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ASSOCIATION OF POLYMORPHISM OF LEPTIN RECEPTOR GENE AND POLYCYSTIC OVARY SYNDROME

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ABSTRACT: The common gynecological disorder "Polycystic ovaries syndrome" (PCOS) is the main cause of infertility in females. It is characterized by chronic oligo-anovulation or anovulation and presence of cysts in ovaries. The etiology and genetic basis of disease is still unknown. The aim of current study is to investigate the association of polymorphism of leptin gene receptor (LEPR) with PCOS. A structured questionnaire was obtained from each subject including their symptoms and medical history. The weight gain was observed in PCOS subjects when compared with their controls. Similarly, the body mass index (BMI) of PCOS women was greater than their age matched controls. DNA of all subjects was isolated and the genetic variant rs1137101of leptin gene was selected. The optimization of primers and copies of DNA fragments was done by Polymerase Chain reaction (PCR) analysis. The products were then digested using restriction fragment length polymorphism (RFLP). The length of Gln223Arg PCR fragment was 481bp and digestion of this fragment occur with Msp I enzyme at 37°C for 6h. The two fragments of length 291bp and 190bp produced after digestion. The frequency of allele "A" was 72 % in PCOS while 46% in their respective control whereas frequency of "G" allele was 28 % in PCOS and 54% in controls (p= 0.001). This study concludes that LEPR gene polymorphism is associated with risk of PCOS in Punjabi population.
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Key word: PCOs, infertility, polymorphism, leptin gene, genetic difference

Introduction: Polycystic ovary syndrome (PCOS) is a complex multifactorial heterogeneous, genetic and hormonal disease. This disease is characterized by irregular menstruation cycle, demonstration of clinical and biochemical hyperandrogenism and polycysts in ovaries (Pusalkar *et al*., 2010). It is recognized as metabolic disorder with certain abnormalities like insulin resistance and has widespread consequences on physiology and metabolism of body (Moghetti, 2020). The clinical symptoms of disease differ among different women with PCOS, these signs and symptoms change over time and can be same within same women. The weight gain leads to develop the signs and symptoms of PCOS. Obese women has more symptoms of disease such as extreme level of androgen, hirutism, oligomenorrhea in comparison to normal women (Allahbadia *et al*., 2011). More than 50% of PCOS women are obese (Pusalkar *et al*., 2010). The prevalence of PCOS is different in different geographical areas and ranges between 2.2% and 26% (Wolf *et al*., 2018). In Southern China the occurrence of disease is 2.4%. In India the prevalence of PCOS in women is about 20% (Irgam *et al*., 2019).There is high prevalence of disease in southern US that is 47.5% while the occurrence of disease in Spain is 6.5% (Wolf *et al*., 2018). The PCOS is becoming highly prevalent in Pakistan with occurrence of 15.7-37% (Naz, 2020). In Korean urban population the prevalence is 14.5% and in Turkey the prevalence is 16% (Zheng et al., 2013). While in Brazil the cases of PCOS and metabolic syndrome are 38.4% (Peppard et al., 2001). Environment and sedentary life style are the main factor that leads toward reproductive dysfunction, obesity, continuous change in level of gonadotropin, hyperandrogenism and insulin resistant. There are many abnormalities associated with PCOS such as biochemical abnormality, metabolic abnormality and reproductive abnormality (Allahbadia *et al*., 2011).Moreover excess level of androgen induces acne, hirsutism, abnormal hair growth on body and weight gain (Zeng *et al*., 2020) The etiology and genetic basis

of PCOS is still unknown (Pusalkar *et al*., 2010). Likely, the pathogenesis of this disease is not clear and controversial but the many changes of neuroendocrine system are the main element of this disease (Allahbadia *et al* ., 2011). The important cause of disease is the disruption of follicle growth. Because in ovarian follicular development, primordial follicle are grouped from which antral follicle is start to ovulate. In PCOS, the disruptions of signal stop follicle growth. The resultantly the menstrual irregularities occur and small antral follicles start accumulation on periphery of ovary and give the appearance of polycyst in ovaries (Gooderzi *et al*., 2011). Leptin is the hormone that causes obesity in women. Adipose tissue and the ovary are the sources that produce leptin and testosterone respectively. These two hormones causes obesity and play role to cause PCOS. Leptin also increase the androgen level by enhancing adipogeneiss that cause obesity (Pusalkar *et al*., 2010). Leptin gene and leptin gene receptors (LEPR) have been identified in human and is present on 7q31.3 and 1p31 chromosomes respectively (Smaism,2016). The studies showed that leptin is the gene that is associated with PCOS. Leptin is a polypeptide hormone and regulates adipose-tissue mass through hypothalamic effects that work on satiety and energy expenditure. It binds to receptor and activates to produce a series of chemical signal. Leptin gene work through specific receptors called LEPR. It is the receptor of cytokine family and single has domain of single tarns-membrane (Abilash, 2016).

Lean female produce more leptin than obese and male counterpart. Many factors cause mutation in LEPR gene that may prevent their binding to receptor. The other reason may be obesity due to excessive eating in childhood begin with LEPR deficiency disorder and hypogonadism that cause increase secretion of hormone help in direct sexual development. Recent studies have showed the association of obesity with PCOS and have elevated serum value of leptin as compared to controls (Daghestani *et al*., 2019).

Various studies showed the heritability of PCOS in various populations such as PCOS families, twins and ethnic groups. There is still no particular pattern of inheritance, therefore it is proved that conjugation of disease in first degree relatives and familial segregation is diagnosed for PCOS and it has been represented (Diamanti-Kandarakis *et al*, 2006). The polymorphism Gln223Arg and Pro101Pro are the polymorphism of LEPR associated with risk of PCOS (Abilash, 2016). These are the different forms of LepR known in which long isoform used as signaling of leptin. The LepRb are expressed in nuclei of brain hypothalamus but it can effect on cells of periphery with nuclei. While, the expression of LepRb is occur in different organs such as adipocutes, lung, endometrium, kidney, endothelial cells, stomach, blood cells, liver, muscle, osteoblast, placenta, pancreatic islets and umbilical cord. Sometimes mutation in leptin gene causes infertility and changes in reproductive dysfunction (Perez- Perez *et al*., 2017).

Aim of study

The purpose of this research study is to elaborate association of polymorphism of leptin gene receptor with polycystic ovaries syndrome in Pakistani Punjabi population.

MATERIAL AND METHOD

Ethical approval of study

This research is approved by the research ethics Committee of Government College University Lahore.

Subjects

The women with reported hormonal problem and infertility due to PCOS were subjected as cases. The blood samples and history of cases were taken from female patients at gynecology ward of civil hospital Chichawatni and Lady Willingdon hospital Lahore. I assessed the medical history on specially assigned data sheets that has height, weight, age, BMI from every subject (Questionnaire Annexure II). The consent form was taken from all subjects (Annexure I). The normal healthy Punjabi women with regular menstrual cycle and spontaneous conception were subjected as controls.

Sample selection

Cases suffering from polycystic ovaries syndrome were taken with controls.

- Fifty(50) women with PCOS
- Fifty (50) healthy women as normal group.

3.1.1 Inclusion criteria

- Age of female was ranged between 15 to 45 years.
- The women with PCOS were proven clinically with developing symptoms (e.g. pelvic pain, coarse hair growth on face and infertility).
- Reproductive age group was selected.

Exclusion criteria

- Any metabolic, cardiovascular or other relevant medical illness
- Inflammation of pelvis and abdomen or abnormal vaginal discharge
- If any medicine were taken including hormonal treatment in last 1 year

On treatment of infertility i.e, IVF

Selection of Controls

- The control with were taken with regular cycle
- The control don't show any symptom of excess androgen e.g. hirutism.
- Women having normal conception
- The women that had not taken any hormonal contraception
- The agreed female were selected as control

Physical examination

Determination of weight, height and Body Mass Index

Portable standiometer was used to determine standing height of each female and height scale was used to determine weight of individuals. Body Mass Index (BMI) of each individual was calculated (Meriem *et al.*,2019)

BMI=Weight in kilogram/ (Height in meter)^2

Physical examinations and interviews

Every subject filled proper questionnaire covering all physical examination and surgical history .The physical examination part involve questions related to Weight and height, family history, level of education, waist and hip ratio (WHR). Ultrasonography was done by doctor prior to physical examination. Every control was matched with respect to their age, living area and was genetically non-related in order to minimize biasedness. Hence, selection of samples was random.

Blood sampling and storage

The blood samples were taken from every patient and they were divided for further processes. Samples were collected in EDTA tubes containing ethylenediaminetetraacetic acid .EDTA act as an anticoagulant. The samples were stored at -20°C.The serum was separated from serum later on. For transportation of samples, ice was used.

Genotyping panel of LEPR gene is presented in table 3.1.

SNPs Selection:

The NCBI website (http://www.ncbi.nlm.nih.gov/sbp/) was used to search SNP. The SNP was selected from "National center for biotechnology information(NCBI)". The SNP was selected relevant to Polycystic ovaries syndrome. The selection was done after searching of databases.

GTGTCCCAAATAGTTTACTTCAATTAGTATTTAGTATCCTGCTTTAAAAGC
CTATCCAGTATTTTCATATCTGTTTTAATATTTAGCTCTTATTTTTCAATAT
AGGCCTGAAGTGTTAGAAGATTCACCTCTGGTTCCCCAAAAAGGCAGTTT
TCAGATGGTTCACTGCAATTGCAGTGTTCATGAATGTTGTGAATGTCTTGT
GCCTGTGCCAACAGCCAAACTCAACGACACTCTCCTTATGTGTTTGAAAA
TCACATCTGGTGGAGTAATTTTCCAGTCACCTCTAATGTCAGTTCAGCCCA
TAAATATGGGTAAGTTATGCACTAAAATGATGATAATAGGTCTAAACATC
AGTCATATATAAAGGTTAAAAATTGCTTACAAAAATATTTGCTAGCTTATC
TCACTTTGCTTAACACTGTAATGATGGTAGATGTAGTACTGGGGGTATTAA
GAGTGGCTTCTAGAAGATTTAACAATGGTATGTATATCTCTGCCATTGTCA
CTTAAATTCTGTTTTGAAAACTG

Figure 3.1: The forward and reverse primers represent by highlighted red and SNP are shown by purple arrows

Restriction fragment length polymorphism (RFLP)

It is the technique used to obtain changes in DNA sequences that are homologous. This method is inexpensive and genotyping is done depending on cleavage of endonucleases. The genotyping of changed DNA sequence is done using RFLP method. Then the amplified DNA part undergo digestion using restriction enzyme which has ability to verify a restriction enzyme site.

Primers

The primer designing is done using online software Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). The primers are designed with forward and reverse pairs. The NCBI BLAST and UCSC In-Silico PCR2 web engine are used to test validity of all primers. The deionized water with concentration of 100uM is used for dilution purposes of Primers. Polymerase chain reaction is used to amplify SNP.

PCR DNA amplifications:

The amplification of DNA for RFLP is done on genomic DNA using Platinum PCR Super Mix. This contains many components such as 22U/ml recombinant Taq DNA polymerase, 55mM KCl, 22mM Tris-HCl (pH 8.4), 1.65 mM MgCl2, 220µM dATP. 220µM dGTP, 220µM dCTP, 220µM dTTP and stabilizers. The 50ul volume mixture is used for PCR amplification. The thermal cycler is used for incubation of PCR mixture. There are three steps occur in PCR such as denaturation, annealing and extension. Denaturation is done at 95°C for 5 minutes. The process followed by 35 cycles of

denaturation at 94°C for 30 seconds. The annealing is done at 58°C for 30 seconds. Then the elongation process takes time of 40 seconds at 95°C and final elongation take five minutes at 72°C. The evaluation of PCR product is done using gel electrophoresis technique. Primers, annealing temperature, PCR program and size of product are presented in table 3.2.

Table 3.1: Genotyping panel for *LEPR* gene

Candidate gene	Symbol of gene	Gene ID	*SNP ID	*Chr	Cytogenetic Position
LEPR(Gln223Arg)	*LEPR*	3953	rs1137101	1	1p31.3

Table 3.2: Primers, annealing temperature, PCR program and product size

Sequence of primers	PCR Program	Annealing temperature	Product size (bp)
F: ACCCTTTAAGCTGGGTGTCC R: GAAGCCACTCTTAATACCCCC	95ºC 5min, 94ºC 45 Sec 58ºC 30 Sec, 72ºC 5 min	58ºC	481

PCR product purification

For accurate results of PCR products, all primers and dNTPs residues are needed to remove. The commercially available kits are used for purification purposes of PCR products. These kits used mini-column spin technology. It removes excess primers, enzyme and excess dye such as ethidium bromide etc.

Assay protocol

PCR purification steps were carried out at room temperature. The concentration of washing buffer was diluted using absolute ethanol of 80ml. The precipitation was dissolved by swirling and mixing with buffer then incubation is done at 55°C -65°C temperature. The PCR is done in a nuclease free sterile microcentrifuge having 100ul DNA sample. Then vortexing is done to dissolve and mix 500ul of PCR DNA binding buffer. Then mixture was transferred to a column at collection tube and centrifugation occurs at 10000×g for 1 minute. Then washing of column was done with 750ul wash buffer and centrifugation done at 10000×g for 1 minute. There the flow was discarded and column is dried again with centrifugation at 10000×g for 1 minute. Then the column was shifted to a new 1.5ml tube in centrifugate. The centrifugation is again done at 10000×g for 1 minute with preheated 50ul elution buffer or deionized water. The eluted DNA was aliquoted and stored at -80°C.

Gel electrophoresis

The quantification of PCR product was done using gel electrophoresis. Agarose gel of different percentage was prepared for DNA quantification, 0.8% of genomic DNA, 1.5% for PCR amplified products, 2% for purified PCR products and 3% for restriction enzyme digested PCR product. In a flask added 1×TAE buffer and weighted agarose. The agarose and TEA buffer is complete dissolve by heating in oven. Then add ethidium bromide to make agarose solution clear by mixing it. Then allowed the solution to cool down and later add on sealed casting tray having combs in it. The casting tray was filled with TAE buffer and placed into electrophoresis chamber, the seal was removed then. The 6× loading dye (3ul) was mixed with 10ul PCR product and loaded in wells. 10ul DNA ladder was loaded in one well. Then power supply was set at 180 volts and electrodes are connected with it. The power is turned off when blue dye move towards the end of gel. Gel documentation system is used to observe gel under UV light.

3.6.7 PCR-RFLP analysis

The PCR-RFLP analysis for genotyping of single nucleotide polymorphism (SNP) Gln223Arg of LEPR gene was done for all samples. The length of Gln223Arg PCR fragment was 481bp and digestion of this fragment occur with Msp I enzyme at 37°C for 6h. The two fragments of length 291bp and 190bp produced after digestion of allele G.

3.7 Statistical analysis

The Hardy-Weinberg equilibrium was used in cases and control study. The other test used was simple chi-square test for analysis. The sigma plot 11.0 was used to calculate confidence intervals and odd ratios. The student t-test was used to compare the means of various parameters. $P \leq 0.05$ was considered significant statistically.

RESULTS

A total of 400 subjects included in the study. Subjects were categorized in married and

unmarried. Age, age of menarche, Height, weight, BMI, Waist to Hip Ratio, Duration of marriage, Diabetes Mellitus, Acne, Hirsutism, Weight gain, Infertility and pattern of cycle were measured among all subjects. Out of 400 subjects including 248 were married and 152 were unmarried (figure 4.1)

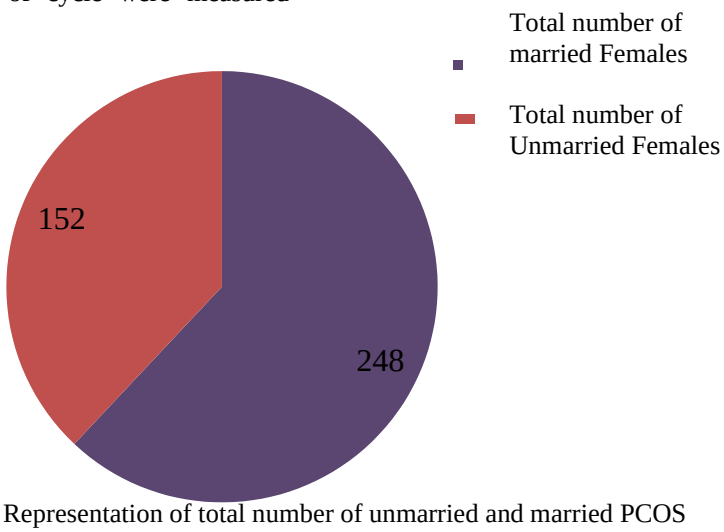

Figure 4.1: Representation of total number of unmarried and married PCOS

Age

The women included in this case study were divided in two categories, Married and unmarried. Married PCOS and unmarried subjects were categorized in four age groups i.e. 15, 15≤ 30; 31≤ 45 and ≤ 45 having different number of PCOS women as in 15 years of no married and 4 unmarried were present. In second category 15≤ 30 unmarried were 141 and 130 married were included. In third category31≤ 45, unmarried were 7 and married were 103. In fourth category≤ 45, married were 14 and no unmarried were included in this category.

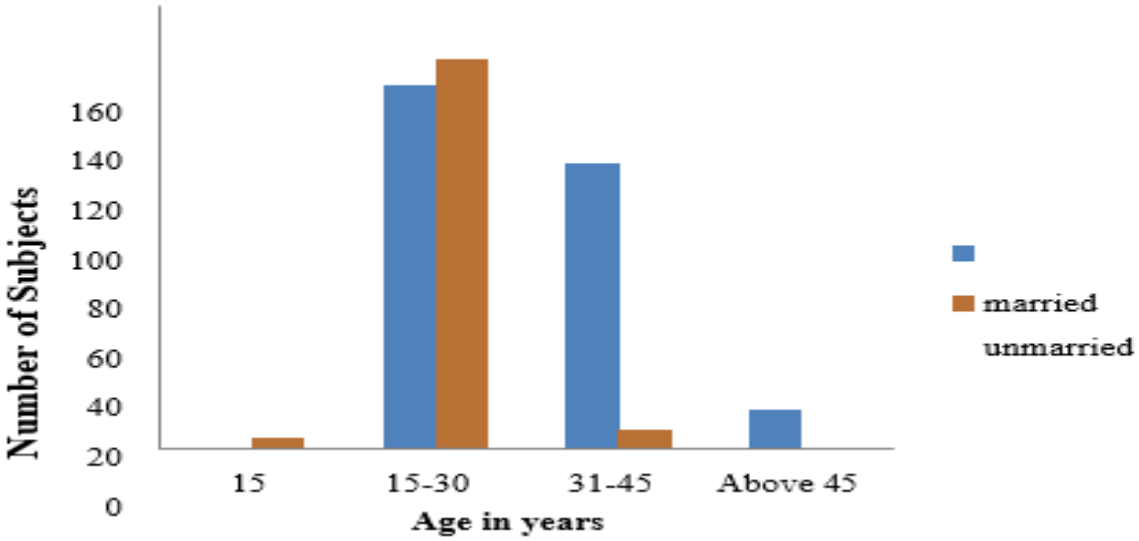

Figure 4.2: Age representation of married and unmarried PCOS

Duration of Marriage

The duration of marriage was noted. All the married PCOS subjects were categorized according marriage duration. The total married PCOS subjects were 248. In first category 1-5 years of marriage duration 100 females were included. In second category of 6-10 years of marriage duration 50 females were included. In third category of 11-15 years of marriage duration 50 females were included. In fourth category of 16 and above years of marriage 48 females were included.

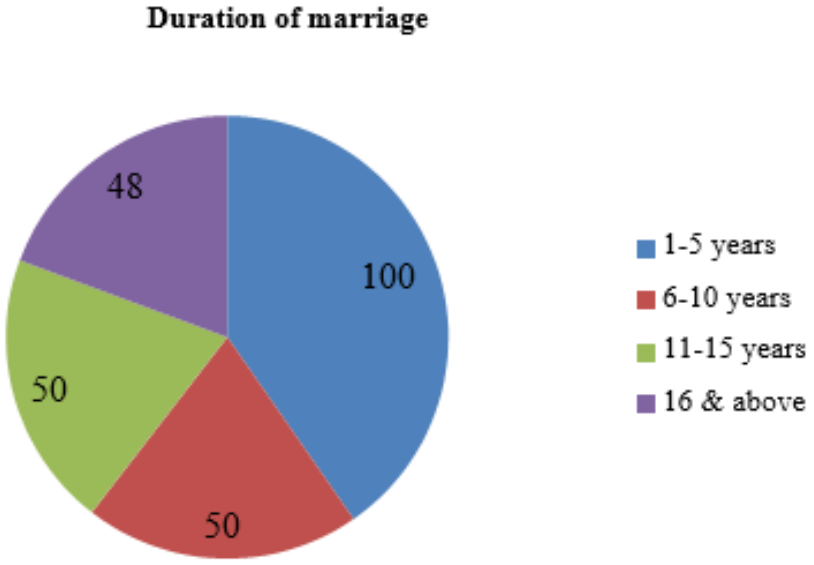

Figure 4.3: Representation of marriage duration of married PCOS

4.3. Hirsutism

In married subjects hirsutism was present in 75 females out of 248 and in unmarried PCOS 43 out of 152 was found with hirsutism.

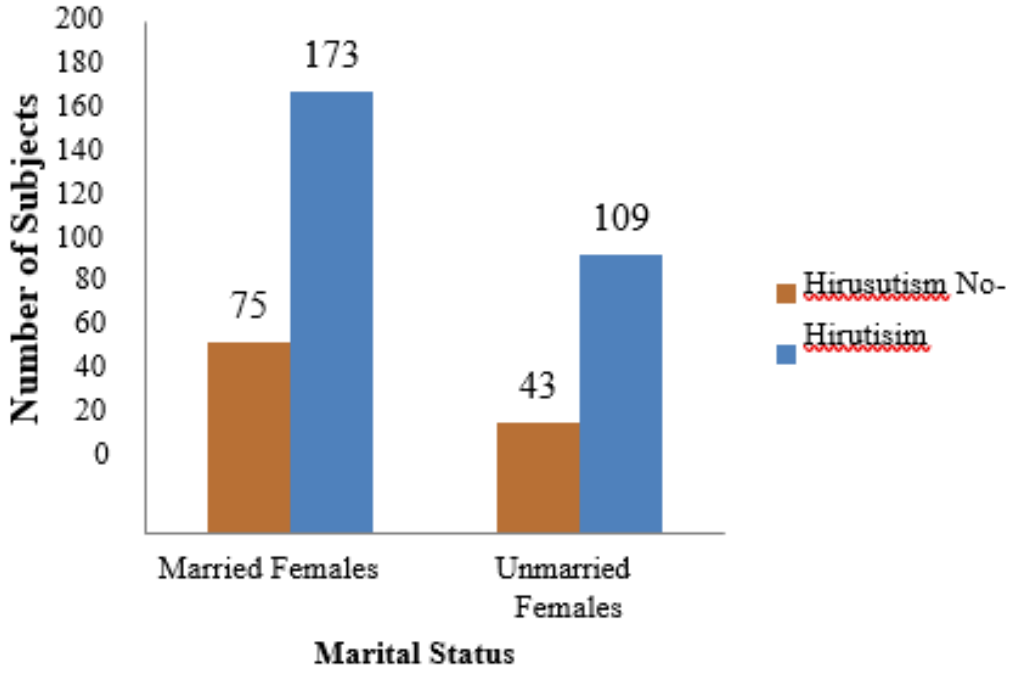

Figure 4.4: Hirsutism in married and unmarried females

4.4 Body Weight

Married and unmarried PCOS showed body weight gain. 204 married PCOS showed weight gain and in unmarried 91 females showed weight gain.

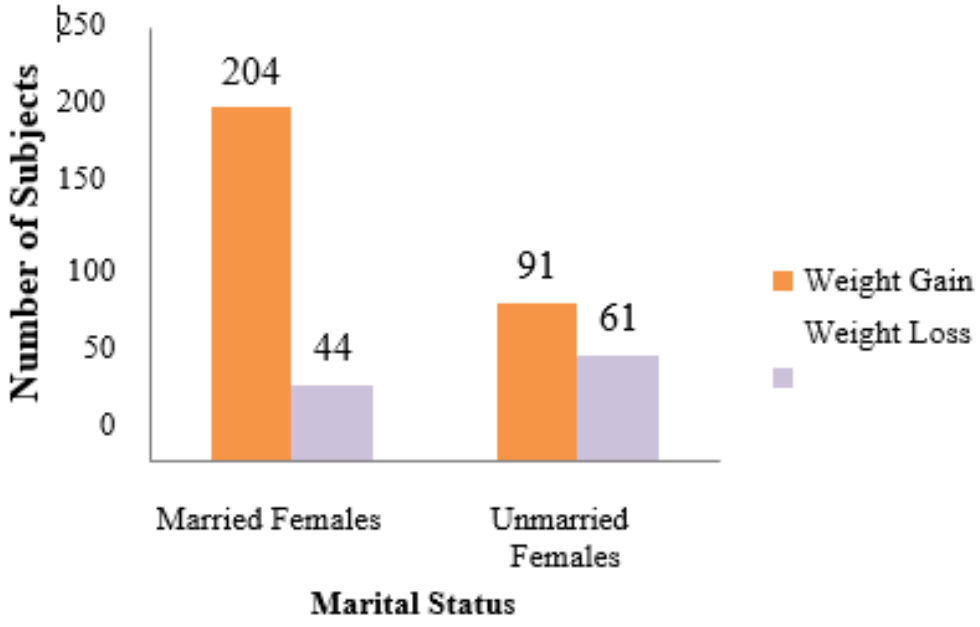

Figure 4.5 Representation of increase Body Weight in Married and unmarried PCOS

4.5 Diabetes Mellitus

Diabetes Mellitus was also noted in few subjects. In case of married PCOS subjects 46 were diabetic and in unmarried 8 were suffering from diabetes.

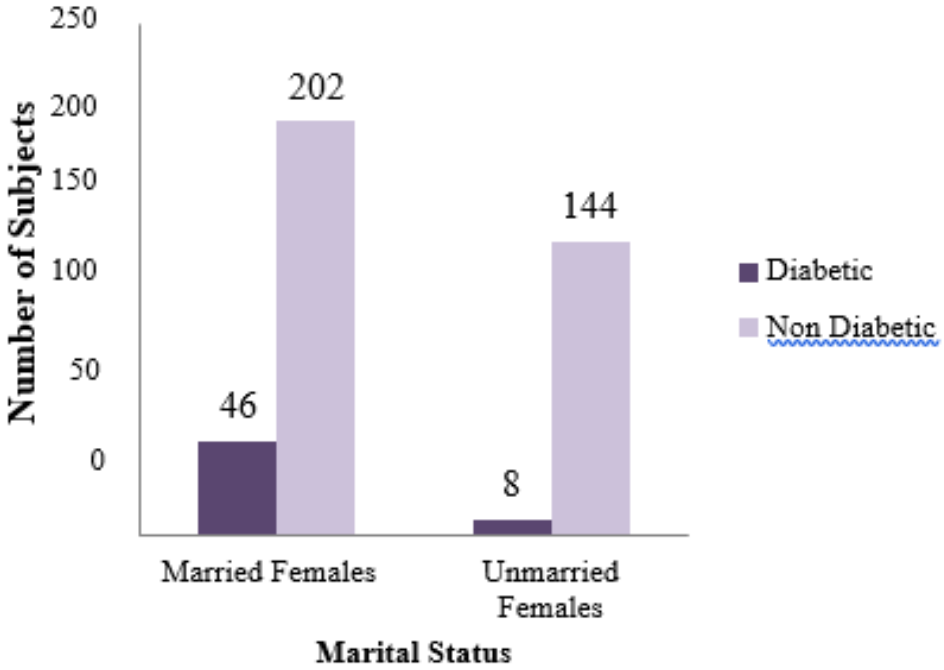

Figure 4.6 Married and unmarried Diabetic PCOS Subjects

4.6. Acne

Acne was also noted among all married and unmarried PCOS. In case of unmarried PCOS subjects 97 and in case of married subjects 58 were found with acne problem.

4.7. BMI

The Body Mass Index (BMI) of every subject was calculated by using the equation demonstrated by BMI = Weight in kilogram / (Height in meter) [2.] Mean BMI of married PCOS were 33.34 and mean BMI of unmarried PCOS were 30.52.

4.8. Infertility

Infertility status was also noted. The married PCOS subjects 30 females have infertility problems. No infertility case found among unmarried subjects.

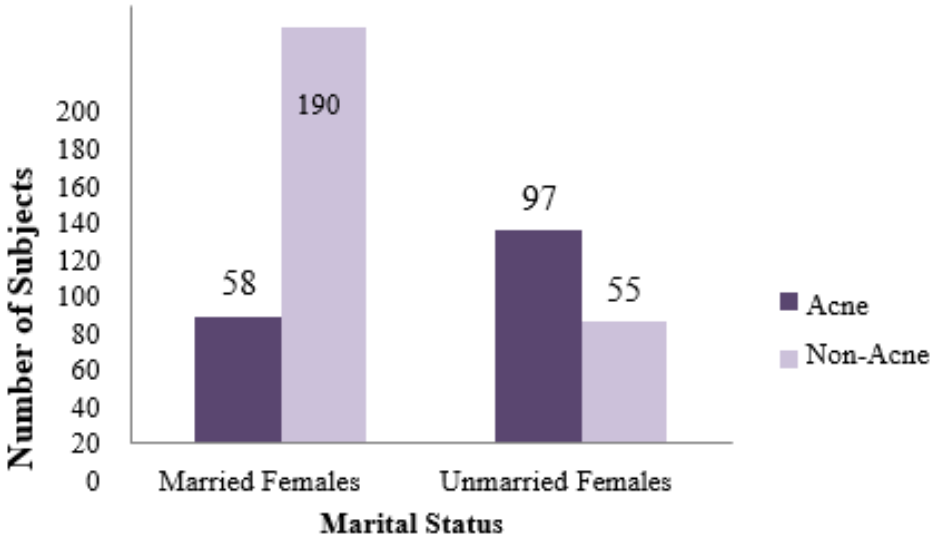

Figure 4.7 Married and unmarried PCOS with Acne

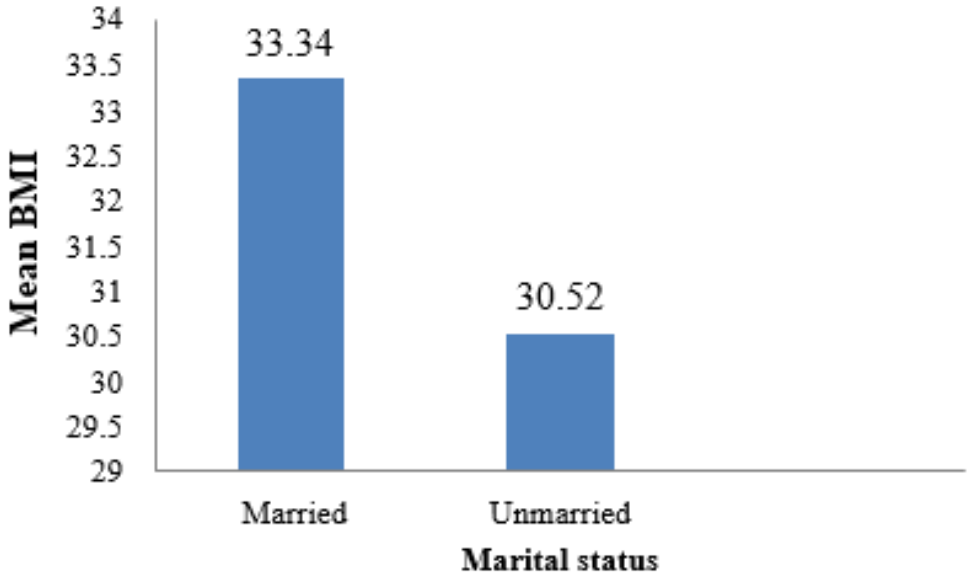

Figure 4.8 Mean BMI values in Married and unmarried PCOS

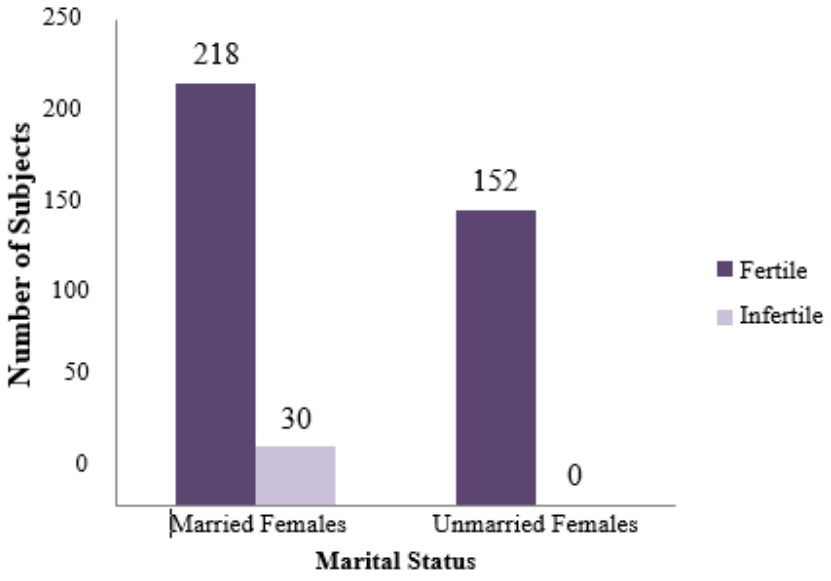

Figure 4.9 Infertility in Married and unmarried PCOS

4.9. Education

The literacy rate was recorded. 16% were illiterate, 37% were up to primary level, 34% were at higher secondary and only 13 % were at graduate level or above it.

4.10 Pattern of cycle

Menstrual pattern of cycle was also noted .In married females 48 were with regular pattern of cycle while 200 were with irregular pattern. In unmarried females 90 were with regular and 62 were with irregular pattern of cycle.

4.11 Duration of cycle

Duration of cycle is also noted. Duration of cycle is categorized into 4 categories 25 days, 25-35 days, 35-60days and variable .In first category of 25 days, 72 married and 57 unmarried were included. In second category of 25-35 days, 108 married and 75 unmarried were included. In third category of 35-60 days, 64 married and 16 unmarried were included. In fourth category of variable 4 married and 4 unmarried were included.

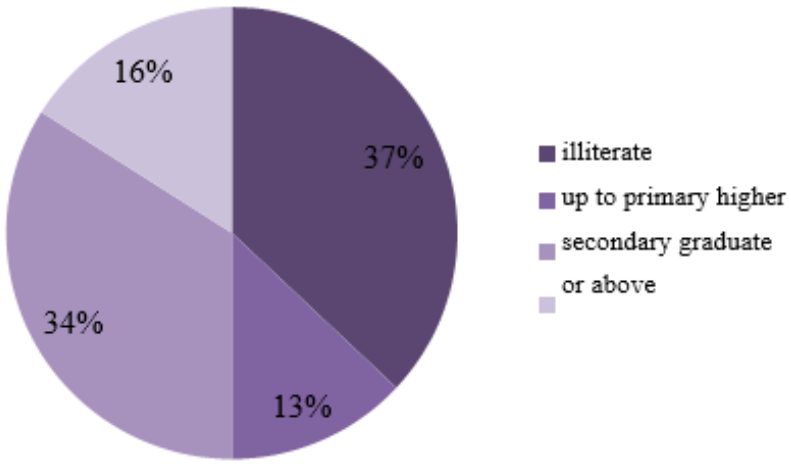

Figure 4.10 Different Education levels in married and unmarried PCOS

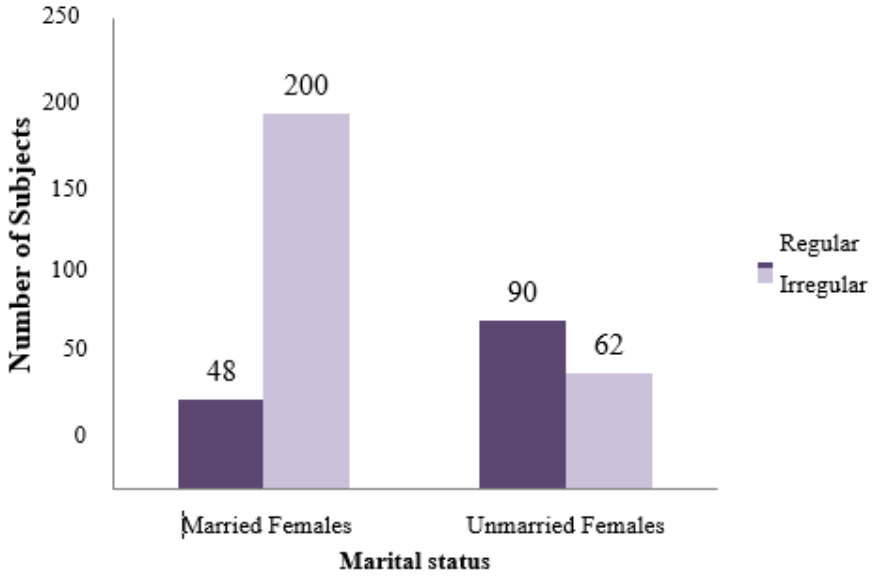

Figure 4.11: Representation of pattern of cycle of married and unmarried PCOS

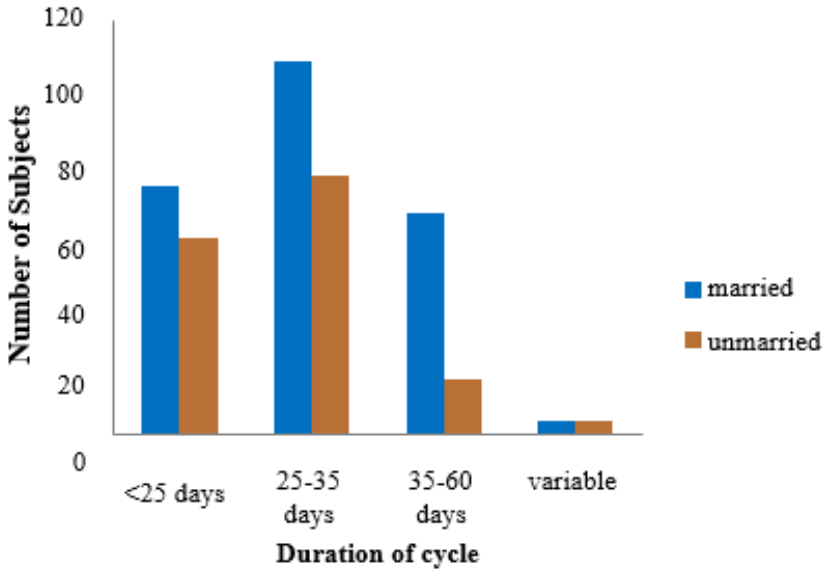

Figure 4.12: Duration of cycle for married and unmarried PCOS

Genetic Analysis

Restriction Fragment Length Polymorphisms (RFLP)

PCR-RFLP was performed on SNP of LEPR Gene. The association of this SNP with PCOS is provided in detail below.

The SNP and the genetic variation of LEP gene polymorphism associated with the obesity in Lahore population.

(a) Alignment of two sequences BLAST (PCOS)

The contribution of the genomic variants of *LEPR* was studied for SNP rs1137101. *LEPR* polymorphism is in exon 2. The rs1137101 is characterized by a A→G transition. The mutations of *LEPR* in two sequences BLAST alignment are shown in figure 4.2. The sequence chromatogram showed homozygous and heterozygous form of A/G base situation of rs1137101 figure 4.3.

Query 93 TCTTCAGCTCCCAGAGTCACCA**G**TGGTTCCACTTACATACTTGTCCCTCTAAGTCATTTA 152

Sbjct 62 TCTTCAGCTCCCAGAGTCACCA**A**TGGTTCCACTTACATACTTGTCCCTCTAAGTCATTTA 121

Query 153 GCCCAAAACTAAAACACAATGTGAAAATGTATCTGAGTATTGAATGATAATTCAGTCTTG 212

Sbjct 122 GCCCAAAACTAAAACACAATGTGAAAATGTATCTGAGTATTGAATGATAATTCAGTCTTG 181

(b) Alignment of two sequences BLAST (Control)

Query 95 TTCAGCTCCCAGAGTCACCA**G**TGGTTCCACTTACATACTTGTCCCTCTAAGTCATTTAGC 154

Sbjct 66 TTCAGCTCCCAGAGTCACCA**G**TGGTTCCACTTACATACTTGTCCCTCTAAGTCATTTAGC 125

Query 155 CCAAAACTAAAACACAATGTGAAAATGTATCTGAGTATTGAATGATAATTCAGTCTTGCC 214

Sbjct 126 CCAAAACTAAAACACAATGTGAAAATGTATCTGAGTATTGAATGATAATTCAGTCTTGCC 185

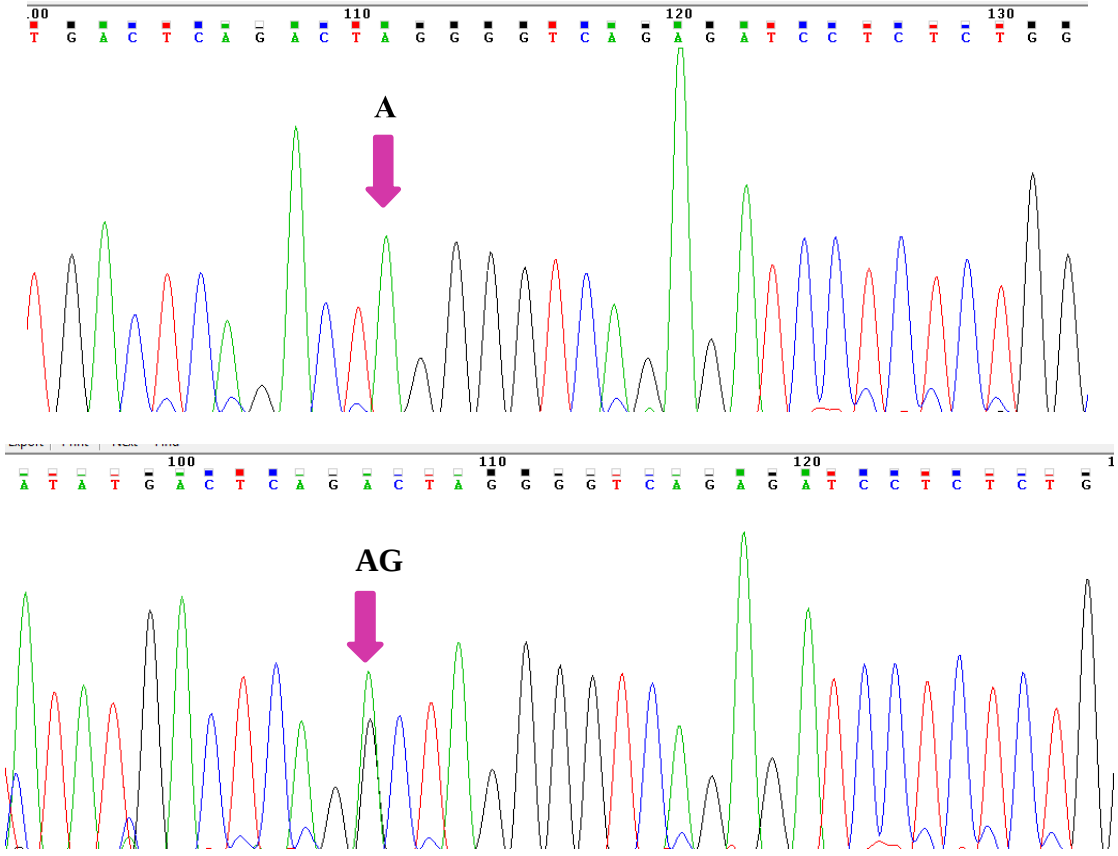

Figure 4.13: Sequence chromatogram showing homozygous and heterozygous form of A/G base substitution of Gln223Arg of *LEPR* gene.

Table 4.3: Frequency distribution of single nucleotide polymorphism of *LEPR* gene polymorphism in obese and non-obese population

SNP ID		**rs1137 101**					***OR* (95% CI)**	**χ^2**	**p**
Genotype/allele frequencies		AA (Gln/Gln)	AG (Arg/Gln)	GG (Arg/Arg)	A allele	G allele			
PCOs (n=50)	**N**	27	18	5	72	28			
	%	54	36	0.1	72	28	2.55(0.94-4.97)	2.44	0.01
Control (n=50)	**N**	12	22	16	46	54			
	%	24	44	32	46	54	22.12(0.15-3.89)	3.17	0.04

The association of polymorphism of LEPR was studied for SNP rs1137101.There is a change from A→G for rs1137101 polymorphism. The BLAST alignment show the mutation in two sequence of LEPR in fig.4.2. A/G base substitution for rs1137101 in homozygous and heterozygous form shown by sequence chromatogram. The relative allele frequencies and genotype distribution for LEPR gene are shown in table 4.3. The rs1137101 frequency "A" in patients is 72% and control group 46%. The clear difference is observed between relative allele frequency and genotype distribution of SNP rs1137101 for obese and non-obese. The statistical values for genotype frequencies of obese are (p=0.01), 95% CI=2.55(0.94-4.97), X^2= (2.44) and statistical value for non-obese were (p=0.04), 95% CI=22.12(0.15-3.89), X^2=(3.17). The PCOS subjects for rs1137101, 27(54%) were homozygous (AA), 18(36%) were heterozygous were AG and there were 5(0.1%) subjects GG. Similarly, genotypic frequency for control was 12(24%) for AA homozygous, for heterozygote the frequency was 22(44%), and for GG the frequency was 16(32%).The frequency for "A" allele of rs1137101 was72% for PCOS and 46% for control. For "G" allele the frequency for PCOS 28% and 54% for control. The frequency of the rs1137101 "A" allele was72% in obese individuals and 46% for non-obese individuals. The statistical values of the obese females for rs1137101 were p=(0.01), 95% Cl=2.55(0.94-4.97), $X^{2=}$(2.44) and the statistical values for the non-obese females were p=(0.04), 95% Cl=(22.12(0.15-3.89), X^2=(3.17).

Discussion

Polycystic ovaries syndrome is the disease that affect women of reproductive age and leads toward infertility. PCOS is becoming highly prevalent in Pakistan with occurrence of 15-37% (Naz, 2020).

Reproductive disorders are the main health problem globally in developing world. Although obesity and insulin resistance is one of the diagnostic criteria of polycystic ovaries syndrome; but both obese and non-obese become equally affected with it. The PCOS women either obese or non-obese have more visceral adipose tissue and elevated androgen levels. This androgen play an important role in PCOS (Zeng *et al.*, 2019).With the help of genetic studies the pathogenecity of disease is better understood. It helps in findings better ways for treatment of disease. The BMI calculated in current study for married PCOS women was 33.34 Kg/m^2 and mean BMI for unmarried PCOS were 30.52 Kg/m^2. Chances of obesity more elevated in adults those face obesity in their childhood. In Egyptian population BMI of children were more closely related to mother's BMI than parental BMI (Hassan *et al.*, 2018). In Pakistani Punjabi population current study was done and observed that in females above age 31 years obesity was more common as compared to females having age 15 to 30 years. This finding of current study was similar with the previous studies that increase in age was more closely associated with weight gain (Hassan *et al.*, 2018; Wasim, 2015). The hirsutism was observed in both married and unmarried PCOS women as reported previously in Han Chinese women (Li *et al.*, 2013; Shen *et al.*, 2015). The present study investigate that most sensitive age range for PCOS occurrence was 15-30 years of age in Punjabi women while in another study the age range slightly varies from 20-40 years of age (Zheng *et al.*,2012). The single nucleotide polymorphism is mainly involved in occurrence of different disease. Polymorphism is actually show variation in different forms and it is present in 1% of the population. Variations that are present in gene maintain the degree of expression and levels of protein (Valdivielso and Fernandez, 2006). Considering the involvement of gene various studies showed that there is association of leptin gene receptor with the occurrence of PCOS. The possible link of association of PCOS with LEPR has been investigated previously (Abilash, 2016). Current study examined significant (p= 0.001) association between LEPR gene polymorphism and PCOS. Similarly, A study was conducted on Korean population and reported an association between SNP of Gln223Arg and Pro101Pro in polycystic ovaries syndrome and LEPR (P = 0.033, OR = 1.523, P= 0.0001) (Li *et al.*, 2013). In this study it was observed that frequency of A allel was high in PCOS female (72%, p=0.001) than normal people (46 %). In Iranian population it was observed that A allele were present more frequently in obese women as compared to G allele (Farzam *et al.*, 2017). So, current study was similar with the Iranian studied population because A allele in Iranian obese population and Punjabi Pakistan obese PCOS population was high as compared to the frequency of G. The PCOS subjects for rs1137101 were 54% homozygous AA and there were 0.1% subjects of GG. Similarly, genotypic frequency for control was 24% for AA homozygous and for GG the frequency was 32%. In Saudi women frequency of AA genotype in obese group were 62.9% and the frequency of AG were 16.13% and the GG were 20.96% (Daghestani *et al.*, 2019). Similarly, in India data analysis had shown that there is a significant linkage between *LEPR* Gln223Arg and increased BMI, and obese people faced diabetes due to this disorder (Subramanian *et al.*, 2010). A previous study found association of LEPR gene with PCOS (Pereira *et al.*, 2011).The findings suggested that leptin is involved in pathogenecity of PCOS. Therefore, current study concluded that the *LEPR* gene polymorphism was associated with higher BMI and risk of PCOS and its related disorders in

Punjabi population

References

[1]. Allahbadia, N.G & Merchant, R. (2011). Polycystic ovary syndrome and impact on health. *Middle East Fertility Society Journal*, 16, 19-37

[2]. Abilash, VG.(2016).Candidate gene associated with polycystic ovary Syndrome in Asian populations: A Research Review. *Asian Journal of Pharmaceutical and Clinical Research.* 9(5), 17-20.

[3]. Ali, S., Kallel, A., Ftouchi, B., Sediri, Y., Slimane, H., Jemma, R & Kaabachi, N. (2009). Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. *Clinical Biochemistry*, 42, 584-588.

[4]. Becer, E., Mehmetcik, G., Bareke, H & Serakinci, N. (2013). Association of leptin receptor gene Q223R polymorphism on lipid profiles in comparison study between obese and non-obese subjects. *Gene*, 5-10

[5]. Daghestani, M. H., Bjørklund, G. & Chirumbolo, S. (2019) . The influence of the rs1137101 genotypes of leptin receptor gene on the demographic and metabolic profile of normal Saudi females and those suffering from polycystic ovarian syndrome. *BMC Women's Health* **, 19,** 10.

[6]. Diamanti-Kandarakis, E & Piperi, C.(2005). Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth. *Human Reproduction Update.*11, 631-643

[7]. Dallel, M., Sghaier, I., Finan, R., Douma, Z., Hachani, F., Letaifa, D., Mahjoub, T & Almawi, W.(2019). Circulating leptin concentration, LEP gene variants and haplotypes, and polycystic ovary syndrome in Bahraini and Tunisian Arab women. *Gene*. 694, 19-25

[8]. ESHRE, TR., Group A-SPCW. (2004). Consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and sterility*. 81(1), 19-25

[9]. Goodarzi, MO & Azziz, R.(2006).Diagnosis, epidemiology, and genetics of the polycystic ovary syndrome. *Best practice & research Clinical endocrinology & metabolism.* 20(2), 193-205.

[10]. *F*arzam, F., Mahmazi, S & Nasseryan, J. (2017). Association of Leptin Receptor Gene Gln223Arg and lys109Arg Polymorphisms with Obesity and Overweight in an Iranian Young Population.*Gene,cell,tissue.*4, 3–8.

[11]. Glintborg, D &Anderson,M. (2017). Morbidility in Polycystic ovaries Syndrome. *European Journal of Endocrinology*.176(2), 53-65

[12]. Hussein, Z & Harun,S.(2009). Construction of a polycystic ovarian syndrome (PCOS) pathway based on the interactions of PCOS-related proteins retrieved from bibliomic data. *Theoretical Biology and Medical Modelling.6, 18.*

[13]. Hashemipour, M., Faghihimani, S., Zolfaghary, B., Hovsepian, S., Ahmadi, F & Haghighi S.(2004). Prevalence of polycystic ovary syndrome in girls aged 14– 18 years in Isfahan, Iran. *Hormone Research in Paediatrics.* 62(6), 278-82.

[14]. Hacivelioglu, S., Gungor, ANC., Gencer, M., Uysal, A., Hizli, D & Koc E . (2013). Acne severity and the Global Acne Grading System in polycystic ovary syndrome. *International Journal of Gynecology & Obstetrics.* 123(1), 33- 6.

[15]. Irgama, K., Reddya, B., Annamanenia, S & Rayabarapub, P.(2019).The genetic susceptibility profile of the South Indian women with polycystic ovary syndrome and the universality of the lack of association of type 2 diabetes genes. 6(10), 873-876

[16]. Joseph, N., Reddy, AG., Joy, D., Patel ,V., Santhosh, P & Das S.(2016). Study on the proportion and determinants of polycystic ovarian syndrome among health sciences students in South India. Journal of natural science, *biology, and medicine*. 7(2), 166

[17]. Joshi, B., Mukherjee, S., Patil, A., Purandare, A., Chauhan, S &Vaidya R.(2014).A cross-sectional study of polycystic ovarian syndrome among adolescent and young girls in Mumbai, India. *Indian journal of endocrinology and metabolism*. 18(3), 317.

[18]. Kitzinger, C., Willmott, J.(2002). 'The thief of womanhood': women's experience of polycystic ovarian syndrome. *Social science & medicine*. 54(3), 349-61

[19]. Kansra ,AR. (2016). Polycystic ovary syndrome in adolescents. *Journal of Clinical Outcomes Management.* 23(5).

[20]. Kamboj, MK &Bonny ,AE. (2017).Polycystic ovary syndrome in adolescence: diagnostic and therapeutic strategies. *Translational pediatrics.* 6(4), 248.

[21]. Li, L., Lee, K., Choi, B & Baek, K. (2013). Relationship between leptin receptor and polycystic ovary syndrome. *Gene*. 527, 71-74.

[22]. Lizneva, D., Suturina, L., Walker, W., Brakta ,S., Gavrilova-Jordan, L & Azziz R.(2016). Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertility and sterility*. 106(1), 6-15.

[23]. Laddad, MM., Kshirsagar, NS., Shinde ,G &Shivade ,VS.(2019). Contribution of Weight Gain and Its Association with Insulin Resistance and Dyslipidaemia in Rural Adolescents in Polycystic Ovarian Syndrome-A Prospective Cross- Sectional Study. *Journal of Evolution of Medical and Dental Sciences.* 8(31), 2442-6.

[24]. Liu, Y., Chen, S., Jing, Z., Hou, X., Chen, Y., Song, X., Lv, W., Wang, R &Wang, Y. (2015). Association of LEPR Gln223Arg Polymorphism with T2DM:A meta-analysis. *Diabetes Research and clinical practice.109,* 21-26

[25]. Musmar, S., Afaneh ,A &Mo'alla H.(2013). Epidemiology of polycystic ovary syndrome: a cross sectional study of university students at An-Najah national university-Palestine. *Reproductive Biology and Endocrinology.* 11(1), 47.

[26]. Memon, T., Channar, M., Wadood Shah, SA., Sheikh, A., Batool, M & Shri, N. (2020). POLYCYSTIC OVARY SYNDROME: RISK FACTORS AND ASSOCIATED FEATURES AMONG UNIVERSITY STUDENTS IN

[27]. PAKISTAN. *Journal of peoples university of Medical & health sciences for women Nawabshah SBA.* 10(1), 12-13.

[28]. Mendoza, JM., Licona, N., Martinez, SE., Cardona, MG., Tremary, R &Corona, J.(2005).Association analysis of the Gln223Arg polymorphism in the human leptin receptor gene and traits related to obesity in Mexican adolescent. *Journal of human hypertension*, 19, 341-34.

[29]. Maslyanskaya, S., Talib, HJ., Northridge ,JL., Jacobs ,AM., Coble,C &Coupey, SM.(2017). Polycystic ovary syndrome: an under-recognized cause of abnormal uterine bleeding in adolescents admitted to a children's hospital. *Journal of pediatric and adolescent gynecology*. 30(3), 349-55. .

[30]. Morreale-Escobar, H.F.,Ramirez-Luque, M & Millan San, J.L.(2005).The Molecular Genetic Basis of Functional hyperandrogenism and the Polycystic Ovary Syndrome. *Endocrine Reviews*, 26(2), 251-282

[31]. Nidhi, R., Padmalatha, V., Nagarathna ,R & Amritanshu, R.(2011). Prevalence of polycystic ovarian syndrome in Indian adolescents. *Journal of pediatric and adolescent gynecology*. 24(4), 223-7.

[32]. Naz, MSG., Tehrani, FR., Majd, HA., Ahmadi, F., Ozgoli, G& Fakari, FR. (2019). The prevalence of polycystic ovary syndrome in adolescents: A systematic review and meta-analysis. *International Journal of Reproductive BioMedicine.* 17(8), 533.

[33]. Oksanen, L., Kaprio J, T., HA, K., Koistinen, S& Kontula, K. (2007).No evidence for mutations of the leptin or leptin receptor genes in women with polycystic ovary syndrome. *Molecular Human Reproduction*. 6(10), 673-675

[34]. Panda, P., Rane, R., Ravichandra, R., Singh, S & Panchal, H.(2016). Genetics of PCOS: A systematic bioinformatics approach to unveil the proteins responsible for PCOS. *Genomic data.* 8(52-60).

[35]. Pusalkar, M., Meherji, P.,Gokral, J., Savardekar, L., Chinnaraj, S & Maitra, A.(2010).Obesity and polycystic ovaries syndrome: Association with androgens, leptin and its genotypes. *Gynecological Endocrinology*. 26(12), 874-882

[36]. Paul, S & Smith, L. (2005). The metabolic syndrome in women: a growing problem for cardiac risk. *Journal of Cardiovascular Nursing*. 20(6), 427-32.

[37]. Pereira, T., Netto, R & Yamada, Y. (2011). ADRB2 and LEPR Gene Polymorphisms: Synergistic Effects on the Risk of Obesity in Japanese. *Obesity*. 10, 322-331

[38]. Perez-Perez, A.,Toro,A.,Vilarino-Garcia, T., Maymo, J., Guadix, P., Duenas,J.L.,Sanchez-Fernandez, M.,Varone,C & Margalet-Sanchez V.(2017).Leptin action in normal and pathological pregnancies. *Journal of Cellular and molecular medicine*.11, 1-12

[39]. Ruan, X., Li ,M 7 Mueck,AO.(2018). Why does Polycystic Ovary Syndrome (PCOS) Need Long-term Management.*Current pharmaceutical design*. 24(39), 4685- 92.

[40]. Rosenfield, RL & Ehrmann, DA.(2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocrine reviews*. 37(5), 467-520.

[41]. Scicchitano, P., Dentamaro ,I., Carbonara ,R., Bulzis, G., Dachille, A &Caputo.(2012). Cardiovascular risk in women with PCOS. *International journal of endocrinology and metabolism*. 10(4), 611.

[42]. Smaism, F. (2016). Assessment of leptin levels

in the different genotypes and leptin receptor genes in the women with polycystic ovary syndrome and diabetes mellitus type 2 in Iraq population. *International journal of Pharm Tech Research.* 9(5), 269-276

[43]. Sarif, E., Rahman, S., Zia ,Y & Rizk ,NM.(2017).The frequency of polycystic ovary syndrome in young reproductive females in Qatar. *International journal of women's health*. 9(1).

[44]. Tee, M., Speek, M., Legeza, B., Modi, B., Teves, M., McAllister,J.,StraussIII,J & Miller, W.(2016).Alternative splicing of DENNDIA, a PCOS candidate gene, generates variant 2.*Molecular and cellular Endocrinology.* 434,25-3

[45]. Tu, X., Yu, C., Gao, M., Zhang, Y., Zhang, Z., He, Y., Yoa, L., Du, J., Sun, Y& Sun, Z. (2017). LEPR gene polymorphism and plasma soluble leptin receptor levels are associated with polycystic ovary syndrome in Han Chinese women. 14(4), 299-307

[46]. Tehrani, FR & Amiri, M.(2019). Polycystic Ovary Syndrome in Adolescents: Challenges in Diagnosis and Treatment. *International journal of endocrinology and metabolism*. 17(3).

[47]. Vrbikova, J., Vondra, K., Cibula, D., Dvorakova, K., Stanicka, S& Sramkova D. (2005). Metabolic syndrome in young Czech women with polycystic ovary syndrome. *Human Reproduction*. 20(12), 3328- 32.

[48]. Wauters, M., Considine, V.R., Chagnon, M., Mertens, I., Rankine, T., Bouchard, C & Gaal, V.(2002). Leptin Levels, Leptin Receptor Gene Polymorphisms, and Energy Metabolism in Women. *Obesity Research*.10, 395.

[49]. Wolf,W.M.,Wattick,R.A.,Kinkade,O.N & Olfert. D.M. (2018).Geographical Prevelance of Polycystic Ovary Syndrom as Determined by Region and Race/Ethinicity, *Environmental Research and Public Health.*

[50]. Xita, N., Georgiou & Tsatsoulis . (2002).The genetic basis of polycystic ovaries syndrome. *European Journal of Endocrinology*,147, 717-725

[51]. Ybarra, M., Franco ,R., Cominato, L., Sampaio, R., Sucena, S & Damiani ,D.(2016). Prevalence and characteristics of polycystic ovary syndrome in obese adolescents. *European Society for Paediatric Endocrinology.*

[52]. Zafar, U., Memon, Z., Moin, K., Agha ,S., Hassan ,JA& Zehra, D. (2019). Prevalence of PCOS with Associated Symptoms and Complications at Tertiary Care Hospital of Karachi. *Journal of Advances in Medicine and Medical Research.* 1-9

[53]. Zandi, S., Farajzadeh, S & Safari, H. Prevalence of polycystic ovary syndrome in women with acne: hormone profiles and clinical findings. (2016). *Journal of Pakistan Association of Dermatology*. 20(4), 194-8.

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