CORRELATION OF XENIN WITH INHERITANCE AND EXISTENCE OF POLYCYSTIC OVARY SYNDROME IN FERTILE WOMEN

DR MISBAH RIAZ

(06-116212-004)

BAHRIA UNIVERSITY ISLAMABAD

PAKISTAN

CORRELATION OF XENIN WITH INHERITANCE AND EXISTENCE OF POLYCYSTIC OVARY SYNDROME IN FERTILE WOMEN

DR MISBAH RIAZ

(06-116212-004)

A thesis submitted in fulfillment of the requirements for the award of the degree of Master of Philosophy (Biochemistry)

DEPARTMENT OF BIOCHEMISTRY

BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

OCTOBER 2023

APPROVAL FOR EXAMINATION

Scholar's Name: **Dr. Misbah Riaz** Registration No: **78867**

Programme of Study: **MPhil Biochemistry** Thesis Title**: "Correlation of Xenin with Inheritance and Existence Of Polycystic Ovary Syndrome in Fertile Women"**.

It is to certify that the above scholar's thesis has been completed to my satisfaction and, to my belief, its standard is appropriate for submission for examination. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index **6%** that is within the permissible limit set by the HEC for the MPhil degree thesis. I have also found the thesis in a format recognized by the BU for the MPhil thesis.

Principal Supervisor's Signature: _____

Prof. Dr. Hasan Ali
Professor & HOD
Biochemistry
BU Health Sciences
Karachi

Date: 13th Oct 2023

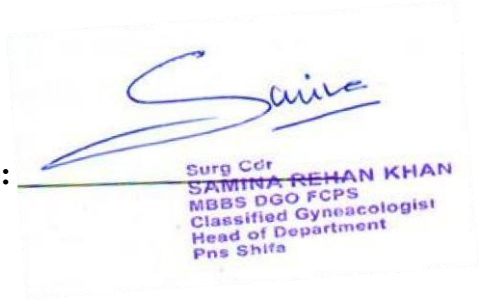
Name: Prof Dr. Hasan Ali

THESIS COMPLETION CERTIFICATE

Student's Name: **Dr. Misbah Riaz** Registration No. **78867**

Programme of Study: **MPhil Biochemistry** Thesis Title: **"Correlation of Xenin with Inheritance and Existence Of Polycystic Ovary Syndrome in Fertile Women".**

It is to certify that the above student's thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index at **6%** that is within the permissible limit set by HEC for the MPhil degree thesis. I have also found the thesis in a format recognized by the BU for the MPhil thesis.

Co-Supervisor's Seal & Signature: 

Date: 13th Oct 2023

Name: Surgeon Cdr Dr. Samina Rehan Khan

AUTHOR'S DECLARATION

I, **Dr. Misbah Riaz** hereby state that my MPhil thesis titled **"Correlation of Xenin with Inheritance and Existence of Polycystic Ovary Syndrome in Fertile Women"** is my own work and has not been submitted previously by me for taking any degree from this university, the **Bahria University** or anywhere else in the country/world.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw/cancel my MPhil degree.

Name of Scholar: **Dr. Misbah Riaz**

Date: **13 Oct 2023**

PLAGIARISM UNDERTAKING

I, solemnly declare that research work presented in the thesis titled **"Correlation of Xenin with Inheritance and Existence of Polycystic Ovary Syndrome in Fertile Women"** is solely my research work with no significant contribution from any other person. Small contribution / help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Bahria University towards plagiarism. Therefore, I as an Author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred / cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MPhil degree, the university reserves the right to withdraw / revoke my MPhil degree and that HEC and the University has the right to publish my name on the HEC / University website on which names of scholars are placed who submitted plagiarized thesis.

Scholar/ Author's Sign: 

Name of Scholar: **Dr. Misbah Riaz**

TO MY BELOVED FAMILY

ACKNOWLEDGEMENTS

Foremost, I am thankful to Allah Almighty for providing me strength and conviction to pursue my higher studies at Bahria University Health Sciences (BUHSCK). I am profoundly thankful to management of BUHSCK especially efforts of Professor Dr. Naseem Karim principal PGPTM for continuous pursuance of MPhil Program thus affording an opportunity to me and other students to undertake postgraduate studies. I humbly express my deepest appreciation to my respected supervisor Professor Dr. Hasan Ali. Without his continuous support, guidance and encouragement successful completion of this research work would not have been possible. I am thankful to him for taking out time from his busy schedule for reviewing, improving and editing my research work. I express my special thanks to all senior faculty members especially Assistant Prof Dr Faraz Anwar, all faculty and staff of Biochemistry Department for their valuable advice and encouragement throughout my thesis. My sincere gratitude to my co-supervisor Surg Cdr Dr Samina Rehan, Head of Gynae & OBS department at PNS SHIFA for her guidance and expert clinical opinion in my research project. I am obliged to Surg Lt Cdr Dr. Nazia Mushtaq and Dr Naila Izhar for their help and support in data collection from Gynae & OBS outpatient department of PNS SHIFA. My humble thanks to Professor Dr. Mehreen Latif, principal BUHSCK and incharge MDRL for helping me out in analysis of ELISA tests of my project. I am thankful to my all MPhil batch mates and colleagues for providing moral support and assistance whenever needed. My humble gratitude goes to my parents Mr. Riaz Raja & Mrs Fehmida Riaz for their moral support and never ending prayers for my success and siblings Iram Riaz, Bilal Riaz and Aqib Riaz. I am grateful to my husband, Cdr. Usman Zafar for his continuous support throughout research work most notably during formatting. Special thanks to my son M. Aarib Usman for providing me the will to keep moving forward. Last but not the least, my humble thanks to all the patients of my study for their cooperation.

Dr. Misbah Riaz

ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a condition in which numerous small cysts are formed in the ovaries of a woman resulting in an increased production of androgens (male sex hormones). National health services (NHS) has reported a few common symptoms like oily skin, weight gain, complications in conceiving and irregular periods. In most cases, it shows irregular hair growth on different areas such as the chest, face and back. Studies have revealed that the main causes or factors that resulted in PCOS prevalence are still unknown. South Asian women, especially Pakistani women, suffers more from PCOS (around 52%) as compared to white population in UK (20 - 25%). This study is an attempt to contribute towards research/ studies that are trying to establish exact cause for prevalence of PCOS. The objectives of the study were to compare the biochemical parameters and plasma xenin levels in PCOS diagnosed group, probable group and control group. This was a case control study. All women between age 15 - 45 years who fulfilled the inclusion criteria were included. The calculated sample size of 105 subjects were divided into three separate study groups (control group, PCOS diagnosed patients and probable group). Venous blood sample was taken for measuring different parameters like FSH, LH, Prolactin, Testosterone, Fasting blood sugar, Fasting Insulin, HbA1c and serum Xenin after taking ethical approval from Bahria University Health Sciences Karachi (BUHSCK). Ultrasound pelvis was done. For statistical analysis SPSS v26 was used. Descriptive statistics were presented in terms of frequency with percentages and mean with standard deviation. ANOA was applied for mean comparison. Cut off values were identified using ROC curve. The P value <0.05 was considered as statistically significant. Mean age for cases, controls and probable group was 30.82±6.60 years, 27.88±6.43 years and 25.94±5.47years. BMI in cases, controls and probable group was 30.91±5.66 kg/m2, 22.69±5.07kg/m2 and 26.08±7.36 kg/m2. Serum FSH in cases, controls and probable groups were 5.68±1.23mIU/ml, 7.59±0.97mIU/ml and 6.56±0.80 mIU/ml S. LH in cases, controls and probable group were 11.35±1.87mIU/ml, 10.19±1.44mIU/ml and 10.70±1.22mIU/ml. Serum LH : FSH was 2.02±0.23, 1.34±0.11 and 1.64±0.18 respectively. Serum Testosterone (nmol/L) was 1.20±0.42, 0.78±0.45 and

1.02±0.33. Serum prolactin (µg/L) in cases, control and probable group was 24.40±9.50, 15.34±3.07 and 17.93±4.18. Fasting blood sugar (nmol/L) was 5.55±0.91, 4.98±0.40 and 4.76±0.38 respectively. Fasting insulin levels (mIU/L) were 11.63±5.65, 5.85±2.91 and 7.85±4.19. HbA1c was 5.54±0.78, 5.09±0.39 and 4.94±0.31 respectively. Mean serum xenin was 31.25±2.86 pg/ml in cases, 23.58±3.36 pg/ml in controls and 26.93±3.94 pg/ml in probable with significant mean difference (p=0.000). By ROC Curve, Cut offs values of serum xenin was 27.18 (Sen=82.9%), 27.41(Sen=82.9%) and 27.96(Sen=82.9%) for cases while the cut offs values for serum xenin for proable group was 22.93(sen=82.9%) and 23.01(sen=80%). Increase levels of Xenin were found in cases and probable group than controls.

Key Words: Polycystic Ovary, Probable group, BMI, FSH, LH, Prolactin, Testosterone, HbA1c, Xenin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMH	Anti-Mullerian Hormone
ANOVA	Analysis of variance
AR	Androgen Receptor Gene
BMI	Body Mass Index
CAPN10	Caplain-10.
CVD	Cardiovascular Disease
CYP	Cytochrome FAMILY P450
DM	Diabetes Mellitus
ELIZA	Enzyme Linked Immunosorbent Assay
FSH	Follicle-Stimulating Hormone
FSHR	Follicle-Stimulating Hormone Receptor
FTO	Fat Mass Obesity
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-Like Peptide-1
GNR	Gonadotropin-Releasing Hormone
HBA1C	Glycated Hemoglobin
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HS-CRP	High-Sensitive C-Reactive Protein
INS	Insulin Gene
INSR	Insulin Receptor
IRS	Insulin Receptor Substrate
LH	Luteinizing Hormone
NHS	National Health Services
NTSR-1	Neurotensin Receptor 1
OCPs	Oral Contraceptives
OD	Optical Density
PAI-1:	Plasminogen Activator Inhibitor 1
PCO	Polycystic Ovary

PCOS	Polycystic Ovarian Syndrome
PEG	Polyethene Glycol
ROC	Receiver Operating Characteristic Curve
RPM	Rotation Per Minute
SHBG	Sex-Hormone-Binding Globulin.
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TGF-β	Transforming Growth of Factors β
WHO	World Health Organization
XRP-1	Xenopsin-Related Peptide-1

LIST OF ANNEXURES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Polycystic ovarian syndrome (PCOS) is an endocrine disorder that is described as the numerous small cysts that are formed in the ovaries of most females. In a similar context, Meng et al. (2016) defined PCOS as a biological condition in which the ovaries generate or produce an abnormal quantity of androgens (male sex hormones) that are usually present in smaller quantities in the women population. PCOS is caused by a deficiency in the process of transcriptional regulation of a genome caused by a polymorphism or even any sequence alteration (Shorakae, Boyle and Teede, 2014). Androgen receptor proteins, LH receptor proteins, FSH receptor proteins, and Leptin receptor proteins are the most common genes involved (Abraham, Divakar and Valsala, 2021). National health services (NHS) have reported a few common symptoms of PCOS like oily skin, weight gain, complications in conceiving and irregular periods (NHS, 2021). In most PCOS cases, the irregular hair growth on different areas such as the chest, face and back is being observed. Past studies have revealed that the main causes or factors that resulted in PCOS prevalence are still unknown. However, differential genetic dictated hormonal dysfunction is considered to be the underlying cause (Casals-Casas and Desvergne, 2011). Moreover, a gene abnormality disrupts the metabolic process, causing ovarian malfunction. (Fig 1.1a, 1.1b)

Figure 1.1(a, b) represents numerous environmental and genetic factors that are more likely to influence PCOS.

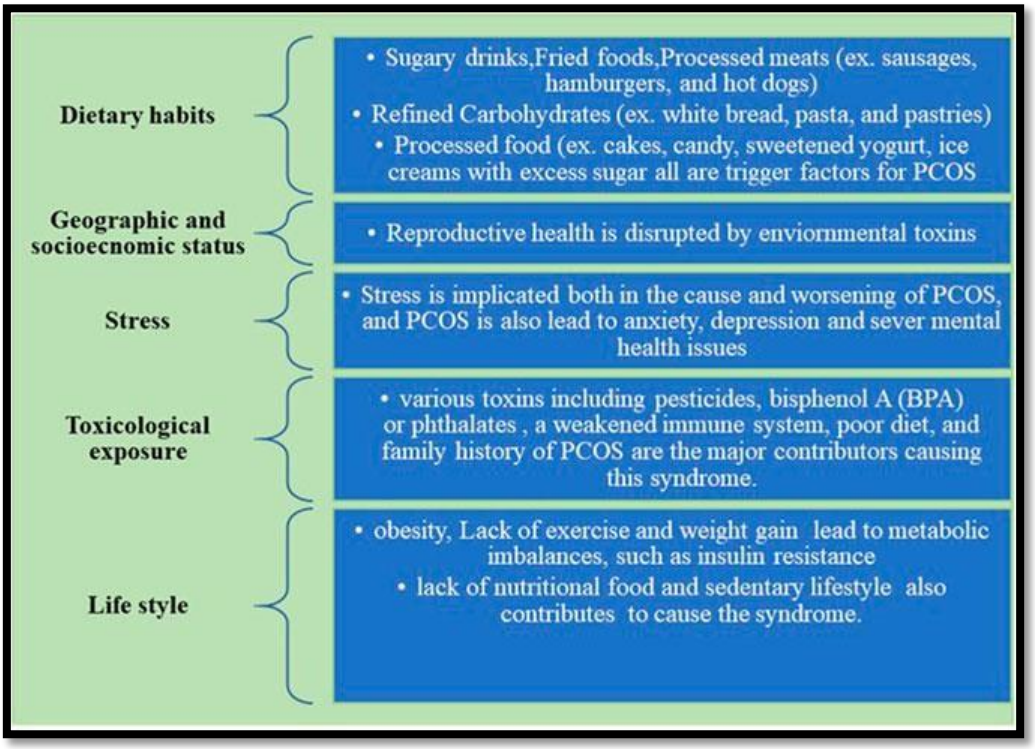


Figure 1.1(a): Environmental factors affecting PCOS (Lydic, & Juturu, 2008)

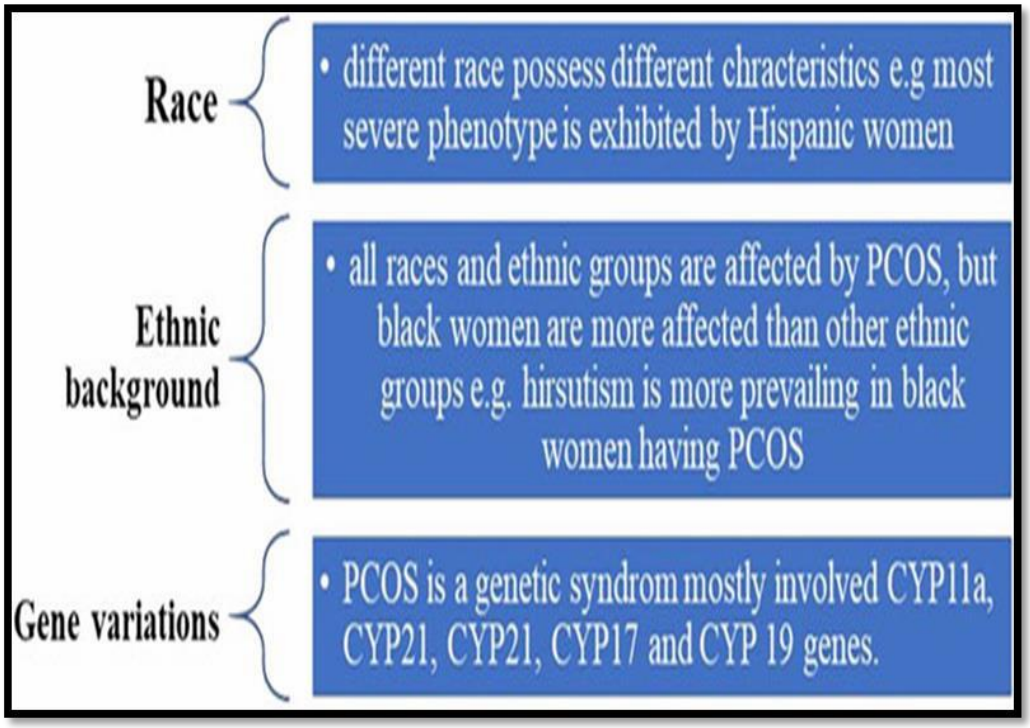


Figure 1.1 (b): Genetic factors associated with PCOS (Lydic, & Juturu, 2008)

According to Christ & Cedars (2023), it is believed that in 1990, National Institute of Child Health and Human Development made a first attempt to define PCOS, and clearly indicated that its presence could be demonstrated by the presence of both such as oligo/amenorrhea and biochemical/ clinical hyperandrogenism signs. But the conflict among leading practices in Europe and United States has been continued till 2003, in a context of defining key features of PCOS. Then, in Rotterdam, 27 PCOS experts met at a conference and developed a joint consensus statement most commonly called "Rotterdam Criteria" for diagnosing PCOS. The study by Thorat et al. (2023) revealed that it was suggested to use modified Rotterdam criteria that might diagnose PCOS by recognising any two of the aspects such as biochemical/clinical hyperandrogenism, polycystic representing-ovarian morphology on the ultrasound, and oligo-anovulation evidences.

Mukerjee (2020) also studied the main features of PCOS and revealed that polycystic ovaries have large sacs in which eggs are matured, but in the case of PCOS patients these sacs are unable to release the eggs. According to Diamanti-Kandarakis and Dunaif (2012), in PCOS the ovaries make higher than-normal amounts of androgens, interfering with egg development and release. Sometimes cysts develop on the ovaries, which are small fluid-filled pouches that can increase in size. In addition, without eggs, fertilisation does not occur. In a similar context Bharathi et al. (2017) evaluated that PCOS is a prevalent hormonal condition. PCOS is an endocrine disorder which has impacted around 20-30% of women of reproductive age (The syndrome is characterised by the presence of multiple ovarian cysts, menstrual irregularities, infertility, excessive hair growth and acne, and a high metabolic disorders risk like obesity and diabetes mellitus (Boyle & Teede, 2012). Regarding the Centres for Disease Control and Prevention report, the prevalence of PCOS is highly seen in the United States. The prevalence of PCOS in South Asian women, especially in Pakistani women, is much

higher (52%) as compared to white population (20 - 25% in UK) (Bharathi et al., 2017). Past studies have revealed that the main causes or factors that resulted in PCOS prevalence are still unknown. (Meng et al., 2016). One of the studies has linked prevalence of PCOS with inheritance issues (Stener and Deng, 2021). Whereas, generally, it is considered to be due to underlying hormonal dysfunction due to different genes and their transcription. In another study, it has been deduced that PCOS is caused by a deficiency in the process of transcriptional regulation of a genome caused by a polymorphism or even any sequence alteration (Strachan and Read, 1999).

Nowadays, the PCOS questionnaire is frequently used to measure the impact of infertility on the quality of life of women with PCOS. It represents five domains to be evaluated such as, infertility, menstrual problems, emotions, body hairs and weight. Now, a recent analysis of the studies forecasts that if obesity becomes a major problem around the world, more women may get PCOS (Shorakae et al, 2014). In females, the occurrence of PCOS is higher in Pakistan and about 52% of females are encountered as compared to different countries (Anjum et al., 2021). This high rate indicates the effect of PCOS on the female reproductive system severely (Liu et al., 2022). PCOS occurs due to an imbalance of hormonal levels that significantly influences the reproductive system and induces abnormality, including weight gain, infertility and excessive hair growth (Haq et al., 2017). However, to recognise the potential harm that is induced in PCOS, different associated factors such as obesity and diabetes mellitus are also checked by evaluating a hormonal profile, fasting sugar level, and fasting insulin level. Moreover, for this purpose, HbA1c is a diagnostic approach that is performed to determine the sugar level over several months (Shahsavari and Bavarsad, 2020).

The study by Kambale et al. (2023) demonstrated that there are multiple parameters that could be observed for diagnosing PCOS such as age, obesity, body mass

index (BMI), hormonal profile (Prolactin, FSH, LH, testosterone), fasting insulin levels, fasting blood sugar, HbA1c, CRP, dyslipidemia. It is also illustrated that Rotterdam criteria could be used for effective PCOS diagnosis but obesity could also be a common finding of PCOS women but is not a part of Rotterdam criteria. Hoeger et al. (2021) reflects that obesity is one of the major concerns among females experiencing PCOs. In a similar context, Makhija et al. (2023) revealed that women with PCOS are at enhanced risks of obesity and the factors contributing to obesity are genetic and environmental factors, such as inactivity and high-calorie meals. It is clearly indicated that there is close association among PCOS and metabolic dysfunction, furthermore BMI could allow for a clear and better appreciation for weight optimization among PCOS women. It is explored that BMI of 25 (kg/m^2) or less, could be defined as lean PCOS, whereas having BMI more than 25 (kg/m^2) could be referred as obese PCOS (Eng et al., 2023).

Additionally, it is believed that exact cause of PCOS is not completely known, but it is possible that obesity and weight gain may also contributes to the PCOS development. Abraham Gnanadass, Divakar Prabhu & Valsala Gopalakrishnan (2021) demonstrated that obesity is considered the main issue in patients with PCOS and almost 80% of individuals with the syndrome being overweight or obese. Obesity cause PCOS is associated with a fact that accumulation of body fat in these women is mainly attributed to resist insulin which is a key feature of PCOS. It has been found that in PCOS the cells of the body become extremely resistant to insulin which ultimately leads to increased levels of glucose in the body and the subsequent production of more insulin by the pancreas, which in turn regulates the ovaries to produce more androgens (male hormones), leading to the symptoms of PCOS (Casals-Casas & Desvergne, 2011). Insulin resistance also promotes the fat to accumulate in abdominal cavity, which can further

worsen insulin resistance and exacerbate the metabolic risks associated with the syndrome (Akbaribazm, Goodarzi, & Rahimi, 2021).

According to Zhang et al. (2019), PCOS has different metabolic characteristics as it is associated with significant defects in beta-cell function and insulin action that promotes the risks of type 2 diabetes and obesity. Moreover, it is demonstrated that there is clear association between PCOS and insulin resistance, though this resistance such as insulin resistance is completely independent of obesity (Shirazi et al., 2021). According to Shorakae et al. (2015), PCOS may produce too much insulin or show peripheral resistance to its action, mainly on insulin receptors present in muscle and body fat, which stimulates their ovaries to release excess male hormones. Having PCOS can be complicated for women's self-esteem since it causes skin problems, body hair or weight gain, among others (Norman et al., 2016). The good news is that even though this syndrome has no cure, it can be treated. Early diagnosis and treatment are essential to try to avoid, in the long term, the development of a multitude of different pathologies, including resistance to the action of insulin and the development of diabetes, alterations in fat metabolism (dyslipidemias) and infertility, since its existence is a risk factor for their development (Lim et al., 2012).

Xenin is referred as a peptide hormone produced within the gastrointestinal tract, and a serum xenin is believed as measurement/ concentration of xenin hormones within the blood. Serum xenin has a significant relationship with PCOS through and this relationship as well as its potential role is highlighted. For example, it is explored that xenin levels are much increased in the women with PCOS as compared to healthy females (Haq et al., 2017). In this section, a genetic factor that influences PCOS and interaction between xenin and infertility in PCOS females is also determined to provide the potential significance of xenin as a therapeutic intervention for females with PCOS (Dabravolski

et al., 2021). The key functions of xenin reveals that its increased concentration in PCOS significantly contributes to impaired glucose metabolism and insulin resistance. Additionally, in women with PCOS, serum xenin levels showed a positive correlation with glucose disposal rate, suggesting a potential function in glucose management (Purwar & Nagpure, 2022). In contrast to that, the study by Kruszewska et al. (2022) revealed that xenin plays an active role in pathogenesis of PCOS and insulin resistance. After considering these essential elements, the appropriate intervention for females with PCOS in Pakistan can be identified. Through this, the prevalence can be reduced efficiently (Aziz et al., 2023)

Women of reproductive age are susceptible to the complex hormonal condition known as PCOS (Stener-Victorin et al., 2020). While irregular periods, high androgen levels, and polycystic ovaries may be used to diagnose PCOS in fertile women, the symptoms and available treatments differ from person to person (Zehravi et al., 2021). In 1935 Stein and Leventhal described a clinical entity consisting of menstrual disorders, sterility, hirsutism and obesity (Shorakae et al., 2015). In addition, the ovaries of these patients presented certain particular morphological characteristics such as enlargement, thickening of the tunica albuginea and multiple microcysts located peripherally in the subcortical ovarian zone (Adashi et al., 2023). With the advent of ultrasonography, it was established that healthy women could present ultrasonographic images suggestive of polycystic ovaries without clinical syndrome.

Mumusoglu, S., & Yildiz, B. O. (2020) demonstrated that two traditional phenotypes, referred to as phenotype A and phenotype B, are included in the National Institutes of Health (NIH) criteria for the diagnosis of polycystic ovarian syndrome (PCOS). The "hyperandrogenic anovulation" phenotype, also known as phenotype A, is characterised by chronic anovulation (irregular or non-existent menstrual cycles),

whereas the "ovulatory" phenotype, also known as phenotypic B, is characterised by the presence of polycystic ovaries and chronic anovulation. So, phenotypes A and B meet NIH criteria and are considered classical forms. At the same time, phenotypes C and D are under discussion (Cao et al., 2019). Therefore, according to the Rotterdam consensus, polycystic ovaries do not necessarily have to be present to define the disease and the presence of polycystic ovaries alone does not establish the diagnosis (Giannouli et al., 2023). In adolescents, there is no established criterion to define this syndrome (Céspedes et al., 2010). Hirsutism, menstrual irregularities, acne and being overweight can represent physiological changes typical of the age. Two-thirds of apparently normal adolescents develop acne, half have menstrual irregularities and a high percentage become obese and of those, a quarter develop metabolic syndrome (Rosenfield, 2020). In addition, during the two years following menarche, girls may physiologically present with multi-follicular ovaries, which, associated with menstrual disorders, may be mistakenly confused with PCOS. Due to these characteristics, it has been suggested that at this age, the diagnosis should be based on biochemical hyperandrogenism associated with menstrual irregularities and the morphology of polycystic ovaries (Conway et al., 2014).

Bednarska and Siejka (2017) proposed a new approach to PCOS diagnosis. Anovulation, oligo-amenorrhea, hyperandrogenism, severe hirsutism and insulin resistance are major criteria for diagnosis. The minor criteria are polycystic ultrasound ovaries, high LH: FSH ratio, acne, mild hirsutism and obesity. The presence of two minor criteria constitutes a mild form of PCOS. A major and a minor criterion, or one or more majors and more than two minors, indicate a moderate or severe form of the disease (Hasan et al., 2022). According to Bednarska and Siejka (2017), this syndrome exists if at least two of the three following criteria are present: polycystic ovary shape, biochemical menstrual abnormalities, or/ and clinical evidence of androgen excess.

Furthermore, insulin resistance and raised blood LH levels are identified as typical hallmarks of this condition, and patients have an increased risk of Type 2 Diabetes Mellitus (DM20 and cardiovascular events. According to Norman et al. (2016), the concept of PCOS has had a considerable influence on scientific study and serious repercussions for each patient. The criteria suggested there are: clinical or biochemical hyperandrogenism, ovarian dysfunction proven by oligo anovulation with or without polycystic ovary morphology, and the exclusion of any other ovulatory disorder or androgen excess (Legro et al., 2013). These criteria were established by the National Institute of Health of the United States in 1990.

Polycystic ovary is a disorder that causes skin problems, body hair or weight gain and predisposes to other diseases (Jabeen et al., 2022). The cause of polycystic ovary is currently unknown and what is observed is an imbalance in the reproductive hormones (estrogen, progesterone and androgens) that the ovaries manufacture and that is responsible for regulating the menstrual cycle and ovulation (the moment when the egg is released) (Walters and Handelsman, 2016). In women, the ovaries make higher than normal amounts of androgens, which leads to interference with egg development and release. According to Walters and Handelsman (2016), sometimes cysts develop on the ovaries, which are small fluid-filled pouches that can increase in size. Thus, women suffering from this syndrome may not ovulate regularly and, therefore, it is common/frequent that they have an absence of menstruation (ammenorrhea) or few menstrual periods a year (oligomenorrhea) (Sirmans and Pate, 2013).

A woman's reproductive and general health may be adversely impacted by PCOS, a prevalent disorder. According to Stener-Victorin et al. (2020), polycystic ovaries are a rather frequent finding in women of reproductive age in the UK, affecting 20–25% of women, however, not all these women suffering from PCOS, experience any symptoms

so it goes unnoticed. The significance of polycystic ovaries in the UK depends on whether they are found in isolation or in conjunction with other signs of PCOS. If a woman has no other symptoms, the presence of polycystic ovaries on an ultrasound scan is usually not a cause for concern and does not require any treatment. Further testing and therapy may be required, according to Conway et al. (2014), if a woman exhibits other PCOS symptoms such as irregular periods or excessive hair growth. According to Shorakae et al. (2015), certain genetic variants may enhance the chance of getting PCOS and there may be a hereditary component to the development of the disorder. It is not yet known whether xenin or other gut hormones are directly connected to the inheritance of PCOS, and further research is still needed to identify the precise genes responsible for PCOS (Craig et al., 2021). It's important to keep in mind that PCOS can affect women of any age, not simply those who are infertile, while discussing its presence in fertile women (Caldwell et al., 2014). Even though infertility is a typical PCOS symptom, not all PCOS sufferers have trouble getting pregnant. Period irregularities, increased hair growth, acne, and weight gain are all potential signs of PCOS. More studies are required to properly comprehend the association between these variables, even though research into the potential involvement of xenin in PCOS is still being conducted (Céspedes et al., 2010).

Polycystic ovaries, despite what it may seem at first, are not directly related to the development of cysts. In reality, we are talking about an alteration in the arrangement of the follicles anatomical and functional structures that are part of the ovaries (Walters and Handelsman, 2016). This often occurs in young women and can be asymptomatic or associated with painful ovulations and irregular periods. It would be more correct to call this type of ovaries 'multicystic' rather than 'polycystic' because in most cases they are asymptomatic and do not require treatment (Stener-Victorin *et al.*, 2020). In cases where one or more of the aforementioned symptoms occur, hormonal treatment adapted to the

needs of each patient will be prescribed (Jonklaas, 2022). With the passage of time, it is normal for this type of polycystic ovaries to cease to be. By decreasing the number of follicles, so do the symptoms that may have manifested (Norman *et al.*, 2016). PCOS symptoms can be very different from patient to patient. Some girls or women may experience symptoms of very mild intensity (Zhou et al., 2017). Others may suffer from a wide variety of symptoms to a greater or lesser degree. Some of them are (Witchel et al., 2015b) (Figure 1.2):

- Irregular menstrual periods, not having it (amenorrhea), or abnormal uterine bleeding. These types of alterations occur in 8 out of 10 women.
- Polycystic and large ovaries.
- Pelvic pain.
- Increased facial and body hair (hirsutism). It occurs in 70% of cases.
- Weight gain and obesity, especially with a distribution of fat in the centre of the body. Obesity appears in 50-60% of cases. However, some women may have excess androgens and other symptoms of PCOS and have weight according to their body and characteristics.
- Acne. It affects a third of women. It may be accompanied by darkening or thickening of the skin (spots or streaks around the neck and in the armpits).
- Hair loss on the top of the head.
- Deep voice (very rarely).

The increased chance of infertility for women who are fertile is one of PCOS's most important effects. Ovulation irregularities in PCOS women can make it more challenging to get pregnant (Liu et al., 2021). According to Witchel et al. (2015b), many PCOS women can conceive and have healthy pregnancies with the right management

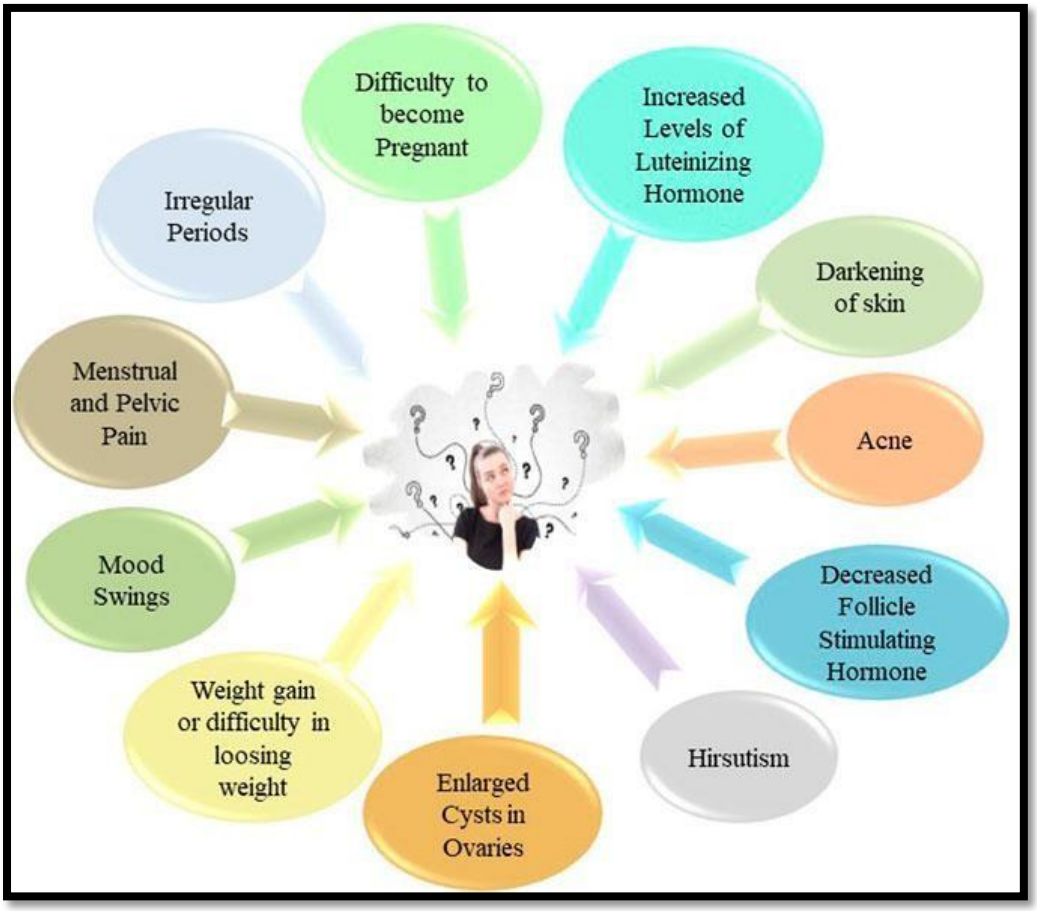


Figure 1.2: Signs and symptoms of PCOS (Source: Witchel et al., 2015)

and therapy. Premature birth, gestational diabetes, and pre-eclampsia are among the pregnancy issues that PCOS can make more likely. To ensure a successful pregnancy and delivery, medical professionals should closely follow PCOS women who become pregnant (Witchel et al., 2015). It's crucial to remember that not all PCOS sufferers have the same symptoms, and that symptom intensity can vary greatly. PCOS can affect a fertile woman's health in other ways besides her reproductive system (Punzón-Jiménez, and Labarta, 2021). According to Aziz et al. (2016), type 2 diabetes, metabolic syndrome, and cardiovascular disease are all more likely to occur in PCOS women.

Wilson et al. (2014) claimed that PCOS women experience excessive hair growth, acne, and weight gain, which can lower their self-esteem and quality of life. The management of these signs and symptoms can enhance general health and quality of life. For fertile women, PCOS can also have psychological effects. Acne and excessive hair growth are two outward signs of PCOS that can cause feelings of humiliation and low self-esteem. Sadness, anxiety, and melancholy can also result from infertility and the difficulties of attempting to conceive (Zhou et al., 2017). Support from family, friends, and healthcare professionals is necessary for PCOS women. The psychological effects of PCOS can also be managed with the aid of counselling and support groups (Witchel et al., 2015). For fertile women, PCOS can have a variety of negative effects on their reproductive, general health, and psychological well-being (Jiskoot et al., 2020). However, many PCOS women can conceive, have successful pregnancies, and lower their risk of developing additional health issues with the right management and medication (Azziz et al., 2016). To treat their illness and improve their health and well-being, PCOS women must collaborate closely with their healthcare providers.

There are certain genes associated with PCOS. One of the primary genes associated with PCOS is the (IRS-1) gene. This gene is responsible for regulating insulin

levels in the body (Abraham Gnanadass et al., 2021). The gene receptor substrate-1 (IRS-1) is known to play a critical role in the progression of PCOS. This gene codes for a protein that is involved in insulin signalling pathways in the body (Bharathi et al., 2017). In individuals with PCOS, there is often dysfunction in how the body responds to insulin, leading to insulin resistance. This, in turn, can cause hormonal imbalances, including an excess of androgens (male hormones), which can contribute to symptoms such as acne, hirsutism and irregular menstrual cycles (Boyle & Teede, 2012).

Another gene closely associated with PCOS is the FTO (Fat mass and obesity-associated) gene. This gene is responsible for regulating body weight and metabolism (Casals-Casas & Desvergne, 2011). PCOS women have been found to have alterations in this gene, which may increase their risk of obesity and the complications associated with it. Other genes that have a significant role in the inheritance of PCOS include the LH receptor gene, the CYP17 gene, and the AMH (anti-Mullerian hormone) gene (Craig, Gault & Irwin, 2018). LH receptor gene helps in the regulation of ovulation, and alterations in this gene may disrupt the normal menstrual cycle. The CYP17 gene is significant for androgens, and alterations in this gene may lead to an overproduction of these hormones, which causes the symptoms of PCOS (Craig, Irwin & Gault, 2021). The AMH gene is responsible for the production of the anti-Mullerian hormone, which regulates the growth of follicles in the ovaries. Alterations in this gene may cause the formation of cysts in the ovaries (Davies, 2016). While these genes increase the risk of PCOS, genetic factors alone may not be sufficient to cause this condition. Other factors such as lifestyle, environment, and diet can also contribute to the onset and severity of PCOS (Witchel et al., 2015). condition than women without such a history.

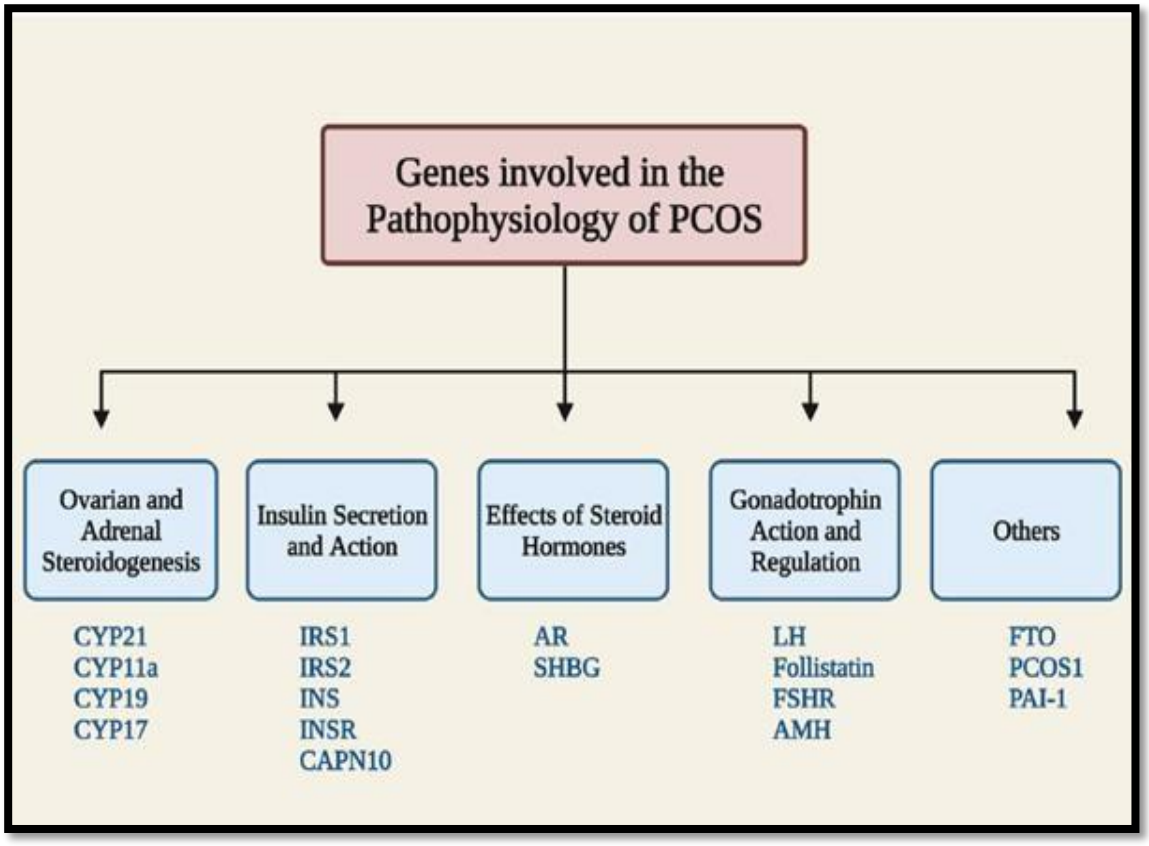


Figure 1.3: Genes involved in pathophysiology of PCOS (Khan, Ullah, & Basit,2019)

Figure 1.3 represent family history are that PCOS is a condition that has a genetic component. It is explored that women with a PCOS more susceptible to develop the condition than women without such a history. The genes associated with PCOS are involved in the regulation of insulin levels, body weight, ovulation, and the production of androgens. However, PCOS is also affected by lifestyle and environmental factors in which females live (Gnanadass, Prabhu & Gopalakrishnan, 2021).

The relationship between PCOS and diabetes mellitus is complex, with both conditions exhibiting several overlapping features. Women who suffer from diabetes are considered to have chances of developing diabetes mellitus (T2DM), with estimates ranging from 3-10 times higher compared to women without PCOS (Craig, Gault & Irwin, 2018). This risk is further increased in individuals who are overweight or obese. The underlying mechanisms for this association are not entirely clear; however, it is believed to be related to insulin resistance, hyperandrogenism, and chronic inflammation (Craig et al., 2018). Insulin resistance is also considered the main reason behind the development of T2DM. A person who has a great level of insulin resistance in the body will be at risk of developing a state of hyperinsulinemia in which the body is subjected to an increased level of insulin secretion by the pancreas beyond a certain limit (Davies, 2016). Over time, the excess insulin secretion leads to pancreatic exhaustion, leading to decreased insulin production and eventually developing into T2DM. In addition to its association with diabetes mellitus, metabolic disturbance is also associated with PCOS, which includes dyslipidaemia, hypertension and increased cardiovascular risk (Guclu Sahin & Aksit, 2019). These metabolic abnormalities can significantly increase cardiovascular diseases and stroke, which is the leading cause of mortality in PCOS women (Amiri & Tehrani, 2020).

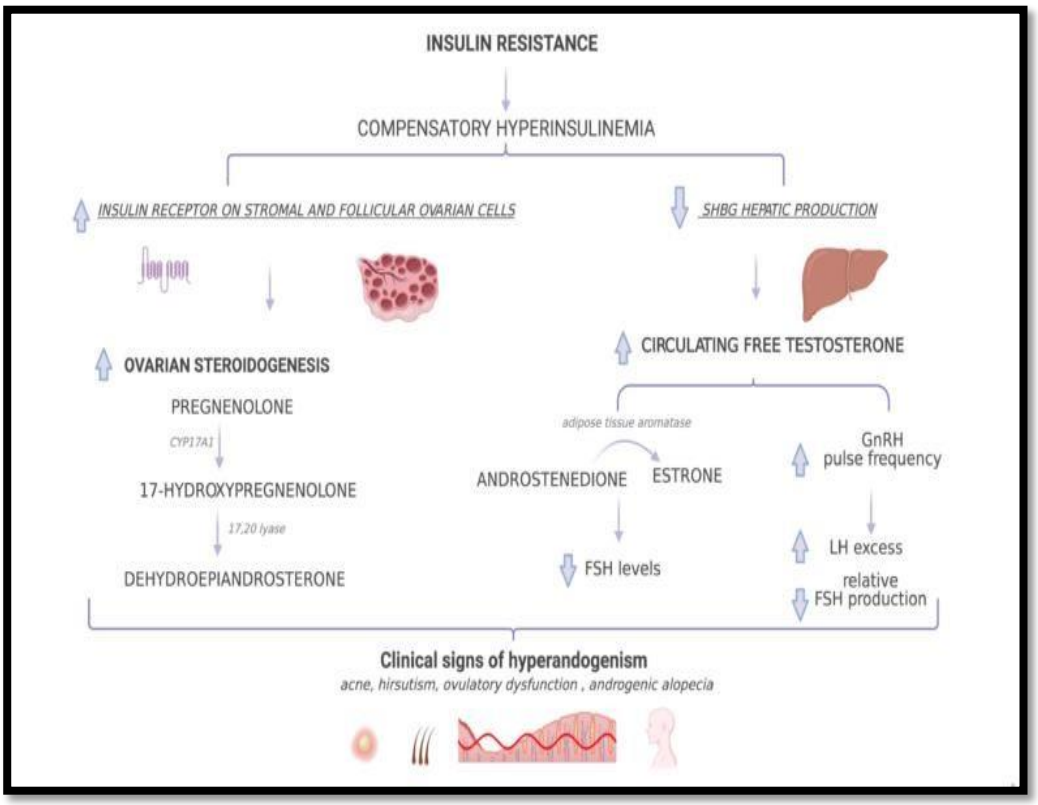


Figure 1.4: Insulin resistance and occurrence of PCOS (Carreau, A.M., 2022)

Figure 1.4 reflected that there is significant association among insulin resistance and the presence/ occurrence of PCOS. In other words, the insulin resistance outcomes in PCOS cases are represented in this figure.

Hence, PCOS is a disorder associated with increased risks of obesity, insulin resistance, and diabetes mellitus. Obesity and insulin resistance, in turn, are critical determinants of the metabolic complications of the syndrome (Anjum et al., 2020). Healthcare professionals should be aware of the close relationship between PCOS, obesity, and diabetes mellitus, and adopt a comprehensive approach to the management of these disorders, with emphasis on lifestyle changes, weight loss, and appropriate pharmacologic interventions (Armanini et al., 2022). Future research should focus on elucidating the underlying mechanisms responsible for the link between PCOS, obesity, and diabetes mellitus and developing more effective treatments to improve the metabolic outcomes and also focused on the improvement of quality of life in women who suffered from PCOS (Aslam et al., 2022).

The hormonal profiles could also be measured in order to demonstrate the presence of PCOS, as it is believed that during this condition the concentration of FSH, LH, prolactin and testosterone are being altered as compared to normal (i.e., in healthy females). Follicle-stimulating hormone (FSH) is referred to as the hormone that plays key roles in sexual functioning and development. Normally, FSH regulates the menstrual cycle as well as is involved in the growth of the eggs within the ovary and has a range of 4-8 among young fertile women. While, in PCOS, the FSH levels are often within the range of about 4-8 while the ratio of LH to FSH is expected to get much higher such as 2:1 or 3:1 (Khmil et al., 2020). (Fig 1.5, 1.6)

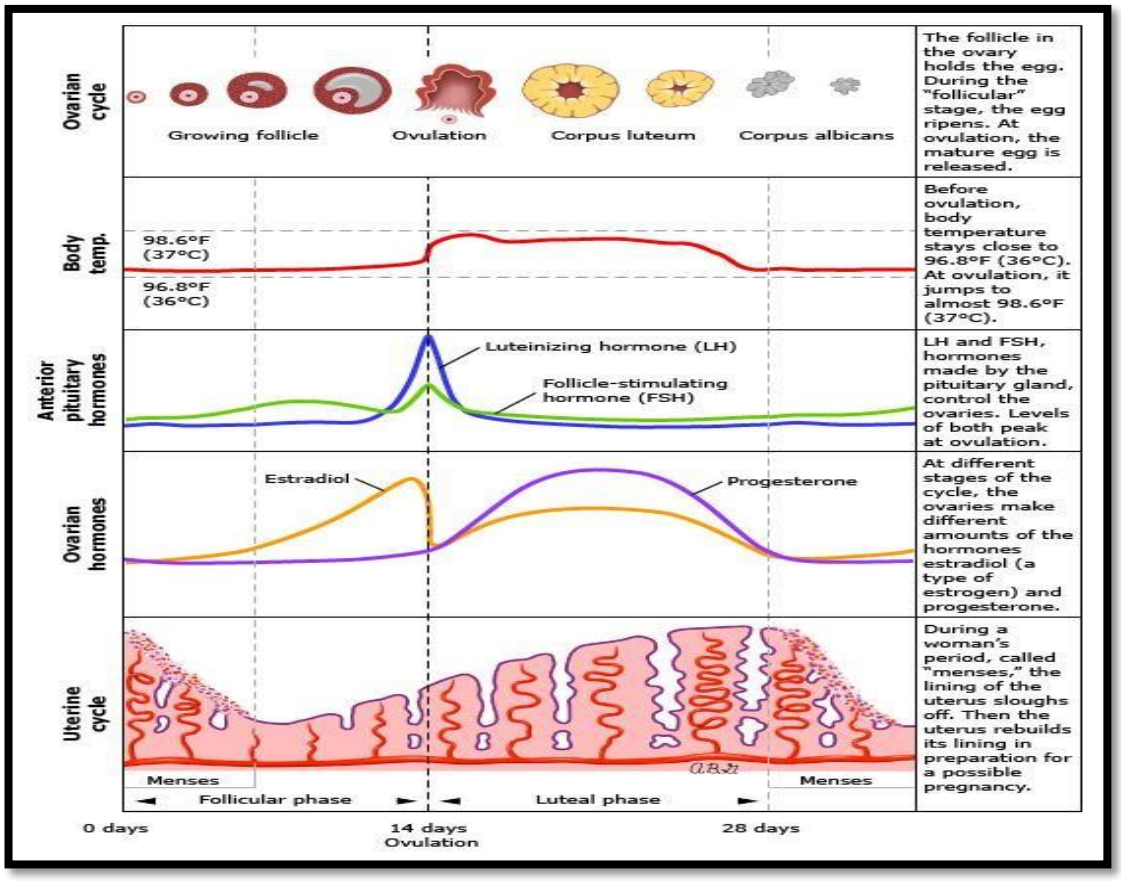


Figure1.5: Hormonal variations during menstrual cycle (Barbieri & Ehrmann, 2018)

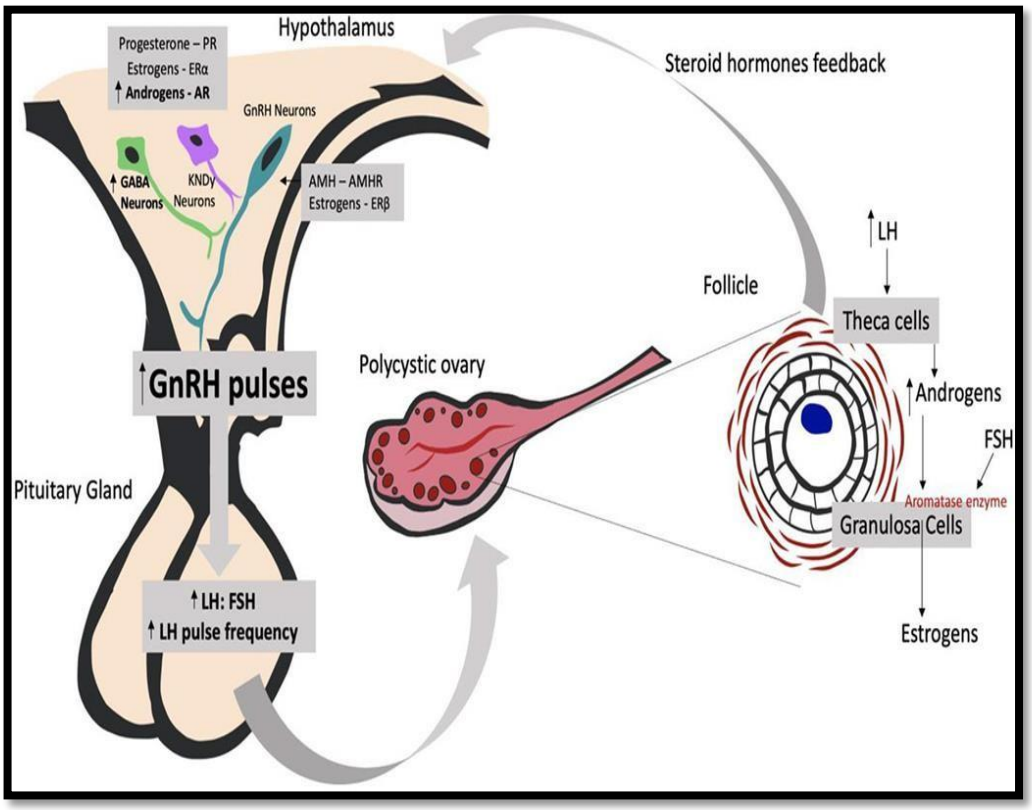


Figure 1.6: Hormonal disruption during PCOS (Doretto et al., 2020)

Luteinizing Hormone (LH) is found in blood and is generated in the pituitary gland. The LH normal range is about 4-8 in young fertile females but is believed to be much enhanced in PCOS. In PCOS, the LH to FSH ratio is around 2:1 or 3:1, referring to an aspect that LH is much elevated. The hypersecretion of LH during PCOS is due to the fact that pituitary sensitivity is increased leading to enhanced gonadotropin-releasing hormone (GnRH), causing increase in LH concentrations (Coutinho & Kauffman, 2019).

Prolactin is a hormone that is considered to regulate the balance among differential body hormones. It is believed to stimulate milk production and breast development among females, while in males, its functions are unknown. The normal Prolactin levels are 25 ng/ml or less than it, while it is believed that in PCOS, prolactin concentration is much enhanced, such as above 25 ng/ml (Davoudi et al., 2021).

Testosterone is a steroidal hormone produced in adrenal glands in both women and men and is controlled by LH. The normal concentration of testosterone is less than 150 ng/dL (≤5.2 nmol/L), while it is much lower in PCOS. For example, testosterone values ≤150 ng/dL (≤5.2 nmol/L) indicate PCOS, while if its values are ≥200 ng/dL (≥6.9 nmol/L), the female might have an adrenal or ovarian tumor (Meczekalski et al., 2021).

There might be a close association between PCOS and fasting blood sugar, as it is believed that women experiencing PCOS are usually insulin resistant. Meanwhile, in PCOS – the female body might make insulin but is incapable of utilising it in effective ways. The inadequate utilisation of insulin among PCOS females is more likely to enhance the risks of enhanced fasting blood sugar or type 2 diabetes (Pani et al., 2020).

Fasting blood sugar is referred to as the simplest and most common blood test that is effective for diagnosing prediabetes, diabetes as well as gestational diabetes (Vijayam et al., 2022). For demonstrating the fasting blood sugar levels, the individuals are requested to stop eating or drinking (except water) for about 8 to 12 hours prior to the

test. The enhanced fasting blood sugar refers to increased glucose concentration or hyperglycaemia, which is a significant indicator of the risks of diabetes incidence (Yang et al., 2019). The fasting blood sugar should be considered for the patients experiencing PCOS as there might be insulin resistance among them (Sun et al., 2020). According to Ishrat and Hussain, (2021), PCOS is much more frequent in women at their reproductive age, and in this condition- the prevalence of hyperinsulinemia or insulin resistance is much more frequent. PCOS is referred to as an endocrine disorder inducing inflammation as well as altering blood sugar levels via insulin resistance. PCOS is considered polygenic or multifactorial in nature and generates meaningful impacts on the insulin and glucose pathways (Maqbool et al., 2019).

Figure 1.7 is representing that insulin reduces blood sugar by appropriate storage of glucose among living cells, meanwhile, if there is a continuous insulin supply, the cells might become resistant, and the signals for lowering blood sugar levels are required in such cases (Joham et al., 2022). In these cases, the resistance goes on, leading to high blood sugar or insulin levels. Both the insulin and glucose levels have close relevance in diabetic outcomes; hence for demonstrating the association between PCOS and fasting sugar levels, the insulin levels are also vital to consider (Barber et al., 2019). According to Modak et al (2019), it is believed that around 40% of women experiencing PCOS are more likely to initiate prediabetes (or enhanced blood sugar levels). In a female patient experiencing PCOS, increased blood sugar levels, as well as increased fasting blood sugar levels, are predicted. It is encountered that around 30 to 40 % of PCOS patients are patient's prediabetic outcomes might progress into type 2 diabetes. Furthermore, prediabetes may also increase the risks of adverse cardiovascular events, in these ways, it is demonstrated that fasting blood sugar levels are influenced by PCOS in significant ways (Allen et al., 2022).

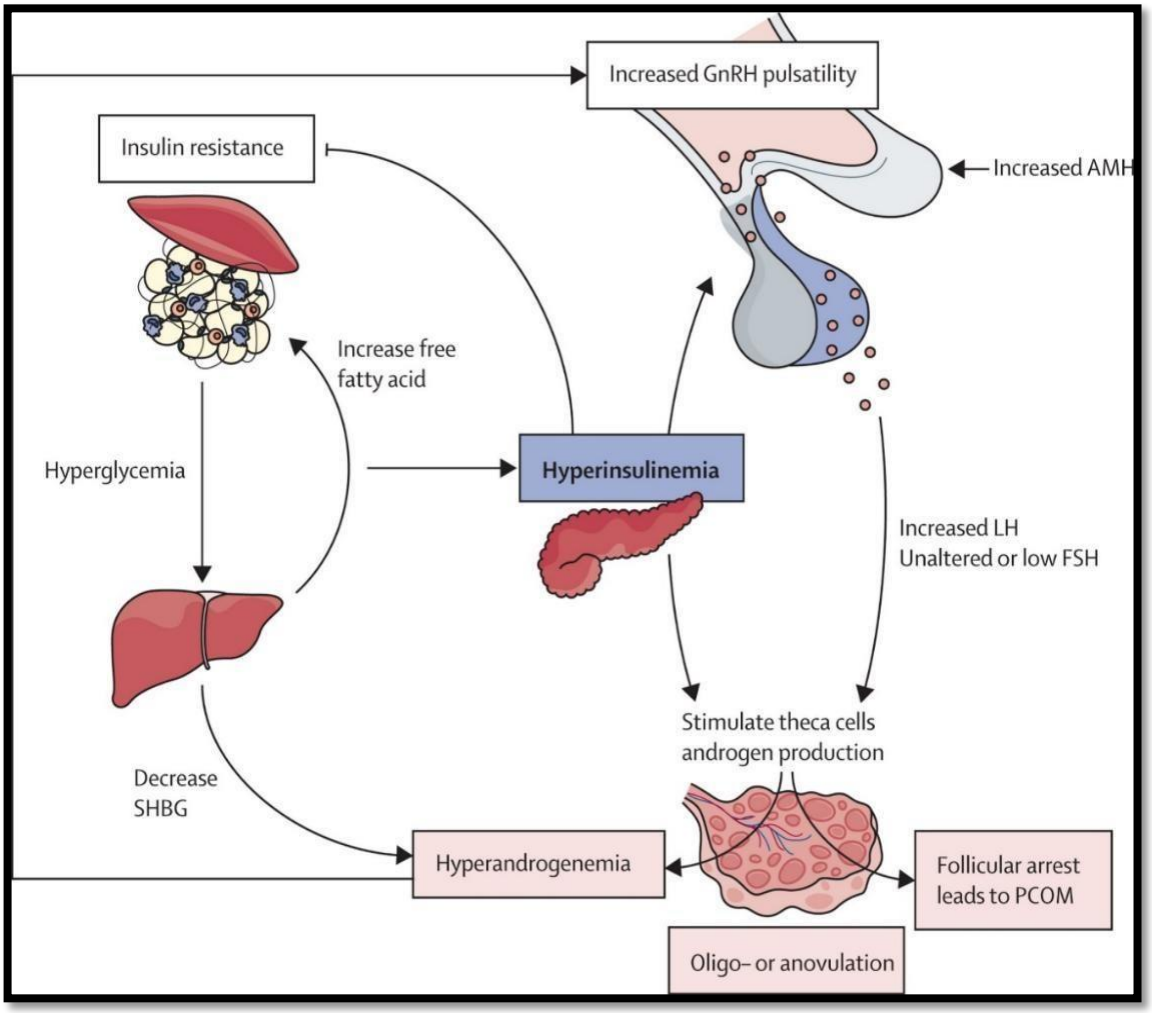


Figure 1.7 PCOS and Metabolic Outcomes *(*Joham et al., 2022*)*

Hartmann and McEwen (2019), also believed that almost 70% of the PCOS also demonstrated that PCOS disrupts normal glucose or insulin pathways, leading to improved fasting blood glucose levels, but meaningful interventions such as nutrition and diet play a significant role in managing PCOS. The specified nutrients and diets have beneficial impacts on the cardiometabolic parameters of PCOS as well as reduce the risks of enhanced blood sugar levels (prediabetes or type 2 diabetes) in PCOS patients. In these ways, it is found that in PCOS females, the fasting blood glucose or sugar levels are more likely to disrupt (Mohamed et al., 2023).

Insulin sensitivity is being accessed by evaluating fasting insulin levels. The fasting insulin levels provide a clear idea regarding the diabetic outcomes, for example- the impaired sensitivity of insulin precedes the intolerance of glucose in type 2 diabetes development. It is believed that elevated fasting insulin levels is the compensatory mechanism for preventing diabetes or glucose tolerance (Al-Beltagi et al., 2022). In the context of PCOS, it is encountered that most people have enhanced insulin levels as well as insulin resistance. Meanwhile, high or reduced insulin or insulin resistance might indicate PCOS or any other underlying physiological conditions (Xu & Qiao, 2022). Insulin resistance is often noticed among women experiencing PCOS as, in this condition, their bodies are incapable of utilising insulin in effective ways. PCOS is a disorder that not only influences the ovulation or ovaries but is also considered a full-body endocrine and metabolic disorder as is closely relevant or tied to insulin resistance (Zeng et al., 2020). The fasting insulin levels are more likely to shoot up to under ten during PCOS meanwhile the fasting glucose levels should be under 90 or so (Risal et al., 2019).

According to Zhao et al. (2023), elevated fasting insulin levels are a major sign of abnormal metabolic outcomes or parameters among women. This study found that the rise in insulin levels in PCOS, as well as other pathophysiological conditions, indicates

that there is insulin resistance within the body. It is also encountered that the PCOS-experiencing women show more pronounced dyslipidemia as compared to the controls when the fasting insulin status is stratified. Almost in least 50% of PCOS patients, insulin resistance is being noticed due to the excessive serine phosphorylation among the insulin receptors (Lewandowski et al., 2022). Usually, clinicians prescribe a natural or dietary treatment for PCOS, along with that they also recommend fasting insulin tests too, such as the HOMA-IR index. The fasting insulin levels are being considered for the evaluation of insulin resistance among the patients, via a glucose challenge test such as a 2-hour insulin glucose challenge test. After fasting for 8 hours, the professional collect blood samples of the respective patients such as PCOS patients, and encounter the patient's insulin levels (Ismayilova and Yaya, 2022). A fasting insulin level is an effective approach for encountering is there is insulin resistance developed due to PCOS among particular patients or not (Livadas et al., 2022).

Ionescu et al. (2023) demonstrated that for proposing promising therapeutic approaches or interventions for PCOS among infertile as well as pregnant women, the fasting insulin, as well as glucose levels, are referred to as encounter. This study reveals that insulin is a pancreatic hormone playing a vital role in glucose metabolism, and due to the most common hyperinsulinemia condition, the PCOS therapeutic approach's goal is to lower insulin resistance for improving fertility or pregnancy outcomes. In these ways, it is encountered fasting insulin levels and the PCOS have close relevance, as insulin levels are expected to be much higher in PCOS.

PCOS is an endocrine disorder in which different functions induce inflammation, alter blood sugar by insulin resistance, enhance the risk of cardiovascular diseases and metabolic abnormality. HbA1c is a standard blood sugar test that is used to measure the level of sugar in the blood in order to identify pre-diabetes and diabetes over three months

(Nazari et al., 2021). The value of HbA1c is about 5.4%, indicating that patients with PCOS have induced high-sensitive C-reactive protein (hs-CRP), which participated in promoting cardiovascular diseases (CVD) (El-Dalou, 2019). The disulphide-linked hetero-diameters are composed of Alpha-subunits or Beta-subunits known as (Inhibin-B), and it is interlinked with the transforming growth of factors β (TGF-β). Moreover, this Inhibin-B is essential for female's normal reproductive system. The regular secretion of inhibin-B in the granulosa cell helps develop the follicles (Wen et al., 2021). Bouzoni et al., (2022) stated that the low level of inhibin-B impacts the ovaries and decreases maturity for fertilisation. In contrast, the increased level of inhibin-B also influences the female body, such as increased body weight, anorexia nervosa and leptin levels. Alteration in the level of inhibin-B is a result of insulin suppression. Hassan and Nasif (2020) stated in the study that hbA1c has a significantly higher level of PCOS patients. In contrast, Inhibin-B is considered a lower level in patients with PCOS. The findings showed that HbA1c has a crucial correlation with PCOS patients also having T2DM. Additionally, through HbA1c, type 2 diabetes is diagnosed in the patient with PCOS.

Furthermore, the occurrence of diabetes is high about 10% of females with PCOS (Lisa Marie Basile, 2020). This means the patient with PCOS encounters a higher level of testosterone, which can lead to insulin resistance. Additionally, females have a low testosterone level, but in PCOS females, this level eventually rises and impacts insulin resistance (Zeng et al., 2020). However, an increased level of insulin may significantly severe PCOS in which the raised insulin influences specialised cells called theca cells to produce more androgen (Han et al., 2023). It is also increasing the number of theca cells in order to produce androgen, which impacts the reproductive system abnormality. About 60% to 80% of androgen is enough to cause menstrual disorders etc (Shankar et al., 2023). Meczekalski et al., (2023) stated that an increased level of androgen helps to diagnose

PCOS. The reason to use HbA1c in the PCOS patient is to evaluate the potential impact on the different vital organs, such as the cardiovascular system. This occurs due to an increased level of hs-CRP. By using HbA1c, healthcare professionals may identify that an elevated level of 5.7% suggests that prediabetes' risk in the patient (Herman et al., 2021). In addition, a lower level of HbA1c is indicated that a PCOS patient has no risk but an increasing level of HbA1c in PCOS indicates that the patient has a risk of cardiovascular disease due to effective substances (Drzewoski and Hanefeld, 2021).

Xenin is a hormone made up of peptides produced in the duodenum's mucous membrane. The peptide has been found in mammals such as rabbits, rats and humans (Haq et al., 2017). Xenin circulates in the plasma of blood in humans (Navarro et al., 2018). Xenin is significant in insulin secretion and its potential for diabetes therapeutics has recently gained a lot of attention, mainly because of its capacity to augment the insulinotropic effects of Gastric Inhibitory Polypeptide (GIP) and its use in dual/triple acting gut hormone therapeutic methods (Helvaci et al., 2017). Because there are currently no clinically licensed medicines for the restoration of type 2 diabetes, xenin could be a promising diabetic treatment (Jasim & Alkareem, 2022). Xenin has several essential biological activities that have already been demonstrated in a variety of animal systems and have been thoroughly discussed recently. Controlling energy intake and gastrointestinal transit, delaying stomach emptying in individuals, suppressing appetite and modulating pancreatic exocrine as well as endocrine functionality are only a few of xenin's main biological functions (Jonard et al., 2003).

PCOS is a complex and multifaceted endocrine. Recent scientific research has identified a new neuropeptide called Xenin in the pathogenesis of PCOS. Xenin is a small, 25 amino-acid peptide that was initially identified in the small intestine and it has a crucial role in regulating appetite and energy metabolism (McCartney & Marshall, 2016). Recent

studies have shown that xenin is also expressed in the ovaries, where it interacts with receptor-coupled guanine-nucleotide-binding (GPCR) proteins to modulate various endocrine functions (Meng et al., 2016). In particular, xenin is considered to be involved in the regulation of secretion of insulin, glucose metabolism, and lipolysis within the ovarian follicles, which are critical processes that can be disrupted in PCOS women (Baldani et al., 2019).

Moffett, Docherty & le Roux, (2021) stated that xenin is involved in ovarian follicle growth and development. For example, a study conducted by Navarro et al., (2018) showed that xenin treatment of mouse ovarian follicles resulted in increased follicle growth and survival, as well as enhanced oocyte maturation and fertilisation. The study by Ndefo, Eaton & Green (2013) shows that xenin is directly involved in the regulation of follicular development and maturation, which may be disrupted in PCOS women.

Xenin is a sequence of a 25-amino acid peptide that is secreted from human jejunal, duodenal and gastric mucosa. Vital organs such as the lung, heart, liver, pancreas, testicles, skin, hypothalamus, kidney and adrenal gland may also produce xenin (Parthsarathy et al., 2016). The concentration of xenin is increased after meals in the blood and affects the exocrine secretion of the pancreas (Ramanand et al., 2013). The electromechanical activity of gastrointestinal smooth muscle known as Migrating Motor Complex also relates to the peaks of plasma xenin concentration. For instance, the infusion of synthetic xenin causes the activity of the third phase of Migrating Motor Complex in fasting individuals. In the postprandial state, xenin infusion increases the frequency of generated contractions (Roland et al., 2010). One of the most important functions of xenin is to significantly improve the disposal of glucose and the secretion of insulin in the human body (Bongrani et al., 2022)

Xenin is identical to an amphibian octa-peptide xenopsin. Xenin does not have its particular binding receptor. Hence, it binds to the neurotensin receptor 1 (NTSR-1) (Shorakae, Boyle & Teede, 2014). However, sequences of both peptides stimulated the secretion of insulin from rodent β-cells. It has been shown in a recent study that xenin may intensify the secretion of glucose-dependent (GIP)-mediated insulin. Since xenin is co-localised with GIP within the endocrine cells of the small intestine, hence, different derivatives of xenin such as xenin-25, xenin-8 and the C-terminal octa-peptide fragment exert an incretin effect (Stener-Victorin, & Deng 2021). Moreover, Xenin-25-Gln maintains glucose homeostasis, enhances metabolic control and increases lipolysis. Hence, these derivatives of xenin can be considered potential drugs that help in treating diabetes mellitus by increasing lipolysis (Temur et al., 2017).

Hence, although the exact role of Xenin in PCOS is not fully understood, it is clear that this neuropeptide is significant in the pathogenesis of the condition (Toosy, Sodi & Pappachan, 2018). Xenin is involved in the insulin secretion regulation, lipolysis in the ovaries and glucose metabolism, as well as the regulation of ovarian follicle growth and development Shorakae, Boyle & Teede, 2014).

Xenin can be activated by neurotensin receptor for the mediation of hormonal activities in the body. In addition, the animal models have shown that Xenin has a prominent role in the maintenance of insulin production. Patients with PCOS show insulin resistance and complications of type 2 diabetes that indicates a relationship between the Xenin abnormality and the PCOS existence. The management of PCOS could be possible by considering weight loss drugs, anti-anxiety drugs, oral contraceptive pills and gonadotropins (Ndefo, Eaton and Green, 2013).

Xenin is a recently discovered hormone produced by the stomach that has been linked to both insulin secretion and appetite regulation. Guclu, Sahin & Aksit (2019) have

shown that xenin levels are elevated in PCOS women, particularly those who are obese or have insulin resistance. The potential role of PCOS with infertility women is still being investigated. Haq et al. (2017) have suggested that xenin helps in the pathogenesis of PCOS by influencing insulin resistance and metabolic dysfunction. Insulin resistance, a hallmark feature of PCOS, is closely related to the development of hyperandrogenism and irregular menstrual cycles. Xenin has been found to enhance the secretion of insulin and suppress the secretion of glucagon, suggesting that it may contribute to the development of insulin resistance (Brennan et al., 2019).

In addition to its effect on insulin resistance, xenin has also been implicated in appetite regulation. It has been highlighted by the Haq et al. (2017) that PCOS women have increment in appetite and an altered response to food intake, which may contribute to the development of obesity and metabolic dysfunction (Helvaci et al., 2017). Xenin has been shown to regulate food intake by stimulating the release of satiety hormones and reducing hunger, which may provide a potential therapeutic target for the treatment of PCOS-related obesity. Infertility in PCOS women is caused by ovulatory dysfunction, which is associated with an imbalance in follicle-stimulating hormone (FSH) and luteinising hormone (LH) secretion (Jasim & Alkareem, 2022).

Hence, Xenin is considered to play an important and significant role in infertility in women. Additionally, xenin may have a direct effect on ovarian function and may have therapeutic potential for improving fertility outcomes in PCOS women (Calcaterra et al., 2021). Higher concentration levels of xenin were present in PCOS patients (Roland et al., 2010). In terms of xenin association with PCOS, it has been demonstrated that levels of xenin-25 were elevated in small set of 32 women diagnosed with PCOS as compared to the healthy and regular menstruating women (Jonard et al., 2003). Moreover, the optimal cut-off value for diagnosing PCOS using xenin-25 as a biomarker was 32.60 pg/mL, with

an 86.7% specificity and 61.3% sensitivity. Approximately 5.6 – 21.3% of women across the globe are affected by PCOS at their reproductive age (McCartney & Marshall, 2016). In Meng et al., (2016), a strong link between xenopsin related peptide-1 and PCOS has been identified with levels of xenopsin considerably higher in people suffering from PCOS as compared to healthy people (Temur et al., 2017). Similarly, serum xenin levels in females with PCOS are considerably higher than in individuals without menstrual cycle disorders, as determined by Guclu et al. (2019). Along the same lines, higher xenin levels (xenopsin-related peptide-1) in the prevalent PCOS group were also identified by Moffett et al. (2021).

Although many authors identified that the level of xenopsin was significantly higher in PCOS patients than in the healthy group; however, it still requires comprehensive analysis (Navarro et al., 2018). For example, it has been reported during previous studies by Ndefo, Eaton & Green (2013) that Xenin levels were not significant in the control group; however, different sex hormones should also have been assessed in these women to confirm the disease condition (Craig, Irwin & Gault, 2021). Xenopsin also results in cellular inflammation. It does so by inducing settings, is not known (Jasim & Alkareem, 2022). Parthsarathy et al. (2016) have shown that xenin macrophages and stimulating histamine levels in mast cells. However, the exact correlation between xenopsin and CRP, which is a systematic inflammation biomarker used mostly in clinical levels are elevated in PCOS women. This suggests that xenin may help in PCOS pathogenesis, potentially making it a useful biomarker or therapeutic target for the disorder (Choudhury & Rajeswari, 2022).

The study Ramanand et al. (2013) explored the role of xenin in PCOS pathogenesis. The study found that xenin levels were significantly elevated in PCOS women compared to healthy controls. Additionally, the study by Roland et al. (2013)

found that xenin levels were positively correlated with BMI and insulin resistance, which are two common features of PCOS. The results shows that xenin may contribute to the metabolic abnormalities associated with PCOS. Another study conducted by Gao et al. (2020) investigated the potential therapeutic effects of xenin on PCOS. The study found that treatment with xenin improved insulin sensitivity, reduced serum testosterone levels, and promoted ovulation in a rat model of PCOS. The results of this study highlight that targeting xenin may be a promising therapeutic strategy for PCOS (Stener-Victorin & Deng, 2021; Shorakae, Boyle & Teede, 2014). In recent years, there has been growing interest in the role of xenopsin-related peptide-1 (XRP-1) and xenin in the pathophysiology of PCOS. XRP-1 and xenin are two closely related peptides that are synthesised in the gastrointestinal tract and released into circulation (Temur et al., 2017). They have been implicated in a range of physiological processes, including appetite regulation, glucose metabolism, and reproductive function. It also investigated the relationship between XRP-1 and PCOS in a case-control study. Temur et al., (2017) also highlighted that serum levels of XRP-1 were higher in PCOS women compared to healthy controls. The study also suggested that XRP-1 may help in the development and progression of PCOS by altering insulin sensitivity and ovarian function. Similarly, Toosy, Sodi & Pappachan (2018) measured serum xenin levels in patients with PCOS and compared them to individuals without menstrual cycle disorders. It was found that xenin levels were significantly higher in the PCOS group. The authors suggested that xenin may be involved in the regulation of menstrual cycle function in PCOS. More recently, Craig, Gault & Irwin (2018) also reported higher levels of XRP-1 in PCOS patients compared to healthy controls. In addition, they found that XRP-1 levels related with body mass index (BMI) in females with PCOS (Helvaci et al., 2017). The authors

suggested that XRP-1 may contribute to the pathophysiology of PCOS by affecting adipose tissue metabolism and insulin sensitivity (Chouhan and Dadhich, 2022).

The findings of Haq et al. (2017) suggest that XRP-1 and xenin are involved in the pathophysiology of PCOS. However, the process by which these peptides contribute to the development and progression of the disorder are not fully understood. Further research is needed to elucidate the role of XRP-1 and xenin in PCOS and to determine whether targeting these peptides could be a viable therapeutic approach for the disorder. Hence, the studies discussed above provide evidence for a link between XRP-1 and xenin levels and PCOS. The results shows that these peptides have an important role in the progression of the disorder, possibly by affecting insulin sensitivity, ovarian function, and adipose tissue metabolism. However, further research is needed to fully comprehend the procedure involved and to determine whether targeting XRP-1 and xenin could be a viable therapeutic approach for PCOS (Davoudi et al., 2021).

In conclusion that prevalence of PCOS has significantly increased in Pakistan. Different factors are involved in PCOS and impacting on different body functions such as reproduction, growing hairs, etc. Aetiology and risk factors that induce PCOS in females as well as signs and symptoms are also evaluated in this section (Deswal et al., 2020). The sign and symptoms include missing periods on time or finding irregularity over months, large ovaries with numerous cysts, weight gain, resistance of insulin and excessive body hair. In addition, PCOS women also confront abnormality in hormonal level, which impacts the other body system such as an increased level of hs-CRP in PCOS by which the cardiovascular system is negative impacted (Dumesic et al., 2021). Glucose level is also raised, and about a significant ratio encountered diabetes. To recognise this level, HbA1c is a proficient approach to diagnosing the indication (Flück et al., 2022).

1.2 RATIONALE

Literature review highlighted that there is a lack of attention and evidence regarding PCOS and Xenin levels in the female population. Hence, this study is undertaken to measure the Xenin in the Pakistani population of females and study its correlation with PCOS. Overall this study will contribute towards global research being undertaken to establish exact cause for prevalence of PCOS.

1.2.1 THEORETICAL GAP

Most of the research undertaken in Pakistan regarding prevalence of PCOS pertains to genetic background and environmental conditions. However, very little research data is available regarding correlation of PCOS and serum Xenin. Even existing research data does not specify any threshold of Xenin level which can be used to predict PCOS.

1.2.2 CONTEXTUAL GAP/ANALYSIS

No research data is available for identification of PCOS probable/ high risk group using serum Xenin levels in fertile women. Hence, this study aims to establish Xenin levels as biomarker in pre-disposed group to early detect PCOS thus enabling health practitioners in management of PCOS at early stages.

1.2.3 METHODOLOGICAL GAP/ANALYSIS

NA

1.3 PROBLEM STATEMENT

PCOS can be managed if detected early. However, there is no screening marker available for early prediction. This study aims to examine serum xenin levels as biomarker for early detection of PCOS in probable group.

1.4 RESEARCH HYPOTHESIS

a. **Null Hypothesis** There is no relationship between serum Xenin and PCOS.

b. **Alternative Hypothesis** There is a significant relationship between serum Xenin and PCOS.

1.5 OBJECTIVES

- To quantify levels of Xenin in PCOS diagnosed group, control group and pre-disposed group.
- To compare serum Xenin levels in PCOS diagnosed group, control group and pre-disposed group.
- To determine the statistical significance between Xenin level and PCOS in PCOS diagnosed group and pre-disposed group.
- To compare biochemical parameters such as serum FSH, LH, prolactin, testosterone, serum fasting levels, serum insulin levels and HbA1c in PCOS diagnosed group, predisposed group and control group.

1.6 SIGNIFICANCE OF STUDY

The significance of this research study is to provide an idea regarding the relationship between PCOS and Xenin in the women population of Pakistan. The current

study was designed to evaluate the serum Xenin levels and relate them to the potential of developing PCOS by testing the Xenin levels among three different study groups (control group, PCOS diagnosed patients, and probable group). This study identified the level of serum Xenin as a biomarker among probable group and will help healthcare practitioners to promote lifestyle modifications in order to reduce the risk of developing PCOS. Hence, the study is of significant value as it has introduced a novel parameter leading towards an effective and timely approach for predicting PCOS. This predictive approach will improve disease management as there are very few studies that have determined the relationship of Xenin and PCOS in combination.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews an endocrine disease such as PCOS that mainly influence young girls and women, leading to major health complications such as infertility, menstrual issues, and other problems. The main focus of this chapter is to explore the key literature focused on biochemical parameters such as FSH, LH, Prolactin, Testosterone, serum fasting levels, serum insulin levels, HbA1c and biomarker Xenin levels in PCOS. Therefore, this review examines previously published research on the prevalence, signs and symptoms of PCOS, and its pathogenesis. Additionally, association of PCOS with obesity and diabetes is discussed.

The term polycystic ovary syndrome (PCOS) refers to the endocrine and metabolic disorder which affects 6 to 21% of women of reproductive age, depending on the mean body mass index (BMI), diagnostic criteria and population used (McCartney and Marshall, 2016). The main characteristic features of PCOS that have been described in medical records of recent times include infertility, hirsutism and menstrual dysfunction. Stein and Leventhal officially described PCOS and associated polycystic ovaries (PCO) with the clinical aspects of obesity, menstrual dysfunction, hirsutism and infertility. These observations were expanded by the researchers in the 1980s to report and explored an association between hyperandrogenism and hyper-insulinaemia and revealed the possible aetiologies and complicated reproductive and metabolic conditions with economic and psychosocial consequences across the lifetime (Ramanand et al., 2013). These revolutionary studies identify the relationship of PCOS with various hormones and chemical mediators of the body that contributes to the health issues and

clinical features. As per Rotterdam consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2003), PCOS can be diagnosed with the presence of atleast two of following three criteria's: oligo-anovulation, hyperandrogenism and polycystic ovaries (≥ 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary). The sonographic criteria agreed in Rotterdam consensus was found to have significantly more follicles in the 2-5 mm range than a control group comprised of women with tubal or male factor infertility (Jonard et al, 2003). In polycystic ovary syndrome (PCOS), the following changes take place in the ovarian tissues: enlarged, sclerotic, and multiple cystic follicles, whole ovarian hypertrophy, thickened capsule >100 μm, increased number of subcapsular follicle cysts, scarcity of corpora lutea or albicantia, hyperplasia and fibrosis of the ovarian stroma, and premature luteinization of theca cells.

The appropriate diagnosis of PCOS is essential as it is referred as body condition when the ovaries start to develop an irregular level of androgens in the female body. However, it is found in a small amount among females (The Johns Hopkins University, 2023). In a similar context, the study by Khan et al. (2019) revealed that the abnormal hormonal changes in the female lead to stopping ovulation. Ovulation can be defined as the production of an egg from female ovary. This mature egg can be fertilised by male sperm. However, if it is not fertilised, then it discharges from the body during periods. In another case, when ovulation does not occur, the various small cyst produced in the ovaries leads to large ovary size, irregular menses, high level of androgen hormones that increase the growth of hair on faces, body, acne, infertility and obesity. Hence, PCOS leads to major health complications in females.

Helvaci et al. (2017) also studied the key features or diagnostic principles of PCOS and evaluated that it has been observed PCOS result in additional health

complication in patients. For example, one of the primary issues in the female ovaries of PCOS patient's is development of numerous cysts in them measuring up to 8 mm in diameter. It is possible for a human ovary to contain more than twelve cysts. Development of cysts in PCOS patients results in around 70 percent of them childless. Apart from this, other main complications of PCOS include diabetes, high blood pressure, cardiovascular disease, endometrial hyperplasia, endometrial cancer, and sleep disorders such as sleep apnoea, depression and anxiety. This research study also illustrated that the concentration of male hormone levels, such as androgen, has been found to be increased, resulting in hirsutism as well as acne. Insulin resistance is also observed to occur in PCOS patients, which contributes to obesity and can also cause Type 2 diabetes mellitus thus causing irregularities in the menstrual period, which ultimately contributes to infertility. Sleep apnoea affects 20 percent of the women, suffering from PCOS, on a regular basis. Moreover, psychological concerns such as anxiety and depression are frequently observed in women with PCOS.

According to Armanini et al. (2020), PCOS in girls and adolescent remain undiagnosed and underreported, which increases the likelihood of PCOS-related comorbidities development. This disorder's late diagnosis leads to infertility, pregnancy-related complications, cardiovascular disease, and metabolic syndrome. Similarly, Liu et al. (2021) also explored that besides that, female with PCOS is likely to develop imbalanced glucose tolerance that leads to type II diabetes. The study by Chen et al., (2021) also represent that the major health complication of PCOS includes gynaecological cancers, which is a serious health concern Therefore, early diagnosis of PCOS and effective biomarker analysis is crucial to reduce major health complications in women.

The prevalence of PCOS was assessed as approximately 20% globally, as per Deswal et al. (2020). According to the World Health Organisation record, about 116 million females are influenced by PCOS worldwide (Jabeen et al., 2022). A high prevalence rate of PCOS is observed in those women who have an Asian and Indigenous background (Boyle and Teede, 2012). However, Aslam et al. (2022) illustrated that the prevalence of PCOS in Pakistan is above 50%, and the progression of the disease is also increasing with time in young girls of Pakistan. Anjum et al. (2020) research was focused or concentrated on estimating the clinical presentation of metabolic syndrome, specifically PCOS presentation in Pakistan. This study was specifically conducted in Karachi, 53 females participated in this research, out of which 33.3% were found effective with PCOS, 46.4% were found affected with MetS, and 82.4% were found obese.

Similarly, Zafar et al. (2019) study also focused on investigating the occurrence of PCOS in the Karachi female population. It was found that PCOS was the most prevalent gynaecological syndrome in Karachi women. About 30 agreed women participants were involved in the research, and the result indicated that about 55.45% of women were affected with PCOS. The female participants experienced higher menstruation irregularities and were also experiencing infertility. Besides that, these patients were also overweight and obese, with 32% and 46.2%.

Another study by Sarosh et al. (2021) also analysed the prevalence of PCOS in Pakistan, specifically in Lahore. A total of 753 females participated in the study, out of which 169 women were found with PCOS. Hence, the incidence rate of PCOS was 22.4% in Lahore females. A higher number of females, 66.86% were obese and were also experiencing subfertility. Hence, PCOS is a common syndrome in Pakistani females, and menstrual irregularities were found to be a major clinical presentation of PCOS. However, the variation in PCOS prevalence in Pakistan may be due to the sample size, study design,

and diagnostic approach. Moreover, Sarosh et al., (2021) demonstrated that the prevalence of PCOS in Pakistan differs in different regions, and the major reason for PCOS or factors associated with PCOS development, such as environment, lifestyle, and genetic factors, are unknown.

PCOS is a higher ovarian, adrenal androgen secretion condition in females. Several internal and external factors are related to the development of PCOS in females including genetic makeup, environmental factor, and hormonal factor (Singh et al., 2020). The study by Flück et al., (2022) revealed that the hormonal factor is related to a higher level of androgens including testosterone which plays a major role in ovulation interference. The ovulation interference leads to cyst development in ovaries. The higher level of androgen level in the body may be a result of the increased enzyme activity associated with the synthesis of adrenal glands. According to Parker et al. (2020), besides other factors, insulin resistance also results in a condition of higher insulin production that abrupts glucose level, stimulate androgen production in the body, higher hair follicles growth by 5 to 8 mm, and also interfere ovulation. Hence, hormonal changes lead to follicle maturation and ovary deregulation in people with PCOS.

The study by Heidarzadehpilehrood et al. (2022) encountered that the genetic factor of PCOS is related to hereditary as a family history of PCOS with first-degree relatives, including siblings or parents indicates the genetic link of the disorder. It is mainly because several genes were found potentially important in the development of PCOS that involves insulin signalling and follicle maturation such as cytochrome P450 enzymes, steroidogenic enzyme, CYP17A1, CYP19A1, and CYP11A1. Hence, genetic factor is also related to the occurence of PCOS.

Study conducted by Shahid et al. (2022) demonstrated that environmental factors including lifestyle and dietary patterns lead to the development of PCOS. For instance,

the consumption of a diet rich in carbohydrates and higher added sugars leads to the disruption level of insulin. Besides that, being overweight and obese also lead to insulin resistance and disrupt androgen level in the body (Amiri and Tehrani, 2020). In a similar context, Heidarzadeh Pilehrood et al. (2022) also claimed the reason behind the development of PCOS. However, stress, obesity, and lack of participation in physical work result in the development of PCOS. Hence, the hereditary, environmental, and hormonal imbalance leads to the development and progression of PCOS.

Riestenberg et al. (2022) demonstrated that PCOS is a prevalent endocrine disorder affecting women of reproductive age. It is characterised by a complicated interaction of hormonal and metabolic disorders. PCOS can cause a variety of signs and symptoms, but the most common ones are irregular menstruation, hyperandrogenism, and ovarian dysfunction (Saeed et al., 2022).

According to a study by Chouhan et al. (2022), menstrual cycle irregularities, which can be infrequent, heavy, or completely missing, are the most typical sign of PCOS. Additionally, hirsutism, or the growth of excessive hair on the face, chest, or back, can also be a symptom of PCOS in women. Male-pattern baldness, acne, and oily skin are some other symptoms of PCOS (Kim et al., 2022).

Zeng et al. (2022), androgen levels, such as testosterone, in PCOS women may be higher than normal. This can result in symptoms like acne and excessive body hair. PCOS is also frequently associated with insulin resistance and weight gain (Armanini et al., 2022). According to a study by Osibogun et al. (2020), women who have PCOS may be more likely to develop type 2 diabetes and cardiovascular disease.

A thickened, darkened region of the skin termed as acanthosis nigricans, which is typically present in the neck, armpit, and groin areas may also be found in PCOS women. (O'Brien et al., 2020). Several small cysts on the ovaries may also be visible on a pelvic

ultrasound, however this does not always indicate PCOS (Zeng et al., 2022). PCOS women may also have ovarian dysfunction, which can lead to infertility or difficulties getting pregnant (Akbaribaz et al., 2022).

Genetic, environmental, and hormonal variables all have a role in the complex and multifaceted link between PCOS, obesity and diabetes mellitus (Neves et al., 2020). According to Mirzohidovna. (2021), approximately 60% of PCOS women are obese or overweight, making obesity one of the main comorbidities linked to the condition. PCOS and obesity have a bidirectional association, with obesity increasing the likelihood of developing PCOS and PCOS aggravating obesity.

Insulin resistance is a critical element of both PCOS and obesity, and it is believed to be a major contributing cause of obesity in PCOS women. Insulin resistance in PCOS is characterised by reduced peripheral glucose absorption and increased hepatic glucose synthesis, resulting in hyperinsulinemia and hyperglycemia (Shirazi, et al., 2021). Additionally, insulin resistance in PCOS is hypothesised to be associated with increased intra-abdominal fat deposition, which has been demonstrated to be an independent predictor of Type 2 Diabetes Mellitus and insulin resistance (Brennan et al., 2019).

There is a complex connection between PCOS and diabetes mellitus. Up to 40% of PCOS women may acquire type 2 diabetes mellitus (T2DM) in their lifetime, which is a known risk factor for the disease (Mohd et al., 2019). According to a study by Livadas et al. (2022), PCOS women have greater levels of AGEs (Advanced Glycation End) products, which are believed to have a role in the development of T2DM and insulin resistance. Non-enzymatic glucation process between reducing proteins and lipids, sugars, or nucleic acids forms highly reactive molecules called AGEs. Its findings imply that increased AGE production may be a mechanism behind the link between PCOS and T2DM (Livadas et al., 2022). As indicated by Mouanness et al., (2022), AGEs can be

developed during any abnormal conditions such as in inflammatory disease and it is also considered a key component in the phenotype and pathophysiology of PCOS.

Another study by Choudhury et al. (2022) discovered that PCOS women who also had gestational diabetes mellitus (GDM) have a greater chance of acquiring T2DM later in life. The study emphasizes how crucial it is to recognise and treat GDM in PCOS-affected women as soon as possible in order to prevent the development of T2DM.

One of the hormones connected to the association between PCOS and obesity is xenin, a gut hormone that is co-secreted with the appetite-regulating hormone ghrelin. According to a study by Guclu et al. (2019), circulating xenin levels are greater in PCOS-affected women than in non-PCOS-affected women, and they are also strongly correlated with both insulin resistance and body mass index (BMI). It has been proposed that xenin may contribute to obesity in PCOS women by increasing hunger and food consumption, as well as affecting glucose homeostasis and insulin sensitivity (Kruszewska et al., 2022).

PCOS (PCO) can be manifested clinically among young women age groups. Menstrual abnormality is considered one of the common presentations of PCOs. In addition, the occurrence of a deranged hormonal profile was found associated with diabetes type 2 and cardiovascular disease risk. Clinically PCOs found to cause metabolic disorders such as glucose intolerance, lipid disorders, diabetes, and resistance against insulin, and escalating the threat of endometrial uterine and breast cancers. Therefore, biochemical test sounds beneficial in diagnosing PCOs among women. Performing biochemical tests such as LH, Testosterone, FSH, and prolactin was found helpful in identifying PCOs through changes in hormone levels (Kruszewska, Laudy-Wiaderny, and Kunicki, 2022).

Lizneva et al. (2019), the study indicates that hormonal imbalance is manifested by the occurrence of multiple small follicles that gather in the ovary. These follicles are

enabled to undergo the ovulation process resulting in an increase in the FSH, estrogen, progesterone and LH level. It is observed that in females with low to normal FSH rate, the ratio of LH hormone was found to be much high that resulting in the inhibition of egg release leading to the occurrence of irregular menstrual cycles. Moreover, it is also reported that with disturbance in the gonadotrophin axis, a reversal ratio of LH/FSH occurs. According to Vaidya et al. (2020), it was observed that changes in the pulsatile nature of the gonadotrophin hormone cause increase in the release of LH to FSH. The study found normal levels around 2 to 1, which become reversed in the condition of PCOs. These findings found correlated with the study as it was observed that 2/3rd of cases of PCO among women presented >= 2 ratios of LH/FSH. It has been studied that FSH motivates the ovulation process and is usually present in the pituitary gland present in the head. The level of FSH was found to be equal to LH during the initial stage of the menstrual duration. However, LH increases before the initiation of ovulation, although LH levels drop back as the egg is released by the ovary. It is also reported in some cases that few women present FSH levels within 5-20 mlU/ml and have PCOs (Malini and George, 2018). Another term named, diminished ovarian reserve (DOR), represents mild FSH increase. The increase in FSH level shows the availability of fewer eggs. The increase in the secretion of FSH during PCOs is due to high sensitivity to gonadotropin-releasing hormone (GnRH) or may be due to changes in the secretion patterns of GnRH. The presentation of high FSH also shows depleted ovaries reserve and indicates changes in the normal working of ovaries (Laven, 2019).

The interference of high LH with the normal function of ovaries causes PCOs among women. It is found that LH binds to the LHR present on the granulosa cells' plasma membrane in the ovary, initiating the production of estrogen, development of corpus luteum, and ovulation. The binding of LH with LHR stimulates androgen production and

causes spermatogenesis. The significant role of LH hormone is found to contribute to regulating ovary function in women. The increase in ratio to about 2 or 3 represents the condition of PCOs in women. (Figure 2.4). The study also discovered the diagnosis of PCOs with polycystic ovarian morphology that can be seen in an ultrasound. (Kumariya et al., 2021). Saadia, (2020), the study showed that the process of ovulation doesn't occur in females with an increased FSH/LH ratio. As the study aimed to investigate the FSH/LH ratio among women with decreased BMI and compare them with women with high BMI women. It was reported that no association between BMI and serum hormone levels was found. The PCOs associated morbidities indicated were insulin resistance, dyslipidemia, and obesity. The results of the study also showed an emphasis on improving healthy dietary intake, as changes in dietary intake cause hormonal imbalance among PCOs women.

In addition, some studies showed the link between LH/FSH ratio with prolactin and TSH among PCOS women (Munaver et al., 2023). Furthermore, it is indicated that anti-Mullerian hormone (AMH) is increased among females with PCOS; however, other forms of hyperandrogenism, such as diabetes Type 1 among PCOS women, are linked with increased AMH levels. It is also detected that females with PCOS and DM1 exhibit normal AMH and FSH/LH levels (Kadiroğulları et al., 2020).

According to Davoudi et al. (2021), study prolactin is considered a pituitary hormone that contributes to milk production in nursing mothers. The level of prolactin was found to be normal in PCOS women, i.e., generally less than 25mg/ml. The increase in prolactin levels was found to be associated with pituitary tumours with the presentation of PCOs-related symptoms. It is also reported that an increase in prolactin levels interferes with progesterone and estrogen level production. The disruption in hormonal levels impacts on ovulation process leading to the occurrence of missed or irregular periods.

Some PCOS women show no symptoms while having a high level of prolactin. The increase in prolactin level was also found to affect the fertility process by inhibiting the ovulation process. It is observed that changes in the dopamine level in the body, either due to medication or other reasons, increase prolactin levels (Yang et al., 2020). Mainly medications such as methyldopa and calcium channel blockers contribute to increasing prolactin levels. To control the prolactin level, dopamine agonists as oral medication as usually used (Gadelha et al., 2022).

Davoudi et al. (2021) indicated the association between increased levels of serum prolactin in PCO patients. The occurrence of an increased level of serum prolactin among 330 PCO patients was found when measured with the polyethene glycol (PEG) precipitation method. 37% of patients were found to have hyperprolactinemia which is considered a serious endocrine issue. The excess of macro prolactin, drugs, and physiologic changes are reported as the main causes of this condition. Through study findings, it was also revealed that the necessity of measuring serum prolactin levels among PCO women was reported. However, the association of high serum prolactin levels with PCOs was not found. Furthermore, it is also observed that the condition of hyperprolactinemia can be occurred due to increase secretion of estrogen in PCO patients (Davoudi et al., 2021). Additionally, it was also described that among PCO patients, the insulin level was found to be greater with normal prolactin, whereas, in the condition of hyperprolactinemia, insulin resistance is found to contribute the PCOs pathogenesis (Overgaard et al., 2020).

According to Bongrani et al. (2022) study, the existence of testosterone in women can be identified by measuring total testosterone level or free testosterone level. Total testosterone level found representing the complete amount of testosterone, together with free testosterone in the body. The level of entire testosterone levels was found to be

between 6.0-86 ng/dl. On the other side, the term free testosterone refers to the unbound testosterone present in the body in active form. The level for free testosterone level is considered in the middle of 0.7-3.6 pg/ml (Kshetrimayum et al., 2019). It is reported that women having PCOs have increased points of both free and total testosterone. Likewise, a minor increase in the level of testosterone was found to the inhibiting occurrence of ovulation and normal menstruation cycle in women's bodies. Usually, during the condition of PCOS, increased production of testosterone is observed among women, which can give rise to issues such as increased hair development, hair loss and bad skin symptoms during PCOs. There is a number of ways through which testosterone level during PCOs can be controlled, such as weight loss, intake of flaxseeds, green tea, mint tea, antiandrogens and combined estrogen-progestin oral contraceptives (COC) (Dumesic et al., 2021).

Adiponectin, leptin, and insulin are some of the other hormones that have been linked to PCOS and obesity. Adiponectin levels have been reported to be lower in PCOS women than in women without PCOS, which may cause insulin resistance and obesity (Calcaterra et al., 2021). Leptin is another adipokine that regulates energy balance and appetite, and its levels are often higher in obesity. Leptin levels were shown to be greater in PCOS-afflicted women than in non-PCOS-afflicted women, and they were also positively correlated with Obesity and insulin resistance (Baldani et al., 2019)

Xenin is a peptide produced in the mucous membrane of the duodenum by a subpopulation of chromogranin A-positive endocrine cells. The peptide has been found in mammals such as rabbits, rats and humans (Navarro et al., 2018). The significance of xenin in insulin secretion and its potential for diabetes therapeutics has recently gained a lot of attention, mainly because of its capacity to augment the insulinotropic effects of Gastric Inhibitory Polypeptide (GIP) and its use in dual/triple acting gut hormone

therapeutic methods. Because there are currently no clinically licenced medicines for restoring GIP bioactivity in type 2 diabetes, xenin could be a promising diabetic treatment (Navarro et al., 2018).

Xenin has a number of essential biological activities that have already been demonstrated in a variety of animal systems and have been thoroughly discussed recently. Controlling energy intake and gastrointestinal transit, delaying stomach emptying in individuals, suppressing appetite and modulating pancreatic exocrine as well as endocrine functionality (Parthsarathy et al., 2016) are only a few of xenin's main biological functions.

Xenin is a 25-amino acid peptide sequence that are secreted from human jejunal, duodenal and gastric mucosa. Vital organs such as the lung, heart, liver, pancreas, testicles, skin, hypothalamus, kidney and adrenal gland may also produce xenin. The human serum has also shown some amount of xenin (Roland et al., 2010). The concentration of xenin is increased after meals in the blood and affects the exocrine secretion of the pancreas. The electromechanical activity of gastrointestinal smooth muscle known as Migrating Motor Complex also relates to the peaks of plasma xenin concentration. For instance, infusion of synthetic xenin causes activity of the third phase of Migrating Motor Complex in fasting individuals. In the postprandial state, xenin infusion increases the frequency of generated contractions. In recent years, it has been established that xenin may perform other functions such as regulation of gut motility, delayed gastric emptying, the contraction of the gallbladder, appetite suppression, inhibition of gastric acid production and electrolyte transport. One of the most important functions of xenin is to significantly improve the disposal of glucose and secretion of insulin in the human body (Moffett et al., 2021).

Xenin is identical to an amphibian octa-peptide xenopsin and 13-amino acid

peptide sequence termed neurotensin. Xenin does not has its own particular binding receptor. Hence, it binds to the neurotensin receptor 1 (NTSR-1). However, sequences of both peptides stimulated the secretion of insulin from rodent β-cells.

Higher concentration levels of xenin were present in the serum of PCOS patients (Roland et al, 2010). In terms of xenin association with PCOS, it has been demonstrated that levels of xenin-25 were considerably increased in a small set of 31 women diagnosed with PCOS as compared to the 30 healthy and regular menstruating women. Moreover, the optimal cut-off value for diagnosing PCOS using xenin-25 as biomarker was 32.60 pg/mL, with an 86.7% specificity and 61.3% sensitivity. Approximately 5.6–21.3% of women across the globe are affected by PCOS at their reproductive age.

In previous studies, an established link between xenopsin related peptide-1 and polycystic ovarian syndrome has been identified with levels of xenopsin considerably higher in PCOS patients than in healthy controls (Temur et al., 2017). Similarly, serum xenin levels in females with PCOS are considerably higher than in individuals without menstrual cycle disorders as determined by Guclu et al. (2019). On the same lines, higher xenin levels (xenopsin related peptide-1) in PCOS prevalent group were also been identified by Jasim et al. (2022).

Although many authors identified that the level of xenopsin was significantly higher in PCOS patients than the healthy group; however, it still requires comprehensive analysis. For example, it has been reported during previous studies that Xenin levels were not significant in control group; however, different sex hormones should also have been assessed in these women to confirm the disease condition (Craig, Irwin and Gault, 2021). Xenopsin also results in cellular inflammation. It does so by inducing macrophages and stimulating histamine levels in mast cells. However, exact correlation between xenopsin and CRP, which is systematic inflammation biomarker used mostly in clinical settings, is

not known (Jasim and Alkareem, 2022).

Very little research data exists regarding relationship between PCOS and xenin. It is considered that pathophysiology of PCOS can be further explored by studying impact of xenin on prevalence of PCOS. Although current literature suggests high levels of xenin in PCOS sufferers, however more research is needed for timely prediction of disease. For example, researchers should study the levels of xenin in individuals with different levels of risk or tendency to develop PCOS and then relate it with PCOS incidence. Such findings will not only improve disease prediction & management but will also open new research areas in the domain of PCOS.

According to Guclu et al. (2019), study xenin is referred to 25-amino acid peptide. It is considered a gut hormone and acts as a biomarker for PCOS. PCOS has been found affecting aged women's reproductive system with an estimation of 5-10% around the world. It is also found that the PCOS condition is linked with insulin resistance, abnormal gonadotrophin secretion and hyperandrogenism.

Furthermore, it is reported that glucose metabolism and insulin secretion are regulated by xenin. Experiments in obese mice showed that infusion of xenin increases glucose tolerance, while on the other side, in healthy humans, insulin secretion is increased by xenin infusion (Craig et al., 2021). On other side, it is reported that xenin depicts an inhibitory effect on glucagon secretion. Glucagon is referred to as a hormone that contributes to glucose regulation. The quantifiable demonstration of hyperandrogenism is reported as hirsutism, menstrual irregularities and acne. It has been studied that role of xenin was also found to be associated with androgen production regulation. The level of testosterone was found to increase by xenin infusion in men. However, in women, it is reported that xenin contributes to producing a stimulating effect on LH secretion that helps in the regulation of ovaries' function. The findings of the

studies helped to indicate the role of xenin in the virulence of PCOs. However, it is also observed that the rate of xenin among PCOs women can be found in an altered manner. In addition, through one example, it was encountered that PCOS women had lower fasting plasma xenin in comparison to healthy controls (Dabravolski et al., 2021).

Moreover, in certain cases, xenin was found to be negatively correlated with insulin resistance markers. It was identified through evidence that a positive correlation with hyperandrogenism, such as LH and testosterone as markers, were found (Jeelani et al., 2019). Therefore, it was evident that xenin acts as an impending biomarker for PCOs; however still, additional evidences need to be done to increase the prognostic value of xenin as a biomarker in PCOs. It is found that people with PCOs are at risk of evolving metabolic disorders and cardiovascular diseases. (Wölk et al., 2021).

The study by Kruszewska et al. (2022) showed that xenin affects the secretion of the exocrine pancreas; however, the concentration of xenin was found to increase after meals in the blood. The function of xenin was found to be involved with delaying gastric emptying, electrolyte transport, appetite suppression and gut motility regulation. It is also found made by further organs such as the heart, kidney, lung, liver, hypothalamus, testicles, skin, pancreas and adrenal glands. It is observed that xenin, a peptide secreted by the small intestine, benefit in PCO diagnosis. Additionally, xenin was also reported to be related with hyperandrogenism and insulin resistance. It is also found that the occurrence of hyperandrogenism is caused due to insulin resistance that subsidises to the hyperandrogenism development by encouraging production of ovarian androgen in PCOS women. It is reported that xenin-25 are fewer susceptible to degradation also shows positive response for lipolysis increase and reduction of lipogenesis (Wölk et al., 2021).

OPERATIONAL DEFINITIONS

POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome (PCOS) is the endocrinopathy condition in which an abnormal number of androgens and male sex hormones are produced by the ovaries that lead to the formation of numerous small fluid-filled cysts determined by ultrasonography (Ndefo et al., 2013).

OLIGOMENNORHEA

Oligomenorrhea is defined as irregular and inconsistent menstrual blood flow in a woman. Some change in menstrual flow is normal at menarche, postpartum, or in the perimenopausal period. But if a woman reports the length of menstrual cycle greater than 35 days or four to nine menstrual cycles in a year, then it is termed as oligomenorrhea. Menstrual flow should be normal before the development of oligomenorrhea (Hennegan J et al., 2020).

AMENNORHEA

Primary amenorrhea is the lifelong absence of menses. Secondary amenorrhea is the cessation of previously regular menses for three months or previously irregular menses for six months and warrants evaluation (Klein DA., 2013)

XENIN

Xenin is a peptide hormone secreted from the K-cells or Chromogranin A-positive enteroendocrine cells present in the mucous of the duodenum and stomach of the upper intestinal tract (Martin et al., 2014).

PEPTIDE

It is the short sequential chain of amino acids that are connected to one another by peptide bonds (Fosgerau & Hoffmann, 2015).

INFERTILITY

It is defined as failure to conceive a baby or get pregnant (Gurunath et al., 2011).

XENOPSIN-RELATED PEPTIDE-1

Xenopsin-related peptide-1 (XP-1) is an octapeptide that shares some specific structural and biological characteristics with neurotensin (NT)/xenopsin/xenin family. (Temur et al., 2017)

OVERWEIGHT

Overweight is defined as abnormal or excessive fat accumulation that may impair health. Overweight is a BMI greater than or equal to 25. (WHO, 2021).

OBESITY

Obesity is defined by excess body fat and is a current health epidemic associated with increased risk for type 2 diabetes and cardiovascular disease (CVD) (Kopelman PG et al., 2000). Obesity is a BMI greater than or equal to 30. (WHO, 2021).

BMI

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2) (WHO, 2021).

PREDISPOSED GROUP

Pre-disposed in our study is the group of individuals with the positive family history of PCOS.

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

This study is designed to be a case control study.

3.2 SUBJECTS

Human females whom diagnosed with PCOS (according to Rotterdam criteria) are selected as subjects for this study.

3.3 SETTING

This case control study, conducted in PNS Shifa hospital Karachi in Obstetrics and gynecological department.

3.4 INCLUSION CRITERIA

3.4.1 PCOS DIAGNOSED GROUP

- Women age 15-45 years
- History of oligomenorrhea and/or anovulation
- Serum testosterone ≥ 2.5nmol/L as clinical sign of hyperandrogenism.
- Ultrasonographic evidence of polycystic ovaries

3.4.2 HEALTHY GROUP

- Women age 15 - 45 years
- Patients not fulfilling Rotterdam's criteria.

3.4.3 PRE-DISPOSED GROUP

- Women age 15 - 45 years
- Females with family history of PCOS

3.5 EXCLUSION CRITERIA

- Women age <15 and > 45 years.
- Patients on management regimes against PCOS.
- Patients with 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing's syndrome, ovarian tumours, thyroid disease and hyperprolactinaemia
- Patients who are on anti-depressants and anxiolytics as they have raised levels of Xenin.

3.6 DURATION OF STUDY

- Individual study period: 3 months
- Total period of study: 6 months
- After approval from Bahria University Health Sciences Karachi Ethical Review Committee (ERC) and Faculty Review Committee (FRC).

3.7 SAMPLE SIZE ESTIMATION

- For the calculation of the sample size, results are calculated from Open Epi, Version 3, open source calculator—SS Propor are utilized. The required sample size was found to be 105 by using the equation
- Sample size n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)]

3.8 SAMPLING TECHNIQUE

Consecutive sampling technique used to recruit samples for this study.

3.9 HUMAN SUBJECTS AND CONSENT

Total number of patients enrolled in the study were 105 subjects (35 patients ineach group). Written informed consent was taken from each participant prior to enrollment in the study. (Appendix- C)

3.10 MATERIALS

3.10.1 DRUGS

N/A

3.10.2 CHEMICALS

N/A

3.10.3 PROFORMA/QUESTIONNAIRE

Subject evaluation proforma/ Questionnaire (Appendix -D)

3.10.4 EQUIPMENT

Syringes, tourniquet, gel top vaccutainer, epenorff, ELISA Washer, ELISA Reader (Figure 3.1 a-f)

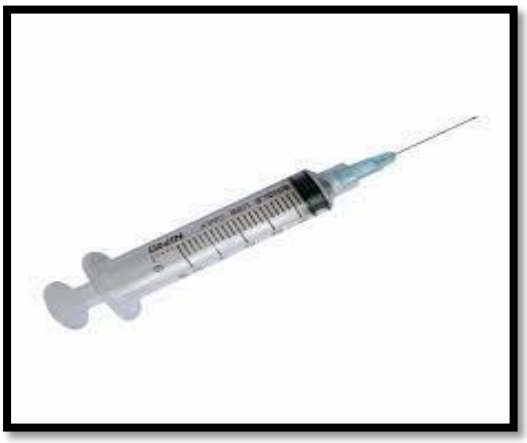


Figure 3.1(a): Syringe

Figure 3.1(b): Tourniquet

Figure 3.1(c): Gel Top Vacutainer

Figure 3.1(d): Ependorff

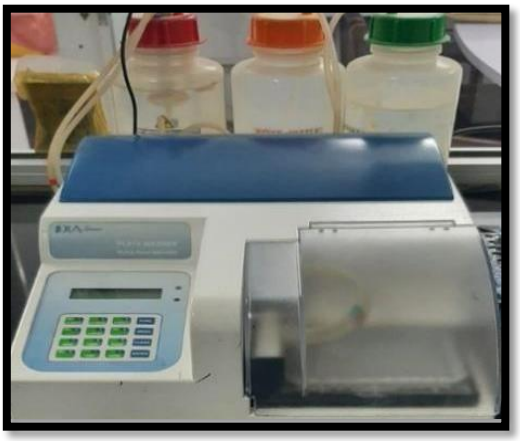


Figure 3.1(e): ELISA washer

Figure 3.1(f): ELISA Reader

3.10.4.1 KIT USED

Human Xenin 25 ELISA Kit was procured from BT LAB. Figure (3.2)

- CATALOGUE NO: EA0208Hu
- Standard Curve Range: 1.88-120ng/ml
- Sensitivity: 1.04ng/ml
- Storage: Store the reagents at 2-8°C. For long term storage refer to the expiration date keep it at -20°C. (Figure 3.3) Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

3.10.4.1.1 INTENDED USE

This competitive ELISA kit is for the quantification of Xenin 25 in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids.

3.10.4.1.2 ASSAY PRINCIPLE

This kit is a Enzyme-Linked Immunosorbent Assay (ELISA). Add samples to the pre-coated 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. (Figure 3.4). 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 Xenin-25 in the sample. The concentration of Xenin-25 in the sample is then determined by comparing the O.D. of the samples to the standard curve.

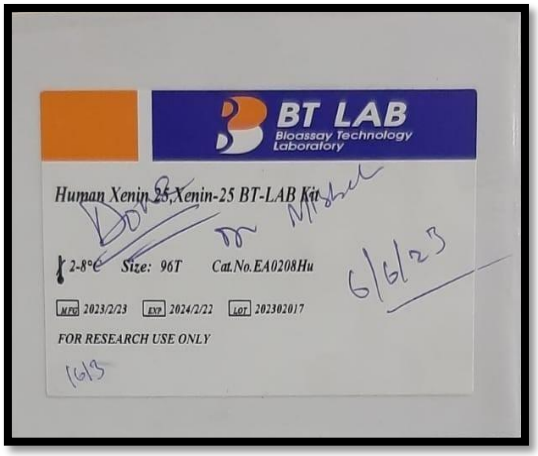


Figure 3.2: Human Xenin 25 ELISA Kit

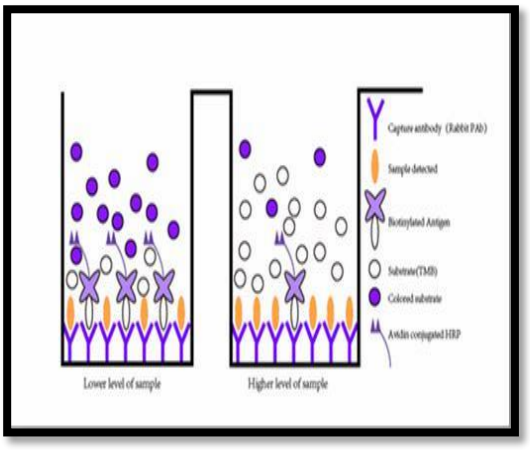


Figure 3.3: Competitive ELISA

Figure 3.4 Reagent Storage Refrigerator+

3.10.4.1.3 REAGENTS

Table 3.1: Components used for estimation of Xenin levels

Component	Quantity
Pre-coated Plate	12 * 8 well strips x 1
Human Xenin-25 Standard, lyophilized	2 vials
Standard/Sample Diluent	6ml ×1 vial
Biotinylated Antigen, lyophilized	1 vial
Avidin-HRP Concentrate	100µl × 1 vial
Biotinylated Antigen Diluent	6ml × 1 vials
Avidin HRP Diluent	5.9ml × 1 vials
Substrate Solution A	6ml ×1 vial
Substrate Solution B	6ml ×1 vial
Stop Solution	6ml ×1 vial
Wash Buffer Concentrate (25x)	20ml × 1 vial
Plate Sealer	2 pcs
Zipper Bag	1
User Instruction	1

3.10.4.1.4 OTHER MATERIALS REQUIRED

- 37°C ± 5°C incubator
- Precision pipette and disposable tip
- Deionized or distilled water
- Clean tube
- Absorbent paper
- Microplate reader with 450 ± 10nm wavelength filter

3.10.4.1.5 PRECAUTIONS

- Prior to running the assay, the kit and sample should be warmed naturally to room temperature 30 minutes.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- There are two vials of standard in the kit for users, please cover the other unused vial and keep refrigerated.
- Do not allow wells to become dry during the assay procedure.
- This instruction should be strictly followed in the experiment.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.

- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

3.10.4.1.6 ASSAY PROCEDURE

- Three ml of venous blood was collected in a yellow top from the participants during the early follicular period of menstruation (second or third days) following minimum 10-12 h of night fasting. (Figure 3.5)
- The samples were centrifuged for 5 min at 3500 rpm separating the sera.(Figure 3.6)
- The serums were stored in ependorff at -80 ^{0}C for batch analysis. (Figure 3.7)
- Prior to use the kit, buffer solution and samples were thawed at room temperature for 30 minutes. (Figure 3.8a, 3.8b)
- Samples stored in ependorff were mixed on a vortex. (Figure 3.8c)
- Diluted standard 50µl were added to standard well. (Figure 3.8d)
- 50 ul sample (Sample recommended dilution: 2-5 times when necessary) to the sample well were added and then 50 µl biotinylated antigen was added to each well.
- Mixed well. Covered the plate with a sealer and incubated for 60 minutes at 37°C. (Figure 3.8e)
- The searler and the liquid in the well were removed, washed five

times with 300 µl wash buffer manually. The plate was inverted each time and the contents were decant, hit 4-5 times on absorbent material to complete remove liquid. For automated washing, aspirate all wells and wash 5 times with wash buffer. Blot the plate on absorbent material.

- 50 µl avidin-HRP was added to the standard well and sample well, the plate was covered with a sealer and incubated for 60 minutes at 37°C. (Figure 3.8f)
- Sealer was removed and washed. (Figure 3.8g)
- 50 µl substrate solution A and then 50 µl substrate solution B was added to each well. (Figure 3.8h, 3.8 i)
- Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
- 50 µl of Stop Solution was added to each well, the blue color was changed into yellow immediately.(Figure 3.8j)
- Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. (Figure 3.8k, 3.8l)
- Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations were performed with computer-based curve-fitting software but can be determined by regression analysis. Figure 3.8m)
- Flow chart of procedure is as follows:

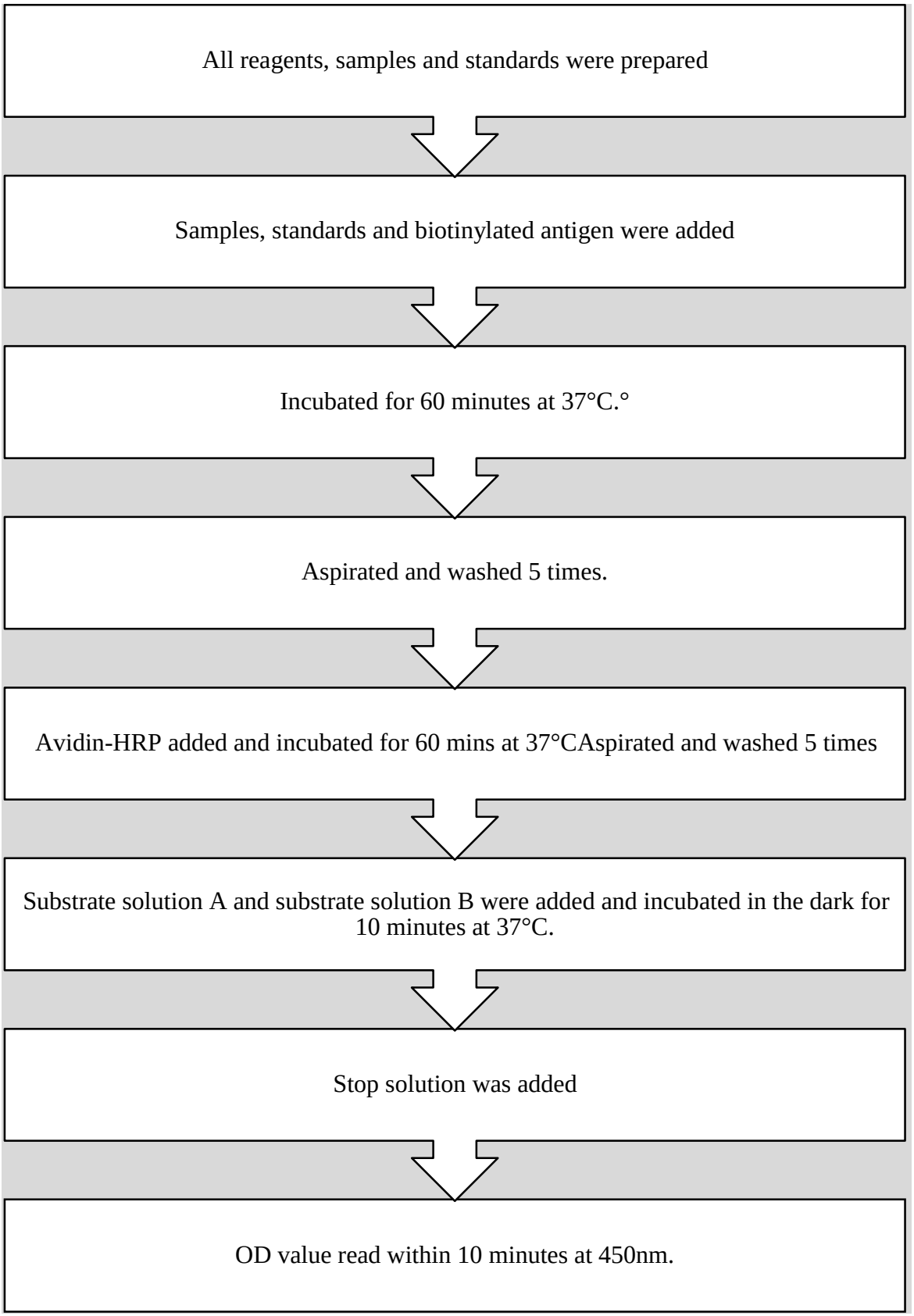

All reagents, samples and standards were prepared
Samples, standards and biotinylated antigen were added
Incubated for 60 minutes at 37°C.°
Aspirated and washed 5 times.
Avidin-HRP added and incubated for 60 mins at 37°CAspirated and washed 5 times
Substrate solution A and substrate solution B were added and incubated in the dark for 10 minutes at 37°C.
Stop solution was added
OD value read within 10 minutes at 450nm.

Figure 3.5: Blood Sampling

Figure 3.6: Sample Centrifugation

Figure 3.7: Sample Storage Freezer

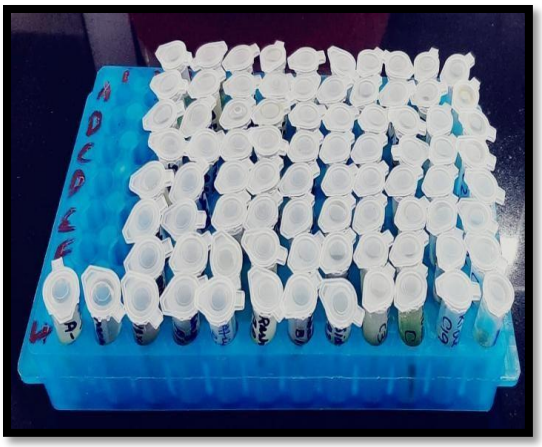


Figure 3.8(a): Samples thawed at room temperature

Figure 3.8 (b): Buffer solutions

Figure 3.8 (c): Mixing of sample

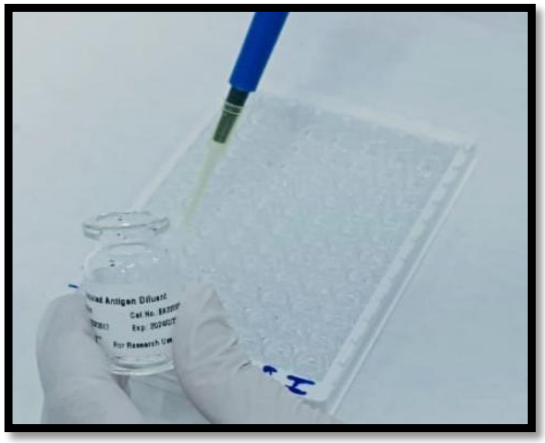


Figure 3.8(d): Standard Antigen Diluent

Figure 3.8(e): 60 Minutes incubation

Figure 3.8(f): Adding Avidin-HRP

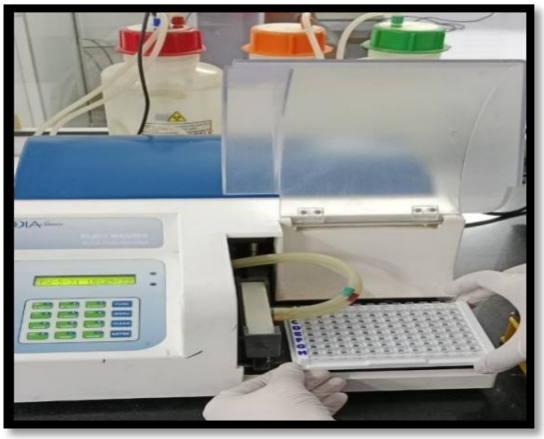


Figure 3.8(g): Sealer removed and washed solution A

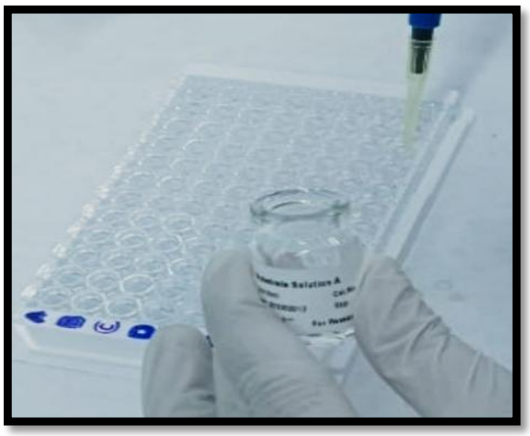


Figure 3.8(h): Adding Substrate

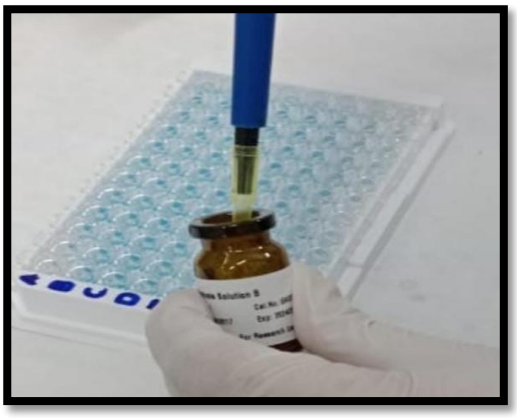


Figure 3.8(i): Substrate solution B

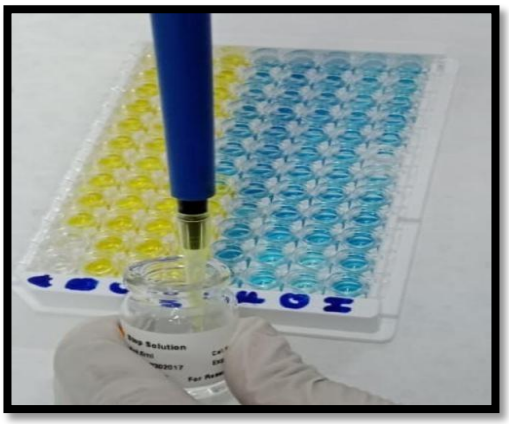


Figure 3.8(j): Stop solution added

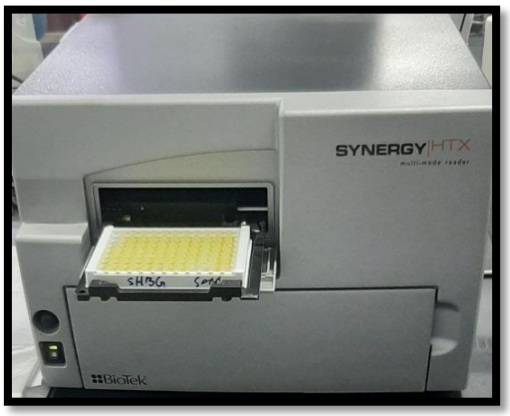


Figure 3.8(k): Microplate reader

Figure 3.8(m): OD determined

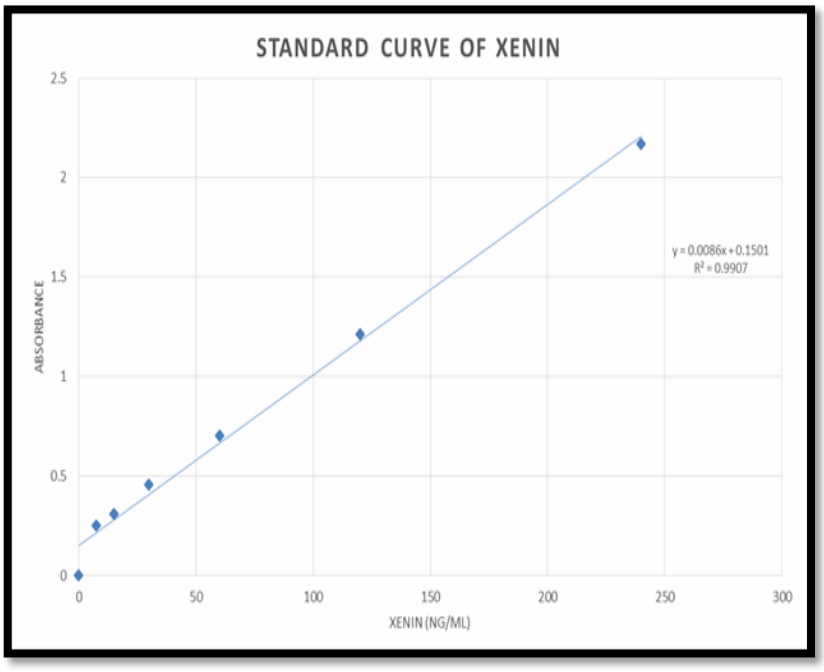


Figure 3.8(n): Standard curve plotted by regression analysis

3.10.5 OTHERS

- Weighing machine for body weight estimation in kilograms (Figure 3.9a).
- Measuring tape for measuring height in metres (Figure3.9b).

3.11 PARAMETERS OF STUDY

3.11.1 CLINICAL PARAMETERS

- Oligomenorrhea/Anovulation
- Hyperandrogenism
- Polycystic ovaries

Patients with PCOS had oligomenorrhea or amenorrhea with clinical or biochemical evidence of hyperandrogenism (serum total testosterone levels greater than female assay maximum) or having polycystic ovaries on transvaginal ultrasound. Control participants had a regular menstrual cycle occurring every 21 to 35 days. They also lacked clinical and biochemical evidence of hyperandrogenism (total testosterone <60 ng/ml). Females in pre-disposed group had a history of regular menstrual cycle and lacked clinical or biochemical evidence of hyperandrogenism or polycystic ovaries on ultrasound. Family history (maternal) of PCOS was present in this group.

3.11.2 ANTHROPOMETRIC PARAMETERS

(1) Height was measured in standing position by utilizing measuring tape.

(2) Weight was measured by utilizing weighing machine and recorded in Kg.

(3) Body mass index (BMI) was calculated using Quetelets index formula:

$$BMI = \frac{\text{Body weight (kg)}}{\text{Body height } (m^2)}$$

BMI ≥ 30 kg/m2 is regarded as obesity (Fatima, Rehman and Chaudhry, 2014)

3.11.3 BIOCHEMICAL PARAMETERS

- **Serum FSH:** Blood samples were collected during the follicular phase (2^{nd} or 3^{rd} day of menstrual cycle) of the individuals, sent to the PNS Shifa hospital laboratory and were measured by using the Roche FSH Elisa kit Cat no 0062576.
- **Serum LH:** Blood samples were collected during the follicular phase (2^{nd} or 3^{rd} day of menstrual cycle) of the individuals, sent to the PNS Shifa hospital laboratory and were measured by using the Roche LH Elisa kit Cat no 00621030.
- **Serum Prolactin:** Blood samples were collected during the follicular phase (2^{nd} or 3^{rd} day of menstrual cycle) of the individuals, sent to the PNS Shifa hospital laboratory and were measured by using the Roche FSH Elisa kit Cat no 00655494.
- **Serum Testosterone**: Blood samples were collected during the follicular phase (2^{nd} or 3^{rd} day of menstrual cycle) of the individuals, sent to the PNS Shifa hospital laboratory and were measured by using the Roche FSH Elisa kit Cat no 00688765.
- **Fasting Blood Sugar:** It was measured by using Roche Elisa kit Cat No. 625672
- **Fasting Insulin Levels:** These were measured by using the electrochemiluminescence immunoassay method (Roche Diagnostics GmbH, Mannheim, Germany Cat No. 682501)
- **HbA1C:** It was was measured by using Roche Elisa kit Cat No. 708797
- **Serum Xenin:** Five ml of venous blood was collected from the participants during the early follicular period of menstruation (second or third days) following minimum 12 h of night fasting. Xenin-25 analyzes were

performed using Human Xenin 25 ELISA Kit was procured from BT LAB. (CATALOGUE NO: EA0208Hu).

3.11.4 RADIOLOGICAL PARAMETERS

Transvaginal ultrasound scans of the ovaries were performed using the 5-MHz transvaginal transducer. (Figure 3.10)

3.12 PROTOCOL OF STUDY

This case control study was conducted after obtaining RRC and ERC approval of Bahria University Health Sciences Karachi (BUHSCK). Patients were recruited from PNS Shifa Patients who fulfilled the inclusion criteria were enrolled in the study. The patients were fully educated about the study. Written informed consent was obtained from all the participants after they agreed to participate in the study All selected individuals were divided into three groups:

- Group A: PCOS diagnosed group according to Rotterdam criteria.
- Group B: Control group (not fulfilling Rotterdam criteria)
- Group C: Pre-disposed group (subjects having positive family history of PCOS)

When the patients attended the out patient department (Obs and gynae opd) and had been assessed by the consultants, they were sent for their lab tests to the PNS Shifa lab. The tests included hormonal profile such as FSH, LH, Prolactin, serum Testosterone levels, fasting blood sugar and fasting insulin and HbA1c. The blood reports were assessed. The patients parameters were recorded and the subject evaluation forms were filled.

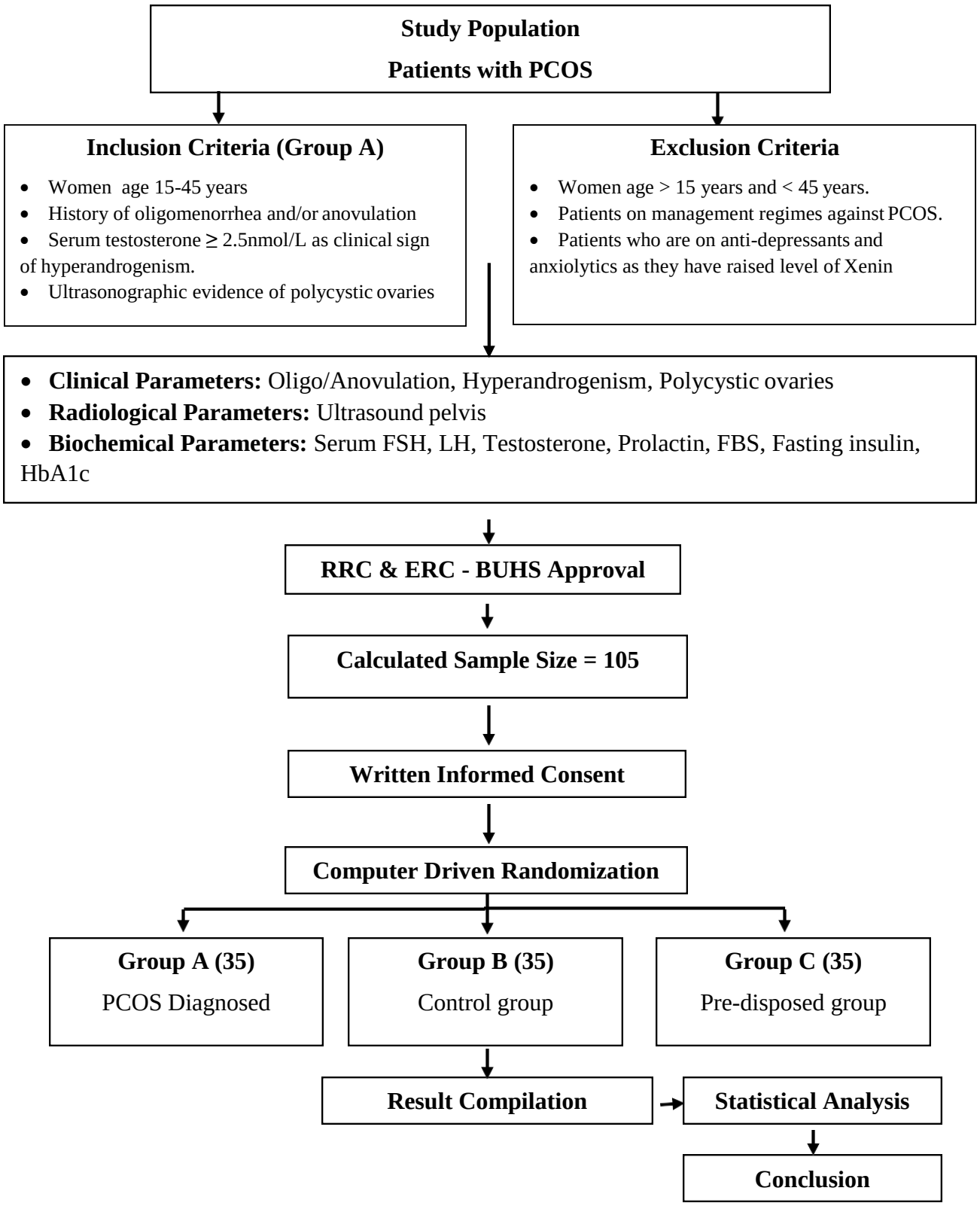
In order to assess BMI, height was measured using measuring tape and weight was determined by means of weighing scale. For biochemical analysis of Xenin 3 ml of venous blood was collected in a gel top vacutainer from the participants during the early follicular period of menstruation (second or third days) following minimum 10-12 h of night fasting. The vacutainers were immediately placed under ice and then after centrifugation serum was transferred to eppendorf and stored at -80°c until analysis by Human Xenin 25 ELISA Kit.

Figure 3.9 (a): Weighing Machine

Figure 3. 9(b): Measuring Tape

Figure 3.10: Ultrasound Machine

3.13 ALGORITHM OF STUDY



3.14 STATISTICAL ANALYSIS

- Data Analysis was performed by IBM SPSS Statistics version 26.
- Qualitative variables like occupation, oligo/anovulation, hyperandrogenism, hirsutism, polycystic ovaries and family history of PCOS were presented in terms of frequency
- Quantitative variables like age, height, weight, body mass index, S.FSH, S.LH, S. testosterone, prolactin, fasting blood glucose, fasting insulin levels, HbA1c and serum Xenin were presented in term of mean and standard deviation.
- Mean comparison of quantitative variable was done by one Way ANOVA test.
- Pearson's correlation coefficient was applied to determine the relationship between quantitative variables while ROC curve was used to determine cut off values.
- $P \leq 0.05$ was considered as significant.

CHAPTER 4

RESULTS

A total of 105 samples of female patients were taken during the study period. These patients were divided into three groups i.e., Cases, Controls and Pre-disposed group. Biochemical parameters as well as seum Xenin levels of these groups were compared in the study.

Table 4.1 shows the distribution of total no of patients in different groups. 105 patients were divided into three groups like case, control and pre-disposed group. Each group comprised of 35 patients of different ages.

Table 4.2 shows the distribution of female patients according to age groups along with the mean age. Among the all three groups from 105 numbers the age range was found to be 16 - 44 years. The mean age was calculated as 28.21±6.45 years and majority of the patients belonged to the age group ≤30 years among all groups.

Table 4.3 shows the distribution of female patients according to the height, weight and body mass index. The table revealed the overall mean height, weight and body mass index as, 143.52±9.94 cm, 55.42± 11.84 kg and 26.56±6.93 kg/m2 respectively. The details of the descriptive statistics of these variables are mentioned in the given table.

Table 4.4 reveals that among 105 females 35(33.3%) had a complaint of oligo/amenorrhea whereas 70(66.75%) females were without this complaints.

Table 4.5 has shown the frequency distribution of Hyperandrogenism. among the involved females. The given table reveals that out of 105 females, 19(18.1%) were presented with hyper-androgenism, whereas 86 (81.9%) were without this complaint.

Table 4.1: Distribution of total no of patients in different groups (n=105)

Groups	No of Patients	% of Groups
Case	35	33.33%
Control	35	33.33%
Pre-disposed group	35	33.33%

Table 4.2: Distribution of age in total no of patients

Distribution of Age in Total No of Patients	
Total No of Patients	105
Age Range	Minimum = 16
	Maximum= 44
Mean Age	28.21±6.45

Table 4.3: Descriptive Statistics of Height (cm), Weight (kg), Mass Index (kg/m^2), N=105

Variables	**Range**	**Mean**
Height(cm)	132 -168	143.52
Weight(kg)	36 - 97	55.42
Mass Index(kg/m^2	17.90 - 44.28	26.56

Table 4. 4: Frequency distribution of Oligo/Ammenorhea, N=105

Occurrence of Oligo/Anovulation	Frequency (%)	(%) of Numbers
Yes	35	(33.3)
No	70	(66.7)
TOTAL	**105**	

Table 4.5: Frequency distribution of Hyperandrogenism, N=105

Occurrence of Hyperandrogenism	Number of patients	Frequency (%)
Yes	19	(18.1)
No	86	(81.9)
TOTAL	**105**	

Table 4.6 reveals the occurence of Hirsutism among all females. It has been estimated that 30(28.6%) presented with hirsutism, and remaining 75(71.4%) had no history of hisutism.

Table 4.7 revealed that 20(19%) out of 105 females had history of polycystic ovaries, whereas 85(81%) were free from this complain.

Table 4.8 compares the frequency distribution of PCOS with family history. It has been estimated that 55(52.4%) females having positive family history of this disorder, whereas 50(47.5%) having no history of PCOS.

Table 4.9 has shown the mean values of Serum FSH, 6.61±1.27 (mIU/ml), Serum LH, 10.75±1.59 (mIU/ml), Serum Testosterone, 1.67±0.33 (nmol/L), Prolactin 1.00±0.43 and Serum LH/FSH ratio, 1.67±0.33 (µg/L) among all females involved in study.

Table 4.10 has shown the serum values of blood sugar, insulin and HbA1c, which had taken from all females in their fasting state. It has been reported that mean Fasting Blood Glucose is about 5.10(mmol/L), the mean Fasting Insulin Levels is 8.44 (Miu/ml) and HbA1c has been recorded as 5.19 (%).

Table 4. 11 has shown the mean values of Serum Xenin that is about 27.25 (pg/ml).The ranges of this serum marker has been taken as 16.93 - 35.88.

Table 4.12 and figure 4.1 demonstrates the frequency distribution and association of age groups according to study groups. Among patients with age <30 years, 31.8% were from cases, 31.8% were from control and 36.4% were from pre-disposed group while patients with age ≥30 years, there were 35.9 % patient from cases 35.9% from controls and 28.2% from pre-disposed group. There was insignificant association of study group with age group i-e (p=0.721)

Table 4.6: Frequency distribution of Hirsutism, N=105

Occurrence of Hirsutism	Number of patients	Frequency(%)
Yes	30	(28.6)
No	75	(71.4)
TOTAL	**105**	

Table 4.7: Frequency distribution of Polycystic Ovaries, N=105

Occurrence of Polycystic Ovaries	Number of patients	Frequency (%)
Yes	20	(19)
No	85	(81)
TOTAL	**105**	

Table 4.8: Frequency distribution of PCOS family history, N=105

Subjects with family history of PCOS	**Number of patients**	**Frequency (%)**
+ve	55	(52.4)
-ve	50	(47.6)
TOTAL	**105**	

Table 4.9: Descriptive statistics of Serum FSH (mIU/ml), Serum LH (mIU/ml), Serum Testosterone (nmol/L) and Prolactin (μg/L), N=105

Serum Markers	Range	Mean
FSH	3.40 - 8.80	6.61
LH	6.80 - 14.50	10.75
Testosterone	1.10 - 2.49	1.67
Prolactin	0.10 -1.90	1.00
LH FSH Ratio	1.10 – 2.49	1.67

Table 4.10: Descriptive statistics of Fasting Blood Glucose (mmol/L),Fasting Insulin Levels (mIU/ml), HbA1c (%), N=105

Serum Markers	Range	Mean
Fasting Blood Glucose	4.10 – 7.20	5.10
Fasting Insulin Levels	0.80 -22.80	8.44
HbA1c	4.52 -7.00	5.19

Table 4.11: Descriptive statistics of Serum Xenin (pg/ml), N=105

Serum Markers	Range	Mean
Serum Xenin	16.93 - 35.88	27.25

Table 4.12: Frequency distribution and association of age group according to study groups, N=105

Age Groups in Years	Study Group			P-VALUE
	Case N (%)	Control N (%)	Probable N (%)	
<30 years	21(31.8)	21(31.8)	24(36.4)	0.721**
≥30 years	14(35.9)	14(35.9)	11(28.2)	
Total	**35**	**35**	**35**	

Chi-Square Test was applied, P<0.05 considered as significant, **: Not Significant.

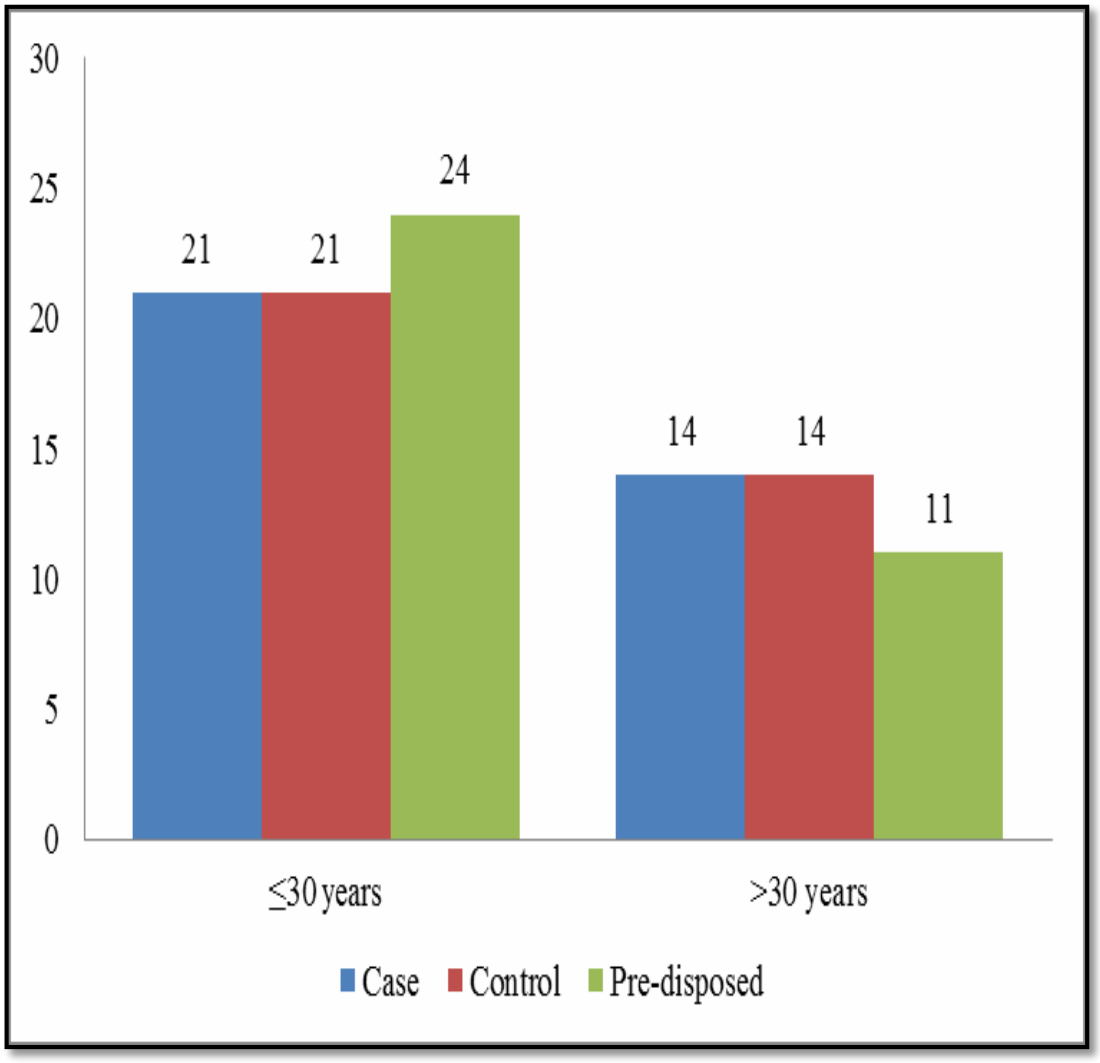


Figure: 4.1: Frequency distribution of age group according to study groups

Table 4.13 and Figure 4.2 compares the correlation of obese and non- obese people groups according to study groups. It has been estimated that there were 13% obese patient from case, 44.9% from control and 42% from pre-disposed group, while among the non – obese people 72.2% from case, 11.1 % from control and 16.7% from pre-disposed group were reported. This table revealed the significant association of obesity (p=0.000) with the study groups.

Table 4.14 and figure 4.3, 4.4, 4.5,4 .6, 4.7, 4.8, 4.9 and 4.10 shows mean comparison of biochemical Parameters according to study groups. In cases mean age, body mass index, serum FSH, serum LH, LH FSH ratio, serum testosterone, prolactin, fasting blood glucose, fasting insulin level, HbAIc and serum xenin was 28.85±6.37years, 30.91±5.66 kg/m^2, 5.68±1.23mIU/ml, 11.35±1.87mIU/ml, 2.02±0.23, 1.20±0.42 nmol/L, 24.40±9.50 µg/L, 5.55±0.91 mmol/L, 11.63±5.65mIU/ml and 5.54±0.78 %, respectively while in control group, mean age, body mass index, serum FSH, serum LH, LH FSH ratio, serum testosterone, prolactin, fasting blood glucose, fasting insulin level, HbAIC and serum xenin was 28.17±7.04 years, 22.69±5.07kg/m^2, 7.59±0.97mIU/ml, 10.19±1.44 mIU/ml, 1.34±0.11, 0.78±0.45nmol/L, 15.34±3.07µg/L, 4.98±0.40 mmol/L, 11.63±5.65 mIU/ml and 5.85±2.91%, respectively. mean age, body mass index, serum FSH, serum LH, LH FSH ratio, serum testosterone, prolactin, fasting blood glucose, fasting insulin level, HbAIC and serum xenin was 25.94±5.47years, 26.08±7.36 kg/m^2, 6.56±0.80 mIU/ml, 10.70±1.22mIU/ml, 1.64±0.18, 1.02±0.33 nmol/L, 17.93±4.18 µg/L, 4.76±0.38 mmol/L, 7.85±4.19 mIU/ml and 4.94±0.31% in pre-disposed group, respectively.

We found significant mean difference for age(p=0.005), body mass index(p=0.000), serum FSH(p=0.000), serum LH(p=0.009), LH FSH ratio(p=0.000), serum testosterone(p=0.000), prolactin(p=0.000), fasting blood glucose(p=0.000), fasting insulin level(p=0.000), HbAIC(p=0.000). By applying One Way ANOVA Test, we found significant mean difference for age(p=0.005), body mass index(p=0.000), serum FSH(p=0.000), serum LH(p=0.009), LH FSH ratio(p=0.000), serum testosterone(p=0.000), prolactin(p=0.000), fasting blood glucose(p=0.000), fasting insulin level(p=0.000), HbAIc(p=0.000), respectively.

Table 4.13: Frequency distribution and association of obesity according to study groups, N=104

BMI Groups	Study Group			P-VALUE
	Case	**Control**	**Probable**	
Non Obese (<30 kg/m^2)	9(13)	31(44.9)	29(42)	0.000*
Obese (≥30 kg/m^2)	26(72.2)	4(11.1)	6(16.7)	
Total	**35**	**35**	**35**	

Chi-Square Test was applied, P<0.05 considered as significant, *: Significant at 0.05 level.

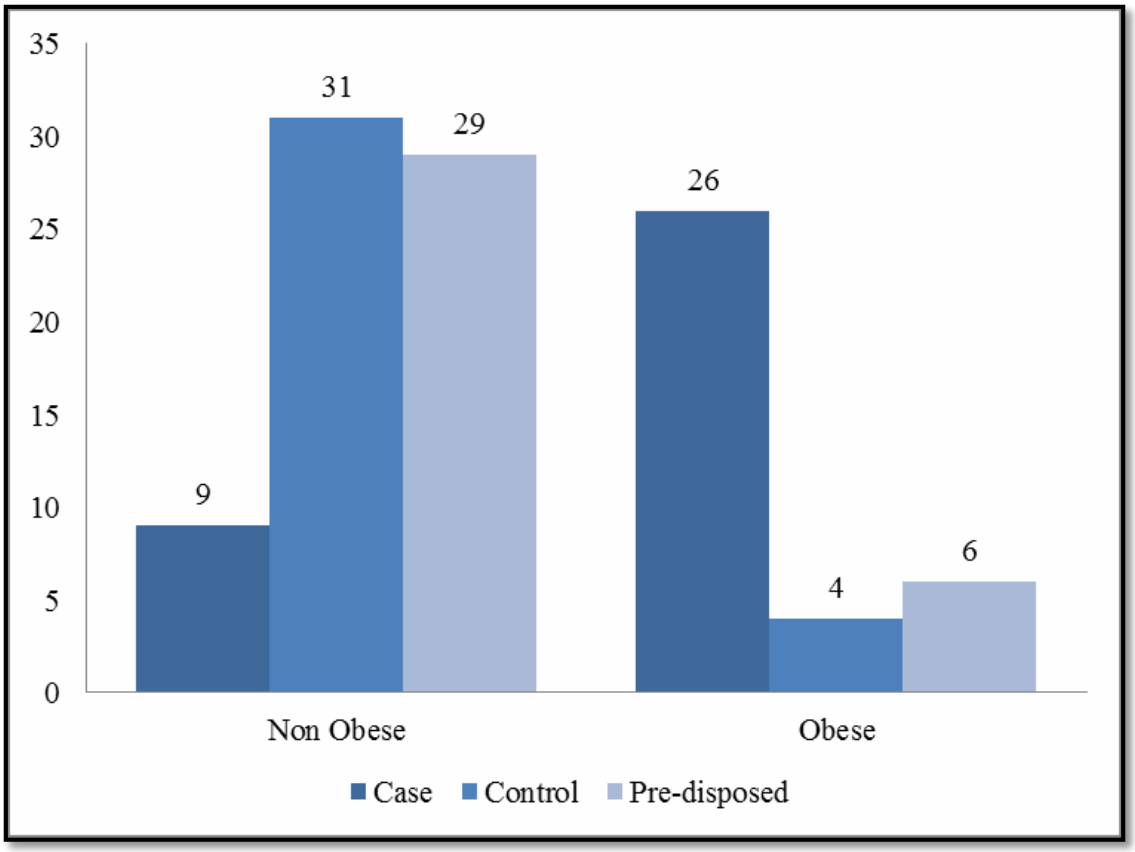


Figure: 4.2: Frequency distribution of obesity according to study groups

Table 4.14: Mean comparison of biochemical parameters according to study groups, n=105

Biochemical Parameters	Study Group Mean± Std. Deviation			P-Value
	Case	Control	Probable	
Age (years)	28.85±6.37	28.17±7.04	25.94±5.47	0.137
Body Mass Index (kg/m^2)	30.91±5.66	22.69±5.07	26.08±7.36	0.000*
S.FSH (mIU/ml)	5.68±1.23	7.59±0.97	6.56±0.80	0.000*
S. LH (mIU/ml)	11.35±1.87	10.19±1.44	10.70±1.22	0.009*
LH FSH Ratio	2.02±0.23	1.34±0.11	1.64±0.18	0.000*
S. Testosterone (nmol/L)	1.20±0.42	0.78±0.45	1.02±0.33	0.000*
Prolactin (µg/L)	24.40±9.50	15.34±3.07	17.93±4.18	0.000*
Fasting Blood Glucose (mmol/L)	5.55±0.91	4.98±0.40	4.76±0.38	0.000*
Fasting Insulin levels (mIU/ml)	11.63±5.65	5.85±2.91	7.85±4.19	0.000*
HbA1c	5.54±0.78	5.09±0.39	4.94±0.31	0.000*

One Way ANOVA Test was applied, P<0.05 considered as significant, *: Significant at 0.05 level.

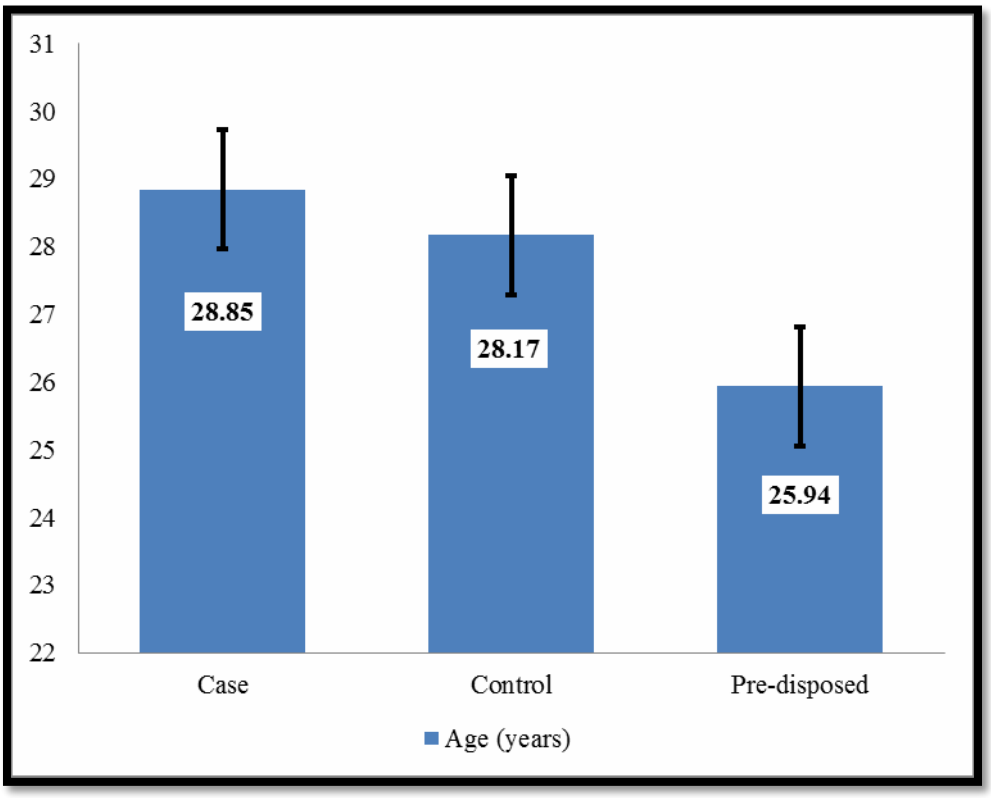


Figure 4.3: Mean age of PCOS in diagnosed group, pre-disposed group and control group

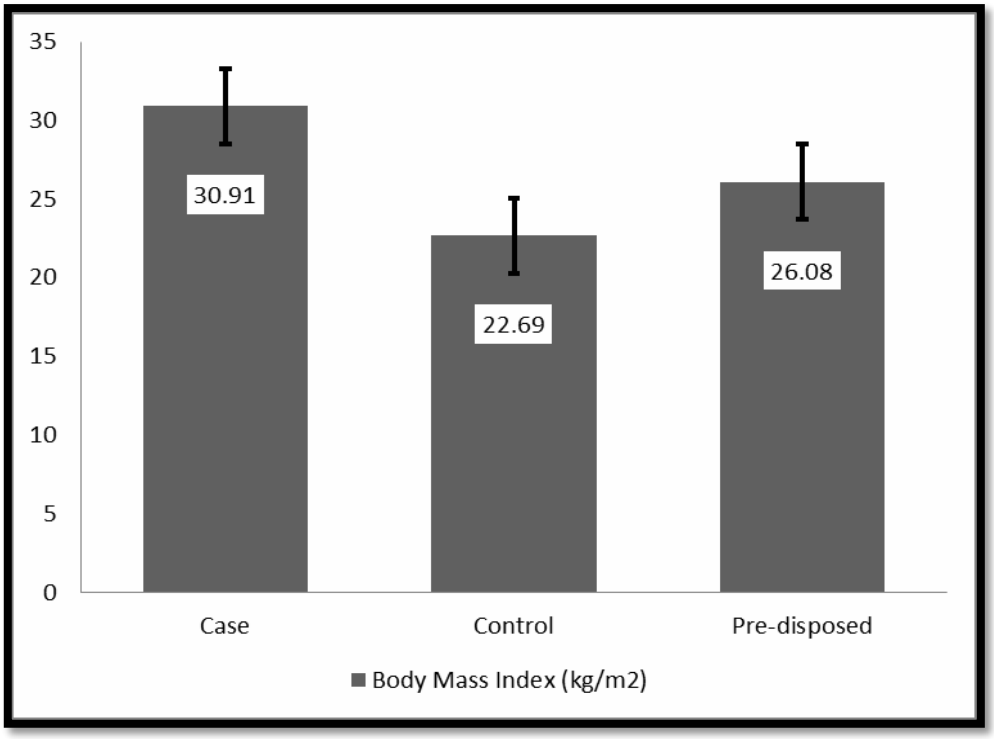


Figure 4.4: Mean body mass index in diagnosed group, pre-disposed group and control group

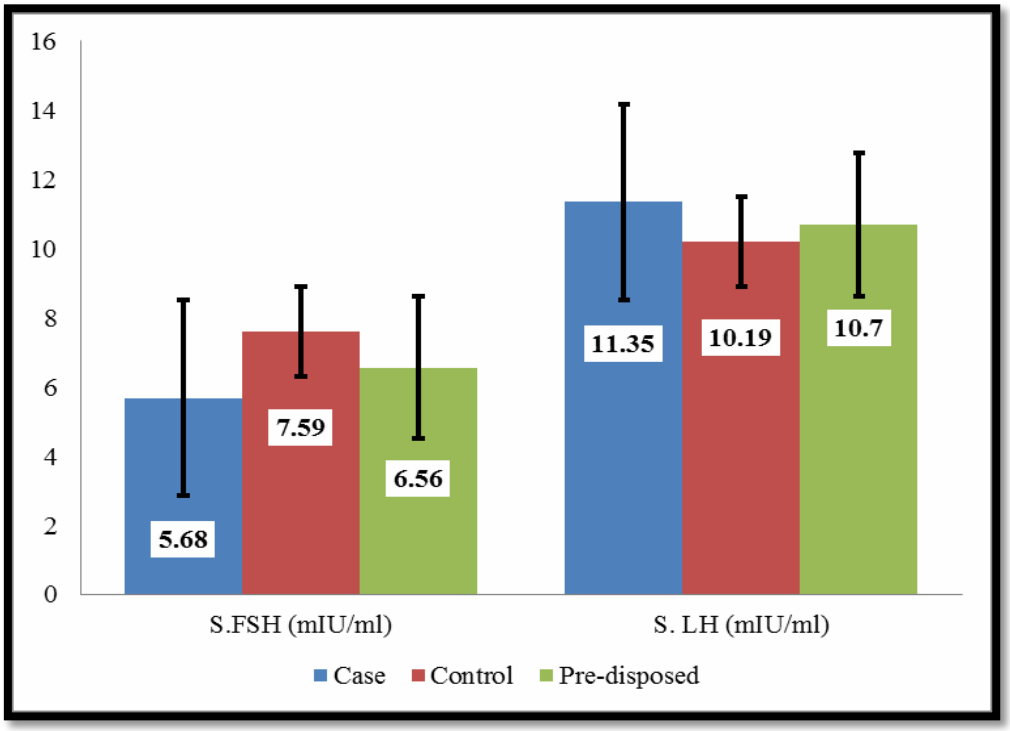


Figure: 4.5 Mean serum FSH and Serum LH in diagnosed group, control group and pre-disposed group

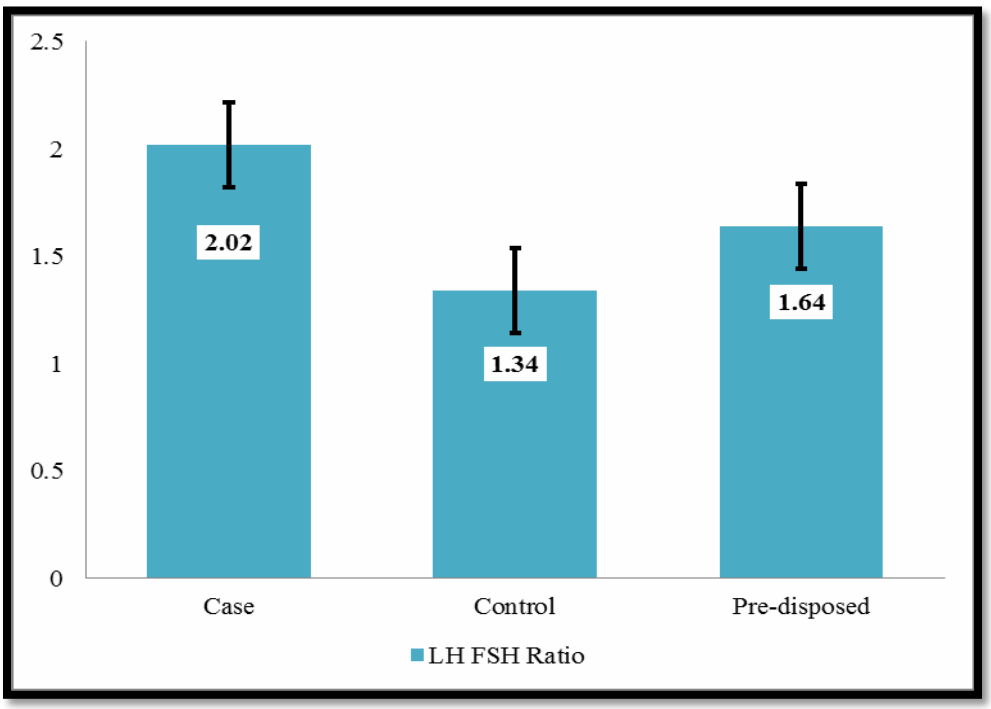


Figure: 4.6 Mean LH FSH ratio in diagnosed group, pre-disposed group and control group

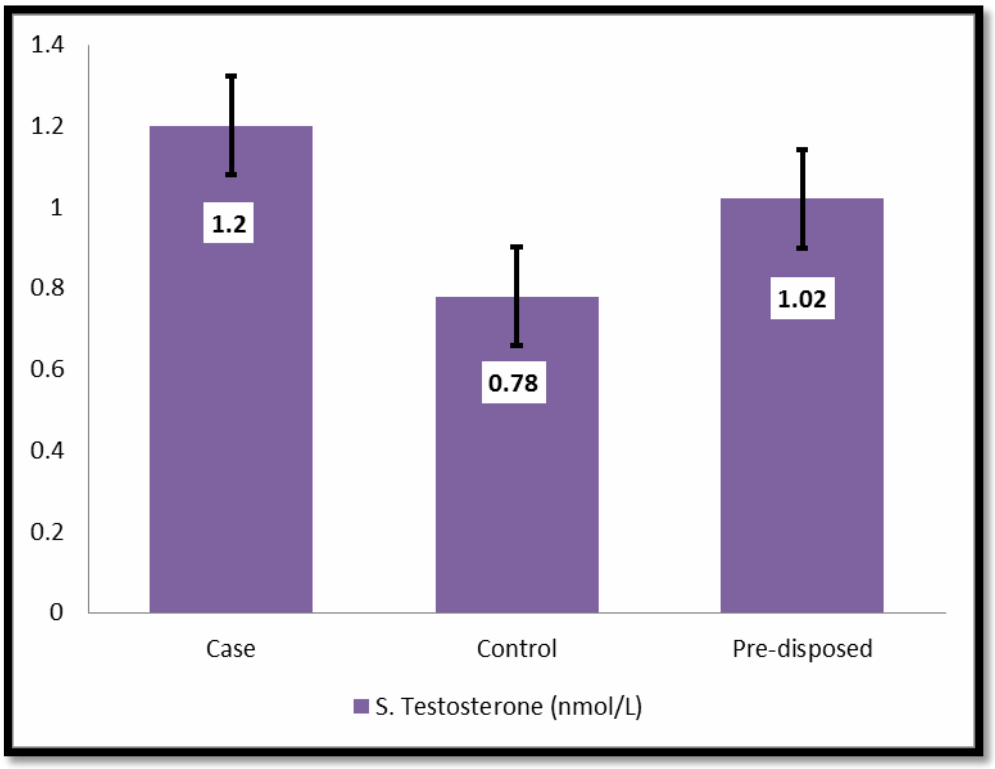


Figure:4.7: Mean serum testosterone in diagnosed group, pre-disposed group and control group

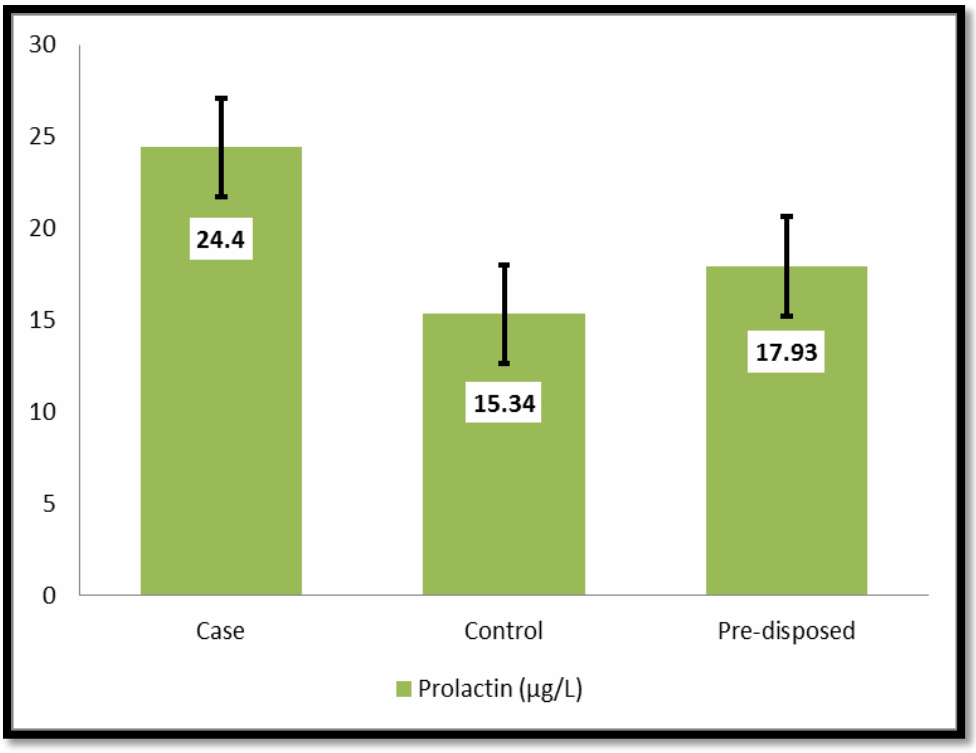


Figure : 4.8 Mean serum prolactin in diagnosed group, pre-disposed group and control group

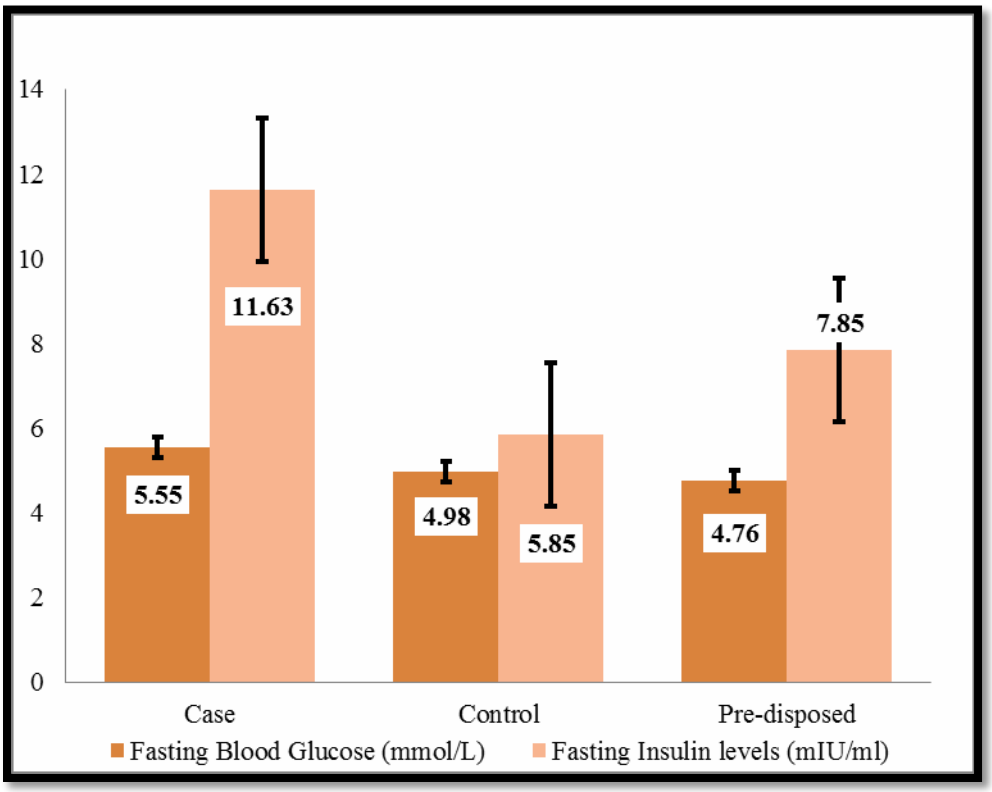


Figure:4.9: Mean fasting blood glucose and fasting insulin levels in diagnosed group, pre-disposed group and control group

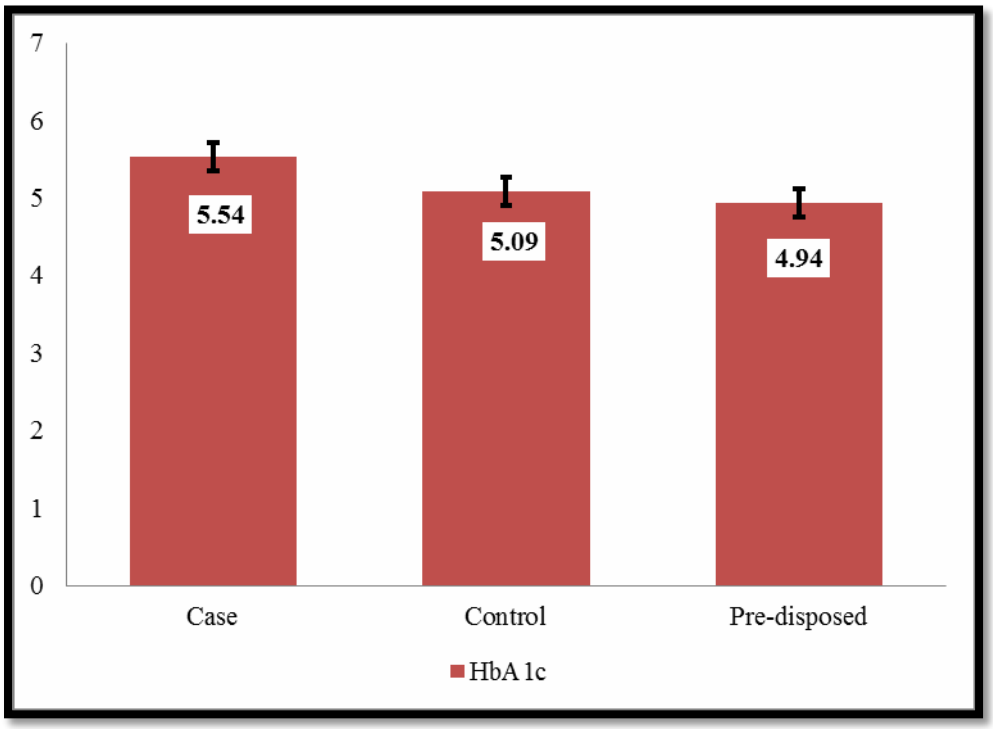


Figure:4.10: Mean serum HbA1c levels in diagnosed group, pre-disposed group and control group

Table 4.15 illustrates the results of mean comparisons with the detail description of all variables like age, BMI, serum FSH, serum LH, serum Testosterone, serum prolactin, LH:FSH, Fasting blood sugar, Fastin insulin levels and HbA1c. Post hock Tukey HSD test was applied. P<0.05 considered as significant

Table -4.16 and figure4.11 reveals that mean serum xenin was 31.25±2.86 pg/ml in cases, 23.58±3.36 pg/ml in control and 26.93±3.94 pg/ml in pre-disposed group with significant mean difference (p=0.000) as presented in given table. One Way ANOVA Test and Post Hoc Tukey HSD test was applied.

Figure 4.12,4.13 4.14,4.15 shows ROC Curves. Cut off values of serum xenin was 27.18 (Sen=82.9%, 27.41(Sen=82.9%) and 27.96(Sen=82.9%) for cases while the cut offs values for serum xenin for proable group was 22.93(sen=82.9%) and 23.01(sen=80%).

We have a good overall model quality of ROC for cases (91%) and for pre-disposed group (62%). Area under curve for cases and pre-disposed group was 0.953 and 0.742 respectively. Detailed results are presented in following and figures

Table 4.17 illustrates the Serum Xenin Relationship with Anthropometric and Bio-Chemical Markers. We found that there was negative weak correlation serum xenin with serum FSH(r=-0.477) and serum LH(r=-0.317) in control group. To find out the association Pearson’s coefficient of correlation has been applied. Detailed results of correlation are presented in following table.

Table 4.15: Multiple mean comparisons of biochemical parameters according to study groups, n=105

Variable	(I) Study Group	(J) Study Group	Mean Difference	P-Value
Age (years)	Case	Control	2.94	0.120
		Probable	4.88*	0.004*
	Control	Case	-2.94	0.120
		Probable	1.94	0.391
	Pre-disposed	Case	-4.88*	0.004*
		Control	-1.94	0.391
Body Mass index (kg/m^2)	Case	Control	8.21*	0.000*
		Pre-disposed	4.82*	0.004*
	Control	Case	-8.21*	0.000*
		Pre-disposed	-3.38	0.058
	Pre-disposed	Case	-4.82*	0.004*
		Control	3.38	0.058
S.FSH (mIU/ml)	Case	Control	-1.90*	0.000*
		Pre-disposed	-0.87*	0.001*
	Control	Case	1.90*	0.000*
		Pre-disposed	1.03*	0.000*
	Pre-disposed	Case	.87*	0.001*
		Control	-1.03*	0.000*
S. LH (mIU/ml)	Case	Control	1.15*	0.006*
		Pre-disposed	0.64	0.191
	Control	Case	-1.15	0.006*
		Pre-disposed	-0.51	0.351
	Pre-disposed	Case	-0.64	0.191
		Control	0.51	0.351
LH FSH ratio	Case	Control	0.68	0.000*

		Pre-disposed	0.38	0.000*
	Control	Case	-0.68	0.000*
		Pre-disposed	-0.29	0.000*
	Pre-disposed	Case	-0.38*	0.000*
		Control	0.29*	0.000*
S.Testosterone (nmol/L)	Case	Control	0.41*	0.000*
		Pre-disposed	0.17	0.191
	Control	Case	-0.41	0.000*
		Pre-disposed	-0.24	0.041*
	Pre-disposed	Case	-0.17	0.191
		Control	0.24	0.041*
Prolactin (µg/L)	Case	Control	9.06	0.000*
		Pre-disposed	6.47	0.000*
	Control	Case	-9.06	0.000*
		Pre-disposed	-2.58	0.200
	Pre-disposed	Case	-6.47	0.000*
		Control	2.58	0.200
Fasting Blood Glucose (nmol/L)	Case	Control	0.57	0.001*
		Pre-disposed	0.78	0.000*
	Control	Case	-0.57	0.001*
		Pre-disposed	0.21	0.333
	Pre-disposed	Case	-0.78	0.000*
		Control	-0.21	0.333
Fasting Insulin levels (Miu/ml)	Case	Control	5.78	0.000*
		Pre-disposed	3.78	0.001*
	Control	Case	-5.78	0.000*
		Pre-disposed	-1.99	0.145
	Pre-disposed	Case	-3.78	0.001*
		Control	1.99	0.145

HbA1c (%)	Case	Control	0.44	0.002*
		Pre-disposed	0.59	0.000*
	Control	Case	-0.44	0.002*
		Pre-disposed	0.15	0.470
	Pre-disposed	Case	-0.59	0.000*
		Control	-0.15	0.470

Post Hoc Tukey HSD test was applied, P<0.05 considered as significant, *: Significant at 0.05 level.

Table 4.16: Mean comparison of serum xenin according to study groups, N=105

	Study Group Mean± Std. Deviation			**P-VALUE**
	Case	**Control**	**Pre-disposed**	
Serum Xenin	31.25±2.86	23.58±3.36	26.93±3.94	0.000*
Multiple Comparison¤				
(I) Study Group	**(J) Study Group**	**Mean Difference**	**P-value**	
Case	Control	7.66	0.000*	
	Pre-disposed	4.31	0.000*	
Control	Case	-7.66	0.000*	
	Pre-disposed	-3.35	0.000*	
Pre-disposed	Case	-4.31	0.000*	
	Control	3.35	0.000*	

One Way ANOVA Test was applied, Post Hoc Tukey HSD test was applied. P<0.05 considered as significant, *: Significant at 0.05 level.

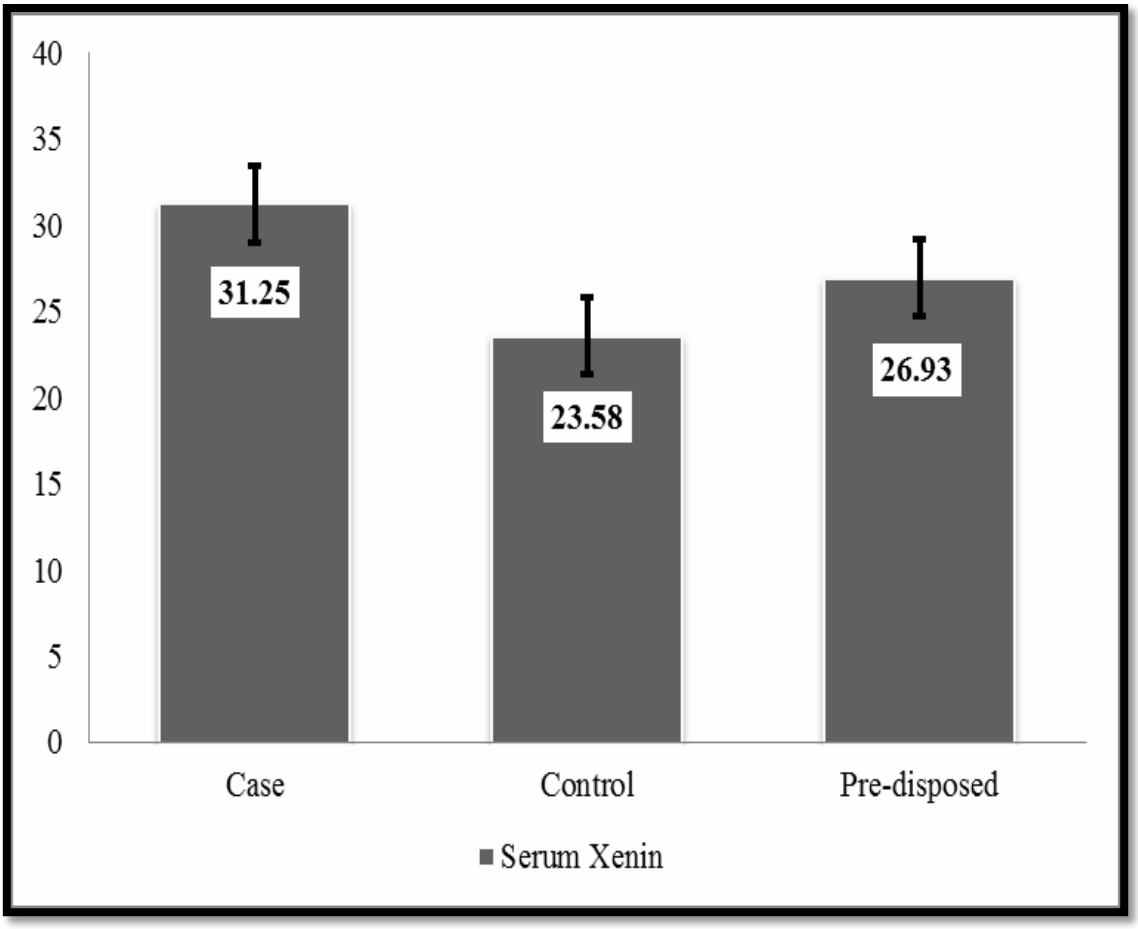


Figure 4.11: Mean Serum Xenin levels in diagnosed group, pre-disposed group and control group

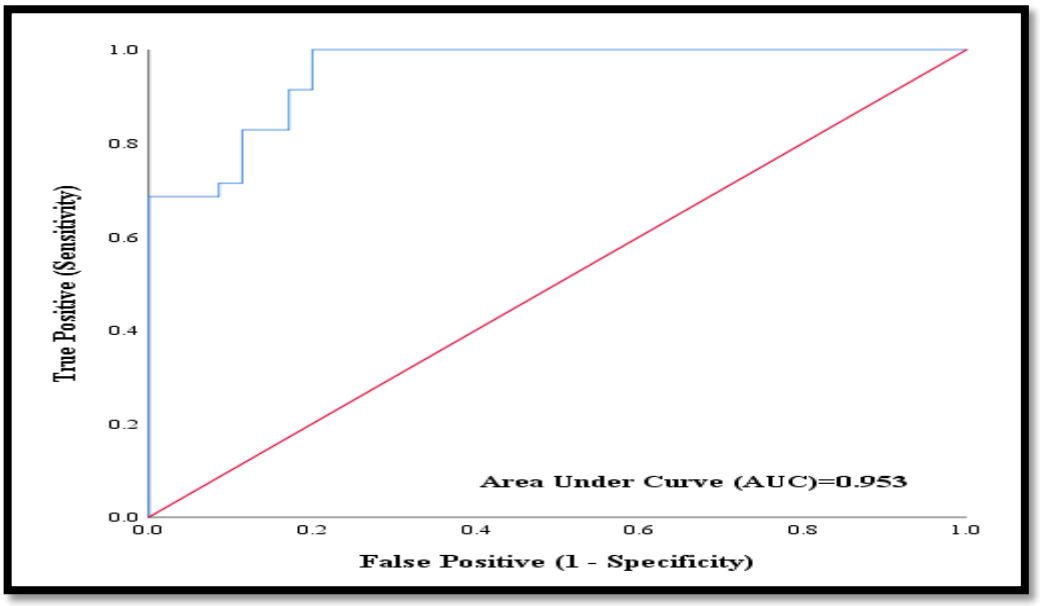


Figure: 4.12 ROC Curve for Serum Xenin in cases

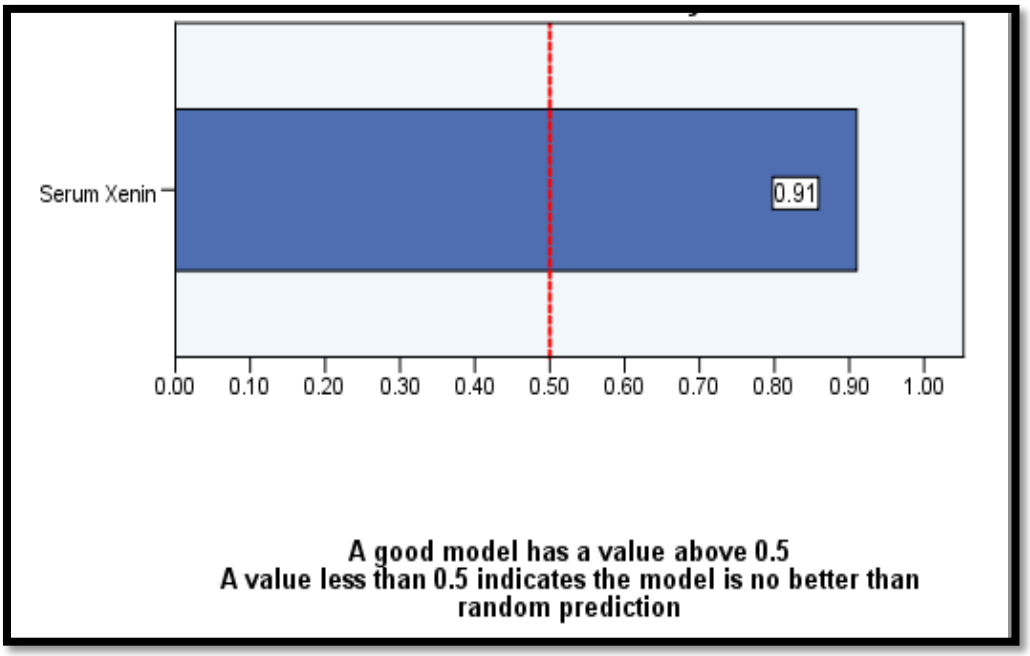


Figure 4.13: Overall Model Quality of ROC Curve for Serum Xenin in cases

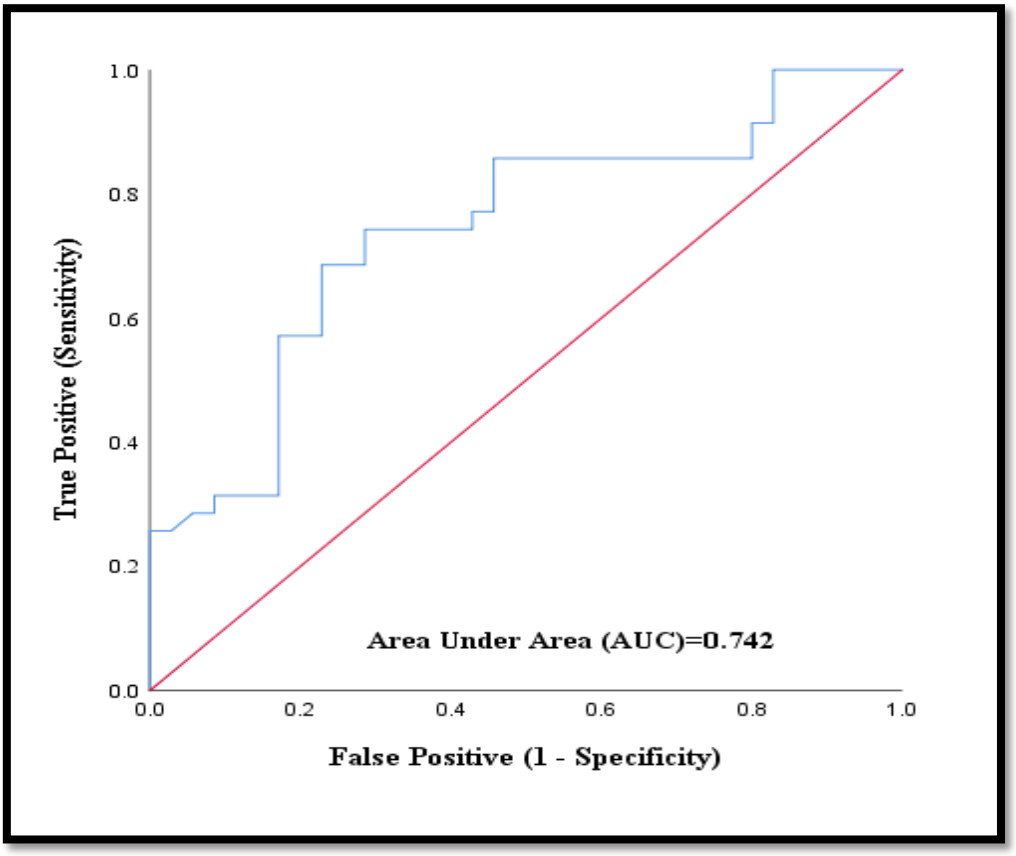


Figure 4.14: ROC Curve for Serum Xenin in pre-disposed group

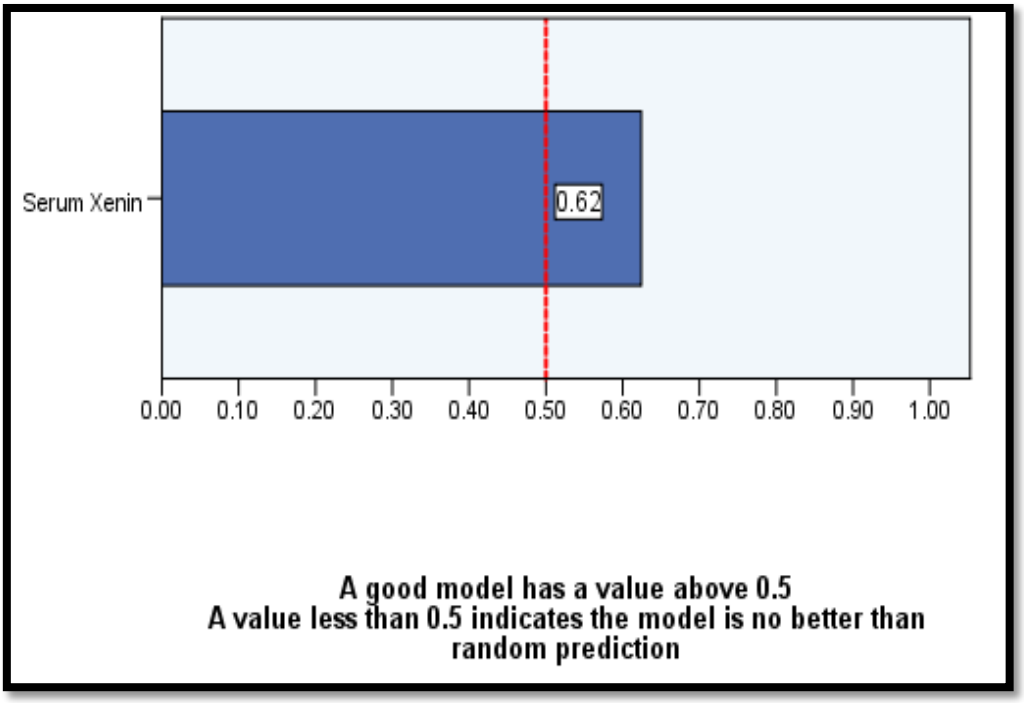


Figure 4.15: Overall model quality of ROC curve for serum xenin in pre-disposed group

Table 4.17: Serum Xenin Relationship with Anthropometric and Bio-Chemical Markers

Anthropometric and Bio-Chemical Markers	**Serum Xenin Correlation coefficient**		
	Case	**Control**	**Pre-disposed**
Age (years)	-0.093	0.17	-0.122
BMI	0.009	0.02	-0.19
S.FSH (mIU/ml)	0.239	-0.477	0.085
S. LH (mIU/ml)	0.136	-0.317	0.155
LH_FSH_ratio	-0.213	0.256	0.059
S.Testosterone (nmol/L)	-0.168	-0.11	0.006
Prolactin (µg/L)	0.136	0.134	-0.178
Fasting Blood Glucose (nmol/L)	-0.279	0.267	0.083
Fasting Insulin levels (Miu/ml)	-0.163	0.192	0.165
HbA1c	0.216	0.181	0.006

Pearson's coefficient of correlation has been applied**.**

CHAPTER 5

DISCUSSION

5.1 SEQUENCE OF DISCUSSION EXPERIMENT

The discussion chapter has covered the elaborate analysis in light of the results of the present study. It has analysed the parameters like age, and BMI based on height and weight. It has evaluated the FSH and LH. It has also continued to weigh the Prolactin and testosterone. They are evaluated one by one in the context of their results for the present study. Further, it compared the Fasting blood sugar and Fasting insulin levels for the specific disease PCOS. It has also valued HbA1c and xenin in the results. The role of the specific parameters has been discussed in the discussion section one by one. The various reviews which are taken from the literature from previous records to compare and contrast the present study output.

PCOS is known to be linked to obesity, insulin resistance, type 2 diabetes, cardiovascular disease, and psychosocial issues. Age affects PCOS symptoms, Diagnostic Criteria, consequences, biochemical and clinical presentations and treatment (Merhi et al., 2019). These changes are likely due to decreased production by the adrenal glands over time or ovarian aging. Likely because of the fact that approximately at 30 years of age ovarian steroid secretion capacity begins to decrease. (Gleicher et al. 2022). It was highlighted by van Keizerswaard et al. (2022). Menstrual irregularities return to normal as androgen levels drop and the morphology of polycystic ovarian tissue improves. The risk of acquiring metabolic diseases and insulin resistance increases with age (Hsu, 2013; Gleicher et al. 2022).Hormonal variations, ovarian function changes, and genetic variables affect PCOS symptoms, although the molecular mechanisms are unknown. Age and PCOS were examined in three groups: case, control, and probable. The case group consisted of female patients with PCOS, the control group of those without PCOS, and the likely group of those with symptoms but no diagnosis. Out of the included females case group had a greater mean age as compared to the control group and probable group. Research showed no significant difference of age between the groups, hence the age was matched amongst all the groups.

A study conducted by Cakir et al. (2012) found that women with PCOS have a higher mean age than controls validating findings of our study showing greater mean age in the case group. Carmina et al. (2012) found that PCOS severity and metabolic abnormalities increased with age at diagnosis. The case group of diagnosed PCOS patients had a greater mean age than the control and probable groups in this study. In contrast Sousa et al. (2010) found no significant difference in mean age between PCOS patients and controls. They found that age may not affect PCOS development or diagnosis. Contrary to the present study's findings, the case group had a greater mean age than the control group. Sidra et al. (2019) observed that age was substantially linked with PCOS only in a subgroup of patients with different clinical characteristics. These patients have more metabolic problems at a later age. The current investigation did not identify a significant age group-study group connection.

PCOS impacts about 20-30% of women of reproductive age (Bharathi et al., 2017). For the described clinical entity and age issues, the consisting menstrual disorders, and sterility are chances for PCOS (Shorakae et al., 2015). In addition, the role of Hirsutism is to menstrual irregularities, and acne at an early age. This may cause overweight which is a representation of physiological changes typical of the age. Two-thirds of apparently normal adolescents develop acne due to hormonal issues. This is a plan for half of the menstrual irregularities in the high percentage for obesity and about a quarter develop metabolic syndrome (Rosenfield, 2020). The young women may be asymptomatic for being associated with painful ovulations. This may also lead to irregular periods and body function imbalance. Indeed, it is proving the issues of some ovaries 'multicystic' rather than 'polycystic' because they are asymptomatic and do not require treatment (Stener-Victorin *et al.*, 2020).

BMI is the mass index for the body. It is a term that shows an impact on polycystic ovary syndrome (PCOS). This also refers to endocrine and metabolic disorders with the levels of 6 to 21% of women of reproductive age depending on the mean body mass index (BMI). This is a diagnostic criterion and population that shows the increasing chances of PCOS in case of BMI imbalance (McCartney and Marshall, 2016) PCOS patients generally have high BMIs. Because excess adipose tissue causes insulin resistance, high BMI is connected to PCOS. Insulin resistance increases insulin levels, which boost androgen production and aggravate PCOS. PCOS and BMI affect clinical presentation, management, and long-term health outcomes. The 105 female inclusion criteria patients

had a mean BMI of 26.56±6.93 kg/m2. The case group had the greatest mean BMI (30.91±5.66 kg/m2) compared to the control (22.69±5.07 kg/m2) and probable groups (26.08±7.36 kg/m2). 72.7 percent of patients under 30 were obese, compared to 11.1% of the control group and 16.7% of the probable group. Obesity was associated with the research group (p=0.000). Pinola et al. (2017) identified a favourable connection between PCOS and higher BMI in a large sample. PCOS patients exhibited considerably higher BMIs than controls. The case group had a higher mean BMI than the control and probable groups, supporting this study's findings. McCook et al. (2015) studied PCOS and obesity indicating that obesity was consistently higher in women with PCOS than in the general population. Obesity was significantly associated with PCOS in this study. On the contrary Kim and Choi. (2019) found no BMI difference between PCOS patients and controls. Their Asian women study revealed that genetic and ethnic variables may explain BMI discrepancies between populations. This contradicts the present study's findings that the case group had a higher mean BMI than the control group. Spinedi & Cardinali (2018), examined PCOS, BMI, and metabolic abnormalities. BMI did not entirely explain metabolic abnormalities in PCOS individuals.

Follicle-stimulating hormone (FSH) has a role in sexual functioning and development. In this context, the studies reveal that FSH regulates the menstrual cycle in addition to the functioning of the growth of the eggs within the ovary. It has specific support for the range of 4-8 fertile women hormones. This is regarded with PCOS, specifically, FSH levels are often within the range of about 4-8 which is the ratio for LH to FSH. This is expected to get much higher such as 2:1 or 3:1 in adults. The effect on insulin resistance is under xenin which needs appetite regulation. Haq et al. (2017) revealed that PCOS women have an increment in appetite which is negative for an altered response to food intake. This mentions the development of obesity and metabolic dysfunction (Helvaci et al., 2017).

Hormonal imbalances play a key role in the development of PCOS, and the levels of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are particularly relevant (Mahmud et al., 2022). FSH grows and matures egg-containing ovarian follicles. It makes biological changes in body which are essential for life functions. Ovulation is triggered by LH. FSH and LH imbalances in PCOS women impair ovulation and cause ovarian cysts. The study found that 36.4% of patients under 30 were in the likely category, followed by 31.8% in the case and control groups. PCOS-related hormonal abnormalities

are more frequent in younger people. The mean serum FSH, LH, and LH/FSH ratios reveal the study participants' hormonal profiles. According to Valsamkis et al. (2019), PCOS and FSH/LH levels are disputed; the altered hypothalamic-pituitary-ovarian feedback system may cause increased FSH levels in PCOS. As a result of this communication breakdown, FSH output rises to compensate for ovulation. However, other studies suggest that FSH levels may not differ between women with PCOS and those without the illness (Azziz et al., 2016). These differing viewpoints suggest more investigation and evaluation of numerous criteria in PCOS diagnosis.

Infertility is a possibility due to PCOS women is caused by ovulatory dysfunction, which is associated with an imbalance in follicle-stimulating hormone (FSH) and luteinising hormone (LH) secretion (Jasim & Alkareem, 2022). Further, the females with low FSH rate has a ratio of LH hormone for high. This is resulting in the inhibition of egg release leading to the occurrence of irregular menstrual cycles. The disturbance in the gonadotrophin axis has a reversal ratio of LH/FSH occurs. Jasim & Alkareem (2022) show that the changes in the pulsatile nature of the gonadotrophin hormone lead to increase production of LH to FSH. The normal levels are around 2 to 1, which become reversed in the condition of PCOs. This is also correlated with the study as it was observed that $2/3^{rd}$ of cases of PCO among women presented >= 2 ratios of LH/FSH.

In addition, the FSH motivates the ovulation process which shows that the pituitary gland is operating it from the brain. The FSH level was found to be equal to LH in the initial stage of the menstrual cycle. LH increases before the initiation of ovulation which is also leading to LH levels dropping back. This can mention the egg release from the ovary. In some cases, few women present FSH levels of 5-20 ml/ml which is accountable for PCOs. Another term for this is diminished ovarian reserve (DOR), which represents mild FSH increase with time. The increase in FSH level shows the availability of fewer eggs which may cause PCO. The increase in the secretion of FSH may happen for PCOs based on high sensitivity to (GnRH) gonadotropin-releasing hormone. This is accountable for changes in the secretion patterns of GnRH. High FSH is also showing the depleted ovaries reserve which is indicating the changes in the normal occupied of ovaries (Jiskoot et al., 2019).

Kadiroğulları et al. (2020) revealed that the process of ovulation does not occur in females by the percentage increase in LH/FSH ratio. The study aimed to investigate the LH/FSH ratio with the help of comparing the declining BMI. This is also in comparison

to the women with high BMI for no association between BMI and serum hormone levels. A review of the link between LH/FSH ratio shows the connection for TSH among PCOS women. It is indicated that anti-Mullerian hormone (AMH) can help out with the increased level of females with PCOS. Hyperandrogenism can cause diabetes Type 1 in women with PCOS women. They are linked with increased AMH levels proving that females with PCOS and DM1 exhibit normal AMH and FSH/LH levels (Kadiroğulları et al., 2020). Luteinizing Hormone (LH) is found in blood and is generated in the pituitary gland which may be impacting FSH as well. The LH normal range is about 4-8 in young fertile which needs to be reviewed for PCOS females but is believed to be much high.

The LH ratio needs to be reviewed as the normal concentration of testosterone, it is less than 150 ng/dL (≤5.2 nmol/L). this seems effective while reviewing the lower level for PCOS. For example, the specific testosterone values ≤150 ng/dL (≤5.2 nmol/L) show an increase in PCOS, but at the same time if its value is ≥200 ng/dL (≥6.9 nmol/L), which is for a female that might have an ovarian tumour (Kadiroğulları et al., 2020). PCOS women show some alterations in the gene for LH, which may increase the risk of obesity and complications in the same link. It has been associated with PCOS. While in the same context, other genes also have a significant role in the inheritance of PCOS. This may include the LH receptor gene and, the CYP17 gene (Craig, Gault & Irwin, 2018). LH receptor gene is enrolled for it also helps in the regulation of ovulation, and some kind of alterations in this gene can also disrupt the normal menstrual cycle in the long run.

The HbA1c (hemoglobin A1c) testing for blood examines the average level of glucose in the blood during the two or three months prior to the test to determine the presence of diabetes and prediabetes (Arzati et al., 2017). It is frequently utilised for the diagnosis and monitoring of diabetes (Yazdanpanah et al., 2017). While PCOS and HbA1c levels are not substantially associated, there may be an indirect relationship between the two disorders.

PCOS and insulin resistance are linked, hence HbA1c values may be important when discussing PCOS (Guler et al., 2014). PCOS causes insulin resistance, which raises blood glucose. Polycystic ovarian syndrome (PCOS) is not diabetes, although it increases the risk of insulin resistance and poor glucose metabolism in women. HbA1c monitoring can help PCOS individuals control their metabolic health and glucose levels (Lerchbaum et al., 2013). PCOS patients had higher fasting insulin levels than controls. The PCOS group had 10.19±1.44 mIU/ml fasting insulin, while the control group had 7.85±4.19

Miu/ml. These findings support PCOS's substantial relationship with insulin resistance. PCOS may be caused by insulin resistance; Hyperinsulinemia results from pancreatic insulin production (Skarra et al., 2017). Zeng et al. (2020) identified in a similar context that insulin increases free testosterone via stimulating ovarian androgen synthesis, disrupting follicular growth, and decreasing SHBG production. Hormonal abnormalities cause PCOS symptoms, including irregular menstrual cycles, hirsutism, and infertility. According to Zhang et al., (2018), women with PCOS could be more likely to experience insulin resistance, which may result in higher levels of glucose in the blood and could lead to a high HbA1c. Insulin resistance is a prevalent symptom of PCOS and can be a risk factor for type 2 diabetes (Rubin et al., 2017). Checking HbA1c levels in women with PCOS can give beneficial data on long-term blood sugar management and the possibility of getting diabetes (Hippisley-Cox et al., 2017).

On the contrary, it was found in a study conducted by Yan et al. (2021) that women with PCOS had higher HbA1c readings than controls, suggesting impaired glucose metabolism. However, GÜNGÖR et al. (2020) found no significant difference in HbA1C between groups. Sample size, study design, population factors, and PCOS diagnostic criteria may explain these discrepancies.

Not every woman with PCOS suffers from elevated HbA1c values. Lee et al., (2018), asserts that the level of HbA1c could be altered by a variety of variables such as nutrition, ways of life, and familial susceptibility. While insulin resistance is common in PCOS, it does not always imply that every woman with PCOS has high levels of glucose or an increased HbA1c (Amisi, 2022). Considering insulin resistance, some women with PCOS may have normal blood sugar management.

The percentage of PCOS women with increased HbA1c levels varies based on the community and specific instances. According to studies, 30-50% of women with PCOS might suffer from difficulty metabolising glucose or diabetes of the type 2 variety, both of which can result in increased HbA1c values (Amisi, 2022). The variation in values lead to trouble hot performing regular body functions and metabolism.

Despite contradicting findings, PCOS is linked to higher HbA1C levels. PCOS patients had increased HbA1C values, supporting the notion of impaired glucose metabolism (Orio et al., 2016). In women with PCOS, HbA1C was positively correlated with androgen levels (Zhang et al., 2021), suggesting a link between hormonal dysregulation and glycemic management. PCOS increases HbA1C, impaired glucose

tolerance, prediabetes, and type 2 diabetes may result from elevated HbA1C (Pelanis et al., 2017). Early detection of dysglycemia in PCOS women allows for lifestyle changes, medication, and glycemic monitoring. Treating insulin resistance, a typical PCOS symptom, may also lower HbA1C and reduce metabolic problems.

PCOS is often linked to insulin resistance, where cells become less responsive to insulin. Pancreatic insulin controls blood sugar; insulin resistance can raise insulin levels in PCOS women (Abuelezz et al., 2020). Due to cell sensitivity reduction, the body produces more insulin. Increased insulin levels cause the ovaries to produce more androgens like testosterone. Hormonal abnormalities, such as increased androgens and insulin resistance, affect the hypothalamus-pituitary-ovary axis and hormone production, creating PCOS symptoms. Ispasoiu et al. (2013) discussed that PCOS patients' fasting insulin levels are monitored and diagnosed. Fasting insulin is blood insulin following an overnight fast while PCOS women have high fasting insulin levels, indicating insulin resistance. Moreover, fasting insulin can assist doctors diagnose insulin resistance in PCOS patients and guide treatment. While the study supports the link between PCOS and fasting insulin levels, additional authors and research studies must be considered.

According to the studies of Xu & Qiao (2022), insulin resistance is a typical symptom of PCOS, in which the body's cells have trouble reacting to the hormone insulin, resulting in elevated blood insulin concentrations. Insulin resistance is common in both obese and lean PCOS patients (Bannigida et al., 2020). Talib et al., (2022), explained in their research that it affects 70-95% of obese PCOS patients and 30-75% of lean PCOS patients. In the conditions of PCOS, most persons have elevated insulin levels in addition to insulin resistance. Furthermore, high or low insulin levels, as well as insulin resistance, may suggest PCOS or similar underlining physiological disorders (Xu & Qiao, 2022). Insulin resistance is common in women with PCOS because their systems are unable to use insulin effectively in this disease (Calcaterra et al., 2021). PCOS is an illness that not just affects ovulation or the ovaries, but it is also a full-body endocrine and metabolic condition that is directly related to or linked to insulin resistance (Azziz, 2018). Fasting insulin levels are more inclined to go below ten with PCOS, although fasting glucose levels need to be about 90.

The study findings revealed HbA1c values were 5.85±2.91%. PCOS patients also had higher blood sugar levels, implying PCOS may impact glucose control. Da Silva Rosa et al. (2020) found that in PCOS, insulin resistance causes abnormal blood sugar levels.

Moreover, insulin resistance occurs when skeletal muscle and adipose tissue respond poorly to insulin. Thus, the pancreas produces more insulin, raising fasting blood sugar. High blood sugar can cause pre-diabetes and type 2 diabetes. Kakoly et al. (2018) also indicated that the supplied study suggests a link between PCOS and blood sugar levels, evaluating other authors' and studies' viewpoints is crucial. PCOS patients have greater incidences of impaired glucose tolerance, pre-diabetes, and diabetes than the general population. Qu et al. (2017) identified that the aetiology of PCOS is linked to insulin resistance, and fasting insulin levels may be a diagnostic indication. There are also contradicting studies, in PCOS, fasting insulin levels may not always indicate insulin resistance. They believe the Homeostatic model assessment of Insulin Resistance (HOMA-IR) or Oral Glucose Tolerance Test (OGTT) may better detect insulin resistance in PCOS. Moghetti and Tosi (2021) argued that PCOS heterogeneity is also relevant; PCOS has many phenotypes, including insulin resistance. This heterogeneity may explain the conflicting findings on fasting insulin levels with PCOS.

PCOS and blood sugar levels had inconsistent outcomes in other investigations. Kusters et al. (2017) explained that after controlling for obesity and insulin resistance, other investigators claim the association is not significant. They suggest that insulin resistance, not PCOS, causes aberrant glucose metabolism in PCOS. Moreover, the mechanisms linking PCOS and blood sugar levels are complex and poorly understood. Notably, genetics, hormone imbalances, chronic inflammation, and lifestyle may affect PCOS and glucose metabolism.

Increased fasting insulin levels are frequently linked to PCOS. Insulin resistance leads the pancreas to generate higher levels of insulin to make up for the insulin-producing cells' decreased sensitivity (Gao et al., 2018). High levels of insulin in the blood can drive the ovaries to create extra androgens (male hormones) and upset the normal equilibrium of female hormones, which leads to the emergence of symptoms associated with PCOS (Witchel et al., 2019).

While increased fasting insulin levels are frequent in PCOS, not all women with the condition will have increased levels. Some women with PCOS have normal fasting insulin levels, whereas other individuals have insulin resistance but not high insulin levels (Tosi et al., 2017). Furthermore, elevated fasting insulin levels are not solely limited to PCOS and can also be seen in those who do not have the syndrome (Polak et al., 2017).

Many women with PCOS have elevated blood sugar levels while fasting than those who do not have PCOS. This high blood sugar level during fasting is frequently associated with insulin resistance, a state that occurs when the cells of the body become less receptive to the actions of insulin (Hansen et al., 2017). Insulin resistance in PCOS can lead to elevated blood insulin levels. Insulin regulates the level of glucose in the blood, and as the body develops resistance to its impacts, the amount of sugar in the blood could increase (Nakrani et al., 2020). Higher fasting blood sugar levels and insulin resistance can set off a chain reaction of biochemical alterations, like increasing ovary secretion of androgens (including testosterone), that adds to symptoms associated with PCOS (Unluhizarci et al., 2021). Controlling and stabilising fasting blood sugar levels in PCOS women is critical for general health and lowering the risk of chronic conditions like type 2 diabetes and heart disease. A nutritious diet, frequent physical activity, and maintaining a healthy weight can all assist enhance insulin sensitivity and regulate the blood's sugar levels (Awuchi et al., 2020).

While insulin resistance and high fasting blood sugar levels are frequent in women with PCOS, these metabolic disorders may not affect all women with PCOS (Maqbool et al., 2019). PCOS is a complex disorder in which the existence and degree of insulin resistance vary from person to person (National Institute of Health, 2019). Some PCOS women possess normal fasting blood sugar levels but have insulin resistance. Other indicators, like postprandial (after meal) levels of glucose or an oral test for glucose tolerance, could be required in certain circumstances to indicate a reduced metabolism of glucose (Lages et al., 2022). Although insulin resistance is closely linked to PCOS, it is crucial to remember that PCOS is a complicated condition with many contributing causes. Not all PCOS women suffer from irregular fasting blood sugar levels or insulin resistance, and a specific association between these variables and PCOS is currently being researched.

Prolactin is a pituitary gland-produced hormone that is mainly accountable for boosting the production of milk in nursing mothers (Blair and Rushton, 2021). Although prolactin has no direct connection to the onset of PCOS, there may be a secondary connection linking the two disorders. Prolactin (PRL) is a multifunctional polypeptide hormone that plays a variety of physiological roles, such as reproduction, development, growth, metabolic processes, immune-regulation, neural activity, and controlling behavior (Bernard et al., 2019). Pituitary lactotroph cells release PRL. Thyrotropin-

releasing hormone (TRH), estrogen, and dopamine receptor antagonists all enhance PRL synthesis (Al-Chalabi et al., 2018). Furthermore, a range of clinical, physiological, and genetic circumstances might cause lactotroph cells to secrete excessive PRL, resulting in hyperprolactinemia. The process of pregnancy, nursing, anxiety, and extreme activity are examples of physiological circumstances (Saei Ghare Naz et al., 2022). Drug-induced hyperprolactinemia (DIH), non-functioning pituitary adenomas (NFPA), primary hypothyroidism, polycystic ovary syndrome (PCOS), chronic renal failure, and liver cirrhosis are all pathological diseases (Wong et al., 2015). However, PCOS women may have hyperprolactinemia. PCOS and prolactin levels are unclear, PCOS may cause increased prolactin due to brain hormone regulation issues, this association's causes are unknown (Sarahian et al., 2022). PCOS-related high prolactin levels might affect the body. Prolactin blocks GnRH, which regulates the menstrual cycle. Thus, elevated prolactin levels may cause irregular or missing menstruation in PCOS women. The case, control, and probable groups in this study had their mean serum prolactin levels measured. Group prolactin levels differed considerably. The case group had the highest mean prolactin concentration, 24.409.50 g/L, followed by the probable group at 17.934.18 g/L and the control group at 15.343.0 g/L. PCOS may be associated with elevated prolactin levels. Prolactin affects reproduction; increased prolactin levels may cause irregular menstrual periods, anovulation, and infertility in PCOS, according to Kriedt et al. (2019). Prolactin suppresses GnRH, which is necessary for reproductive axis function. The GnRH-pituitary-ovarian axis can disrupt ovulation and sex hormone production, causing PCOS symptoms. While the study suggests a link between PCOS and increased prolactin levels, PCOS patients have greater prolactin levels than controls in several studies. These findings demonstrate that PCOS-related hypothalamic-pituitary-ovarian axis disruption may cause increased prolactin levels. Prolactin levels and PCOS are disputed. PCOS and controls have similar prolactin levels in a study conducted by Mahboobifard et al. (2022). These findings demonstrate that PCOS may not continuously have increased prolactin levels; some authors suggest that BMI and insulin resistance may explain prolactin variations (Glezer et al., 2023). PCOS heterogeneity may affect prolactin levels; PCOS phenotypes differ in hormonal and metabolic characteristics. This heterogeneity may explain why studies disagree about prolactin levels with PCOS.

Androgen levels in PCOS women can be sometimes higher than normal. It is also a result of the symptoms like acne and excessive body hair (Han et al., 2023). PCOS is

leading to increase insulin resistance and increase body weight leading to weight gain. In addition, it is also a claim that women with PCOS may be more likely to develop type 2 diabetes and cardiovascular disease in the long run due to an imbalance in body weight. The metabolic issues might be accountable to lead to diabetes in the long run. Women with diabetes tendency are considered to have chances of developing diabetes mellitus (T2DM), 3-10 times higher as compared to the women without PCOS (Craig, Gault & Irwin, 2018). It shows weight gain as a risk which is further increased in individuals with overweight or obesity challenges. The underlying mechanisms are also for this association are not entirely clear. This is also linked with specific insulin resistance and hyperandrogenism (Craig et al., 2018).

Women with PCOS frequently have higher testosterone levels. This is referred to as hyperandrogenism or an overabundance of masculine hormones in the body of the female. According to the studies of Rostamtabar et al., (2021), increased levels of testosterone in PCOS are associated with the occurrence of indications like abnormal cycles of menstruation, skin conditions like acne and pimples, hirsutism (extensive hair development), and male-pattern alopecia. An elevated level of testosterone can result in the production of cysts in the ovaries, which is a marker of PCOS (Morales-Ledesma et al., 2017). Treatments for PCOS frequently include correcting hormonal abnormalities, such as lowering testosterone levels (Armanini et al., 2022). To balance the levels of hormones and treat symptoms associated with PCOS, medicines such as contraceptive pills and anti-androgen can be used (Ibáñez et al., 2017).

Hyperandrogenism high blood androgen levels, especially testosterone is a hallmark of PCOS (Ashraf et al., 2019). Hyperandrogenism causes PCOS symptoms include hirsutism, acne, and monthly abnormalities. PCOS hyperandrogenism involves increased testosterone levels. This hormonal imbalance causes PCOS symptoms like hirsutism, acne, and monthly abnormalities. This study examined serum testosterone levels in PCOS patients, controls, and probables. Current study shows a mean blood testosterone level of 1.00±0.43 nmol/L. Compared to the probable and control groups, the case group had the highest mean serum testosterone level (1.20±0.42 nmol/L). Serum testosterone levels varied significantly between groups ($p=0.000$). These findings indicate that the case group has the highest testosterone level. Zhang et al. (2009) examined serum testosterone levels in PCOS and non-PCOS women. This study found that PCOS patients had greater testosterone levels than controls. PCOS patients' high testosterone levels

encourage hyperandrogenism. Sendur et al. (2021) reviewed studies on androgen levels in PCOS women and controls. PCOS patients had greater testosterone levels across all middle easter women and Australian women, according to the analysis. These findings support the present study's observation of higher serum testosterone levels in the case group than the control group. In contrast, Jaenes & Reeves (2017), found no significant testosterone difference between PCOS patients and controls. Their study population of slender women with PCOS had no obesity or insulin resistance, which may have explained the lack of testosterone differential. Contrary to the present study's findings, the case group had greater testosterone levels than the control group. Moreover, Guastella et al. (2010) examined androgen levels in PCOS phenotypes. Only PCOS patients with a typical phenotype hyperandrogenism and polycystic ovaries had higher testosterone levels. The present study's case group, which included all PCOS patients, had greater testosterone levels than the control group.

PCOS patients have greater testosterone levels than controls in a study conducted by Umayal et al. (2019). PCOS is diagnosed by elevated testosterone, and the size and clinical importance of testosterone increase are disputed. Some research reveals PCOS patients' testosterone levels fluctuate considerably. Some researchers believe that PCOS is caused by causes other than increased testosterone levels, such as insulin resistance and obesity (Al-Iefout et al., 2017). In PCOS, testosterone levels fluctuate during the menstrual cycle; therefore, one measurement may not capture the whole hormonal picture. PCOS heterogeneity, hormone measurement timing, and confounding factors can affect testosterone interpretation. PCOS may be linked to high testosterone levels, according to Xu and Qiao (2022). However, the literature's conflicting viewpoints and agreements must be considered. High testosterone levels characterise PCOS. The clinical importance of testosterone increase may differ among PCOS patients.

Xenin, a stomach-secreted peptide hormone, regulates several physiological processes (Kruszewska et al., 2022). Incretin hormones regulate appetite and glucose homeostasis, The study compares mean serum xenin levels in cases, controls, and predisposed groups. The mean serum xenin level in cases was 31.25±2.86 units, significantly higher than the control group (23.58±3.36 units) and the probable group (26.93±3.94 units) (p=0.000). ROC Curve analysis was performed to set serum xenin cut-off values. The cases group cut-off values were 27.18, 27.41, and 27.96 units, with sensitivities of 82.9%. The probable group cut-off values were 22.93 and 23.01 units,

with 82.9% and 80% sensitivities, respectively. ROC curve model quality was good in the cases (91%) and likely (62%). The cases group had an AUC of 0.953, demonstrating that serum xenin can discriminate patients from controls. The probable group had 0.742 AUC, indicating modest discriminating power. These findings suggest serum xenin may be a biomarker for PCOS. The researchers' work parallels and contrasts with Temur et al. (2017) and Guclu et al. (2019). Three studies examined xenin-related peptides with PCOS. While Temur et al. (2017) explored xenopsin-related peptide-1 (XP-1) and Guclu et al. (2019) examined xenin-25, the researchers examined serum xenin levels. PCOS women had higher peptide levels than controls in all investigations.

XP-1's cut-off value was 5.87 ng/mL, while xenin-25's was 32.60 pg/mL. The study did not set serum xenin cut-off values. Study demographics, sample sizes, and test techniques explain’s these cut-off value variances (Malini & George, 2018). While Temur et al. (2017) and Guclu et al. (2019) employed ROC curve analysis to assess the diagnostic accuracy of XP-1 and xenin-25, the researchers' study compared mean serum xenin levels and assessed their potential discriminative power between groups. Although the researchers did not directly analyse XP-1 or xenin-25 levels, the cases group's elevated serum xenin levels are consistent with other studies findings. The findings suggest that XP-1 and Xenin-25 may be altered in PCOS women. The study did not measure XP-1 or xenin-25 levels. Future research should directly examine these peptides and compare their diagnostic accuracy and cut-off values to confirm their potential as PCOS biomarkers.

According to the study, young women (mean age 28.21) have PCOS. This reinforces findings demonstrating hormonal imbalances and metabolic abnormalities cause PCOS in adolescence or early adulthood. Some research suggests that age may improve or aggravate PCOS symptoms. Age and PCOS development and progression need further study.

The study population had a mean BMI of 26.56 kg/m^2, indicating overweight. PCOS is more common in people with higher BMIs. Obesity can cause PCOS, and PCOS can cause obesity. Lifestyle and genetics affect this relationship. FSH, LH, testosterone, and prolactin levels differed across the case, control, and probable groups, highlighting hormonal anomalies. PCOS patients showed greater FSH, LH, and testosterone levels than controls, indicating hypothalamic-pituitary-ovarian axis abnormalities and increased androgen production. PCOS patients had higher prolactin levels, suggesting a connection

between the two. PCOS hormonal abnormalities' causes and clinical effects need further study.

According to the studies 30% of PCOS patients have a little increase in blood prolactin levels throughout the entire follicular and luteal phases (Melgar et al., 2016). Although the cause of the link between increased levels of prolactin and PCOS is unknown, higher concentrations of prolactin may block ovulation and contribute to the appearance of polycystic ovarian morphology (Saei Ghare Naz et al., 2022). PCOS, instead of prolactin, is a condition marked by hormonal abnormalities including excessive androgens (male hormones) and insulin resistance (Khashchenko et al., 2020). High levels of prolactin could be related to conditions other than PCOS, like prescription medications, thyroid diseases, or benign tumors of the pituitary gland (prolactinomas) (Michail et al., 2019).

Hyperprolactinemia, or high prolactin levels, can occasionally develop in women with PCOS. Hyperprolactinemia can cause disruption in the normally occurring equilibrium of reproductive hormones, resulting in abnormal menstrual periods, anovulation (lack of ovulation), and reproductive concerns (Levine & Muneyyirci-Delale, 2018). It is crucial to recognise, however, that increased prolactin levels do not constitute an identifying indicator of PCOS and are not observed in all women who suffer from the disorder.

HbA1c, fasting insulin, and blood sugar were also assessed. PCOS patients showed greater HbA1c and fasting insulin than controls, indicating glucose metabolism and insulin resistance. PCOS patients had increased fasting blood sugar levels, suggesting a risk for impaired glucose tolerance, prediabetes, or type 2 diabetes. PCOS and blood sugar levels are still debated, with inconsistent studies. Xenin levels differed significantly between PCOS patients and controls, suggesting it may be a biomarker for PCOS. It is needed to directly measure xenin-related peptides and determine their diagnostic accuracy and cut-off values.

In our current study correlation of Xenin was found with BMI, Prolactin and HbA1c. There were no similar or contradictory studies found with regard to this.

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATION

Present study has established a relationship between serum Xenin level and PCOS in Pakistani population. Through careful analysis of patient's data collected from PNS SHIFA, it has been proved that PCOS patients have increased Xenin levels. Though, further studies are required to establish exact cause and effect relationship between Xenin Levels and PCOS to further explore that whether PCOS increases xenin levels or vice versa i-e increased Xenin levels results in PCOS, however, it is considered that this research work has stong theoretical implication in establishing Xenin as biomarker for diagnosis of PCOS. Moreover, another novel aspect of present study is examining serum Xenin levels in Probable Group (individuals with family history of PCOS and are likely to contract PCOS in future) which is attempted for the very first time. In this regard, research data has revealed that Xenin levels in Probable group are also increased. Thus, implicating that Xenin levels can also be used for successful prognosis of PCOS in individuals with family history of PCOS.

5.2.2 PRACTICAL IMPLICATIONS

It has a strong practical implications. Medical practitioner may advice Xenin levels to be evaluated in the laboratory to diagnose PCOS as the current study has proved that its levels are increased in PCOS patients. Moreover, it can also aid medical practitioners for prognosis of PCOS as levels are also increased in probable group compared to healthy group shown in the current study.

5.2.3 POLICY IMPLICATIONS

Further studies are required to use results of this research work in policy formulation and implementation.

5. 3 LIMITATIONS & STRENGTHS OF STUDY

5.3.1 LIMITATIONS

- Self-funded study with no allocation of research funds from any institute or organization

- Single centred study at BUHSCK
- Short duration of study of six months as part of MPhil degree requirement
- Small sample size.
- Lack of relevant literature as no one has investigated this concept earlier in Pakistan

5.3.2 STRENGTHS

- Serum xenin levels are being detected first time in Pakistani population.
- New associations were discovered that could be useful in theory and practice.
- Sample size is recruited statistically in agreement with the prevalence of PCOS in Pakistani population.
- Detailed statistical analysis provided a complete description of the data and the results.

5.4 FUTURE RESEARCH DIRECTIONS / RECOMMENDATIONS

- Future prospective studies are recommended to fully understand role of Xenin in pathogenesis of PCOS
- Role of Xenin should be explored as a putative therapeutic target.
- Precise threshold of Xenin levels to diagnose PCOS or probability to develop PCOS in future needs to be established
- Establish exact cause and effect relationship between Xenin levels and PCOS.

5.5 CONCLUSION

It has been concluded through current research work that serum Xenin levels in PCOS patients are significantly higher than healthy control group. Thus, suggesting a strong likelihood of Xenin-25 participating in pathophysiology of PCOS. However,

further studies are required to determine exact cause and effect relationship between Xenin and PCOS so that role of xenin in pathogenesis of PCOS can be fully understood/ explored. Moreover, Xenin levels in Probable Group (i.e women with family history of PCOS and which are likely to contract PCOS in future) were studied for the first time as part of this study. It has been observed that Xenin levels in Probable Group are also on higher side as compared to control group (though less as compared to PCOS patients) whereas other biochemical parameters did not altered significantly in probable group. Hence, increased Xenin levels may aid medical practitioners for prognosis of PCOS in women with family history of PCOS. Thus, present study has strong theoretical and practical implications. Serum xenin levels are being detected first time in Pakistani population.

REFERENCES

Abraham Gnanadass, S., Divakar Prabhu, Y. and Valsala Gopalakrishnan, A. (2021). Association of metabolic and inflammatory markers with polycystic ovarian syndrome (PCOS): an update. Archives of Gynecology and Obstetrics, 303(3), 631-643.

Abuelezz, N. Z., Shabana, M. E., Abdel-Mageed, H. M., Rashed, L., & Morcos, G. N. (2020). Nanocurcumin alleviates insulin resistance and pancreatic deficits in polycystic ovary syndrome rats: Insights on PI3K/AkT/mTOR and TNF-α modulations. Life sciences, 256, 118003

Adashi, E.Y., Cibula, D., Peterson, M. and Azziz, R., 2023. The polycystic ovary syndrome: the first 150 years of study. *F&S Reports*, *4*(1), pp.2-18.

Al-Beltagi, M., Bediwy, A.S. and Saeed, N.K., 2022. Insulin-resistance in paediatric age: Its magnitude and implications. *World Journal of Diabetes*, *13*(4), p.282.

Al-Chalabi, M., Bass, A.N. and Alsalman, I., 2018. Physiology, prolactin. https://europepmc.org/article/NBK/nbk507829

Allen, L.A., Shrikrishnapalasuriyar, N. and Rees, D.A., 2022. Long-term health outcomes in young women with polycystic ovary syndrome: a narrative review. *Clinical Endocrinology*, *97*(2), pp.187-198.

Amisi, C. A. (2022). Markers of insulin resistance in Polycystic ovary syndrome women: an update. World journal of diabetes, 13(3), 129.

Arzati, M. M., Honarvar, N. M., Saedisomeolia, A., Anvari, S., Effatpanah, M., Arzati, R. M., ... & Djalali, M. (2017). The effects of ginger on fasting blood sugar, hemoglobin A1c, & lipid profiles in patients with type 2 diabetes. International journal of endocrinology & metabolism, 15(4). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5750786/https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8984569/

Ashraf, S., Nabi, M., Rashid, F. and Amin, S., 2019. Hyperandrogenism in polycystic ovarian syndrome and role of CYP gene variants: a review. Egyptian Journal of Medical Human Genetics, 20(1), pp.1-10.

Awuchi, C. G., Echeta, C. K., & Igwe, V. S. (2020). Diabetes and the nutrition and diets for its prevention and treatment: a systematic review and dietetic perspective. Health Sciences Research, 6(1), 5-19.

Azziz, R., Carmina, E., Chen, Z., Dunaif, A., Laven, J.S., Legro, R.S., Lizneva, D., Natterson-Horowtiz, B., Teede, H.J. & Yildiz, BO, (2016). Polycystic ovary syndrome. Nature reviews Disease primers, 2(1), pp.1-18.

Aziz, U., Afzal, M., Yousaf, R.T., Imran, S., Sarfraz, T., Javed, H.R. and Sultan, W., 2023. DEALING WITH INSULIN RESISTANCE AMONG WOMEN WITH POLYCYSTIC OVARIAN SYNDROME. Biological and Clinical Sciences Research Journal, 2023(1), pp.276-276.

Barber, T.M., Hanson, P., Weickert, M.O. and Franks, S., 2019. Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies. *Clinical Medicine Insights: Reproductive Health*, *13*, p.1179558119874042.

Bernard, V., Young, J., & Binart, N. (2019). Prolactin—a pleiotropic factor in health and disease. Nature Reviews Endocrinology, 15(6), 356-365.

Bharathi, R.V., Swetha, S., Neerajaa, J., Madhavica, J.V., Janani, D.M., Rekha, S.N., Ramya, S. and Usha, B. (2017). An epidemiological survey: Effect of predisposing factors for PCOS in Indian urban and rural population. Middle East Fertility Society Journal, 22(4), 313-316

Blair, S. and Rushton, L., 2021. The Endocrine System. Infobase Holdings, Inc.

Boyle, J. and Teede, H.J., 2012. Polycystic ovary syndrome: an update. Australian family physician, 41(10), pp.752-756.

Cakir, E., Sahin, M., Topaloglu, O., Colak, N. B., Karbek, B., Gungunes, A., ... & Delibasi, T. (2012). The relationship between LH and thyroid volume in patients with PCOS. Journal of ovarian research, 5(1), 1-6.

Cao, N.T., Le, M.T., Nguyen, V.Q.H., Pilgrim, J., Le, V.N.S., Le, D.D., Pham, C.K., Aharon, D. and Hill, M.J., 2019. Defining polycystic ovary syndrome phenotype in Vietnamese women. *Journal of Obstetrics and Gynaecology Research*, *45*(11), pp.2209-2219.

Carmina, E., Campagna, A. M., & Lobo, R. A. (2012). A 20-year follow-up of young women with polycystic ovary syndrome. Obstetrics & Gynecology, 119(2 Part 1), 263-269.

Casals-Casas, C. and Desvergne, B. (2011). Endocrine disruptors: from endocrine to metabolic disruption. Annual review of physiology, 73, 135-162

Cochrane DE, Carraway RE, Boucher W. Generation of xenopsin-related peptides from tissue precursors by media conditioned by endotoxin-stimulated rat peritoneal macrophages. Inflammation 1991:15(5):381-90.

Craig, S.L., Gault, V.A. and Irwin, N., 2018. Emerging therapeutic potential for xenin and related peptides in obesity and diabetes. Diabetes/Metabolism Research and Reviews, 34(6), p.e3006.

Craig, S.L., Irwin, N. and Gault, V.A., 2021. Xenin and Related Peptides: Potential Therapeutic Role in Diabetes and Related Metabolic Disorders. *Clinical Medicine Insights: Endocrinology and Diabetes*, *14*, p.11795514211043868.

Craig, S.L., Irwin, N. and Gault, V.A., 2021. Xenin and Related Peptides: Potential Therapeutic Role in Diabetes and Related Metabolic Disorders. *Clinical Medicine Insights: Endocrinology and Diabetes*, *14*, p.11795514211043868.

Craig, S.L., Irwin, N. and Gault, V.A., 2021. Xenin and Related Peptides: Potential Therapeutic Role in Diabetes and Related Metabolic Disorders. Clinical Medicine Insights: Endocrinology and Diabetes, 14, p.11795514211043868.

Dabravolski, S.A., Nikiforov, N.G., Eid, A.H., Nedosugova, L.V., Starodubova, A.V., Popkova, T.V., Bezsonov, E.E. and Orekhov, A.N., 2021. Mitochondrial dysfunction and chronic inflammation in polycystic ovary syndrome. *International Journal of Molecular Sciences*, *22*(8), p.3923.

Dabravolski, S.A., Nikiforov, N.G., Eid, A.H., Nedosugova, L.V., Starodubova, A.V., Popkova, T.V., Bezsonov, E.E. and Orekhov, A.N., 2021. Mitochondrial dysfunction and chronic inflammation in polycystic ovary syndrome. International Journal of Molecular Sciences, 22(8), p.3923.

Da Silva Rosa, S.C., Nayak, N., Caymo, A.M. & Gordon, J.W., (2020). Mechanisms of muscle insulin resistance & the cross-talk with liver & adipose tissue. Physiological Reports, 8(19), p.e14607

Davies, K.J., 2016. Adaptive homeostasis. Molecular aspects of medicine, 49, pp.1-7.

Drzewoski, J. and Hanefeld, M., 2021. The current and potential therapeutic use of metformin—the good old drug. Pharmaceuticals, 14(2), p.122.

Fosgerau, K. and Hoffmann, T., 2015. Peptide therapeutics: current status and future directions. Drug discovery today, 20(1), pp.122-128.

Gadelha, M.R., Wildemberg, L.E. and Kasuki, L., 2022. Drugs and Pituitary Function. In *The Pituitary* (pp. 413-427). Content Repository Only!.

Gao, L. R., Zhang, N. K., Zhang, Y., Chen, Y., Wang, L., Zhu, Y., & Tang, H. H. (2018). Overexpression of apelin in Wharton'jelly mesenchymal stem cell reverses insulin resistance & promotes pancreatic β cell proliferation in type 2 diabetic rats. Stem cell research & therapy, 9(1), 1-14. https://stemcellres.biomedcentral.com/articles/10.1186/s13287-018-1084-x

Giannouli, A., Efthymiou, V., Konidari, M., Mani, I., Aravantinos, L., Dourakis, S.P., Antoniou, A., Deligeoroglou, E. and Bacopoulou, F., 2023. The Burden of Non-Alcoholic Fatty Liver Disease in Adolescents with Polycystic Ovary Syndrome: A Case–Control Study. *Journal of Clinical Medicine*, *12*(2), p.557.

Glezer, A., Santana, M.R., Bronstein, M.D., Donato Jr, J. & Jallad, R.S., (2023). The interplay between Prolactin & cardiovascular disease. Frontiers in Endocrinology, 13, p.1018090

Guastella, E., Longo, R. A., & Carmina, E. (2010). Clinical and endocrine characteristics of the main polycystic ovary syndrome phenotypes. Fertility and sterility, 94(6), 2197-2201.

Guclu, Y.A., Sahin, E. and Aksit, M., 2019. The relationship between elevated serum xenin and insulin resistance in women with polycystic ovary syndrome: a case-control study. Gynaecological Endocrinology, 35(11), pp.960-964.

Guler, I., Himmetoglu, O., Turp, A., Erdem, A., Erdem, M., Onan, M. A., ... & Guner, H. (2014). Zinc and homocysteine levels in polycystic ovarian syndrome patients with insulin resistance. Biological trace element research, 158, 297-304.

GÜNGÖR, N.D., Gürbüz, T. & OKÇU, N.T., (2020). Correlation between HbA1c & fibrocystic breast disease among polycystic ovary syndrome. Cumhuriyet Medical Journal, 42(3), pp.383-389.

Han, Y., Wu, H., Sun, S., Zhao, R., Deng, Y., Zeng, S. and Chen, J., 2023. Effect of High Fat Diet on Disease Development of Polycystic Ovary Syndrome and Lifestyle Intervention Strategies. Nutrients, 15(9), p.2230.

Hansen, D., De Strijcker, D., & Calders, P. (2017). Impact of endurance exercise training in the fasted state on muscle biochemistry & metabolism in healthy subjects: can these effects be of particular clinical benefit to type 2 diabetes mellitus & insulin-resistant patients?. Sports Medicine, 47, 415-428

Haq, N., Khan, Z., Riaz, S., Nasim, A., Shahwani, R. and Tahir, M. (2017). Prevalence and knowledge of polycystic ovary syndrome (PCOS) among female science

students of different public Universities of Quetta, Pakistan. Imperial Journal of Interdisciplinary Research, 35(6), 385-92.

Hasan, M., Sultana, S., Sohan, M., Parvin, S., Rahman, M.A., Hossain, M.J., Rahman, M.S. and Islam, M.R., 2022. Prevalence and associated risk factors for mental health problems among patients with polycystic ovary syndrome in Bangladesh: A nationwide cross—Sectional study. *PloS one*, *17*(6), p.e0270102.

Helvaci, N., Karabulut, E., Demir, A.U. and Yildiz, B.O., 2017. Polycystic ovary syndrome and the risk of obstructive sleep apnea: a meta-analysis and review of the literature. Endocrine Connections, 6(7), pp.437-445.

Hippisley-Cox, J., & Coupland, C. (2017). Development and validation of QDiabetes-2018 risk prediction algorithm to estimate future risk of type 2 diabetes: cohort study. bmj, 359. https://www.bmj.com/content/359/bmj.j5019.full

Invitra, 2019, https://www.invitra.com/en/female-hormone-check/

Ismayilova, M. and Yaya, S., 2022. "I felt like she didn't take me seriously": a multi-methods study examining patient satisfaction and experiences with polycystic ovary syndrome (PCOS) in Canada. *BMC women's health*, *22*(1), pp.1-21.

Ispasoiu, C. A., Chicea, R., Stamatian, F. V., & Ispasoiu, F. (2013). High fasting insulin levels and insulin resistance may be linked to idiopathic recurrent pregnancy loss: a case-control study. International Journal of Endocrinology, 2013

Jabeen, A., Yamini, V., Amberina, A.R., Eshwar, M.D., Vadakedath, S., Begum, G.S. and Kandi, V., 2022. Polycystic Ovarian Syndrome: Prevalence, Predisposing Factors, and Awareness Among Adolescent and Young Girls of South India. *Cureus*, *14*(8).

Jasim, R.N. and Alkareem, I.H.A. 2022. Elevated circulating level of xenopsin related 1-are associated with PCOS (Polycystic Ovarian Syndrome).

Jeelani, H., Ganie, M.A., Masood, A., Amin, S., Kawa, I.A., Fatima, Q., Manzoor, S., Parvez, T., Naikoo, N.A. and Rashid, F., 2019. Assessment of PON1 activity and circulating TF levels in relation to BMI, testosterone, HOMA-IR, HDL-C, LDL-C, CHO, SOD activity and TAC in women with PCOS: An observational study. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *13*(5), pp.2907-2915.

Jiskoot, G., Dietz de Loos, A., Beerthuizen, A., Timman, R., Busschbach, J. and Laven, J., 2020. Long-term effects of a three-component lifestyle intervention on

emotional well-being in women with Polycystic Ovary Syndrome (PCOS): A secondary analysis of a randomized controlled trial. *PLoS One*, *15*(6), p.e0233876.

Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C,Dewailly D, 2003. Ultrasound examination of polycystic ovaries: is itworth counting the follicles. Hum Reprod 2003; 18(3): 598–603

Jonklaas, J., 2022. Optimal thyroid hormone replacement. *Endocrine reviews*, *43*(2), pp.366-404.

Kadiroğulları, P., Demir, E., Bahat, P.Y., Kıyak, H. and Seckin, K.D., 2020. Evaluation of relationship between HbA1c levels and ovarian reserve in patients with type 1 diabetes mellitus. *Gynecological Endocrinology*, *36*(5), pp.426-430.

Kakoly, N.S., Khomami, M.B., Joham, A.E., Cooray, S.D., Misso, M.L., Norman, R.J., Harrison, C.L., Ranasinha, S., Teede, H.J. & Moran, L.J., (2018). Ethnicity, obesity & the prevalence of impaired glucose tolerance & type 2 diabetes in PCOS: a systematic review & meta-regression. Human reproduction update, 24(4), pp.455-467

Khashchenko, E., Uvarova, E., Vysokikh, M., Ivanets, T., Krechetova, L., Tarasova, N., ... & Sukhikh, G. (2020). The relevant hormonal levels & diagnostic features of polycystic ovary syndrome in adolescents. Journal of Clinical Medicine, 9(6), 1831. file:///C:/Users/Dell/Downloads/jcm-09-01831-v2%20(1).pdf

Kim, J.J. and Choi, Y.M., 2019. Phenotype and genotype of polycystic ovary syndrome in Asia: ethnic differences. Journal of Obstetrics and Gynaecology Research, 45(12), pp.2330-2337

Kruszewska, J., Laudy-Wiaderny, H. and Kunicki, M., 2022. Review of Novel Potential Insulin Resistance Biomarkers in PCOS Patients—The Debate Is Still Open. *International journal of environmental research and public health*, *19*(4), p.2099.

Kumariya, S., Ubba, V., Jha, R.K. and Gayen, J.R., 2021. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective. *Autophagy*, *17*(10), pp.2706-2733.

Kuhre, R.E., Albrechtsen, N.J.W., Hartmann, B., Deacon, C.F. and Holst, J.J., 2015. Measurement of the incretin hormones: glucagon-like peptide-1 and glucose-

dependent insulinotropic peptide. Journal of Diabetes and its Complications, 29(3), pp.445-450

Kusters, Y.H., Schalkwijk, C.G., Houben, A.J., Kooi, M.E., Lindeboom, L., Op't Roodt, J., Joris, P.J., Plat, J., Mensink, R.P., Barrett, E.J. & Stehouwer, C.D., (2017). Independent tissue contributors to obesity-associated insulin resistance. JCI insight, 2(13).

Lages, M., Barros, R., Moreira, P., & Guarino, M. P. (2022). Metabolic Effects of an Oral Glucose Tolerance Test Compared to the Mixed Meal Tolerance Tests: A Narrative Review. Nutrients, 14(10), 2032

Lee, Y. H., Shin, M. H., Nam, H. S., Park, K. S., Choi, S. W., Ryu, S. Y., & Kweon, S. S. (2018). Effect of family history of diabetes on hemoglobin A1c levels among individuals with & without diabetes: the dong-gu study. Yonsei medical journal, 59(1), 92-100. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725370/

Le, M.T., Nguyen, V.Q.H., Truong, Q.V., Le, D.D., Le, V.N.S. & Cao, N.T., (2018). Metabolic syndrome & insulin resistance syndrome among infertile women with polycystic ovary syndrome: a cross-sectional study from Central Vietnam. Endocrinology & Metabolism, 33(4), pp.447-458

Lerchbaum, E., Schwetz, V., Giuliani, A., & Obermayer-Pietsch, B. (2013). Assessment of glucose metabolism in polycystic ovary syndrome: HbA1c or fasting glucose compared with the oral glucose tolerance test as a screening method. Human Reproduction, 28(9), 2537-2544

Liu, Y., Hao, H., Tingting, L., Jia, R., Cao, M., Liang, Z., Zhao, Z. and Pan, W., 2022. Physiological and pathological roles of Ang II and Ang-(1-7) in the female reproductive system. Frontiers in Endocrinology, 13, p.3349.

Liu, Y., Li, J., Yan, Z., Liu, D., Ma, J. and Tong, N., 2021. Improvement of insulin sensitivity increases pregnancy rate in infertile PCOS women: a systemic review. *Frontiers in Endocrinology*, *12*, p.657889.

Mahboobifard, F., Rahmati, M., Amiri, M., Azizi, F. & Tehrani, F.R., (2022). To what extent does polycystic ovary syndrome influence the cut-off value of Prolactin? Findings of a community-based study. Advances in Medical Sciences, 67(1), pp.79-86.

Mahmud, A. A., Anu, U. H., Foysal, K. A., Hasan, M., Sazib, S. M., Ragib, A. A., & Emran, T. B. (2022). Elevated serum malondialdehyde (MDA), insulin, follicle-

stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and reduced antioxidant vitamins in polycystic ovarian syndrome patients. Narra J, 2(1).

Malini, N. A., & George, K. R. (2018). Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)–Clinical based case control study. General & comparative endocrinology, 260, 51-57.

Maqbool, M., Dar, M. A., Gani, I., & Geer, M. I. (2019). Insulin resistance & polycystic ovary syndrome: a review. Journal of drug delivery & therapeutics, 9(1-s), 433-436.

Martin, C.M., Parthasarathy, V., Pathak, V., Gault, V.A., Flatt, P.R. and Irwin, N., 2014. Characterisation of the biological activity of xenin-25 degradation fragment peptides. J Endocrinol, 221(2), pp.193-200.

McCartney, C.R. and Marshall, J.C., 2016. Polycystic ovary syndrome. New England Journal of Medicine, 375(1), pp.54-64.

McCook, J. G., Bailey, B. A., Williams, S. L., Anand, S., & Reame, N. E. (2015). Differential contributions of polycystic ovary syndrome (PCOS) manifestations to psychological symptoms. The journal of behavioral health services & research, 42, 383-394.

Meng, Y., Chen, X., Peng, Z., Liu, X., Sun, Y. and Dai, S., 2016. Association between high serum homocysteine levels and biochemical characteristics in women with polycystic ovarian syndrome: A systematic review and meta-analysis. PLoS One, 11(6), p.e0157389.

Merhi, Z., Kandaraki, E. A., & Diamanti-Kandarakis, E. (2019). Implications and future perspectives of AGEs in PCOS pathophysiology. Trends in Endocrinology & Metabolism, 30(3), 150-162.

Michail, M., Ioannis, K., Charoula, M., Alexandra, T., & Eleftheria, H. (2019). Clinical manifestations, evaluation and management of hyperprolactinemia in adolescent and young girls: a brief review. Acta Bio Medica: Atenei Parmensis, 90(1), 149. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6502148/

Moffett, R.C., Docherty, N.G. and le Roux, C.W., 2021. The altered enteroendocrine reportoire following roux-en-Y-gastric bypass as an effector of weight loss and improved glycaemic control. Appetite, 156, p.104807

Moghetti, P. & Tosi, F., (2021). Insulin resistance & PCOS: chicken or egg?. Journal of endocrinological investigation, 44, pp.233-244.

Mohamed, N., Hendawy, A.A., Abdelsalam, H.M. and Hussien, M., 2023. Association between polycystic ovary syndrome and Insulin resistance. *Bulletin of Faculty of Science, Zagazig University*, *2023*(1), pp.96-99.

Mouanness, M., Nava, H., Dagher, C. and Merhi, Z., 2022. Contribution of Advanced Glycation End Products to PCOS Key Elements: A Narrative Review. *Nutrients*, *14*(17), p.3578.

Nakrani, M. N., Winel&, R. H., & Anjum, F. (2020). Physiology, glucose metabolism. https://www.ncbi.nlm.nih.gov/books/NBK560599/

National Institute of Health . (2019). Polycystic Ovary/Ovarian Syndrome (PCOS). https://orwh.od.nih.gov/sites/orwh/files/docs/PCOS_Booklet_508.pdf

Navarro, G., Allard, C., Morford, J.J., Xu, W., Liu, S., Molinas, A.J., Butcher, S.M., Fine, N.H., Blandino-Rosano, M., Sure, V.N. and Yu, S., 2018. Androgen excess in pancreatic β cells and neurons predisposes female mice to type 2 diabetes. JCI insight, 3(12).

Ndefo, U.A., Eaton, A. and Green, M.R., 2013. Polycystic ovary syndrome: a review of treatment options with a focus on pharmacological approaches. Pharmacy and Therapeutics, 38(6), p.336.

Orio, F., Muscogiuri, G., Nese, C., Palomba, S., Savastano, S., Tafuri, D., Colarieti, G., La Sala, G., Colao, A. & Yildiz, B.O., (2016). Obesity, type 2 diabetes mellitus & cardiovascular disease risk: an uptodate in the management of polycystic ovary syndrome. European Journal of Obstetrics & Gynecology & Reproductive Biology, 207, pp.214-219.

Parthsarathy, V., Irwin, N., Hasib, A., Martin, C.M., McClean, S., Bhat, V.K., Ng, M.T., Flatt, P.R. and Gault, V.A., 2016. A novel chemically modified analogue of xenin-25 exhibits improved glucose-lowering and insulin-releasing properties. Biochimica et Biophysica Acta (BBA)-General Subjects, 1860(4), pp.757-764.

Pinola, P., Puukka, K., Piltonen, T. T., Puurunen, J., Vanky, E., Sundström-Poromaa, I., ... & Morin-Papunen, L. C. (2017). Normo-and hyperandrogenic women with

polycystic ovary syndrome exhibit an adverse metabolic profile through life. Fertility and sterility, 107(3), 788-795.

Polak, K., Czyzyk, A., Simoncini, T., & Meczekalski, B. (2017). New markers of insulin resistance in polycystic ovary syndrome. Journal of endocrinological investigation, 40, 1-8. https://link.springer.com/article/10.1007/s40618-016-0523-8

Punzón-Jiménez, P. and Labarta, E., 2021. The impact of the female genital tract microbiome in women health and reproduction: A review. *Journal of Assisted Reproduction and Genetics*, *38*(10), pp.2519-2541.

Qu, Q., Zhao, D., Zhang, F., Bao, H. & Yang, Q., (2017). Serum betatrophin levels are increased & associated with insulin resistance in patients with polycystic ovary syndrome. Journal of International Medical Research, 45(1), pp.193-202.

Ramanand, S.J., Ghongane, B.B., Ramanand, J.B., Patwardhan, M.H., Ghanghas, R.R. and Jain, S.S., 2013. Clinical characteristics of polycystic ovary syndrome in Indian women. Indian journal of endocrinology and metabolism, 17(1), p.138.

Risal, S., Pei, Y., Lu, H., Manti, M., Fornes, R., Pui, H.P., Zhao, Z., Massart, J., Ohlsson, C., Lindgren, E. and Crisosto, N., 2019. Prenatal androgen exposure and transgenerational susceptibility to polycystic ovary syndrome. *Nature Medicine*, *25*(12), pp.1894-1904.

Roland, A.V., Nunemaker, C.S., Keller, S.R. and Moenter, S.M., 2010. Prenatal androgen exposure programs metabolic dysfunction in female mice. Journal of Endocrinology, 207(2), pp.213-223.

Rosenfield, R.L., 2020. Current concepts of polycystic ovary syndrome pathogenesis. *Current opinion in pediatrics*, *32*(5), p.698.

Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19(1): 41–7

Rubin, K. H., Glintborg, D., Nybo, M., Abrahamsen, B., & &ersen, M. (2017). Development & risk factors of type 2 diabetes in a nationwide population of women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism, 102(10), 3848-3857. https://academic.oup.com/jcem/article/102/10/3848/4096783

Saei Ghare Naz, M., Mousavi, M., Mahboobifard, F., Niknam, A., & Ramezani Tehrani, F. (2022). A Meta-Analysis of Observational Studies on Prolactin Levels in Women with Polycystic Ovary Syndrome. Diagnostics, 12(12), 2924.

Sarahian, N., Noroozzadeh, M., Saei Ghare Naz, M., Eskandari-Roozbahani, N., Mahboobifard, F., & Ramezani Tehrani, F. (2022). Is there any association between migraine headache and polycystic ovary syndrome (PCOS)? A review article. Molecular biology reports, 1-9.

Sendur, S. N., & Yildiz, B. O. (2021). Influence of ethnicity on different aspects of polycystic ovary syndrome: a systematic review. Reproductive biomedicine online, 42(4), 799-818.

Shahsavari, A. and Bavarsad, M.B., 2020. Is telenursing an effective method to control BMI and HbA1c in illiterate patients aged 50 years and older with type 2 diabetes? A randomized controlled clinical trial. Journal of Caring Sciences, 9(2), p.73.

Shankar, D.Y., Lokesh, G., Chaitanya, P.K. and Sujitha, S., 2023. Overview Of Polycystic Ovary Syndrome (PCOS). World Journal of Advanced Engineering Technology and Sciences, 8(2), pp.011-022.

Shorakae, S., Boyle, J. and Teede, (2014). Polycystic ovary syndrome: a common hormonal condition with major metabolic sequelae that physicians should know about. Internal medicine journal, 44(8), 720-726.

Sidra, S., Tariq, M. H., Farrukh, M. J., & Mohsin, M. (2019). Evaluation of clinical manifestations, health risks, and quality of life among women with polycystic ovary syndrome. PloS one, 14(10), e0223329.

Skarra, D.V., Hernández-Carretero, A., Rivera, A.J., Anvar, A.R. & Thackray, V.G., (2017). Hyper&rogenemia induced by letrozole treatment of pubertal female mice results in hyperinsulinemia prior to weight gain & insulin resistance. Endocrinology, 158(9), pp.2988-3003

Spinedi, E., & Cardinali, D. P. (2018). The polycystic ovary syndrome and the metabolic syndrome: a possible chronobiotic-cytoprotective adjuvant therapy. International journal of endocrinology, 2018.

Stener-Victorin, E. and Deng, Q., 2021. Transmission of Polycystic Ovary Syndrome via Epigenetic Inheritance. Trends in molecular medicine, 27(8), pp.723-724.

Strachan, T. and Read, A.P., 1999. Instability of the human genome: mutation and DNA repair. Human Molecular Genetics, 2, pp.209-217.

Sun, Y.F., Zhang, J., Xu, Y.M., Cao, Z.Y., Wang, Y.Z., Hao, G.M. and Gao, B.L., 2020. High BMI and insulin resistance are risk factors for spontaneous abortion in patients with polycystic ovary syndrome undergoing assisted reproductive treatment: a systematic review and meta-analysis. *Frontiers in endocrinology*, *11*, p.592495.

Temur, M., Özün Özbay, P., Aksun, S., Yilmaz, Ö., Çift, T., Üstünel, S. and Calan, M., 2017. Elevated circulating levels of xenopsin-related peptide-1 are associated with polycystic ovary syndrome. Archives of gynaecology and obstetrics, 296(4), pp.841-846.

Toosy, S., Sodi, R. and Pappachan, J.M. (2018). Lean polycystic ovary syndrome (PCOS): an evidence-based practical approach. Journal of Diabetes & Metabolic Disorders, 17(2), 277-285.

Tosi, F., Bonora, E., & Moghetti, P. (2017). Insulin resistance in a large cohort of women with polycystic ovary syndrome: a comparison between euglycaemic-hyperinsulinaemic clamp & surrogate indexes. Human Reproduction, 32(12), 2515-2521. https://academic.oup.com/humrep/article/32/12/2515/4523637

Umayal, B., Jayakody, S.N., Ch&rasekharan, N.V., Wijesundera, W.S.S. & Wijeyaratne, C.N., (2019). Polycystic ovary syndrome (PCOS) & kisspeptin–A Sri Lankan study. Journal of postgraduate medicine, 65(1), p.18.

Unluhizarci, K., Karaca, Z., & Kelestimur, F. (2021). Role of insulin & insulin resistance in &rogen excess disorders. World Journal of Diabetes, 12(5), 616. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8107978/

Wen, J., Huang, K., Du, X., Zhang, H., Ding, T., Zhang, C., Ma, W., Zhong, Y., Qu, W., Liu, Y. and Li, Z., 2021. Can inhibin B reflect ovarian reserve of healthy repr 60

White, J.F., Noinaj, N., Shibata, Y., Love, J., Kloss, B., Xu, F., Gvozdenovic-Jeremic, J., Shah, P., Shiloach, J., Tate, C.G. and Grisshammer, R., 2012. Structure of the agonist-bound neurotensin receptor. Nature, 490(7421), pp.508-513.oductive age women effectively? Frontiers in Endocrinology, 12, p.626534.

Witchel, S. F., Oberfield, S. E., & Peña, A. S. (2019). Polycystic ovary syndrome: pathophysiology, presentation, & treatment with emphasis on adolescent girls. Journal of the Endocrine Society, 3(8), 1545-1573. https://academic.oup.com/jes/article/3/8/1545/5518341

Xu, Y. & Qiao, J., (2022). Association of insulin resistance & elevated &rogen levels with polycystic ovarian syndrome (PCOS): a review of literature. Journal of healthcare engineering, 2022.

Yang, I.P., Miao, Z.F., Huang, C.W., Tsai, H.L., Yeh, Y.S., Su, W.C., Chang, T.K., Chang, S.F. and Wang, J.Y., 2019. High blood sugar levels but not diabetes mellitus significantly enhance oxaliplatin chemoresistance in patients with stage III colorectal cancer receiving adjuvant FOLFOX6 chemotherapy. *Therapeutic Advances in Medical Oncology*, *11*, p.1758835919866964.

Yazdanpanah, S., Rabiee, M., Tahriri, M., Abdolrahim, M., Rajab, A., Jazayeri, H. E., & Tayebi, L. (2017). Evaluation of glycated albumin (GA) and GA/HbA1c ratio for diagnosis of diabetes and glycemic control: A comprehensive review. Critical reviews in clinical laboratory sciences, 54(4), 219-232

Yesiladali, M., Yazici, M.G., Attar, E. and Kelestimur, F., 2022. Differentiating Polycystic Ovary Syndrome from Adrenal Disorders. *Diagnostics*, *12*(9), p.2045.

Zehravi, M., Maqbool, M. and Ara, I., 2021. Polycystic ovary syndrome and infertility: an update. *International journal of adolescent medicine and health*, *34*(2), pp.1-9.

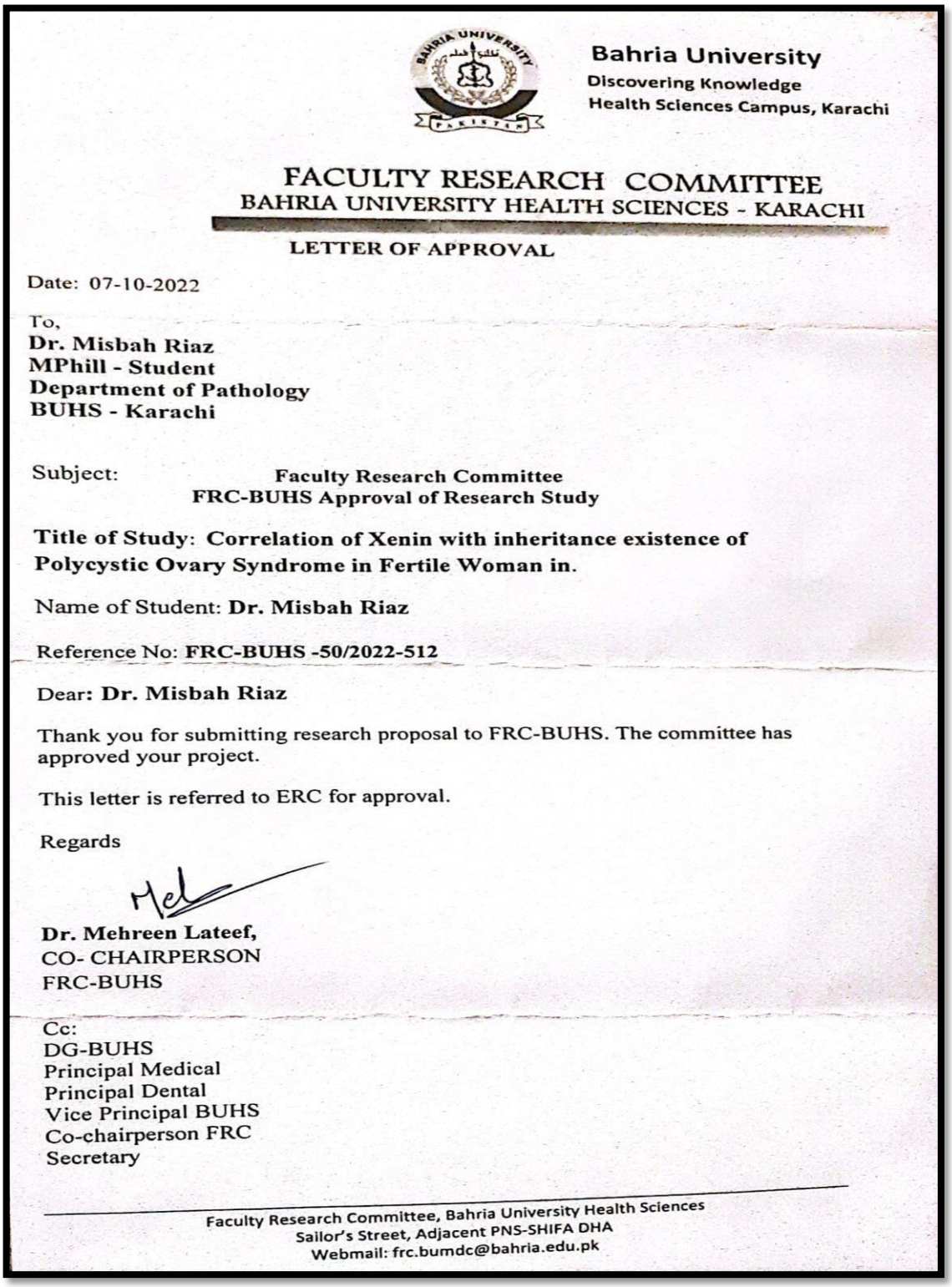
Zeng, X., Xie, Y.J., Liu, Y.T., Long, S.L. & Mo, ZC, (2020). Polycystic ovarian syndrome: correlation between hyper&rogenism, insulin resistance & obesity. Clinica chimica acta, 502, pp.214-221.

Zhang, B., Wang, J., Shen, S., Liu, J., Sun, J., Gu, T., & Bi, Y. (2018). Association of &rogen excess with glucose intolerance in women with polycystic ovary syndrome. BioMed research international, 2018. https://www.hindawi.com/journals/bmri/2018/6869705/

Zhang, H. Y., Zhu, F. F., Xiong, J., Shi, X. B., & Fu, S. X. (2009). Characteristics of different phenotypes of polycystic ovary syndrome based on the Rotterdam criteria in a large-scale Chinese population. BJOG: An International Journal of Obstetrics & Gynaecology, 116(12), 1633-1639.

Zhang, J., Chen, L. & Ye, J., (2021). Correlation analysis of myonectin levels with metabolic & hormonal disorders in patients with polycystic ovary syndrome. Ann Palliat Med, 10(3), pp.3404-9.

(A) BUMDC FRC Approval Letter



Bahria University
Discovering Knowledge
Health Sciences Campus, Karachi

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY HEALTH SCIENCES - KARACHI

LETTER OF APPROVAL

Date: 07-10-2022

To,
Dr. Misbah Riaz
MPhill - Student
Department of Pathology
BUHS - Karachi

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

Title of Study: Correlation of Xenin with inheritance existence of Polycystic Ovary Syndrome in Fertile Woman in.

Name of Student: **Dr. Misbah Riaz**

Reference No: **FRC-BUHS -50/2022-512**

Dear**: Dr. Misbah Riaz**

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

This letter is referred to ERC for approval.

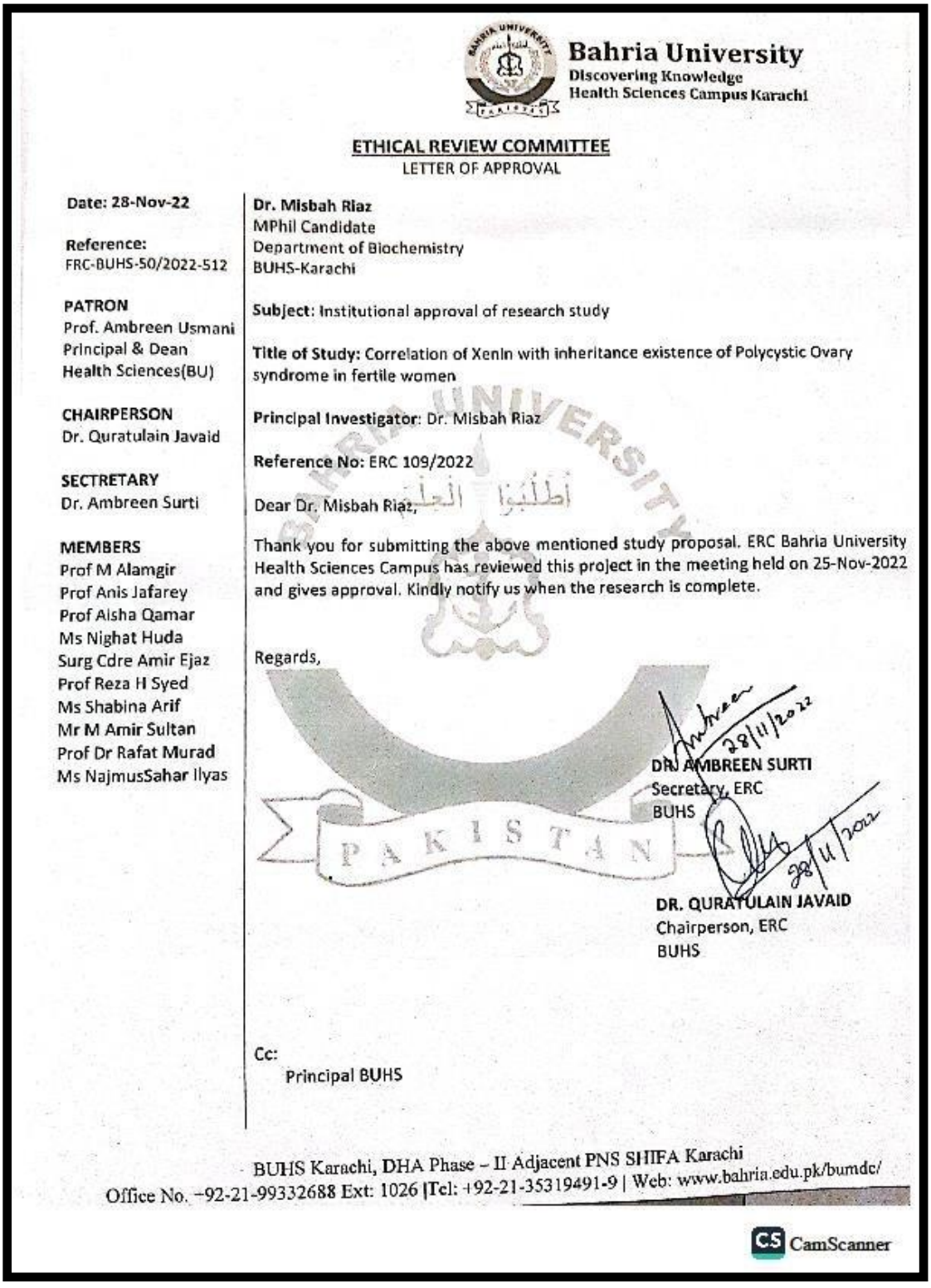
Regards

Dr. Mehreen Lateef,
CO- CHAIRPERSON
FRC-BUHS

Cc:
DG-BUHS
Principal Medical
Principal Dental
Vice Principal BUHS
Co-chairperson FRC
Secretary

Faculty Research Committee, Bahria University Health Sciences
Sailor's Street, Adjacent PNS-SHIFA DHA
Webmail: frc.bumdc@bahria.edu.pk

(B) BUMDC ERC Approval Letter



Bahria University
Discovering Knowledge
Health Sciences Campus Karachi

ETHICAL REVIEW COMMITTEE
LETTER OF APPROVAL

Date: 28-Nov-22

Reference:
FRC-BUHS-50/2022-512

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Dr. Quratulain Javaid

SECTRETARY
Dr. Ambreen Surti

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
Prof Aisha Qamar
Ms Nighat Huda
Surg Cdre Amir Ejaz
Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Prof Dr Rafat Murad
Ms NajmusSahar Ilyas

Dr. Misbah Riaz
MPhil Candidate
Department of Biochemistry
BUHS-Karachi

Subject: Institutional approval of research study

Title of Study: Correlation of XenIn with inheritance existence of Polycystic Ovary syndrome in fertile women

Principal Investigator: Dr. Misbah Riaz

Reference No: ERC 109/2022

Dear Dr. Misbah Riaz,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Health Sciences Campus has reviewed this project in the meeting held on 25-Nov-2022 and gives approval. Kindly notify us when the research is complete.

Regards,

28/11/2022
DR. AMBREEN SURTI
Secretary, ERC
BUHS

28/11/2022
DR. QURATULAIN JAVAID
Chairperson, ERC
BUHS

Cc:
Principal BUHS

BUHS Karachi, DHA Phase – II Adjacent PNS SHIFA Karachi
Office No. +92-21-99332688 Ext: 1026 |Tel: +92-21-35319491-9 | Web: www.bahria.edu.pk/bumdc/

CamScanner

(C) Consent Form (English Version)

This study is a clinical trial with an aim to compare conventional with a new use of existing drug (metformin) for dental caries. By participating in the study, you are giving consent for the placement of filling material in your carious tooth and the results obtained will be used for the benefit of the community. You are agreeing to participate voluntarily and at your own will in this research study where you will be exposed to any one of the following:

a) Placement of traditional filling material inside your carious tooth

OR

b) Placement of traditional filling material plus research drug inside your carious tooth

You have been told the possible side effects of the drug. It may cause nausea, vomiting, diarrhea and flatulence. If you suffer from any above mentioned complains or any other, you will inform the doctor immediately.

You have been explained in detail the nature and significance of participating in the research project and you fully understand the provided explanation. You understand that findings of your disease and data will be kept strictly confidential and will be used only for the benefit of community, thesis writing, publications and paper presentations.

You have been explained the laboratory and radiographic investigations that will be conducted for diagnosis and at start of treatment. For this purpose, you agree to give your dentinal samples at the beginning and end of the study.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified that no incentive will be provided to you for participating in the study. The cost of material, lab and radiographic investigations will be borne by the researcher whereas you do have the right to withdraw from the study at any time. You are advised to contact Dr. Urwa Naseem Akhtar on mobile number: 0323-5564687 or visit Bahria University Dental College Hospital in case of any query/ emergency related to the treatment. In case of any untoward effect resulting from the given drug it will be the responsibility of the researcher to effectively treat the patient at her own expense. In light of above discussion, I agree to be a part of this study.

Name of Patient: _____ Phone no of Patient: _____

Signature / Thumb impression of patient: _____

Name of Researcher: _____ Phone no of researcher: _____

Signature of Researcher: _____ Date: _____

(C) Consent Form (Urdu Version)

مریض کا اجازت نامہ

اس طبی آزمائش کا مقصد (Metformin) اور روایتی دوا کا موازنہ ہے جو کہ دانتوں کے خلا کو پُر کرنے میں استعمال کی جاتی ہے۔ (اس سے حاصل ہونے والے نتائج کا استعمال معاشرے کی فلاح کے لئے استعمال کئے جائیں گے)۔

آپ رضا کارانہ طور پر حصہ لینے اور اپنی مرضی کے مطابق اس تحقیقاتی عمل میں اپنی رضامندی دے رہے ہیں۔ اس تحقیقاتی عمل میں آپ کے دانت کے اندر موجود خلا کو روایتی دوا سے یا اس کے ساتھ تحقیقاتی دوا کو شامل کر کے بھرا جا سکتا ہے۔

آپکو دی گئی دوا، Metformin سے متلی، قے، پیٹ کا پھولنا اور دست کی شکایت ہو سکتی ہے۔ متذکرہ بالا کسی بھی شکایات کے رونما ہونے پر آپ ڈاکٹر صاحبہ کو بروقت مطلع کریں گے۔

آپکو اس تحقیقاتی عمل میں حصہ لینے کی فطرت اور اہمیت کے بارے میں تفصیل سے بیان کیا گیا ہے اور آپ نے فراہم شدہ وضاحت سمجھ لی ہے۔

آپ کو بتایا گیا ہے کہ آپ کی بیماری کے نتائج اور معلومات (Data) کو خفیہ رکھا جائے گا اور معاشرے میں دانتوں پر مشتمل بیماری کے فائدے کیلئے تحقیقی مقالہ لکھنے، پڑھنے اور اشاعت کے لئے استعمال کیا جائے گا۔

آپ کو وضاحت کی گئی ہے کہ تحقیقی عمل کے آغاز سے قبل اور اختتام پر لیبارٹری اور Radiographic جانچ کی جائے گی۔ نیز اس مقصد کے لئے آپ کے Sample کے نمونے تحقیقی عمل شروع کرنے سے پہلے اور اختتام پر لیں گے۔

میں پوری طرح سے تمام ضروری معلومات محقق کو دینے پر رضامند ہوں۔ مجھے یہ واضح کیا گیا ہے کہ اس تحقیق میں حصہ لینے کے لئے مجھے کوئی معاوضہ نہیں دیا جائے گا۔ لیبارٹری کی تحقیقات، خلا پر کرنے والی دوا کی لاگت محقق کی جانب سے ادا کی جائے گی۔ نیز میرے پاس کسی بھی وقت تحقیق سے دست بردار ہونے کا حق ہے۔

مجھے مشورہ دیا گیا ہے کہ ڈاکٹر اروا نسیم اختر سے موبائل نمبر 0323-5564687 پر یا میری بیماری کے متعلق کسی بھی سوال یا ہنگامی صورتحال میں بحریہ یونیورسٹی ڈینٹل ہسپتال سے رابطہ کریں۔ خلا پر کرنے والی ادویات کے استعمال کے نتیجے میں کسی بھی غیر معمولی اثر کی صورت میں یہ محقق کی ذمہ داری ہوگی کہ وہ مریض کا اپنے اخراجات سے مؤثر طور پر علاج مہیا کروائیں گی۔

مریض کا نام: ----------

مریض کا فون نمبر: ----------

تاریخ: ----------

مریض کے دستخط: ----------

ڈاکٹر کے دستخط: ----------

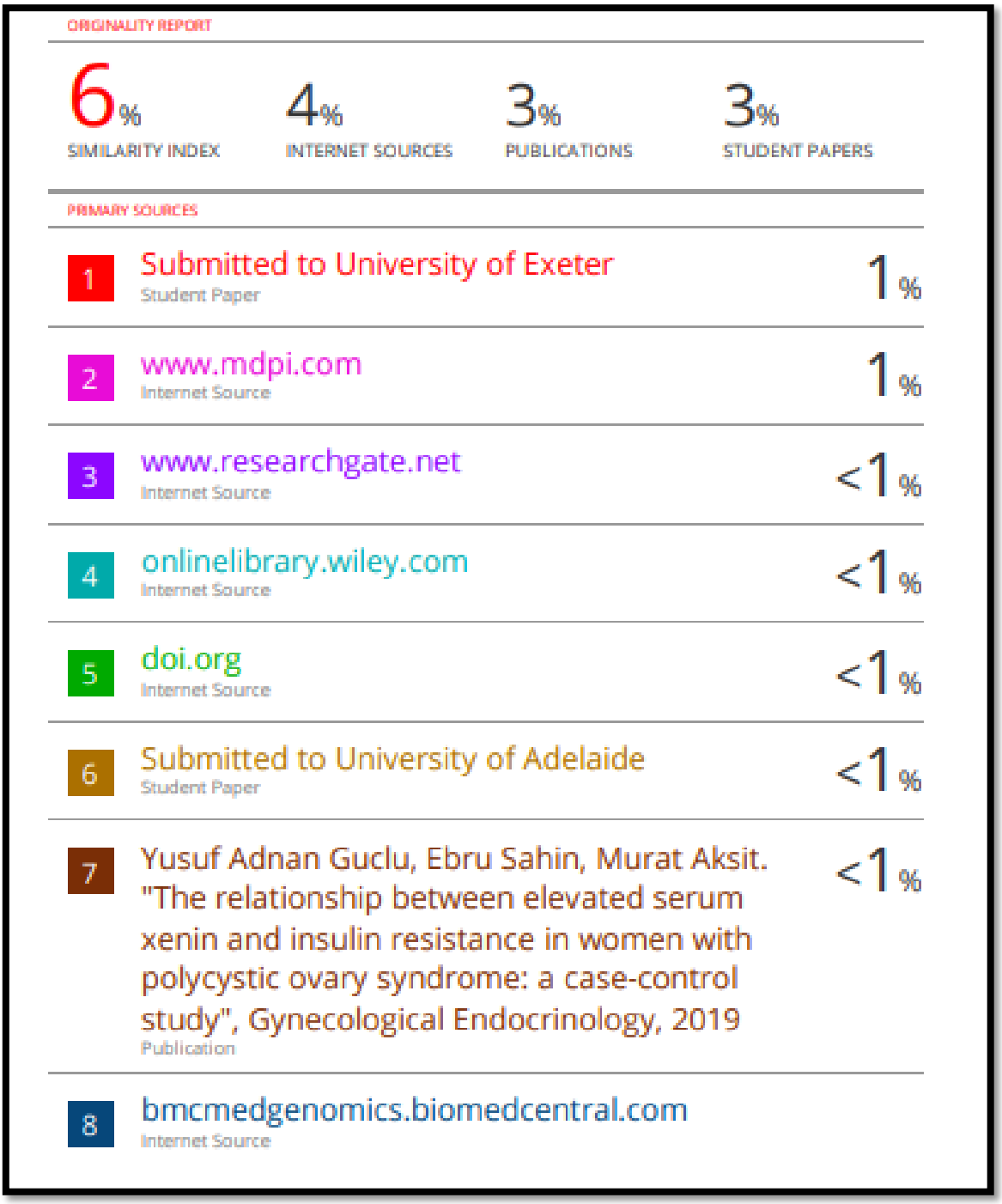
(D) Subject Evaluation Form

Serial no:_____ Registration no._____

Demographics	
Name	
D/O, W/O	
Age	
Marital status	
Education	
Address	
Contact no	
Rotterdam criteria	
Oligo-anovulation,	
Hyperandrogenism	
Polycystic ovaries	
Anthropometric measurements	
Height(meters)	
Weight (kg)	
BMI	
Others	
Family history of PCOS	
Laboratory investigations	
Serum FSH	
Serum LH	
Serum testosterone	
Prolactin	
Fasting blood sugar	
Fasting insulin levels	
HbA1c	
To be evaluated	
Plasma Xenin	

(E) Hospital / Institute Card

(F) Turnitin Plagiarism Check report



ORIGINALITY REPORT

6%	4%	3%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Exeter Student Paper	1%
2	www.mdpi.com Internet Source	1%
3	www.researchgate.net Internet Source	<1%
4	onlinelibrary.wiley.com Internet Source	<1%
5	doi.org Internet Source	<1%
6	Submitted to University of Adelaide Student Paper	<1%
7	Yusuf Adnan Guclu, Ebru Sahin, Murat Aksit. "The relationship between elevated serum xenin and insulin resistance in women with polycystic ovary syndrome: a case-control study", Gynecological Endocrinology, 2019 Publication	<1%
8	bmcmedgenomics.biomedcentral.com Internet Source	