Abstract:

Polycystic syndrome (PCOS) is a considerate infertility disorder in adolescents and adult women in reproductive age. Obesity is a vigorous risk factor related to PCOS. This study aims to evaluate the association of obesity and PCOS by investigating several parameters including: anthropological, biochemical (lipid profile, fasting blood sugar, glucose tolerance test, and hormone levels (LH, FSH, LH/FSH ratio, Estradiol2 and Testosterone), and genetic parameters (Fat mass and Obesity associated gene (FTO) polymorphism at rs17817449) in 63 obese and non-obese PCOS women. The biochemical tests were investigated by colorimetric methods while FTO gene polymorphism was detected by PCR–RFLP. Lipid profile, FBS, GTT, hormones (LH, LH/FSH ratio) in obese PCOS patients were significantly higher than non-obese non PCOS patients. It was found that the FTO variant TT risk genotype is a predisposing factor to obesity but not for PCOS. The study substantiated a possible familial risk factor for developing obesity among women in the same family.

Key words: FTO, Obesity, PCOS, Polymorphism, rs17817449.

Introduction:

Polycystic ovary syndrome (PCOS) is a complex infertility disorder in adolescents (1) and adult women in the reproductive age (2). Stein and Leventhal who were firstly linked between PCOS and obesity in 1935 when 3/7 of first PCOS diagnosed patients were obese (3). The main features of this syndrome are oligo/anovulation, and disturbance in androgen hormones leading to different ranges of hirsutism, weight gain, acne, and amenorrhea according to Rotterdam criteria (4, 5). Overweight or being obese with body mass index (BMI) greater than 30 kg/m² is a vigorous risk factor leading to PCOS women in around the age of 30 (5, 6, 7), in addition to represent a risk factor to cardiovascular disease, diabetes mellitus type 2, hypertension and several types of cancers (8). Obesity and PCOS are multifactorial diseases resulting from the interaction of genetics, metabolic, hormones, patient’s life style, eating habits and psychological behavior(9). The potential genetic investigations focused on the expression, mutations and polymorphism in metabolism related genes, action related genes and genes that encode for reproductive controlling hormones and their receptor(10). Structural chromosomal aberration such as X chromosome aberration leading to abnormal follicular behavior and deletion of the loge arm of chromosomes 2, 11, 16, 19 were reported in some PCOS cases (11, 12, 13, 14 and 15). It is not clear whether obesity leads to PCOS or vice versa. The cornerstone for both PCOS and obesity is insulin resistance that leads to excess levels of insulin and glucose uptake failure by adipose and muscle cells, increasing the risk of
diabetes type II (16), dyslipidemia, decrease in high density lipoprotein cholesterol, hypertriglyceridemia and hypertension which are highly associated with cardiovascular diseases (17). Fat mass and obesity association gene was discovered in 2007 by 3 independent wide genomic association studies in association with polygenic forms of obesity (11). It is highly expressed in neurons of hypothalamus and mesenteric, liver, pancreas and adipose tissues(18,19) to promote the neonatal growth (20) and adults by inducing appetite, food intake, increase hunger /lower satiety and energy balance (21), leading to increase in body weight and hip/circumference ratio (22). FTO gene expands about 40 kb on chromosome 16q12.2, with 9 exons, encoding for 2-oxoglutarate dependent dioxygenase with DNA/RNA demethylase activity (23). The most important polymorphic sites are located at the first introns and affect their expression level (24). Numerous single nucleotide polymorphic sites were proved in association to obesity such as rs7202116, rs9930506, rs1421085, rs3751812, rs9939609 and rs17817449 (25).

This work aims to illustrate the relations of biochemical features and FTO polymorphism in women with obesity and polycystic ovary syndrome.

Material and methods:
Study subjects
This study includes 63 Iraqi women at age 18-38 years, who visit the infertility center for infertility problems during 2014-2016. The studied subjects were diagnosed in the infertility center as PCOS according to Rotterdam criteria (4), 39 women with PCOS (23 obese and 16 non-obese) and 24 non-PCOS (15 obese and 9 non-obese). Several parameters were investigated: anthropological parameters covered (age, height, weight body mass index (BMI) as weight by kg/height by m², Waist/hip circumference ratio and waist /hip circumference ratio), biochemical parameters covered (fasting blood sugar test, glucose tolerance test and lipid profile), and hormonal parameters (follicle stimulating hormone (FSH), luteinizing hormone (LH) , LH/FSH ratio, estrogen (E) and testosterone). Genetic investigation for the polymorphism in FTO gene also was performed.

Blood sample collection for the biochemical and hormonal investigations
Fasting blood samples were collected from all the participants in this study, (4 ml) was poured in plane tubes and sera were separated by centrifuging the samples for 15 minutes at 3000rpm for the biochemical and hormonal investigations. Serum hormonal analysis of LH, FSH, E2 and total testosterone were measured by using MiniVidas instrument. Serum levels of Glucose, Total Cholesterol, Triglycerides, and High-Density Lipoprotein Cholesterol (HDLC) were measured by a commercially available kit (all kits from Biomerieux, Sa, France) and they were measured spectrophotometrically, according to manufacturer instructions. Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) levels were calculated using the formulae supplied by the manufacturer. The biochemical study was done at the biochemical laboratory at the High institute of infertility diagnosis and assisted reproductive technologies, Al Nahrin University, Baghdad, Iraq.

DNA extraction and PCR reaction
Total DNA was extracted from 1mL of peripheral blood of each patient by Relia Prep DNA extraction kit/ Promega /USA, according to manufacturer instructions.

Primers and PCR Conditions
Forward and reverse primers for rs17817449 were designed by Primer3plus software, and provided by Macrogen/Korea. The forward primer sequence 5’AGGACCTCCTATTTGGGACA3’ and the revers: 5’TAATGCTAGCCATGGAAGC3’. Polymerase chain reactions were performed in Applied Biosystem Thermostation (USA). The PCR products were successfully amplified by mixing 1µL of each primer, 12.5µL of Go Tag Green Master Mix/ Promega. The reaction mixture composed of 2 µL of patient’s DNA, 8.5 µL of D.W. with final volume 25 µL. Amplification conditions were: the initial denaturation was single cycle for 7 minutes at 95 ºC, 35 cycles each one includes denaturation at 95 ºC for 30 seconds, annealing at 60 ºC for 45 seconds and extensions at 72 ºC for 30 seconds, then final cycle extension at 72 ºC for 7 minutes. The size of PCR product is 828 bp.
Restriction fragment length polymorphism detection was done by incubating 10μl of PCR products with 0.25μl of AlwN1(New England Biolabs, UK) for 30 minutes at 37 °C for all samples, and then products were electrophoresed in 2% Agarose gel for 75 minutes at 80 volts. Bands with 828bp for the homozygous dominant alleles (G/G), 500bp and 328bp bands for the homozygous recessive alleles (T/T) and three bands ,800bp,500bp and 328bp for the heterozygous (T/G) were visualized and documentation by UV transilluminator (Optima/ Japan).

Statistical analysis
The Statistical Analysis System (according to SSPS version 21 software) was used to assess the differences among different parameters in this study. Independent t-test was applied to compare the data of anthropological and biochemical tests results. Chi-square test was used to analyze alleles and genotypes frequencies among the studied groups as well as to evaluate the studied population genetic equilibrium by Hardy-Weinberg Equation.

Results and discussion:
Anthropological and Biochemical Investigations
Polycystic syndrome is a complicated infertility problem; it may develop due to metabolic or hormonal causes. The results of our investigations are summarized in Tables 1, 2, 3 and 4. The anthropological parameters including age, height, weight body mass index (BMI as weight by kg/height by m²), Waist /hip circumference ratio and waist /thigh circumference ratio data were subjected to statistical analysis among the obese PCOS, non-obese PCOS, obese non-PCOS and non-obese- non PCOS groups as shown in Table 1. There were no significant differences in the age of women (26.35 ± 4.11, 23.67 ± 4.17, and 28.81± 4.49 and 26.82 ± 3.97) years, of the obese PCOS, non-obese PCOS, obese non-PCOS and non-obese non-PCOS groups respectively. There were significant differences at P-value ≥0.05, in BMI means between obese PCOS and non-obese PCOS women as they were (32.51 ± 4.91 and 23.28 ± 1.73) kg/m² respectively, also there were significant differences between the means of BMI between obese non-PCOS and non-obese non-PCOS women (29.97 ± 2.91 and 23.67 ± 1.34) kg/m², and significant differences of the means of BMI between obese PCOS and obese non-PCOS (32.51 ± 4.91 and 29.97 ± 2.91) kg/m², but there were no significant differences between non-obese PCOS and non-obese non-PCOS women. The only differences in Waist/Hip ratio means were seen between obese PCOS and non-obese PCOS (0.84 ± 0.06 and 0.74 ± 0.09) and obese-PCOS and obese non-PCOS (32.51 ± 4.91 and 29.97 ± 2.91) respectively. Women with high mean of waist /high ratio were significantly higher in women with obese PCOS (1.43 ± 0.15) than non-obese PCOS women (1.28 ± 0.18); and there were no significant differences between means of obese non-PCOS women and non-obese non-PCOS women (1.35 ± 0.15 and 1.39 ± 0.16) respectively.

Anthropological parameters had great attention in PCOS as its measurable diagnostic markers. In this study the age had no significant association in the PCOS because all 68 participants were in the same age, even though PCOS can affect women over wide range of their fertility life (12-55) years. In this study it has been found that 39 of women were obese, 23 (58.9%) of them had PCOS with significantly high BMI than those non-obese. Gain of weight or high BMI may be a sign with predicted value of PCOS (26). Body mass index, waist/ hip and waist circumference were found high in Asian women according to WHO report at 2008 (8) and associated with accumulation of adipose in visceral leading to abdominal obesity in PCOS women rather than non-PCOS women (27).

Table 2 shows the levels of fasting blood sugar and glucose tolerance test among the four studied groups. A significant difference was seen in the means of fasting blood sugar between obese women with PCOS and non-obese women with PCOS(101.22 ± 12.02 and 88.36 ± 14.85) mg/dl such differences were seen in the means of FBS between obese women with PCOS and obese women without PCOS (101.22 ± 12.02 and 85.93 ± 17.32) mg/dl, respectively. But there were no significant differences between the means of obese non-PCOS and non-obese non PCOS women. Moreover no significant differences were noticed in the means between non-obese PCOS and non-obese non-PCOS women. The results of glucose tolerance test showed significant differences in the means of the glucose levels after 1/2hour, between obese women with PCOS and non-obese PCOS women (170.40 ± 30.92 , 134.00 ± 34.41) mg/dl; such a significant difference was seen in the sugar level between obese women with PCOS (170.40 ± 30.92) and obsess non-PCOS women (128.00 ± 30.38) mg/dl. No significant differences were seen in the serum sugar level after 1/2 hr. between obese non-PCOS women and non-obess, non-PCOS women. Furthermore, no significant differences were remarked between non-obese PCOS and non-obese non PCOS women. The only significant differences in blood sugar level, after one hour of taking 100 ml of glucose solution, was between obese PCOS women and obese non-PCOS women which they
were (156.96 ± 31.57 and 117.86 ± 27.65) mg/dl, respectively. Blood sugar level after 2 hr. was significantly high between obese PCOS women and obese non-PCOS women (124.74 ± 30.91 and 100.08 ± 19.23) mg/dl, respectively as shown in Table 2.

The results of fasting blood sugar and 2-h glucose tolerance test significantly high obese women rather than had PCOS or not. These results might relate to insulin resistance that is considered as a causative of hyperglycemia (28). High level of serum glucose may induce FTO expression in hypothalamic neurons (29), starting of sequential events leading to eating disorder behavior and obesity (7).

Sex hormones that control the ovulation, menstruation and fertility were investigated in this study and hormones levels are shown in Table 3. There were no significant differences among the four groups in FSH level, obese PCOS, non-obese PCOS, obese non-PCOS and non-obese non-PCOS. Significant differences noted in LH levels between obese PCOS women (7.67 ± 2.50) and non-obese PCOS (5.35 ± 3.08). Additionally, there were significant differences in LH level between obese PCOS women (7.67 ± 2.50) and obese non-PCOS women (3.45 ± 2.01) and non-obese PCOS (5.35 ± 3.08) and non-obese non-PCOS women (2.95 ± 1.10). No significant differences were found in LH level between obese non-PCOS.

The ratio of LH/FSH is an indicator of cystic ovary (30); there were no significant differences in the means of the ratios between obese women with PCOS and non-obese women with PCOS, and no differences between the means of obese non-PCOS women and non-obese non-PCOS women. The differences were significantly high between means of the ratio of obese women with PCOS (1.41 ± 0.41) and obese non-PCOS women (0.48 ± 0.15), Addition to the high significant differences obtained between women non-obese PCOS (1.11 ± 0.45) and non-obese non-PCOS women (0.42 ± 0.19). The means of Estradiol 2(E2) level significantly differ in obese PCOS women (76.29 ± 29.45) than non-obese PCOS women (47.29 ± 18.10) as well as the means of E2 in obese PCOS(76.29 ± 29.45) and obese non-PCOS (47.88 ± 16.15). Estradiol 2 levels in obese non-PCOS (47.88 ±16.15) women and non-obese non-PCOS (52.33±14.86) did not differ significantly; and no significant differences in E2 levels between non-obese PCOS women and non-obese non PCOS women were given. Testosterone levels did not significantly differ between obese PCOS women and non-obese PCOS women and obese non-PCOS women, but there were significant differences in testosterone levels between obese PCOS (1.02 ±0.67) and obese non-PCOS (0.31 ± 0.17), and no differences where shown between non-obese PCOS and non-obese non-PCOS women.

Imbalance in androgen levels is the most common diagnostic characteristic of PCOS. Although the initial reason of PCOS is unclear, yet it may be justified by reducing FSH releasing for long time in association with elevation in LH levels (31); FSH naturally promotes the growth of ovarian follicles, and its reduction will lead to prevent the maturation of the follicles which become small cysts, then the ovulation is prevented. The abnormal secretion of gonadotropin in PCOS women is indicated by the ratio of LH/FSH which is 2/1 to 3/1 in PCOS women (32). The suggested mechanism to clarify the elevated LH secretion is the neuroendocrine abnormalities involved in the continual excretion of gonadotropin releasing hormone and dysfunction of gonadotropin–inhibiting hormone in PCOS women (33). In this study, the results of estradiol 2 showed that women with PCOS are even being obese or not, they had shown a lower level than that of non-PCOS women. This result is a common feature in PCOS (34).

Table 4 shows the results of lipid profile in the studied groups to illustrate the role of serum cholesterol, Triglycerides, VLDL, LDL and HDL in obesity and PCOS. Serum Cholesterol concentrations significantly differ in obese PCOS (155.70 ± 27.25) mg/dl than non-obese PCOS (126.57 ± 27.91) mg/dl. Also cholesterol concentration in obese PCOS women (155.70 ± 27.25) mg/dl was significantly different than obese non-PCOS women (187.06 ± 51.40) mg/dl, but no significant differences in cholesterol concentration between obese non-PCOS women and non-obese non-PCOS women were found. No differences were noticed between non-obese PCOS and non-obese non-PCOS. Triglyceride concentrations were significantly different between obese PCOS women (126.26 ± 63.84) mg/dl and obese non-PCOS women (94.64 ± 28.18) mg/dl. No such differences were seen between obese non-PCOS and non-obese non-PCOS or between obese PCOS and obese non-PCOS nor between non-obese PCOS and non-obese non-PCOS. The means of very low density lipoprotein were not different significantly in all the studied groups and they were within the normal values. The only significant results of serum low density lipoprotein means were seen between the obese PCOS women.
### Table 1. Anthropometric parameters comparison among the obese PCOS, non-obese PCOS, obese non PCOS and non-obese non PCOS groups.

| Parameters          | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 15) (mean ± SD) | P-value | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 9) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 15) (mean ± SD) | Non-obese PCOS (n= 9) (mean ± SD) | P-value |
|---------------------|---------------------------------|------------------------------------|---------|---------------------------------|------------------------------------|---------|-----------------------------|------------------------------------|---------|-------------------------------|------------------------------------|---------|
| Age (yrs.)          | 26.35 ± 4.11                    | 23.67 ± 4.17                       | 0.060   | 28.81 ± 4.49                    | 26.82 ± 3.97                       | 0.236   | 26.35 ± 4.11                | 28.81 ± 4.49                       | 0.090   | 23.67 ± 4.17                  | 26.82 ± 3.97                       | 0.062   |
| BMI (kg/m2)         | 32.51 ± 4.91                    | 23.28 ± 4.91                       | <0.001* | 32.51 ± 2.91                    | 29.97 ± 2.97                       | <0.001* | 32.51 ± 2.91                | 29.97 ± 2.97                       | 0.050*  | 23.28 ± 4.91                  | 29.97 ± 2.97                       | 0.519   |
| Waist/Hip ratio     | 0.84 ± 0.06                     | 0.74 ± 0.09                        | <0.001* | 0.09                            | 0.06                               | 0.0823  | 0.84 ± 0.06                 | 0.78 ± 0.09                        | 0.002*  | 0.09                           | 0.06                               | 0.115   |
| Waist/Thigh ratio   | 1.43 ± 0.15                     | 1.28 ± 0.18                        | 0.011*  | 1.35 ± 0.15                     | 1.39 ± 0.16                        | 0.467   | 1.43 ± 0.15                 | 1.35 ± 0.15                        | 0.090   | 1.28 ± 0.18                   | 1.39 ± 0.16                        | 0.107   |

BMI=Body Mass Index, * Significant at P-value ≥ 0.05.

### Table 2. Fasting blood sugar and glucose tolerance test among the obese PCOS, non-obese PCOS, obese non PCOS and non-obese non PCOS groups.

| Parameters          | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 15) (mean ± SD) | P-value | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 9) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 15) (mean ± SD) | Non-obese PCOS (n= 9) (mean ± SD) | P-value |
|---------------------|---------------------------------|------------------------------------|---------|---------------------------------|------------------------------------|---------|-----------------------------|------------------------------------|---------|-------------------------------|------------------------------------|---------|
| FBS (mg/dl)         | 101.22 ± 12.02                  | 89.36 ± 14.85                      | 0.011*  | 89.53 ± 17.32                   | 89.64 ± 10.39                      | 0.504   | 101.22 ± 12.02              | 85.93 ± 17.32                      | 0.006*  | 88.36 ± 14.85                  | 89.64 ± 10.39                      | 0.802   |
| PP (after ½ hr.) (mg/dl) | 170.40 ± 30.92                  | 134.00 ± 34.41                     | 0.003*  | 128.00 ± 30.38                  | 132.45 ± 12.07                     | 0.633   | 170.40 ± 30.92              | 128.00 ± 30.38                     | <0.001* | 134.00 ± 34.41                | 132.45 ± 12.07                     | 0.877   |
| PP (after 1 hr.) (mg/dl) | 156.96 ± 31.57                  | 136.93 ± 30.59                     | 0.066   | 117.86 ± 27.65                  | 122.27 ± 15.61                     | 0.619   | 156.96 ± 31.57              | 117.86 ± 27.65                     | <0.001* | 136.93 ± 30.59                | 122.27 ± 15.61                     | 0.135   |
| PP(after 2 hrs.) (mg/dl) | 124.74 ± 30.91                  | 108.00 ± 21.20                     | 0.059   | 100.08 ± 19.23                  | 100.55 ± 13.89                     | 0.945   | 124.74 ± 30.91              | 100.08 ± 19.23                     | 0.005*  | 108.00 ± 21.20                | 100.55 ± 13.89                     | 0.301   |

* Significant at P-value ≥ 0.05. PP: Post prandial.

### Table 3. comparisons of Sex hormone level and LH/FSH ratio among the Obese PCOS, Non-obese PCOS, Obese non PCOS and non-obese non PCOS group.

| Parameters          | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 15) (mean ± SD) | P-value | Obese PCOS (n= 23) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 9) (mean ± SD) | Non-obese PCOS (n= 16) (mean ± SD) | P-value | Obese PCOS (n= 15) (mean ± SD) | Non-obese PCOS (n= 9) (mean ± SD) | P-value |
|---------------------|---------------------------------|------------------------------------|---------|---------------------------------|------------------------------------|---------|-----------------------------|------------------------------------|---------|-------------------------------|------------------------------------|---------|
| FSH (mIU/mL)        | 5.66 ± 1.61                     | 5.22 ± 1.79                        | 0.454   | 7.10 ± 3.01                     | 7.22 ± 1.32                        | 0.903   | 5.66 ± 1.61                 | 7.10 ± 3.01                        | 0.143   | 5.22 ± 1.79                    | 7.22 ± 1.32                        | 0.003*  |
| LH (mIU/mL)         | 7.67 ± 2.50                     | 5.35 ± 3.08                        | 0.025*  | 3.45 ± 2.01                     | 2.95 ± 1.10                        | 0.468   | 7.67 ± 2.50                 | 3.45 ± 2.01                        | 0.001*  | 5.35 ± 3.08                    | 2.95 ± 1.10                        | 0.015*  |
| LH/FSH ratio        | 1.41 ± 0.41                     | 1.11 ± 0.45                        | 0.058   | 0.48 ± 0.15                     | 0.42 ± 0.19                        | 0.425   | 1.41 ± 0.41                 | 0.48 ± 0.19                        | <0.001* | 1.11 ± 0.45                    | 0.42 ± 0.19                        | <0.01*  |
| E2 (pg/mL)          | 76.29 ± 29.45                   | 47.29 ± 18.10                      | 0.005*  | 47.88 ± 16.15                   | 52.33 ± 14.86                      | 0.518   | 76.29 ± 29.45              | 47.88 ± 16.15                      | 0.004*  | 47.29 ± 18.10                  | 52.33 ± 14.86                      | 0.505   |
| Testosterone (ng/mL)| 1.02 ± 0.67                     | 0.90 ± 0.67                        | 0.672   | 0.31 ± 0.17                     | 0.22 ± 0.11                        | 0.180   | 1.02 ± 0.67                 | 0.31 ± 0.17                        | <0.001* | 0.90 ± 0.67                    | 0.22 ± 0.11                        | 0.020*  |

PCOS = polycystic ovary syndrome, FSH = follicular stimulating hormone, LH = luteinizing hormone, E2 = estradiol, FBS = fasting blood sugar, BS = blood sugar, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein, * Significant at P value ≤0.05.
Table 4. Comparison of Cholesterol, TG, VLDLC, LDLC, and HDLC among the obese PCOS, non-obese PCOS, non-obese non PCOS group.

| Parameters     | Obese PCOS (n=23) (mean ± SD) | Non-obese PCOS (n=15) (mean ± SD) | P-value | Obese PCOS (n=16) (mean ± SD) | Non-obese PCOS (n=9) (mean ± SD) | P-value | Obese PCOS (n=23) (mean ± SD) | Non-obese PCOS (n=15) (mean ± SD) | P-value | Obese PCOS (n=9) (mean ± SD) | Non-obese PCOS (n=16) (mean ± SD) | P-value |
|----------------|-----------------------------|----------------------------------|--------|-------------------------------|---------------------------------|--------|-------------------------------|---------------------------------|--------|-------------------------------|---------------------------------|--------|
| Cholesterol    | 155.70 ± 27.25              | 126.57 ± 27.91                   | 0.004* | 187.06 ± 51.40               | 155.45 ± 40.45                 | 0.086  | 155.70 ± 27.25               | 187.06 ± 51.40                 | 0.036* | 126.57 ± 27.91               | 155.45 ± 40.45                 | 0.059  |
| TG (mg/dl)     |                             |                                  |        |                               |                                 |        |                               |                                 |        |                               |                                 |        |
| VLDLC (mg/dl)  | 24.91 ± 12.77               | 18.71 ± 6.59                     | 0.046* | 32.83 ± 6.17                 | 23.89 ± 8.96                   | 0.243  | 24.91 ± 12.77               | 32.83 ± 6.17                 | 0.298  | 18.71 ± 6.59                  | 23.89 ± 8.96                   | 0.116  |
| HDLC (mg/dl)   | 39.86 ± 4.52                | 40.21 ± 3.12                     | 0.784  | 39.38 ± 3.01                 | 37.82 ± 3.03                   | 0.201  | 39.86 ± 4.52                | 39.38 ± 3.01                 | 0.691  | 40.21 ± 3.12                  | 37.82 ± 3.03                   | 0.065  |

TG= Triglyceride, VLDLC = very low density lipoproteinC, LDLC = low density lipoprotein, HDLC = high density lipoproteinC, * Significant at P value ≥ 0.05.

(93.32 ± 30.15) mg/dl and non-obese PCOS women (67.36 ± 30.90) mg/dl. While other groups mean did not differ significantly. The means of high density lipoproteins did not significantly differ in all studied groups and they were within the normal values. Lipid profile represented by total cholesterol, triglyceride, HDL, LDL and VLDL was investigated in this study. Cholesterol was found significantly higher in obese PCOS than obese non-PCOS women; while the cholesterol triglyceride and LDL were found significantly higher in PCOS regardless of whether they were obese or not. Elevated TC, and HDL but not in TRG were found significantly high in a pervious Iraqi study, performed in Karbala governorate, and referred to that high lipid profile in PCOS women may predicts to metabolic syndromes and cardiovascular complications (35). In another Polish study, significantly high TC, TRG and LDL concentrations but not the HDL concentrations were found in obese or overweight PCOS Polish women, compared to lean/normal women (36).

**Gene Polymorphism investigation by RFLP-PCR**

This study included the detection of the possible role of polymorphism in FTO gene rs17817449 in obesity and polycystic ovarian syndrome. The DNA was successfully extracted by Promega genomic DNA extraction kit, concentration of DNA ranged between (23-112) ng/μl. Successful PCR products were electrophoresed in 2% agarose gel is showed in Fig. 1.

![PCR products of FTO rs17817449, band size 828 bp, 2% agarose gel electrophoresis for 45min, at 80 v. Lane represents the 1500 bp ladder, 1-13 samples of PCR products.](image)

**Figure 1.** PCR products of FTO rs17817449, band size 828 bp, 2% agarose gel electrophoresis for 45min, at 80 v. Lane represents the 1500 bp ladder, 1-13 samples of PCR products.

**Figure 2.** RFLP analysis of FTO rs17817449, Homozygous alleles GG seen as 828 bp band, the homozygous alleles for TG seen as two bands 500 and 328 bp and heterozygous alleles seen as three bands 828bp for G allele and 500bp and 328bp for T allele

The genotype analysis of FTO gene by restriction fragment length polymorphism technique is shown in Fig. 2. The digestion of PCR products of FTO gene rs17817449 by AlwNI restriction
enzyme showed a single fragment 828 bp represents the dominant homozygous genotype. Two bands with size 500 bp and 328 bp represent the recessive homozygous genotype. The heterozygous genotype is seen as three bands with size 828, 500 and 328 bp.

Table 5. Allele frequency of the G and T alleles in the studied groups.

| Allele | Non PCOS | PCOS | Obese | Non Obese | P-value |
|--------|----------|------|-------|-----------|---------|
| Frequency | 25 (39.68%) | 38 (60.32%) | 39 (61.9%) | 24 (38.1%) | NS |
| G       | 0.47     | 0.52  | 0.53   | 0.44      | NS      |
| T       | 0.53     | 0.48  | 0.47   | 0.56      | NS      |

Allele frequency was predicted by Hardy-Weinberg equation as shown in Table 5, the wild type G allele was slightly less expressed in PCOS women (0.47) than in non PCOS women (0.52), but there are no significant differences between the two groups. The recessive allele T was more frequent in PCOS women (0.53) than in non PCOS women (0.48); and there were no significant differences between the two groups. The dominant G allele was more expressed in obese women (0.53) than non-obese women (0.44) but there are no significant differences between the two groups. In opposite manner the allele T was less observed in Obese (0.47) than non-obese women (0.56) without significant differences.

Table 6. The percentage of genotypes distribution in obese PCOS and obese non-PCOS groups.

| Genotype | PCOS NO=23 | Non PCOS NO=16 | P-value | Odd ratio |
|----------|------------|----------------|---------|-----------|
| GG       | 4 (17.4%)  | 3 (18.75%)     | 0.864 NS| 0.006     |
| GT       | 17 (73.9%) | 10 (62.25%)    | 0.042*  | 0.573     |
| TT       | 2 (8.7%)   | 3 (18.75%)     | 0.047** | 0.581     |
| P-value  | 0.0001**   | 0.0001**       |         |           |

The distribution of genotypes in obese PCOS and obese non-PCOS women are clarified in Table 6. No significant differences were observed in obese women carrying GG genotype even though they had PCOS (4, 17.4%) or non PCOS (3, 18.75%), but there was significant differences at P<0.05 in the distribution of GT genotype between PCOS (17, 73.9%) and (10, 62.2%) in obese non-PCOS women. The recessive TT genotype was significantly at P <0.05 different between obese women with PCOS (2, 8.7%) and non PCOS (3, 18.7%).

Table 7 shows the distribution of genotypes in the non-obese women with and without PCOS. In vertical comparison, the genotype GG seems to be less apparent in non-obese PCOS women rather than the TT genotype in the same category with significant differences at (P<0.01). In a reverse manner GG genotype was significantly higher in non-obese, non PCOS women than non-obese PCOS women, than the TT genotype at (P<0.01). In horizontal comparison, slim or non-obese women and non-PCOS was significantly high at GG genotype which appeared in (3, 33.3%) than non-obese PCOS women (1, 6.7%), at P<0.001. High significant differences between non obese women with PCOS (9, 60%) and non-PCOS (4, 44.4%) those carried the GT genotype at P<0.001. The rare genotype (TT) was represented in non-obese women with PCOS (5, 33.3%) which was significantly higher than in non-obese and non PCOS women (2, 22.2%) at P<0.05.

Table 7. The percentage of genotypes distribution in non-obese PCOS and non-PCOS groups.

| Genotype | PCOS NO=15 | Non PCOS NO=9 | P-value | Odd ratio |
|----------|------------|---------------|---------|-----------|
| GG       | 1 (6.79%)  | 3 (33.3%)     | 0.0071**| 1.276     |
| GT       | 9 (60%)    | 4 (44.4%)     | 0.0149**| 0.794     |
| TT       | 5 (33.3%)  | 2 (22.2%)     | 0.0472* | 0.559     |
| P-value  | 0.0001**   | 0.0001**      |         |           |

NS= Non-significant at P-value < 0.05, * (P< 0.05), ** (P< 0.01)
Table 8 shows the distribution of genotypes in PCOS obese and non-obese women. In vertical comparison, the genotype GG seems to be higher apparent in PCOS obese women than the TT genotype in the same category, but the GG genotype was less apparent than TT genotype in PCOS non-obese women at P<0.05. In horizontal manner, GG genotype significantly higher in PCOS obese women (4, 17.4%) than (1, 6.7%) in PCOS non-obese women at P<0.05. Also high significant differences were seen also in the TT genotype frequencies between PCOS obese (2, 8.7%) and PCOS non-obese women (5, 33.3%) at P≤0.01.

Table 8. The percentage of genotypes distribution in PCOS, obese and non-obese groups.

| Genotype | Obese (No=23) | Non obese (No=15) | P-value | Odd ratio |
|----------|---------------|-------------------|---------|-----------|
| GG       | 4 (17.4%)     | 1 (6.7%)          | 0.049 * | 0.521     |
| GT       | 17 (73.9%)    | 9 (60%)           | 0.042 * | 0.586     |
| TT       | 2 (8.7%)      | 5 (33.3%)         | 0.0136 ** | 0.802 |
| P-value  | 0.0001**      | 0.00836**         |         |           |

P-value < 0.05, * (P<0.05), ** (P<0.01)

Fat mass obesity associated gene polymorphism was extensively studied in obesity and metabolic disorder as it has a high effect on the health and psychological behavior related to obesity. Most important polymorphic site located in the first intron of the gene which may associate with the regulation of the FTO gene expression in different tissues, disorders and ethnic groups (37, 38). In this study rs17817449 polymorphism was analyzed in to determine its effect in obesity and PCOS.

The results of this study illustrate no significant differences in allele frequency T and G alleles in PCOS and non PCOS women. Furthermore, no such differences were noticed between the two alleles in obese and non-obese which may indicate, in the first place, the homogenous distribution of the alleles in the studied population. Secondly, the small size of the studied population may not give sharp differences in the allelic distribution.

Tables (6, 7, 8, and 9) show the results of genotypes distribution (GG, GT and TT) among the 4 studied groups; it is found that obese women tends to carry the GG genotype (rather than PCOS or not) with high odd ration more than 1.2 than the TT genotype, while the PCOS women (rather than being obese or not) tends to carry the TT genotype.
This study may indicate to the cooperative effect of both T and G alleles in the heterozygous genotype GT in obese PCOS women, that may lead to support the persistent of metabolic disorders associated with both PCOS and obesity, such as insulin resistant, glycaemia and lipedema.

Conclusion:
Genetic variation in rs17817449 could be the cause underlying the persistence of obesity and difficulty in weight loss in PCOS women leading to increase in the metabolic dysfunction represented by significant increase in BMI, as a result of high fasting blood sugar, glucose tolerance, androgen hormone imbalance and lipid profile in obese PCOS women who carry the TT and TG genotypes.

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Reference:
1. Patel SS, Truong U, King M, Ferland A, Moreau KL, Doros J, et al. Obese adolescents with polycystic ovarian syndrome have elevated cardiovascular disease risk markers. Vasc Med. 2017 Apr; 22(2):85-95.
2. Valkenburg O. Polycystic Ovary Syndrome Genetic Determinants of The Phenotype, Thesis, Erasmus University Rotterdam, The Netherlands. 2015.
3. Aziz R, Adashi EY. Stein and Leventhal: 80 years on. Am J Obstet Gynecol. 2016 Feb 1; 214(2):247-211.
4. Wang R, Mol BW. The Rotterdam criteria for polycystic ovary syndrome: evidence-based criteria? Hum Reprod. 2017 Feb 1; 32(2):261-4.
5. Peña AS, Witchel SF, Hoeger KM, Oberfield SE, Vogiatzi MG, Misso M, et al. Adolescent polycystic ovary syndrome according to the international evidence-based guideline. BMC Med. 2020 Dec; 18(1):1-6.
6. Wojciechowski P, Lipowska A, Rys P, Ewens KG, Franks S, Tan S, et al. Impact of FTO genotypes on BMI and weight in polycystic ovary syndrome: a systematic review and meta-analysis. Diabetologia. 2012 Oct 1; 55(10):2636-45.
7. Rodino IS, Byrne S, Sanders KA. Obesity and psychological wellbeing in patients undergoing fertility treatment. Reproductive biomedicine online. 2016 Jan 1;32(1):104-12.
8. Hernández-Caballeró ME, Sierra-Ramírez JA. Single nucleotide polymorphisms of the FTO gene and cancer risk: an overview. Mol Biol Rep. 2015 Mar 1;42(3):699-704.
9. Berulava T, Horsthemme B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. Eur J Med Genet. 2010 Sep;18(9):1054-6.
10. Kalantari N, Doaei S, Keshavarz-Mohammadi N, Gholamalizadeh M, Pazar N. Review of studies on the fat mass and obesity-associated (FTO) gene interactions with environmental factors affecting on obesity and its impact on lifestyle interventions. ARYA Atheroscl. 2016 Nov; 12(6):281.290.
11. Chen Y, Fang SY. Potential genetic polymorphisms predicting polycystic ovary syndrome. Endocr. 2018 May 1; 7(5):R187-95.
12. Liu T, Wang Q, Huang W, Tan J, Liu D, Pei T, et al. Anthropometric indices to predict insulin resistance in women with polycystic ovary syndrome in China. Reprod. Biomed. Online. 2019 Jan 1;38(1):101-7.
13. Bellver J, Rodríguez-Tabernero L, Robles A, Muñoz E, Martín F, Landeras J, et al. Polycystic ovary syndrome throughout a woman’s life. J Assist Reprod Gen 2018 Jan 1; 35(1):25-39.
14. Ronkainen J. Role of FTO in the Gene and Microrna Expression of Mouse Adipose Tissues In Response To High-Fat Diet. University of Oulu, Finland 2016.
15. Sinha P, Chitra T, Papa D, Nandeesha H. Laparoscopic ovarian drilling reduces testosterone and luteinizing hormone/follicle-stimulating hormone ratio and improves clinical outcome in women with polycystic ovary syndrome J. Hum. Reprod. Sci. 2019 Jul; 12(3):224-8.
16. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr. Rev. 2016 Oct 1; 37(5):467-520.
17. De Melo AS, Dias SV, de Carvalho Cavalli R, Cardoso VC, Bettiol H, Barbieri MA, et al. Pathogenesis of polycystic ovary syndrome: multifactorial assessment from the foetal stage to menopause. Reprod. 2015 Jul 1; 150(1):R11-24.
18. Döael S, Kalantari N, Mohammadi NK, Tabesh GA, Gholamalizadeh M. Macronutrients and the FTO gene polymorphism (rs9939609) and lipid profile in type 2 diabetes. J. Med. Food. 2016 Nov; 19(11):1392-4.
19. Homer MV, Rosencrantz MA, Shaya RF, Chang RJ. The effect of estradiol on granulosa cell responses to FSH in women with polycystic ovary syndrome. Reprod Biol Endocr. 2017 Dec 1;15(1):13:1-6.
20. Al-Tu’ma FJ, Obed KH. Association between Fat Mass and Obesity Associated (FTO) gene polymorphism (rs9939609) and lipid profile in type 2 diabetic obese Iraqi male. IMJ. 2018 Mar 26;21(1):15-9.
21. Milnerowicz H, Madej P. The effect of abdominal obesity in patients with polycystic ovary syndrome on metabolic parameters. Eur Rev Med Pharmacol.2017; 21:4755-61.
Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Yuan H, Zhu G, Wang F, Wang X, Guo H, Shen M. Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.

Interaction between common variants of FTO and MC4R is associated with risk of PCOS. Reprod Biol Endocrin . 2015 Dec;13(1):55:1-6.

Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci. 2017; 13(5):615-629.