Polymorphism of insulin receptor gene His1058 and its association with insulin resistance among women with polycystic ovary syndrome

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Abstract

Insulin resistance is a central key element contributing to polycystic ovary syndrome (PCOS) pathogenesis and is further aggravated by obesity. Various mutations with the insulin receptor (INSR) gene can result in the clinical manifestation of insulin resistance. This study aimed to assess insulin receptor gene rs-1799817 polymorphisms and its association with fertility hormones among women with PCOS. **Methods**: This case control study was based on (150) Sudanese women with PCOS and (150) healthy females (control group). ASYS Expert Plus Microplate was used to quantify serum luteinizing (LH), follicle-stimulating hormone (FSH) levels in fasting blood specimen drawn during the follicular phase of the menstrual cycle of women with PCOS. The serum insulin was measured using ELISA and glucose concentrations measured using Cobas 311c, Insulin resistance was calculated by the homeostasis model (HOMA-IR). The guanidine extraction procedure was used to extract genomic DNA from EDTA whole blood. Genotyping for insulin receptor gene (rs-1799817) polymorphism by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The data obtained were subjected to analysis using statistical package for social science computer program (SPSS version 26). **Results:** The results revealed PCOS women had a significant increase in insulin hormone, HOMA-IR (P-value=0.000), while there was insignificant difference in FBG (P-value=0.513) when compared with non-PCOS group. Study revealed that there was insignificant variation in genotypes of (rs-1799817) between case and control groups. Although, 9.7% (14/144) of PCOS group had mutant genotypes (TT) compared to 6.5% (9/139) in control group. **Conclusion:** The study showed a high level of LH, FSH and insulin in PCOS patients, associated with increased incidence of TT genotype (rs-1799817) polymorphism among women with PCOS.

Keywords: PCOS, insulin hormone, HOM-IR, Insulin receptor gene His1058, Genotype

Introduction:

PCOS is the most common endocrinological problem among women of reproductive age, is characterized by chronic ovulatory dysfunction, hyperandrogenism, and infertility. PCOS, affecting (8–13%) of women of reproductive age globally[1].

Although the etiology of PCOS is still not well known[2], it seems that insulin resistance is a major pathophysiologic mechanism in developing clinical symptoms and other metabolic complications of PCOS[3]. Resistance to insulin leads to an increase in free androgen availability, and consequently alteration in follicular development and granulosa cell function [(4-5)]. Insulin resistance is universally accepted as a key pathophysiologic feature of PCOS and metabolic syndrome, regardless of obesity[6].

Different genes have been implicated in insulin action and are used as biomarkers for PCOS. INSR is considered as a candidate gene for PCOS susceptibility[7]. The INSR gene is located on chromosome (19) and it consists of (22 exons and 21 introns). The exons (17–21) encode the tyrosine kinase domain of the receptor, which is necessary for insulin signal transduction [8]. Mutations in these exons can cause insulin resistance and hyperinsulinemia[(9-10)].

Materials and Methods:

Study participants:

This was comparative case control hospital-based study. It was conducted in Saad Abu-alela Hospital, Banoon Medical Center, Nile Fertility Center, Royal care hospital, East-Nile hospital, Sudan Assisted Reproductive Clinic and Dr. Elsir Abu-Elhassan Center among (PCOS) patients. It was carried out over (2 years).

Ethical clearance

The study was approved by the internal review research board at Sudan University of Science and Technology. The objectives of the study were explained to all individuals participating in this study. Verbal consent was obtained from all participants in the study.

A total of (150) patients diagnosed with polycystic ovary syndrome, and (150) controls (age was matched) were enrolled in the study, after obtaining written informed consent.

Polycystic ovary syndrome women who are clinically diagnosed according to Rotterdam criteria enrolled in this study. All patients previously diagnosed with disorders that can result in menstrual irregularity and hyperandrogenism, including adrenal or ovarian tumors, thyroid

dysfunction, congenital adrenal hyperplasia, hyperprolactinemia, acromegaly, type 2 Diabetes M mellitus and Cushing's syndrome were excluded.

Biochemical measurements:

After obtaining informed consent from each participant a sample of venous blood (5 mL) was collected and then divided into three containers, (2 ml) of blood was collected in plain container to obtain serum after centrifugation for insulin hormone, (2 ml) of blood was collected in fluoride oxalate for glucose and (2 ml) of blood was collected in EDTA container (whole blood) used for DNA extraction (glucose and insulin hormone from fasting patients).

The collected blood samples were immediately centrifuged, and the serum was stored at (-80 °C) until further analysis. ASYS (model: Expert Plus; type G020150; serial nr: 28382; Austria) was used to quantify serum LH, FSH by indirect method. The insulin levels were measured by ELISA. Cobas c311Analyzer was used to measure plasma glucose levels. HOMA-IR was calculated using the formula: glucose (mmol/L x insulin (mU/L)/22.5.

Genetic analysis:

Polymerase Chain Reaction (PCR) was used to amplify INSR gene SNP (His1058) and the amplified products were analyzed by restriction fragment length polymorphism.

Genotyping of SNP (His1058)

Genomic DNA was extracted from peripheral blood samples by standard procedures G. spin TM and then stored at (-20 °C) till use.

The fragment of interest was amplified by PCR, using primers.

The primer sequences used were:

F: 5'-ACCTGCCGAACTACAACTGG-3'

R: 5'-TGAAGGAACAGGCGGTTAGT-3'

PCR amplification was carried out in a total volume of (25 µl) containing (50 ng) of genomic DNA, (6.25 pmol) of each primer, (25 µl) short tandem repeat (STR), 10x buffer (STR 10x buffer, Promega, Madison, Wl, USA). The PCR was performed in a PTC-100 (convergys TCL) thermocycler as follows: initial denaturation at (95 °C) for (15 min), followed by (34 cycles) of denaturation at (95 °C) for (1 min), annealing at (55 °C) for (1 min), and final extension at (72 °C) for (1 min), with the final extension of (10 mins) at (72 °C)

Aliquots of the amplified PCR products of (317bp) was digested by restriction enzyme Pml1 at (37 °C) for (16 hours). A single (317bp) band indicates homozygosity for the CC genotype. Two

bands of (274bp and 43bp) indicates homozygosity for the TT genotype and combination of (317bp, 274bp and 43bp) bands indicates heterozygosity for the CT genotype. The recognition site was identified by ethidium bromide staining of fragments separated in a (2%) agarose gel.

Statistical analysis

Appropriate descriptive and analytical statistical procedure was followed using Statistic Package for Social Science (SPSS) version 26 on programmed computer. t-test applied to compare biochemical quantitative data between case and the control groups. Chi- square test was used to find the association between genotypes and biochemical variables. The level of significance was expressed as $P < 0.05$.

Results

Glucose, insulin hormone, and HOMA-IR parameters of women from PCOS and control groups were summarized in (Table I). There was statistically in-significant difference in glucose between the two groups, while BMI, LH/FSH ratio and insulin hormone were significantly higher in women with PCOS (P=0.000) when compared with non-PCOS. In the PCOS group, there was higher frequency of TT genotype. However; there was insignificant variation between two groups in genotypes frequency (Table 2). In PCOS women, there were insignificant differences in BMI, fertility hormones, glucose, insulin levels, and HOMA-IR based on His1058 genotypes, when compared with non-PCOS women (Table 3).

(Table 1): comparison between mean of age, BMI, plasma levels of LH, FSH, LH/FSH ratio, glucose, insulin and HOMA-IR in polycystic ovary syndrome group and non-polycystic ovary syndrome group.

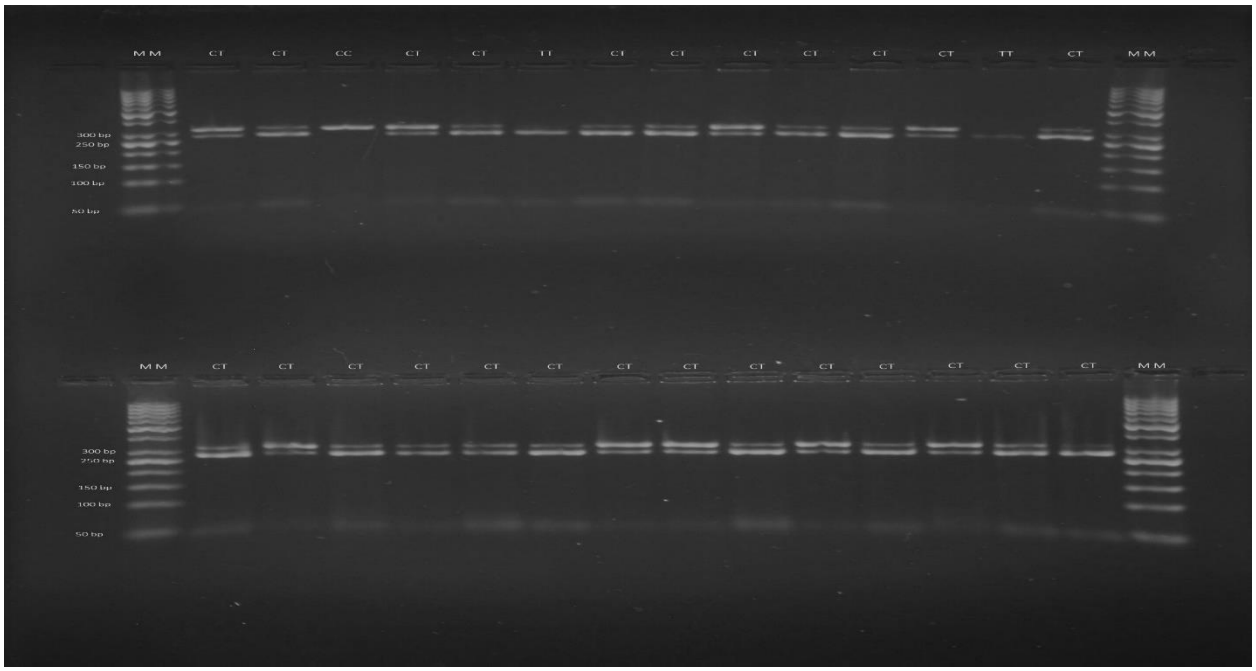
Variable	PCOS (Mean± SD)	Non PCOS (Mean ± SD)	P-value
Age/year	29.0± 6.0	28.5± 5.5	0.432
BMI kg/m^2	28.6±4.8	22.9±1.3	0.000
LH IU/l	10.5±6.2	4.4±1.4	0.000
FSHIu/L	6.6±2.9	8.2± 2.2	0.000
LH/FSH ratio	1.8±1.4	0.5±0.1	0.000
Glucose (mg/dl)	89.9±12.5	89.7±12.6	0.916
Insulin (µIU/ml)	4.48±3.60	0.71±0.72	0.000
HOMA-IR	0.981±.79	0.157±0.16	0.000

- Independent t- test was used to compare between the groups
- P- value considered significant at level ≤0.05

(Table 2): Genotypes frequencies of rs-1799817 in PCOS and control groups**.**

Genotyping	PCOS No.= 144	Non-PCOS No. = 139	P- value
CC(Normal)	2 (1.4%)†	2 (1.4%)	0.607
CT(Hetero)	128 (88.9 %)	128 (92.1 %)	
TT(Mutant)	14 (9.7%)	9 (6.5 %)	

- † Frequency and percentage of genotypes. P- value was obtain by Chi- square test.



(Figure I): Electrophoretic gel represent genotyping alleles of insulin receptor gene (SNP rs1799817) polymorphism TT (mutant), CT (hetero) and CC (wild) types.

(Table 3): Comparison between rs-1799817 genotypes and BMI, fertility hormones, insulin, FBG, and HOMA-IR

Variables	Genotypes			
	CC	CT	TT	P- value
BMI kg/m^2	28.1± 1.4	28.5± 4.8	29.2± 6.0	0.871
LH IU/L	12.5± 9.7	10.3± 6.2	11.2± 6.3	0.802
FSH IU/l	6.5± 6.3	6.7± 2.9	6.0± 3.1	0.657
LH/FSH ratio	1.9± 1.6	1.8± 1.4	2.2± 1.7	0.538
Glucose (mg/dL)	99.0± 4.2	90.1± 12.7	87.7± 12.7	0.491
Insulin (µIU/ml)	4.7± 4.8	4.6± 3.6	3.6± 3.7	0.597
HOMA-IR	1.1± 1.1	1.0± 0.7	0.7± 0.8	0.594

- One way ANOVA was used to compared between groups.

Discussion:

PCOS is the most common endocrinopathy among women in the reproductive age [11]. In this study, mean age of the studied patients was as 29.0 (6.0) years This is comparable to the study done by Krithika *et al*., where mean age of PCOS patients was 27 (7.1) years[12].

In this current study, serum LH, FSH levels and LH-FSH ratio were significantly increased in women with PCOS when compared with non PCOS. Disturbance in the pulsatile nature of gonadotrophin releasing hormone (GnRH) results in the relative increase in LH to FSH release[13]. An abnormal feedback mechanism by ovarian estrogen is blamed to play role in this discriminated increase in LH release[14]. As a result of this derangement, the ratio between FSH and LH levels which is normally around (2 to 1), become reversed and sometimes even more (2 or 3 to 1) in approximately (60%) of the patients with PCOS[15]

This current performed study revealed significant increase of insulin hormone, and HOMA-IR in women with PCOS compared to non-PCOS group (P-value= 0.000). This result similar to another result carried out by Shirazi and Jeddi, which showed that, a high incidence of insulin resistance in PCOS patients[16]. Another study showed that, all extreme insulin-resistance states are associated with PCOS. The compensatory hyperinsulinemia of insulin resistance is closely associated with the un- ovulation of PCOS[17].

In this present study, there was insignificant variation in His1058 (C/T) genotypes distribution among study groups (P- value >0.05). however; there was increased frequency of T allele in PCOS women. Other researchers' results were in line with the current study, Urbanek *et al.* [18], Tehrani *et al.*,[19]Bagheri *et al.*,[20] and Xu *et al.*,[21] on Han Chinese. Rasool *et al.*, on Kashmiri women which found no association of His1058 (rs1799817) polymorphism in the INSR gene, insulin resistance, and PCOS risk[22]. Kumar *et a*l found an increased frequency of INSR His1058H T allele in PCOS patients from China and India[23].Tucci et al. on Canadian population and Tucci *et al* on Caucasian population had found an association between rs1799817 and PCOS[24]. Siegel *et al* on Americans argued for the first time that the INSR gene itself took part in the development of insulin resistance as the main cause of PCOS[25]. This variation of findings may be due to different ethnicity.

This study had some limitations. As this was case control study, long term complications and sequel of PCOS could not be studied.

Conclusions: PCOS has varying clinical manifestation including endocrine manifestations, most commonly affecting the young women of reproductive age group and can lead to reproductive and metabolic complications in the long term. Majority of the patients present with elevated LH/ FSH ratio and some of them revealed mutant allelic variation. Also, the study revealed a high incidence of insulin resistance in PCOS patients, suggests that other polymorphisms of insulin His1058 (rs-1799817) may be associated with the pathogenesis of PCOS.

Conflicts of Interest: authors declare no conflicts of interest.

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