.78	2.18 ± 1.51	0.448
Fasting Insulin	Control	14.02 ± 5.31	12.70 ± 3.18	14.85 ± 7.02	0.935
	Case	12.43 ± 6.44	13.76 ± 4.68	21.30 ± 11.74	0.007
TSH	Control	2.64 ± 0.97	2.20 ± 1.16	2.46 ± 0.99	0.647
	Case	16.72 ± 31.34	8.35 ± 7.93	6.97 ± 3.65	0.997

three BMI groups for case group and control group. However, the p-value for the fasting insulin (case group) is less than 0.05, so we reject the null hypothesis and conclude that there is a significant difference in the fasting insulin among the three BMI groups for case group.

4.9 TEST OF INDEPENDENCE: ON BASIS OF ROTTERDAM CRITERIA AND BMI

The chi-square test of independence is most commonly used technique for testing the association between the two categorical variables. The chi-square test of independence is applied here for four different pairs of variables and a level of 5% significance level is considered for all hypothesis tests.

Table 4.32 showed the results of test of independence of four different categorical variables including regularity of cycle, excessive hair, PCO on ultrasound and BMI groups with the patient type. It can be seen that p-values of the test of independence of the patient type with excessive hair and PCO on ultrasound are greater than 0.05, so we fail to reject the null hypothesis and conclude that there is no association between the patient type (case or control) with the excessive hair and PCO on ultrasound. However, the p-values of the test of independence of the patient type with regularity of cycle and BMI groups are less than 0.05, so we reject the null hypothesis and conclude that there is an association between the patient type (case or control) with the regularity of cycle and BMI groups.

4.10 BIVARIATE RELATIONSHIP: TSH WITH AMH

To examine the linear relationship between the two quantitative variable, Pearson's coefficient of correlation is most widely used technique. The Pearson's correlation coefficient measures the strength and direction of the linear relationship between the two variables. The value of the correlation coefficient lies between -1 and 1, where a value close to ± 1 indicates a strong relationship and a value close to 0 indicates no relationship

Table 4.32: Chi-Square Test of Independence for Rotterdam Criteria and BMI

Pair of Variables	Chi-Square	P-value
Type * Regularity of Cycle	4.850	0.028
Type * Excessive Hair	0.000	1.000
Type * PCO on Ultrasound	2.049	0.152
Type * BMI	8.411	0.015

Table 4.33: Correlation Coefficient of AMH

Variable	Correlation Coefficient (r)	P-value
TSH	-0.0061	0.579
Fasting Insulin	0.055	0.618

Table 4.34: Regression Model of TSH

Model	B	Std. Error	t	Sig.
(Constant)	7.616	3.044	2.50	0.014
AMH	-0.360	0.647	-0.557	0.579

among the two variables. Then regression analysis is also used to build a regression model to estimate the mean TSH level on the basis of AMH level

It can be seen from the table 4.33 that the correlation coefficient between the AMH and TSH levels is -0.0061, which indicates a very weak negative relationship among the two variables. Also, the p-value for the correlation between the AMH and TSH levels is greater than 0.05, so we fail to reject the null hypothesis and conclude that there is no significant relation between the AMH and TSH levels.

Table 4.34 showed the results of regression model. The slope coefficient of regression line is -0.360, which means that for each one unit increase in the AMH, the expected TSH will decrease by -0.360 unit. The intercept of the model is at 7.616, which means that when the AMH is 0, the expected TSH will be 7.616. The p-value of the slope coefficient is 0.579, which is greater than 0.05, indicating that there is no significant impact of AMH on the levels of TSH. The R-square of the model is 0.002, which is very low indicating that this model is not a good fit.

4.11 LOGISTIC REGRESSION: TSH WITH AMH

For situations involving binary classification, the technique most commonly used in the medical research is binary logistic regression. The dependent variable, also known as the outcome or target variable in this type of regression is a binary variable and only have one of two potential values, commonly expressed as 0 (Control) or 1 (Case). The objective of binary logistic regression is to forecast the likelihood that one of the two outcomes will occur by modeling the relationship between the independent variables (predictors) and the dependent binary variable. For the current research analysis, the variable TSH is transformed into a binary variable by dividing the TSH into two groups; first group is control group with ($TSH < 4.20$) and second group is case group with ($TSH \geq 4.20$). The dependent variable is coded as 0 for the control and 1 for the case. The independent variable for the logistic regression model is AMH, which is a continuous predictor**.** The results of the binary logistic regression have been shown in table 4.35.

The overall model is tested using the Omnibus test of model coefficients and it can be seen from the table 4.35 that chi-square test statistic is 0.173 along with the p-value of 0.678. Since the p-value is greater than 5%, so we fail to reject the null hypothesis and conclude that overall model is not statistically significant at 5% level of significance.

This -2 Log likelihood statistic shown in Table 4.36 gauges how poorly the model forecasts the choices; the lower the value, the more accurate the model. The score of -2 Log likelihood seems to be high, indicating poor model forecasting decisions. The values for the explained variation are determined by the Cox & Snell and Nagelkerke R Square techniques. It can be seen from the above table that the range of the explained variation in the dependent variable based on our model measured by Cox & Snell and Nagelkerke R Square is between 0.2% and 0.3%, which is very low or very close to zero and indicates that AMH is not at all effective in explaining the variation in the TSH.
The Hosmer and Lemeshow test is applied to test the hypothesis that observed group memberships fit perfectly with the predictions made by the model. Chi-square test statistic is calculated for comparing the observed counts with the expected counts under the linear model. The chi-square test statistic is 7.871 with p-value of 0.446. Since the p-value of the test is greater than 5%, so we fail to reject the null hypothesis and conclude that the data does not fit the model well.

Logistic regression estimates the probability of an event (case) occurring. If the estimated probability of the event occurring is greater than or equal to 0.5 (better than even chance), it is classified as the occurring event (case) and if the probability is less than 0.5, it is classified as the non-occurring event (control). The binary logistic regression used to predict whether cases can be correctly classified (predicted) from the independent variables. Therefore, it becomes necessary to have a method to assess the effectiveness of the predicted classification against the actual classification. By adding the independent variable to the model, the model now correctly classifies 54.8% of participants overall. We have 95.3% of participants who were cases also predicted by the model as cases and 12.2% of the controls were also correctly predicted by the model as control.

To test the statistical significance of an individual independent variable, the Wald test is most commonly used. It can be seen from the above table that the Wald statistic for AMH is 0.170 with p-value of 0.680. Since the p-value of the AMH is greater than 5%, so we

fail to reject the null hypothesis and conclude that AMH did not contribute significantly to the model. The odds ratio for the AMH is 1.048, which is higher than one and indicates that for one unit increase in the AMH, the odds for TSH increase by 0.047. The 95% confidence interval for the odds ratio is 0.827 to 1.313, which includes the value 1 and also indicates that AMH is not statistically significant.

So, the above results of logistic regression revealed that Omnibus test, Hosmer and Lemeshow test and Wald test are statistically not significant at 5% level of significance. Hence, we concluded that AMH does not contribute significantly in the model or model is not statistically significant overall. The overall model correctly classified 54.8% of the overall participant and Cox & Snell and Nagelkerke R Square explained only 0.2% to 0.3% variation. The odds are increasing by 0.047 for TSH by each one unit increase in the AMH.

Table No. 4.35: Omnibus Test of Mode

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Omnibus Tests of Model Coefficients			
	Chi-square	df	Sig.
Step	0.173	1	0.678
Block	0.173	1	0.678
Model	0.173	1	0.678

Table No. 4.36: Model Summary

Model Summary		
-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
116.228	0.002	0.003

Table No. 4.37: Hosmer and Lemeshow Test

Hosmer and Lemeshow Test		
Chi-square	df	Sig.
7.871	8	0.446

Table No. 4.38: Classification Table (logistic regression)

Classification Table				
		Predicted		
		TSH		
Observed		Control	Case	Percentage Correct
TSH	Control	5	36	12.2
	Case	2	41	95.3
	Overall Percentage			54.8

Table No. 4.39: Variables in the logistic regression model

Variables in the Equation								
							95% C.I. for EXP(B)	
	B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
AMH	0.047	0.115	0.170	1	0.680	1.048	0.837	1.313
Constant	-0.155	0.537	0.083	1	0.773	0.856		

CHAPTER 5

DISCUSSION

Polycystic ovary syndrome (PCOS) is a highly prevalent condition in women or reproductive age worldwide. The main objective of this study was to assess the reliability of serum AMH in diagnosing women with PCOS, given that they were suffering from an additional condition of hypothyroidism.

Our study divided the subjects into two groups; namely, cases and controls. Cases consisted of patients who were diagnosed with PCOS (according to Rotterdam criteria, 2003), and hypothyroidism (TSH>4.2mIU/L). Controls were patients that were diagnosed with PCOS only (according to Rotterdam criteria, 2003).

The general parameters that were assessed between the two groups included; age, weight, height, BMI, marital status, duration of infertility (if married), temperature at the time of presentation, pulse rate, and blood pressure. Among these parameters the two that are of utmost importance and significance in this study are that of age, and BMI.

Among the entire study population, 52 (61.9%) subjects were aged between 18-29 years, and 32 (38.1%) were aged between 30-40 years. Thus the study population in majority was of younger females of reproductive age, this finding is consistent with that of a study by Tabassum, R. et al. (2013) who found that mean age of PCOS patients at presentation was 27 years, and Sidra, S. et al (2019) who in their study found that 62.3% of the patients were aged 15–30 years, indicating a higher prevalence of PCOS among younger patients in Paksitan. Both groups in current study were age matched. 26 (61.9%) subjects in the case, as well as control group were aged between 18-29 years; and 16 (38.1%) subjects in the case, as well as control group were aged between 3040years.

A high BMI is a frequently observed sign in PCOS patients, with a significant percentage falling into the overweight or obese BMI categories (Zhao H. et al., 2023) . This finding agrees with our study, as 37 (44.0%) subjects fell into the obese category (BMI>30), while 24 (28.6%) subjects were overweight (BMI 25-29.9), and only 23 (27.4%) subjects were of normal weight. This finding is consistent with findings of Sidra, S. et al. (2019) and Anjum, S. et a. (2020) who in their study on Pakistani women with PCOS found that a high percentage of them were overweight.

The differences in BMI in the case and control group is such that among cases, 9 (21.4%) were of normal weight, 18 (42.9%) were overweight, and 15 (35.7%) were obese. Among controls, 14 (33.3%) were of normal weight, 6 (14.3%) were overweight, and 22 (52.4%) were obese. Thus among cases, 33(78.5%) subjects were overweight compared to 28 (66.6%) controls. This finding agrees with a study by Glintborg, D. et al. (2019), where it was found that patients with PCOS as well as hypothyroidism tend to have a higher BMI compared to patients with PCOS alone.

Since 95.2% (n = 80) of the patients were married, and 4.8% (n = 4) were unmarried, it can be presumed that most subjects had presented to the clinic for fertility related issues. This is confirmed when the duration of infertility was assessed in the subjects. Average duration of infertility in the case group was 4.62 years, while in the control group it was 3.86 years. Average age among the case and control groups were 28.19 and 28.55 years respectively. Most patients of PCOS in Pakistan tend to present with infertility, this finding agrees with a study conducted by Baqai Z. et al. (2010), and Akram, M. et al., (2015), where the mean age of presentation was 26.71 years.

The vitals recorded at the time of presentation were temperature, pulse, and blood pressure. The mean temperature was 97.6 degrees Farenheit in both groups. While mean pulse was 93 beats/minute in cases and 101 beats/minute in controls, pulse rate was higher among the controls and there is no logical explanation or supportive studies to understand why this was the case. Mean blood pressure in the case group was 124/82mmHg, while in the control group it was 130/82mmHg. The higher readings can be attributed to white coat phenomenon. That being said, the readings were within range in all subjects.

The Rotterdam criteria (2003) is based on presence of two of three criterion; namely, oligomenorrhea/amenorrhea, hirsutism, and polycystic ovarian morphology on ultrasound. In the current study, this criteria was used to diagnose PCOS. It was found in

the current study that 72.6% (61) of the patients have an irregular cycle, these findings are consistent with Sidra, S. et al. (2019) who found that irregular menstruation was present in 71.8% of subjects. Hirsutism was present 88.1% (74) subjects; this finding of hirsutism is similar to findings of Nazir, F. et al (2011) who found an 88% prevalence, and Sidra, S. et al (2019) who reported a 68% prevalence of hirsutism in Pakistani females with PCOS. Polycystic ovaries in ultrasound were found in 100% (84) subjects in the current study, while in the study by Sidra, S. et al. (2019) PCO on ultrasound was reported in 61% of patients. It should be considered however, that even though there is a significant difference in percentage relating to PCO on ultrasound between the current and referenced study, it could be attributed to the fact ultrasound is an unreliable tool to access PCOM and is subject to variability depending on skill of technician and the method (whether transvaginal ultrasound or abdominal) was performed. This is why a diagnostic tool such as level of serum AMH can prove more reliable.

The mean FSH for control group is 6.78 µIU/mL with average variation of 1.67, whereas the mean FSH for the case group is 5.67 µIU/mL with average variation of 3.99. These readings are consistent with fifndings of Anjum, S. et al. (2020) who found the average reading of FSH in their study on Pakistani women with PCOS to be 5.3 µIU/mL. The average FSH is a little lower for the case group as compared to control group. These findings are consistent with Al-Jaff, S. (2018), who in his study on Iraqi women found that FSH levels were significantly lower in PCOS women with hypothyroidism compared to women who only had PCOS (1.748 ± 0.179 vs 4.172 ± 0.219). In the current study however, while there is a small difference between FSH levels, the difference is statistically insignificant.

The mean LH for control group is 6.43 mIU/ml with average variation of 3.33, which is consistent with study by Anjum, S. et al. (2020) on Pakistani women who found the average LH level in PCOS to be 7.4 (4.9-14.4) mIU/ml; whereas the mean LH for the case group is 9.26 mIU/ml with average variation of 5.02. So, the average LH is higher for the case group as compared to control group. These findings are in contradiction with Al-Jaff, S. (2018) who in his study on Iraqi women found the LH levels in PCOS women with hypothyroidism to be significantly lower than those with PCOS alone (11.961 ± 0.644 vs 14.560 ± 0.477).

The mean fasting insulin for control group is 14.26 µU/ml with average variation of 6.00, this finding agrees with study by Tabassum, R. et al. (2013) and Anjum, S. et al. (2020) on Pakistani women who found the average fasting insulin level in PCOS to be 13.7 µU/l and 15 (10-24) µU/ml respectively. The mean fasting insulin for the case group is 16.17 µU/ml with average variation of 8.91. The average fasting insulin is found to be higher for the case group as compared to the control group. These findings are consistent with a review by Brenta, G. (2011) who discussed the impact of hypothyroidism on fasting insulin levels; Zhao H. et al. (2021) who in their study found patients with Hashimoto's thyroiditis as well as PCOS had a higher fasting insulin level compared to subjects with PCOS alone (12.47±8.03 vs 10.9±7.18)

Mean fasting insulin for ages between 18-29 years is 15.47±6.87 µU/ml, whereas the mean fasting insulin for ages between 30-40 years is 12.30±3.63 µU/ml. So, the average fasting insulin is higher for the 18-29 years group. Similarly, in the case group, mean fasting insulin for 18-29 years age group is 16.87±10.19 µU/ml, whereas the mean fasting insulin for the 30-40 years group is 15.02±6.46 µU/ml. So, the average fasting insulin is higher for the 18-29 years group. So, for both control and case group, average fasting insulin is higher for 18-29 years group**.** These findings of a higher fasting insulin level in younger age groups is consistent with findings of Mueller A. et al. (2009), and that of Tohidi, M. et al. (2014) who in their study found that fasting insulin was negatively correlated with age.

Mean fasting insulin for control group with BMI<25 is 14.02±5.31kg/m²; the mean fasting insulin for BMI between 25-29.99 is 12.70±3.18 kg/m² and the mean fasting insulin for the group with BMI of 30 or above is 14.85±7.02 kg/m². So, the average fasting insulin is higher for the control group with BMI >30. Similarly, in the case group, mean fasting insulin for group with BMI<25 is 12.43±6.44 kg/m², the mean fasting insulin for the group with BMI between 25-29.99 is 13.76 ±4.68 kg/m² and the mean fasting insulin for the group with BMI of 30 or above is 21.30±11.74 kg/m². As is apparent, the average fasting insulin is higher in both groups with BMI>30. These findings are consistent with those of Mueller A. et al. (2009) and Garelli, S. et al. (2013) who found a positive correlation of fasting insulin with BMI.

However, in the case group, the level of fasting insulin in subjects with BMI>30 is significantly higher than that of control (21.30±11.74 vs 14.85±7.02). This finding is

supported by Mueller, A. et al. (2009) who found higher HOMA-IR scores in high BMI subjects who were diagnosed with PCOS as well as TSH>2. High HOMA-IR scores would mean that these subjects also had a high fasting insulin level. Another study by Enzevaei A, et al. (2014) also showed that subjects with PCOS as well as hypothyroidism have a higher fasting insulin when the subjects are overweight.

AMH is slightly higher for the case group as compared to the control group. The mean AMH for control group is 4.34±1.86 ng/ml, whereas the mean AMH for the case group is 4.49±2.05ng/ml. So, the average AMH is little higher for the case group as compared to the control group. This difference is minute however, and statistically insignificant. Thus, it is safe to conclude that there is no difference in AMH levels between the case and control groups. This finding in comparable to those by Garelli S. (2011) who found that there was no difference in the levels of AMH in PCOS patients with, and without Auto Immune Thyroiditis (AIT). These findings are contradictory to those of Al-Jaff, S. (2018) who in his study found the AMH levels of patients with PCOS + hypothyroidism to be lower than those of PCOS alone (7.034 ± 0.317 vs 11.871 ± 0.495) ng/ml. The absence of differences in AMH levels among the two groups makes serum AMH a reliable marker for PCOS assessment, as the level of TSH tends to have no confounding effect as is seen in the current study.

With regards to effect of age on AMH levels among the two groups, mean AMH for 1829 years age group in controls is 4.43±2.31ng/ml, whereas the mean AMH for the 30-40 years group in controls is 3.81±0.55ng/ml. So, the average AMH is higher for the 18-29 years group in controls. This finding is consistent with findings of Ran Y. et al. (2021). Similarly, in the case group, mean AMH for 18-29 years group is 4.30± with average variation of 1.98, whereas the mean AMH for the 30-40 years group is 4.55± with average variation of 2.21. So, the average AMH is higher for the 30-40 years group. However this difference is minute, so it is safe to assume that no significant difference in AMH was found in both age groups in the cases. This finding also agrees Ran Y. et al. (2021) who have stated that AMH levels become inconsistent after 30 years of age. In the current study, while a difference in AMH levels in both age groups exist, within the cases as well as controls, the difference is small enough as to not impact the diagnostic potential of serum AMH in PCOS.

In the control group, mean AMH for group with BMI<25 is 4.36±2.18ng/ml, the mean AMH for controls with BMI between 25-29.99 is 3.87±0.32 and the mean AMH for controls with BMI≥30 is 4.17±1.93. So, the average AMH is higher for the group with BMI of less than 25. These findings are consistent with Hirschberg A. L., (2009) and Sahmay, S. et al., (2013); and are contradictory to findings by Kloos, J., Coyne, K., & Weinerman, R., (2022) who in their review article concluded no significant correlation between BMI and AMH levels.

In the case group, mean AMH for group with BMI<25 is 3.81±0.34ng/ml, the mean AMH for the group with BMI between 25-29.99 is 4.86±2.78ng/ml and the mean AMH for the group with BMI≥30 is 4.18±1.51. So, the average AMH is higher for the group with BMI between 25-29.99.

It should be noted, however, that these differences are minimal and with hypothesis testing it can be concluded that there were no differences in AMH levels with respect to BMI. These findings are consistent with a current review by Kloos, J., Coyne, K., & Weinerman, R., (2022).

Results of hypotheses testing for the mean difference among the control and case group for several variables including age (28.55 ± 5.72 vs 28.19±4.7), marriage duration (6.24±5.28 vs 5.27±4.37), years of infertility (3.86±3.04 vs 4.62±3.87), BMI (29.39±6.09 vs 28.14±5.12), FSH (6.78±1.67 vs 11.67±21.87), LH (6.43±3.33vs 9.26±5.02), fasting insulin (14.26±6 vs 16.17±8.91), and AMH (4.19±1.86 vs 4.39±2.05) are given.

Since none of the p-value is less than 0.05, we can conclude that there is insufficient evidence to say that there is a significant difference among the case and control groups for all the above variables. Each individual variable has already been discussed under separate headings previously with supportive as well as contradictory studies to evaluate the findings of this study. The result of the hypothesis testing can be backed by the fact that insignificant differences, especially among levels of Fasting insulin, LH, FSH, and AMH can be attributed to the fact that the participants of this study had similar ages as well as BMI, and perhaps these two factors are responsible in turn for affecting Fasting insulin, LH, FSH, and AMH levels in both groups.

The findings of the current study suggests that differences in TSH levels do not affect the fasting insulin, LH, FSH, and AMH levels. Thus, in patients with thyroid abnormalities, all these markers will not be confounded and can aid in diagnosis. The focus of the current study is serum AMH levels, and the hypothesis testing has concluded that AMH can prove to be a promising marker in the evaluation of PCOS, even in women with thyroid abnormalities.

The three variables, AMH, fasting insulin, and TSH were tested for differences by age among the case and control groups. As far as intergroup variability among cases and controls is concerned, there was no significant difference in AMH in the 18-29 years group for controls and cases ($p=0.297$); and there was no significant difference in AMH in the 30-40 years group either ($p=0.323$). Similarly, if we look at intragroup variability, AMH showed no difference in the 18-29years and 30-40 years group among the controls ($p=0.959$), nor cases ($p=0.876$). These findings are contradictory to findings by Ran Y. et al. (2021) who showed that AMH is highest at age 25, and then has a declining trend after the age of 30. Therefore while there is no intergroup variability in AMH, which means that AMH is not affected by hypothyroidism in PCOS patients, the intragroup variability needs to be pondered over. AMH starts to decline after the age of 30, most of the subjects in the current study were younger (mean age=28 years) therefore AMH did not show significant intragroup variability since the subjects in the 30-40years age group were in their early thirties.

Intergroup variability in fasting insulin among cases and controls was insignificant. Difference in fasting insulin in the 18-29 years group for controls and cases was $p=1.0$; there was no significant difference in fasting insulin in the 30-40 years group either ($p=0.423$). Similarly, if we look at intragroup variability, fasting insulin showed no difference in the 18-29years and 30-40 years group among the controls ($p=0.423$), nor cases ($p=0.657$). Mueller A. et al. (2009) and Tohidi, M. et al. (2014) in their study found that fasting insulin was negatively correlated with age. The intragroup differences in pvalues does show that there is a difference, but since the p values are >0.05, the difference is insignificant. And so it is concluded that the above studies show contradictory results to the current study as fasting insulin has shown no intergroup, or intragroup variability with age. This again can be attributed to the age bracket in the current study being nearer to early thirties in the 30-40 years age group.

Inter group (case and control) variability with respect to the three groups of BMI, (normal, overweight and obese) was found in fasting insulin in normal weight subjects (p=0.026), and TSH in all three BMI groups (p=0.00 in all three). The author has not found any study that has evaluated these parameters (in PCOS patients with and without hypothyroidism) with different groups of BMI, therefore no studies that support or contradict these findings could be found.

As far as intragroup differences are concerned, fasting insulin increased with increasing BMI significantly in the case group (p=0.007). This means that in patients with both conditions (PCOS as well as hypothyroidism), an increased BMI significantly raises the serum fasting insulin levels. Thus patients of PCOS with hypothyroidism that have a higher BMI should have regular follow-ups and should be advised lifestyle modifications to reduce cardiovascular disease risk. These findings are consistent with Enzevaei A, et al. (2014), Mueller A. et al. (2009), and Tohidi, M. et al. (2014).

The results of test of independence show that among cases (PCOS+hypothyroidism), irregular cycles and a higher BMI was more prevalent. While it was found that among both groups, PCOS with, and without hypothyroidism, PCO morphology on ultrasound as well as hirsutism was comparable. This is to infer that on presentation, those patients that present with irregular cycles and a higher BMI should be evaluated for TSH.

The correlation coefficient between the AMH and TSH levels is -0.0061, which indicates a very weak negative relationship among the two variables. Also, the p-value for the correlation between the AMH and TSH levels is greater than 0.05, so we conclude that there is no correlation between AMH and TSH levels. This finding is comparable to that by Garelli S. (2011) who found that there was no difference in the levels of AMH in PCOS patients with, and without Auto Immune Thyroiditis (AIT). This finding is in contradiction with Al-Jaff S. (2018) who found a negative correlation of TSH with AMH.

In the linear Regression model, the p-value of the slope coefficient is 0.579, which is greater than 0.05, indicating that there is no significant impact of AMH on the levels of TSH. The R-square of the model is 0.002, which is very low indicating that this model is not a good fit.

The results of logistic regression revealed that Omnibus test, Hosmer and Lemeshow test and Wald test are statistically not significant at 5% level of significance. Hence, we concluded that AMH does not contribute significantly in the model or model is not statistically significant overall. The overall model correctly classified 54.8% of the overall participant and Cox & Snell and Nagelkerke R Square explained only 0.2% to 0.3% variation. The odds are increasing by 0.047 for TSH by each one unit increase in the AMH. Thus there is no effect of TSH on levels of AMH according to the current study, this means that differences in TSH levels in PCOS patients will not act as confounders in the variation in levels of AMH. This author therefore supports the use of AMH as a diagnostic marker in PCOS.

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATIONS

There is limited data available on Pakistani females with PCOS. Ethnic differences in the syndrome with respect to various criterion used to diagnose PCOS vary ethnicity wise as reported by Bozdag G. et al. (2016). The current study also documented the prevalence of various Rotterdam criterion and found that the classical phenotype was most prevalent. It was also found that patients with PCOS as well as hypothyroidism presented earlier with irregular cycles as well as a higher BMI.

5.2.2 PRACTICAL IMPLICATIONS

Unmarried females undergoing a workup for PCOS are unwilling to undergo a transvaginal scan which is most accurate in determining polycystic ovarian morphology on ultrasound. AMH can be used therefore, in replacement of the ultrasound scan as a more reliable to tool for assessment of PCO morphology since it gives an accurate measure of follicular count. There is no major cost difference in both these tests (ultrasound and serum AMH). Since this study has shown that TSH levels do not impact serum AMH levels, AMH can be used in place of ultrasound for determination of PCO morphology, which is also a part of Rotterdam criteria 2003.

5.2.3 POLICY IMPLICATIONS

Mean AMH levels in the current study were high for both cases and controls. The current study has demonstrated that since there is no significant difference in AMH levels of PCOS patients with and without hypothyroidism, AMH is a reliable marker in the assessment of PCOS.

5.3 LIMITATIONS AND STRENGTHS OF STUDY

(A) LIMITATIONS:

- Single center study
- Hypothyroidism was diagnosed only on the basis of TSH levels
- Free T3 levels were not measured
- Free T4 levels were not measured
- Androgen levels were not measured
- Instead of measuring fasting insulin alone, HOMA-IR should have been calculated
- Hirsutism was assessed by researcher and was therefore subject to bias
- Ferriman-Gallwey score for hirsutism was used, but the values were not recorded or assessed in results of the study.
- Age of diagnosis of PCOS was not recorded or assessed

(B) Strengths

- There are no studies on Pakistani females comparing subjects with PCOS alone and PCOS with hypothyroidism, this is the first
- Parameters of AMH and Fasting insulin have been assessed on the basis of age
- Parameters of AMH and Fasting insulin have been assessed on the basis of BMI • Prevalence of the individual Rotterdam criterion has been conducted

5.4 FUTURE RESEARCH DIRECTIONS / RECOMMENDATIONS

A similar study that includes measurement of the following should be conducted:

- FT3
- FT4
- HOMA-IR
- Free androgen level
- SHBG

The current study only has two groups, PCOS with hypothyroidism, and PCOS without hypothyroidism. However a better approach would have been to have 4 groups namely:

- Normal subjects
- Subjects with PCOS alone
- Subjects with hypothyroidism alone
- Subjects with PCOS as well as hypothyroidism

All these groups should then be assessed for intergroup as well as intragroup variability of parameters that have been assessed by current study as well as the additional parameters stated above with respect to age and BMI. This should be done using a multicentre approach (with a large sample size) from various cities across Pakistan in order to assess the reliability of AMH as a diagnostic marker in PCOS in patients of this region.

A study comparing the sensitivity and specificity of serum AMH alone, and serum AMH as an alternative to PCO morphology on ultrasound in the Rotterdam criteria (2003) can also be conducted. The author believes that serum AMH could be more accurate as a diagnostic tool if it was part of a criteria.

5.5 CONCLUSION

Serum AMH is being considered as a diagnostic and prognostic tool in the assessment of PCOS. The level of AMH significantly increases in PCOS, making it a possible assessment tool in PCOS. Since most studies show that AMH levels decrease in hypothyroidism; this study assessed the differences in AMH levels in PCOS patients with, and without hypothyroidism. In the current study, levels of fasting insulin, BMI, years or infertility and age were recorded in both groups along with the level of serum AMH. Fasting insulin, BMI, years of infertility, and age were comparable in both groups. The results of this study show that serum AMH can prove to be a reliable tool in the assessment of PCOS. This is because, according to the findings of this study, there is no significant difference in serum AMH levels in PCOS patients with and without hypothyroidism.

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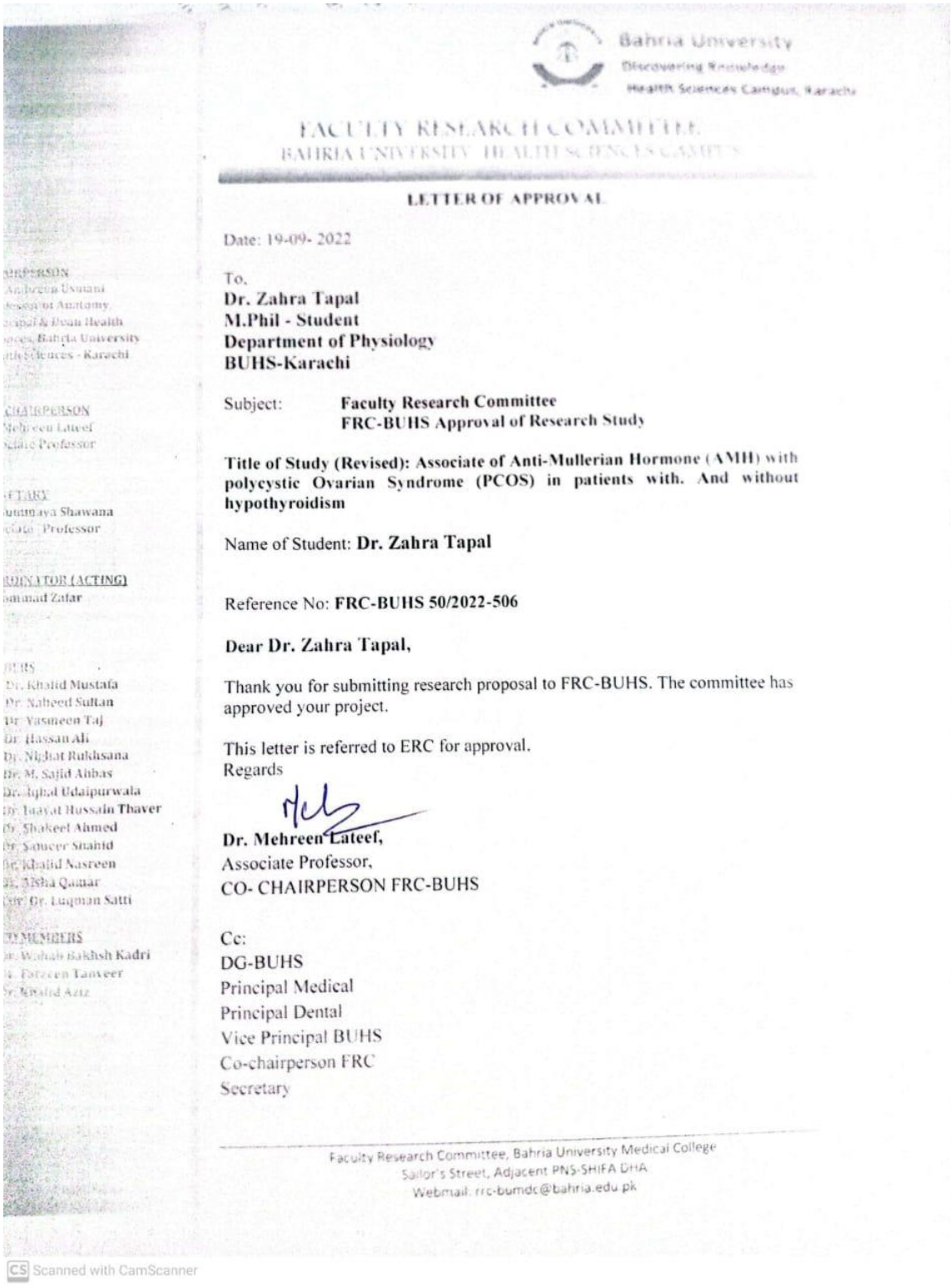
A



Bahria University
Discovering Knowledge
Health Sciences Campus, Karachi

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

LETTER OF APPROVAL

Date: 19-09- 2022

To,
Dr. Zahra Tapal
M.Phil - Student
Department of Physiology
BUHS-Karachi

Subject: **Faculty Research Committee**
FRC-BUHS Approval of Research Study

Title of Study (Revised): Associate of Anti-Mullerian Hormone (AMH) with polycystic Ovarian Syndrome (PCOS) in patients with. And without hypothyroidism

Name of Student: **Dr. Zahra Tapal**

Reference No: **FRC-BUHS 50/2022-506**

Dear Dr. Zahra Tapal,

Thank you for submitting research proposal to FRC-BUHS. The committee has approved your project.

This letter is referred to ERC for approval.
Regards

Dr. Mehreen Lateef,
Associate Professor,
CO- CHAIRPERSON FRC-BUHS

Cc:
DG-BUHS
Principal Medical
Principal Dental
Vice Principal BUHS
Co-chairperson FRC
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Bahria University
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ETHICAL REVIEW COMMITTEE
LETTER OF APPROVAL

Date: 16-Nov-22

Reference:
FRC-BUHS-50/2022-506

Dr. Zahra Tapal
MPhil Candidate
Department of Physiology
BUHS-Karachi

Subject: Institutional approval of research study

Title of Study: Association of anti-Mullerian hormone (AMH) with polycystic ovarian syndrome (PCOS) in patients with, and without hypothyroidism

Principal Investigator: Dr. Zahra Tapal

Reference No: ERC 85/2022

Dear Dr. Zahra Tapal,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Health Sciences Campus has reviewed this project in the meeting held on 15-Nov-2022 and gives approval. Kindly notify us when the research is complete.

Regards,

16/11/2022

DR. AMBREEN SURTI
Secretary, ERC
BUHS

16/11/2022

DR. QURATULAIN JAVAID
Chairperson, ERC
BUHS

Cc:
Principal BUHS

C

INFORMED CONSENT FORM

PURPOSE OF THIS RESEARCH: Is to measure a hormone present in the blood called AMH.

RISK OR DISCOMFORT: blood sample will be taken by trained laboratory staff at the hospital using proper hygienic techniques, however, the participant may feel pain/discomfort when the blood is being drawn

COMPENSATION: if a participant is injured during blood sampling the cost of first aid will be borne by the researcher

BENEFITS: The blood test (AMH) shall be done free of cost and the result will be shared with you

YOUR ROLE IN THIS STUDY:

- You will be asked questions regarding your medical and gynecological history. This process will take up roughly 10 minutes of your time
- You will be asked to give a sample of your blood for the measurement of the hormone AMH, the result of this report will be shared with you via the contact information you give. This process will require 5 minutes of your time
- PLEASE CHECK ☐ THE BOXES BELOW IF IT APPLIES TO YOU

The researcher has also clearly explained the purpose of the study to me

I have had an opportunity to ask questions regarding this study

FREEDOM TO WITHDRAW:

- You can refuse to take part at any time; you can ask questions at any time

I understand I can leave the study at any time without giving a reason

Please note that your name, contact information, and the details you share with us in the questionnaire shall remain confidential and will not be shared with your name in the published study. For any queries, you may contact:

DR. ZAHRA TAPAL **CELL: 0345-2474962** **E-mail: zahratapal@hotmail.com**

I UNDERSTAND CLEARLY THE ABOVE INFORMATION PROVIDED AND I AM WILLING TO PARTICIPATE IN THIS STUDY (YES/NO)

DATE: _____ ***NAME:*** _____ ***SIGN:*** _____

C

باخبر رضامندی کا فارم

اس تحقیق کا مقصد: خون میں موجود ہارمو ن(ا ے-ایم-ایچ) کی پیمائش کرنا ہے ۔

خطرہ یا تکلیف: خون کا نمونہ ہسپتال میں تربیت یافتہ لیبارٹری کے عملے کے ذریعے مناسب حفظانصحت کی تکنیکوں کا استعمال کرتے ہوئے لیا جائے گا، تاہم، خون نکالتے وقت حصہ لینے والے کو درد/تکلیف محسوس ہو سکتی ہے ۔

عاوضہ: اگر کوئی شریک خون کے نمونے لینے کے دوران زخمی ہو جاتا ہے تو ابتدائی طبی امداد کا خرچ محقق برداشت کرے گا ۔

فوائد: خون کا ٹیس ٹ(ا ے-ایم-ایچ) مفت کیا جائے گا اور نتیجہ آپ کے ساتھ شیئر کیا جائےگا ۔

اس مطالعہ میں آپ کا کردار:

آپ سے آپ کی طبی اور امراض نسواں کی تاریخ سے متعلق سوالات پوچھے جائیں گے۔ اس عمل میں آپ کے وقت کے تقریباً 10 منٹ لگیں گے ۔

آپ سے ہارمو نای-ایم-ایچ کی پیمائش کے لیے اپنے خون کا نمونہ دینے کے لیے کہا جائے گا، اس رپورٹ کا نتیجہ آپ کے ساتھ رابطہ کی معلومات کے ذریعے شیئر کیا جائے گا۔ اس عمل میں آپ کے 5 منٹ کا وقت درکار ہوگا ۔

اگر یہ آپ پر لاگو ہوتا ہے تو براہ کرم نیچے دیے گئے خانوں کو چیک ✔ کریں

محقق نے میرے سامنے مطالعہ کا مقصد بھی واضح طور پر بیان کیا ہے۔ ☐

مجھے اس مطالعہ کے حوالے سے سوالات پوچھنے کا موقع ملا ہ ے ☐

حصہ لینے کی آزادی:

آپ کسی بھی وقت حصہ لینے سے انکار کر سکتے ہیں اور آپ کسی بھی وقت سوالات پوچھ سکتے ہیں۔

میں سمجھتی ہوں کہ میں بغیر وجہ بتائے کسی بھی وقت مطالعہ چھوڑ سکت ی ہوں. ☐

براہ کرم نوٹ کریں کہ سوالنامے میں آپ کا نام، رابطے کی معلومات، اور جو تفصیلات آپ ہمارے ساتھشیئر کرتے ہیں وہ خفیہ رہیں گی اور شائع شدہ مطالعہ میں آپ کے نام کے ساتھ شیئر نہیں کی جائیں گی۔ کسی بھی سوال کے لیے، آپ رابطہ کر سکتے ہیں:

ڈاکٹر زہرہ ٹپا ل E-mail: zahratapal@hotmail.com CELL: 0345-2474962

میں اوپر دی گئی معلومات کو واضح طور پر سمجھتا ہوں اور میں اس مطالعہ میں حصہ لینے کے لیے تیار ہوں

- ہاں ☐ - نہیں ☐

نام: _____ **تاریک** _____ **دستخط** _____

D

SUBJECT EVALUATION FORM

PATIENT CODE NUMBER:_____ **DATE:**_____

DEMOGRAPHICS	
NAME	
AGE	
CELL	
EMAIL	
HISTORY	
Marital status	
Married since	
Years of infertility	
Is your menstrual cycle regular?	
Do you have excessive hair on face and body?	
Has PCO been detected on ultrasound?	
Do you suffer from thyroid dysfunction?	
Have you been treated/under treatment for thyroid dysfunction?	
Do you have an ovarian tumor?	
Have you had ovarian surgery?	
Do you have a history of liver disease?	
Do you have history of kidney disease?	
Do you have diabetes?	
Do you smoke?	
PHYSICAL EXAMINATION	
WEIGHT (Kg)	
HEIGHT (m)	
PULSE	
TEMPERATURE	
BLOOD PRESSURE	
LAB INVESTIGATIONS	
FSH	
LH	
TSH	
FASTING INSULIN	
RADIOLOGICAL INVESTIGATIONS	
U/S PELVIS	
TO BE EVALUATED:	
SERUM AMH LEVELS	

E

National Medical Centre

A-5/A, National Highway, Defence Housing Authority, Phase I,
Near Kalapul, Karachi, Pakistan.
UAN: 111-222-662 (NMC) Tel: 35380000-3 Lines Fax: 35805022
E-mail: info@nmc.net.pk Website: www.nmc.net.pk

O.P.D PRESCRIPTION

Patient's Name _____

Age _____ Sex _____ Date _____

℞

.0012

F

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