Study the C/T Single Nucleotide Polymorphism at Tyrosine Kinase Domain of Insulin Receptor Gene in Patients with Polycystic Ovary Syndrome in Babylon Province

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Abstract

Polycystic ovary syndrome (PCOS) is the commonest gynecological endocrinopathy, is characterized by chronic anovulation and hyperandrogenism and is also associated with increase insulin secretion and metabolic disease and insulin resistance. Present study aims to examine the association between the single nucleotide polymorphism (rs1799817) of exon 17 of the insulin receptor (INSR) gene and polycystic ovary syndrome (PCOS) in a Babylon women. Fifty women with PCOS and fifty healthy Iraqi women, age and BMI matching were achieved between study groups. Biochemical parameters that estimated were fasting glucose, fasting insulin, luteinizing hormone, testosterone. His 1058 C/T polymorphism at the tyrosine kinase domain in the INSR gene was analyzed by restriction fragment length polymorphism (RFLP-PCR). Significant increase in the levels of fasting glucose fasting insulin, HOMA-IR, LH, testosterone were noticed in patient when compared with control. TT genotype frequency was higher in PCOS patients whereas CC genotype was higher in control women. All parameters that estimate in this study were higher in TT genotype of patient group. In conclusion there are an association of C/T polymorphism at His1058 of INSR in patients women with PCOS in Babylon Province. Insulin resistance in PCOS patients might differ from these that result from obesity.

Keywords: *Polycystic ovary syndrome, insulin resistance, insulin receptor, tyrosine kinase domain, His 1058 C/T.*

Introduction

The present concept of polycystic ovary syndrome (PCOS) is a disorder that has ovarian dysfunction and endocrine issues and is also associated with increase insulin secretion (hyperinsulinaemia) and metabolic disease [(1)]. PCOS was noted for first time by Stein and Leventhal in modern medical literature in 1935, they described seven women have amenorrhea, hirsutism, and magnify ovaries with multiple cysts [(2)].

PCOS is a very prevalent and complicated endocrine disease of females [(3)], affecting 4-18% of females of reproductive age, based on the diagnostic criteria used [(4)]. PCOS is the most common cause of oligoanovulatory infertility which is characterized by insulin resistance (IR), while hyperinsulinemia is observed in 50–70 % of women diagnosed with PCOS [(3)]. The cause of polycystic ovary syndrome remains unknown, although, like most complex heterogeneous diseases [(5)].

The pathophysiology is complicated and is thought to be a yield from interactions between genetics, epigenetics, ovarian dysfunction, endocrine, neuroendocrine and metabolic alterations, amongst other changes [(6)]. IR is a hallmark of the classic and the ovulatory phenotypes of PCOS [(7)]. IR can be present in 60%-80% of all females with PCOS and present in 95% of obese female with PCOS, IR is not the only metabolic disorder present in PCOS patients; there is also an high rate of impaired glucose tolerance, gestational diabetes (GDM), as well as T2DM [(8)]. Insulin resistance may result from any functional and structural defects in

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insulin receptor might impair the biological response to insulin, The human insulin receptor INSR gene is found on short arm of chromosome 19 (19p13.2) . Its long about 120,000 bp, composed of 22 exons and 21 introns. It plays an important role in insulin metabolism [(9)]. The INSR gene has several genetic polymorphisms and was recognized to be associated with PCOS and insulin resistance (IR) [(10)]. One of these polymorphism is single nucleotide polymorphism at (rs1799817) His1058 [(11)], which encodes the partial tyrosine kinase domain containing the ATP binding site of INSR [(12)].

Materials and Methods

Current study is case control study includes 100 females, which were divided into two groups, the 50 females with PCOS and 50 apparently healthy females, the age was ranged between (17 - 35) years. Assessment of obesity status of all female was done by the body mass index (BMI), [BMI= weight (kg) / height (m)²] and select just normal weight and overweight(BMI < 30) [(13)].

Four ml of venous blood were drawn from all participants at second day of menstrual cycle by using disposable syringe. All participants were fasting during the time of blood samples collection.

The blood specimen was divided into two tubes (2 ml blood in EDTA tube for genetic study and 2ml blood in gel tube). The latter two ml of blood were centrifuged at 2000 rpm for 15 minutes, then the serum are divided into two parts in labeled Eppendorf tube and given serial number with patient's name then stored at -20 ˚C .[(14)]

Fasting serum glucose was analyzed by spectrophotometer. Fasting serum insulin, LH, testosterone were analyzed by enzyme linked immunosorbent assay kit (ELISA kit). Insulin resistance was calculate by homeostasis model assessment insulin sensitivity index (HOMA) as fasting insulin x fasting glucose /405

Genetic Analysis

Genomic DNA extracted from whole blood sample were collected in EDTA tube, its extracted by using gene extraction kit supplied by intron biotechnology company (Korea). Exon 17 was amplified using the following primers for forward and reverse respectively: 5-CCAAGGATGCTGTGTAGATAAG-3 and 5-TCAGGAAAGCCAGCCCATGTC-3 according to Siegel *et al.* [(11)]. A total volume of 50 µl containing genomic DNA 10 µl was used as template in the reaction mixture, 6 µl of each primer and 25 µl of green master mix (Promega, USA), amplification condition was 94 °C for 5 minute to initial denaturation, 35 cycles with 94℃ for 45 seconds, 55 °C for 40 seconds, 72 °C for 60 seconds, and 72 °C for 10 min. PCR products (317-bp) digested with *PmlI* (Thermo Scientific, USA) for 3 hours at 37 °C. A 2% agarose gel containing ethidium bromide was used to electrophoresed the digested DNA fragments and visualized by UV trans-illuminator spectroline (USA). Hence, the CC genotype represented by a single 317-bp band indicates homozygosity. TT genotype represented by two fragments, 274-bp and 43-bp bands, indicates homozygosity. The presence of three fragments, 317-, 274-, and 43-bp bands, indicates heterozygosity for the CT genotype, as shown in Figure1.

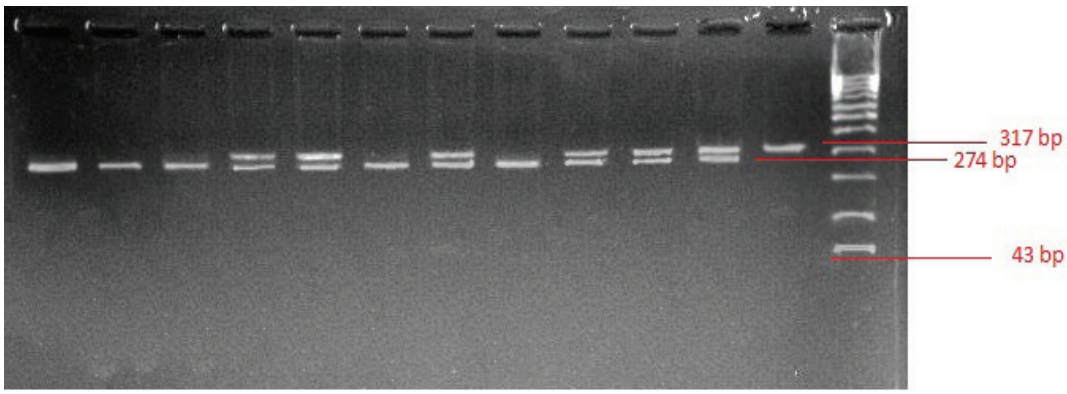

Figure 1 : Restriction fragment length polymorphism analysis of the C/T polymorphism of exon 17 in the INSR gene. Agarose gel (2%) electrophoresis after *PmlI* digestion of the PCR.

Results

Age and BMI matching between patient and control group were achieved to eliminate difference in parameter between group, so there were no significant difference in BMI, age as shown in Table 1.

Table 1 Age and BMI of Studied Groups

Variable	Study groups	N	Mean ± SD	Range	P-value
Age (years)	PCOS	50	26.42 ± 3.94	(17-35)	0.396
	control	50	25.52 ± 4.36	(18-36)	
BMI (kg/m2)	PCOS	50	25.35 ± 2.13	(19.74 – 29.39)	0.713
	control	50	24.00 ± 1.98	(20.31 – 28.6)	

Biochemically, there were significant difference ($p<0.05$) in mean of fasting blood glucose, fasting insulin, HOMA in patient groups in compare with control groups, also significant difference in mean of LH, testosterone levels between patient and control groups as shown as in Table 2.

Table 2 Fasting Blood Glucose, Fasting Insulin and HOMA index, Luteinizing hormone, Testosterone hormone of Studied Groups.

Variable	Study groups	N	Mean± SD	P-value
Fasting glucose level (mg/dl)	PCOS	50	115.84 ± 13.09	0.036*
	control	50	90.70 ± 9.23	
Fasting insulin level (ng/ml)	PCOS	50	11.08 ± 1.81	0.001*
	control	50	7.03 ± 1.11	
Insulin resistance (HOMA)	PCOS	50	3.16 ± 0.72	0.001*
	control	50	1.57 ± 0.26	
LH	PCOS	50	7.150 ± 2.159	0.002*
	control	50	3.230 ± 1.394	
Testosterone	PCOS	50	1.622 ± 1.045	0.001*
	control	50	0.733 ± 0.346	

*significant

The frequency of the CC, CT, and TT genotypes of the INSR His 1058 C/T single nucleotide polymorphism, in patient TT genotype is common one, whereas CT genotype is common in control, as shown in Table 3.

Table 3 Genotypes of the INSR 1058 C/T Single Nucleotide Polymorphism, in Patients and Control groups

	CC	%	CT	%	TT	%
Patient n= 50	9	18 %	20	40 %	21	42 %
Control n=50	11	22 %	36	72 %	3	6 %

In Table 4, the frequency of the C allele (i.e., the CC, CT genotypes) was significantly increased in patients with PCOS compared with controls. Thirty-one (62%) of patients with PCOS but only twenty- two (42 %) of controls.

Table 4 Number and Percentage of Alleles Frequency in both Patient and Control Group.

	Patients		Controls		OR(CI)	P-value
Allele	Count	Proportion	Count	Proportion		
T	31	62%	21	42 %	2.25 (1.01 - 5.01)	0.04
C	19	38%	29	58%	0.44 (0.19 to 0.98)	

The correlations among studied parameter with genotyping of patient group and control groups were shown in Table 5.

Table 5 Correlation between Luteinizing Hormone, Testosterone and Insulin Resistance Using HOMA-IR Index Results in Each Genotype of Patient and Control Groups.

Parameter	Groups	CC	CT	TT
LH	Patient	7.25 ± 1.58	5.93± 1.73	8.45 ± 1.726
	Control	3.39 ± 0.808	3.36 ± 0.233	2.74 ± 0.422
Testosterone	Patient	1.10 ± 0.53	1.35 ± 0.90	2.21 ± 1.231
	Control	1.49 ± 0.97	1.58 ± 0.29	1.55 ± 0.20
HOMA-IR	Patient	2.54 ± 0.61	3.04 ± 0.65	3.54 ± 0.63 *
	Control	0.66 ± 0.34	0.77 ± 0.35	0.55 ± 0.75

Discussion

Most of patient with PCOs were young because of typical PCOS appeared to diminish with increasing age. Similarly, menstrual cycles of PCOS women become normalized with increasing age [15]. The blood glucose that not utilized by all tissue leading to hyperglycemia [16], PCOS is associated with pre-diabetes, GDM (with around a 2-3 fold risk, independent of obesity), T2DM (4-6 fold risk independent of obesity) and these occur at a younger age [17].

Hyperinsulinemia means a constant high level of plasma insulin in the fasting condition [18], Hyperinsulinemia can result from a decrease in insulin clearance as well as from increased insulin secretion [19]. It is believed that hyperinsulinemia result from insulin resistance when β-islets try to control of blood glucose by produce a large amount of insulin [18].

The common feature of PCOS is IR, its affect about 50–80% of patients. IR have clearly relationship with obesity, IR in lean PCOS women may cause by genetic disorders [20], IR, a substantial etiological factor of PCOS, was traditionally refer primarily to obesity. Moreover, insulin-signaling abnormalities is one of evidences that support the presence of an intrinsic IR in PCOS, independent of obesity [15].

Common feature of PCOS disorder is LH excess and a high rate of PCOS patients have an elevated LH/ follicle stimulating hormone (FSH) ratio perhaps due to elevated levels of LH. LH is important for the expression of gonadal steroidogenic enzymes [20], ovarian theca cells need High LH levels to synthesis androgen. reduce in oestradiol synthesis through the conversion of androgens and High LH combined with low FSH levels lead to anovulation due to the absence of a dominant follicle [21].

Biochemical feature of PCOS is hyperandrogenism. About 80–90% of women with PCOS have elevated circulating androgen levels. derangements of androgen production and metabolism lead to hyperandrogenism [20], through several mechanisms the insulin resistance might contribute to hyperandrogenism and gonadotropin abnormalities [5].

The frequency of the T allele (i.e., the TT, CT genotypes) was significantly increased in patients with PCOS compared with controls. Thirty-one (62%) of patients with PCOS but only twenty- two (42 %) of controls, so this support that there were association between pathogensis of PCOS and His 1058 C/T polymorphism at the tyrosine kinase domain of the INSR gene, similar to finding of a studies done by Siegel, Sheera, *et al.* 2002 [11], Kashima, Katsunori *et al.* [22], Mutib, Manal T, *et al.* 2014 [23], Mukherjee, Srabani, *et al.* 2009 [12]. While Lee, Eung-Ji, *et al.* 2006 reported no association in Korea population [24]. There are difference in result between researches, this difference can be attribute to ethnic difference, lifestyle and environment between the group that been studied.

Conclusions

In conclusion current study was noticed that there are an association between C/T polymorphism at His1058 of exon 17 of INSR and PCOS and may paly role in pathogenesis of this disease in share with other factors. Women who have T allele may be susceptible to development PCOS more than others who have C allele. Insulin resistance in PCOS might differ from these that result from obesity.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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References

1. Edmonds, D. Keith, Christoph Lees and THB. Dewhurst's textbook of obstetrics and gynaecology. 2018.
2. Lakkakula BVKS, Thangavelu M, Godla UR. Genetic variants associated with insulin signaling and glucose homeostasis in the pathogenesis of insulin resistance in polycystic ovary syndrome: a systematic review. J Assist Reprod Genet. 2013, 30,7:883-895.
3. Dennett CC, Simon J. The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. Diabetes Spectr. 2015,28,2:116–120.

4. Stein IF. Amenorrhea associated with bilateral polycystic ovaries. Am J Obs Gynecol. 1935;29:181–91.
5. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet, 2007, 370, 8599, 685–697.
6. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update. 2016, 22, 6:687–708.
7. Polak K, Czyzyk A, Simoncini T, Meczekalski B. New markers of insulin resistance in polycystic ovary syndrome. J Endocrinol Invest. 2017, 40, 1:1–8.
8. Kashan IS, Alta'ee AH, Albayati AH, Study of insulin and counter regulatory hormones in patients with type II diabetes mellitus: A case control study, Biochemical and Cellular Archives, 2018, 18 ,1: 77-83.
9. Gangopadhyay S, Agrawal N, Batra A, Kabi BC, Gupta A. Single-Nucleotide Polymorphism on Exon 17 of Insulin Receptor Gene Influences Insulin Resistance in PCOS: A Pilot Study on North Indian Women. Biochem Genet. 2016, 54, 2:158–168.
10. Bagheri M, Abdi-Rad I, Hosseini-Jazani N, Zarrin R, Nanbakhsh F, Mohammadzaie N. An association study between INSR/NsiI (rs2059806) and INSR/ PmlI (rs1799817) SNPs in women with polycystic ovary syndrome from West Azerbaijan Province, Iran. J Reprod Infertil. 2015, 16, 2109.
11. Siegel S, Futterweit W, Davies TF, Concepcion ES, Greenberg DA, Villanueva R, et al. AC/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor gene is associated with polycystic ovary syndrome. Fertil Steril., 2002, 78, 6:1240–1243.
12. Mukherjee S, Shaikh N, Khavale S, Shinde G, Meherji P, Shah N, et al. Genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with polycystic ovary syndrome. Eur J Endocrinol. 2009,160, 5:855–62.
13. Alta'ee AH, Hadwan MH, Almashhedy LA, The Balance between Superoxide Dismutase and Catalase Activities in Sera of Obese Iraqi Men, EuroMed Biomed J, 2015, 10: 197-202.
14. Al-Mokhtar AM, Alta'ee AH, Al-Hattab MK: Leptin and Resistin Induce Oxidative Stress in Patients with Chronic Plaque Psoriatic, Res J Pharm Biol Chem Sci, 2017, 8: 135-142.
15. Palomba S, Santagni S, Falbo A, La Sala GB. Complications and challenges associated with polycystic ovary syndrome: current perspectives. Int J Womens Health. 2015;7:745–763.
16. Kushner RF, Ryan DH. Assessment and lifestyle management of patients with obesity: clinical recommendations from systematic reviews. Jama. 2014;312(9):943–52.
17. Shorakae, Soulmaz and Boyle, Jacqueline and Teede H. PCOS – A common hormonal condition with major metabolic sequelae that physicians should know about. Intern Med J. 2014;44:720--726.
18. Ye J. Mechanisms of insulin resistance in obesity. Front Med. 2013;7(1):14–24.
19. Koivunen RM. Endocrine and metabolic changes in women with polycystic ovaries and polycystic ovary syndrome. 2003;
20. Delitala AP, Capobianco G, Delitala G, Cherchi PL, Dessole S. Polycystic ovary syndrome, adipose tissue and metabolic syndrome. Arch Gynecol Obstet. 2017;296(3):405–419.
21. Altoma FJ, Mohammad KS, Alta'ee AH, Ewadh MJ, Alhamdani KJ, Alsalihy AA and Mkhlof ST, Diabetes Mellitus Correlation with Free Radicals and Creatine Kinase Isoenzymes Activity. Med. J. Babylon, 2006 2, 2, 241-247.
22. Kashima K, Yahata T, Fujita K, Tanaka K. Polycystic ovary syndrome: association of a C/T single nucleotide polymorphism at tyrosine kinase domain of insulin receptor gene with pathogenesis among lean Japanese women. J Reprod Med. 2013;58(11–12):491-496.
23. Mutib MT, Hamdan FB, Al-Salihi AR. INSR gene variation is associated with decreased insulin sensitivity in Iraqi women with PCOs. Iran J Reprod Med. 2014,12, 7:499-506.
24. Lee E-J, Yoo K-J, Kim S-J, Lee S-H, Cha KY, Baek K-H. Single nucleotide polymorphism in exon 17 of the insulin receptor gene is not associated with polycystic ovary syndrome in a Korean population. Fertil Steril. 2006;86, 2:380-384.