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RESEARCH PAPER

Association Study of HSD11B1 rs12086634 (T>G) Gene Polymorphism with Polycystic Ovarian Syndrome in Erbil Province.

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A B S T R A C T:

Background: Polycystic ovarian syndrome (PCOS) is one of the most prevalent gynaecological imbalances of endocrine hormones and metabolic disorders that affect women during puberty, with a prevalence of up to 17.8%.
Objective: The current study focused on the presence of rs12086634 T>G genetic variant of 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) gene polymorphism in Erbil province PCOS women.
Participants and methods: The present study was conducted on 104 PCOS women and 94 control women to investigate the association HSD11B1 rs12086634 (T>G) gene polymorphism with PCOS. The extracted genomic DNA from whole blood according to the protocol provided by the manufacturer company. HSD11B1 rs12086634 T>G gene polymorphism was detected by tetra primer-amplification refractory mutation system based polymerase chain reaction (T-ARMS-PCR) method.
Result: The obtained results suggest that the frequency of the HSD11B1 TG and GG genotypes in women with PCOS increased 3.60-fold and 4.39-fold compared to the control group (odds ratio [OR]; 3.60 and 4.39, 95% confidence interval [CI], 1.951-6.668 and 0.175-110.2; P-value <0.0001 and 0.22) respectively, the body mass index of women with G-allele was significantly greater than women with T-Allele (28.32 kg/ mt2 vs. 26.20 kg/ mt2,and p = 0.0256).
Conclusions: Our findings indicated and proved that the findings indicate that both homozygous and heterozygous genotypes of HSD11B1 rs12086634 T>G gene polymorphism was associated with PCOS in Erbil province.

KEY WORDS: Polycystic ovarian syndrome; HSD11B1; single nucleotide polymorphism; T-ARMS-PCR.
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1.INTRODUCTION :

Polycystic ovarian syndrome (PCOS) is one of the most prevalent gynaecological imbalance of endocrine hormones and metabolic disorders that affect women during puberty (Legro *et al.*, 2013, Mohammed and Ameen, 2017), with an incidence of up to 17.8%. The PCOS is characterized by hyperandrogenism, irregular cycles and polycystic ovaries (Azziz *et al.*, 2016).

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these worsen the symptoms of hyperandrogenemia (Teede *et al.*, 2010, Baptiste *et al.*, 2010).
Despite the aetiological uncertainty of PCOS, it has been suggested that it is a multifactorial disorder, in which genetic factors (including *CYP1A1, CYP19, HSD11B1, AR, SHBG, INSR, PPARG, CAPN10, ADIPOQ, FTO, FSHR, LHCGR, and AMHR2*) interfere with environmental factors leading to develop genetic variations and subsequently genetic disorder (Jain *et al.*, 2015, Azziz *et al.*, 2016). One such candidate gene is *HSD11B1* gene that is located at 1q32.2-41, consisted of six exons and five introns that encode 11β-hydroxysteroid dehydrogenase

type 1(11β-HSD1) (Tomlinson *et al*., 2004, Dujic *et al*., 2012). This keto reductase catalyses the reduction of cortisone to its active form cortisol, hence amplify the activation of the glucocorticoid receptor in different tissues including liver and adipose tissues (Devang *et al*., 2018). The decrease 11β-HSD1 expression leads to impairment of cortisol recreation and also elevates the clearance rate of metabolic cortisol; the excessive level of adrenal androgen detected in some PCOS patients might be a resulted from the compensated activity of the hypothalamic–pituitary–adrenal axis (Rodin *et al*., 1994, Draper et al., 2003). Previous studies on *HSD11B1* gene polymorphism demonstrated that it also has a pathogenic role in metabolic diseases and showed a positive association with Type-II Diabetes Mellitus, insulin resistance, hyperandrogenemia, and hypertension (Nair *et al*., 2004, Gambineri *et al*., 2006, Hughes *et al*., 2008, Liu *et al*., 2008, Cooper and Stewart, 2009, Shimodaira *et al*., 2013, Devang *et al*., 2016).

In addition to nutrition and hormones, genetic factors play an effective role in the regulation of 11β-HSD1 expression. The G allele of rs12086634(T>G) SNP (single nucleotide polymorphism) was studied to have association with PCOS in south Indian women (Devang *et al*., 2018) and specifically with hyperandrogenism among PCOS lean Caucasian women (Gambineri *et al*., 2006), but these associations were not detected in all PCOS patients (Draper *et al*., 2006). Because of insisting of most studies to investigate these associations among different population groups, the current study was managed to investigate the role of *HSD11B1* gene polymorphism in the predisposition of women at Erbil province.

2. MATERIALS AND METHODS

2.1. Participants

The current study was conducted on 104 PCOS women with the mean age 27.16± 0.73 and 94 control women with the mean age 27.36±0.76 recruited from Erbil province general population during May 2018. PCOS patients were chosen based on the presence of at least two respective features namely: chronic oligoanovulation, clinical signs of hyperandrogenism, and polycystic ovarian morphology on ultrasound, those features were selected depending on Rotterdam criteria (Eshre and Group, 2004).

2.2. DNA Analysis

The genetic analysis was performed at Salahaddin University-Erbil (SUE), College of Science, Biology Department. Five millilitres of peripheral blood were collected from both PCOS and control women. Blood samples were kept, in anticoagulant tubes at 4°C. Thermo Fisher DNA Blood Kit (Thermo Fisher Scientific, USA) was used to isolate genomic DNA from peripheral leukocyte. The isolation was made according to the manufacturer protocol. Quality and integrity of DNA were checked by NanoDrop™ (Thermo Scientific, USA).

Genotyping of the SNP rs12086634 T>G *HSD11B1* gene was performed by Tetra-amplification refractory mutation system-polymerase chain reaction (TETRA-ARMS-PCR) technique, which is a rapid and cost-effective technique for SNP detection (Hashemi *et al*., 2012, Khoshnaw, 2018). Four primers were used, two of them were external primers (forward outer: 5′-TTTCTGCTGTATCACTGCAGGTGGTATC-3′, reverse outer: 5′- CAGC TACAGTCAGGACC ACGTAACTGAG -3′), while the other two were internal allele-specific primers (forward inner specified for {G-allele}: 5′- CCTGCAAGAGATG GCTATATTAAGAAACCC -3′, and reverse inner specified for {T-allele}: 5′- AGAATGGGAAAG ATCAACCCCAAAT -3′) (Devang *et al*., 2016).

The PCR cycling program was 5 minutes at 95°C for initial denaturation, then the next 35 cycles of three repeated steps were: denaturation (95°C for 30 seconds), annealing (60°C for 1 minute), and extension (72°C for 1 minute), with a final step at 72°C for 10 minutes to extend all PCR fragments. Separation of the products amplified DNA amplicons were performed by horizontal gel electrophoresis on 2.5% agarose gel, and then visualized by UV-transilluminator after staining with ethidium bromide(Joseph and David, 2001).

2.3. Statistical Analysis

Statistical analysis was done using Graph Pad Prism 6 statistical software. Two sample t-test was used to compare the BMI between women with PCOS and healthy control, and G-allele carriers and TT-Genotype. Genotype and allele frequencies of cases and controls were analyzed using the Chi-square ($\chi 2$) test and both genotype and allelic odds ratio (ORs) and 95% confidence interval (CI) were calculated to determine the association of *HSD11B1* gene polymorphisms with PCOS. A *p*-value of less than 5% ($p<0.05$) was set to be statistically significant.

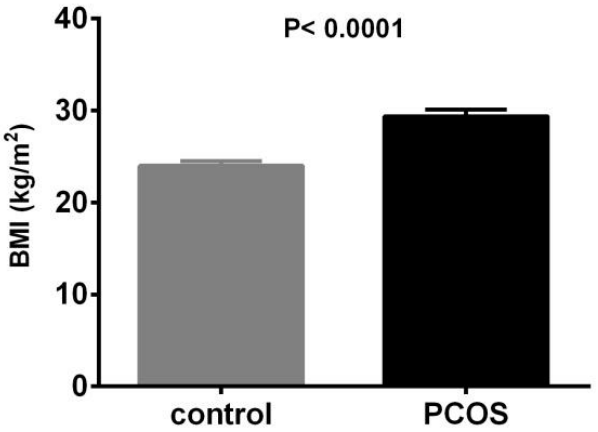

Figure 1: BMI comparison between PCOS and Control. PCOS: Polycystic ovary syndrome, BMI: body mass index.

3. RESULTS

An inspection of (Figure 1) indicates that a highly significant difference of BMI ($p < 0.0001$) was observed among PCOS women with a mean value (29.39 ± 0.7154 kg/mt^2) when compared to healthy control women with a mean value (24.01 ± 0.4915 kg/mt^2).

Genotypes expressed as TT in normal homozygote, TG in the heterozygote, while GG in homozygote polymorphic genotype. In normal TT genotype, two bands of 385 bp and 185 bp were produced. In heterozygote genotype, three bands of 385 bp, 185 bp indicate for T-allele, and 255 bp indicate for G-allele, in homozygote genotype two bands of 385 bp and 255 bp were produced (Figure 2).

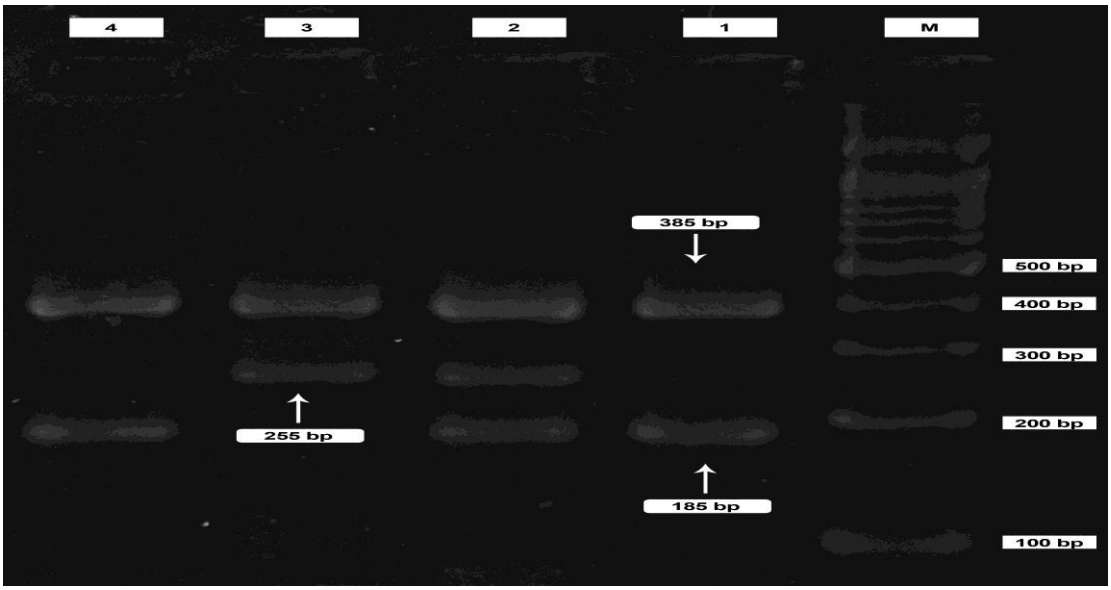

Figure 2: Agarose gel electrophoresis showing results of Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction of four PCOS cases illustrating an amplified 385-bp fragment of *HSD11B1* gene as a control, and two other fragments of 255-bp for T-Allele and 140 bp for G-Allele. Lane M: 100 bp DNA ladder, lane 1 and 4; TT-Genotype, lane 2; TG-Genotype, and lane 3; GG-Genotype.

The allele and genotype frequencies of the SNP rs12086634 detected by T-ARMS-PCR method and were compared between PCOS and healthy women (control). The G-allele frequency of rs12086634 was considerably higher in PCOS than control (27% vs 11.7%). Those patients carrying G allele of rs12086634 showed a higher susceptibility to develop PCOS by 2.78-fold (OR 2.78; 95% CI, 1.620-4.771; P-value <0.0001). TG genotype was 3.607-fold higher (p=0.0001), and GG genotype was 4.394-fold higher (p=0.22) among PCOS women (Table 1).

Table 1: Distribution of genotypes and allele frequencies of *HSD11B1* rs12086634 gene polymorphism in the study population: PCOS women and controls.

Polymorphism	PCOS (n=104)		Control (n=94)		OR	95% CI	p value
	No.	%	No.	%			
TT	49	47.0	72	76.6	1.0	-	-
TG	54	51.9	22	23.4	3.607	1.951-6.668	0.0001
GG	1	0.96	0	0	4.394	0.175-110.2	0.22
TG+GG	55	52.8	22	23.4	3.673	1.989-6.785	0.0001
T-Allele	152	73	166	88.3	2.780	1.620-4.771	0.0001
G-Allele	56	27	22	11.7			

PCOS: Polycystic ovary syndrome; OR: Odds ratio; CI: Confidence interval.

Regarding BMI, women with *HSD11B1* rs12086634 G-allele carriers (TG, and GG) polymorphic genotypes were significantly higher BMI (28.32 ± 0.7385 kg/mt^2 vs. 26.20 ± 0.5863 kg/mt^2, p = 0.0256) when compared with wild type TT genotype (Figure 3).

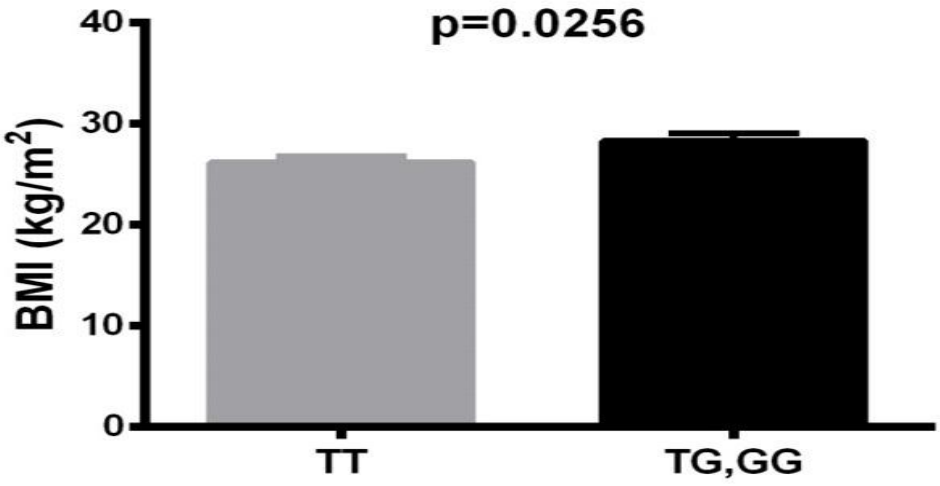

Figure 3: BMI comparison between G-allele carriers vs TT-Genotype. BMI: Body mass index.

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4. DISCUSSION

Polycystic ovarian syndrome is one of the most prevalent gynaecological imbalance of endocrine hormones and metabolic disorders that affect women during puberty (Legro *et al*., 2013). The current understanding is that PCOS is not only a gynaecological condition but a metabolic syndrome with related disorders including; obesity, hormonal disturbance, dyslipidemia and, insulin resistance (Teede *et al*., 2010). It is a multifactorial disorder in which many genes with environmental factors together interact with it (Jain *et al*., 2015). Not just a single gene but alterations in the expression of a number of genes were studied, that inform us PCOS genetic abnormality deteriorates signal transduction pathways regulating the expression of these genes (Jakubowski, 2005).

The results of the current study showed the positive association HSD11B1 rs12086634 T > G gene polymorphism with PCOS. The polymorphic TG-genotype frequency was significantly higher while polymorphic GG-genotype frequency was not significantly greater in PCOS compared to control women. The conflicting results (positive and negative association) from different populations were reported. In the Caucasian population, a positive association of rs12086634 polymorphism that increased by 1.95 fold in women with PCOS have been reported (Gambineri *et al*., 2006). In the south Indian population, the same positive association was confirmed with a G-Allelic OR:1.95 (Devang *et al*., 2018). Whereas the conflicting results with no association were achieved such as the studies in non-Hispanic, Caucasian, and Dallas resident populations that were done by Chua *et al*., San Millan *et al*., and White et al. respectively (White, 2005, San Millán *et al.*, 2005, Chua *et al.*, 2012). However, in the study of Chua et al, and San Millan the number of case and control were not matched, the case numbers were higher than controls. San Millan *et al*. and White they did not follow the all criteria of Rotterdam consensus for diagnosis of PCOS in their study.

Regarding BMI, there was a significant difference in women with G-allele carriers (TG, and GG) genotypes when compared with women with TT genotype. The positive association of BMI marker indicates that this T>G polymorphism might increase the predisposition possibility of obesity in PCOS women. Our results agree with previous reports which recommended that women with TG genotype are at high risk to develop obesity (Devang et al., 2018). This might be related to the overexpression of 11β-HSD1 and increased levels of cortisol within the adipose tissue in obese cases (Masuzaki *et al.*, 2001, Kannisto *et al.*, 2004). The *HSD11B1* rs12086634 T>G polymorphism may increase the chance of individuals to visceral obesity by increasing the 11β-HSD1 activity within visceral adipose tissue (Devang *et al*., 2017).

Previous studies have reported the impact of several genetic polymorphisms in the development of PCOS. Eleven genetic loci were detected to have a role with PCOS such as: DENND1A, THADA, LHCGR, FSHR, YAP1, and RAB5/SUOX (Welt and Duran, 2014) . Previously, genetic polymorphisms in the thyroid peroxidase (TPO) gene, insulin (INS) gene, CYP17, Calpain-10 (capn10) gene, Peroxisome Proliferator Activated Receptors Gamma (PPAR-γ), follicle stimulating hormone receptor (FSHR) gene, and methylenetetrahydrofolate reductase (MTHFR) gene, were associated with PCOS women in Iraqi population (Nader and Aziz, 2014, AlFaisal *et al.*, 2014, Mohammed *et al.*, 2015, Ali, 2016, Ramadhan, 2018, Al Hayawi *et al.*, 2018). Until now there were no studies to examine whether *HSD11B1* gene polymorphism has any association with PCOS in Erbil population. It would be the first study in Erbil province for evaluation of association and frequency of this gene polymorphism among women with PCOS.

5. CONCLUSION

The present study demonstrated and confirmed that the SNP rs12086634 (T>G) of the *HSD11B1* gene is significantly associated with PCOS in Erbil province. Additionally the G-allele of this SNP represented a positive association with obesity.

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