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Involvement of β3-Adrenergic Gene Polymorphism in Insulin Resistance in Iraqi Type 2 Diabetic Patients

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Abstract
A tryptophan to arginine substitution (TGG↔CGG) in codon 64 (Trp 64 Arg) of β3-adrenergic receptor is thought to be important for binding of noradrenaline and G proteins with β3-adrenergic receptor in adipose cells. β3-adrenergic receptor polymorphism may lead to a decrease in thermogenesis and lipolysis in adipose tissue. Therefore, an impairment of β3-adrenergic receptor function may lead to obesity and insulin resistance. The present study was designed to estimate prevalence and association of β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP in insulin resistance type 2 diabetic patients in Iraq. To achieve this aim, 103 of type 2 diabetic patients and 57 apparently healthy control group were subjected to the study. The results of present study show that the heterozygous genotype (TC) of β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP was significantly increased (OR=4.12, CI 95% 1.14-15.86, P < 0.05) the risk of type 2 diabetes mellitus four folds with respect to those of the wild genotype (TT). Also, the results revealed that significant increase (P < 0.05) in fasting insulin, HOMA, BMI and significant decrease (P < 0.05) in HDL-cholesterol of heterozygous genotype (TC) when compared with wild genotype (TT). Also, there are no significant differences in other clinical characteristics between wild genotype (TT) and heterozygous genotype (TC). The study concluded that β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP are associated and involved in the pathogenesis of insulin resistant type 2 diabetes mellitus.
Keyword: Diabetes Mellitus, Insulin resistance, Obesity, β3-adrenergic receptor, Trp 64 Arg

1. Introduction

Type 2 diabetes mellitus is a non autoimmune, heterogeneous and polygenic metabolic diseases characterized by hyperglycemia, resulting from impairment of insulin secretion and/or action (1). Insulin resistance, the reduction of insulin sensitivity by insulin responsive tissues lead to decrease the ability of insulin to inhibit the production of glucose by the liver and decrease peripheral glucose utilization. Consequently, blood glucose level would be rise in insulin resistance and increase the secretion of insulin to overcome insulin resistance (2).

Obesity is the main risk cause for type 2 diabetes mellitus and it coupled with glucose intolerance, particularly when obesity is centrally distributed. Although the environmental factors participate in the obesity, the genetic are also affect on both total adiposity and body fat distribution. Several studies suggested that a low rate of energy expenditure and reduced rates of fat oxidation may contribute to obesity (3, 4, 5). The β3-adrenergic receptor is expressed in visceral fat in humans and it is responsible for increase the lipolysis and the delivery of free fatty acid into the portal vein (6). An increase in visceral fat mass, in turn, correlates with insulin resistance in skeletal muscle. There is an evidence that molecular abnormalities in the β3-adrenergic receptor may lead to obesity and type 2 diabetes mellitus (7).

1.1. Aims of the study

The aims of this study to estimate prevalence and association of β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP in insulin resistant type 2 diabetic patients in Iraqi population.

2. Materials and Methods

2.1. Materials

2.1.1. Subjects

The study included type 2 diabetic patients and control group. All samples were collected from February 2013 till May 2013. The work was carried out in the biochemistry department laboratory in College of Medicine/University of Kufa. The study was performed on 103 of type 2 diabetic patients and 57 apparently healthy control group. Any subject suffered from problems such as, renal dysfunction, heart diseases, hypertension, patient on insulin therapy and drug dependency such as glucocorticoid were excluded from the current study.

2.1.2. Blood Sampling

Five milliliters of blood was taken from all subjects by vein puncture in fasting status and the blood was divided into two parts, the first part include three milliliters of blood placed in plain tube for estimation of insulin, glucose, total cholesterol, HDL-cholesterol, TGs, VLDL-cholesterol and LDL-cholesterol concentrations. The second part for gene analysis includes two milliliters of blood will be collected in EDTA containing tube.

2.2. Methods

Serum glucose, total cholesterol, triglycerides (TGs) and HDL-cholesterol concentration determined by spectrophotometric methods. While serum insulin concentration determined by enzyme linked immunosorbant assay (ELISA) method. Insulin resistance was evaluated by homeostatic model assessment (HOMA) method.
Genotyping of β3-adrenergic receptor gene T↔C (Trp 64 Arg) was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA extracted from frozen blood by genomic DNA mini kit (Geneaid) (8). The DNA was amplified by PCR. A 255 bp DNA fragment containing the polymorphic site T↔C (Trp 64 Arg) of β3-adrenergic receptor gene was amplified by using specific primers (forward primer 5'-CCA GTG GGC TGC CAG GGG-3' and reverse primer 5'-GCC AGT GGC GCC CAA CGG-3') (9). PCR products were digested with *Msp*I (Biolabs/England). The wild genotype (TT) remains uncut (255 bp) where as the homozygous genotype (CC) is digested into 158 and 97 bp fragments. The heterozygous genotype (TC) contained three bands sized 255, 158 and 97 bp. The restriction digestion products were analyzed on 2% agarose gel electrophoresis.

2.3. Statistical Analysis

The results of phenotypes data were expressed as mean ± SD. Student's t-test was used for the evaluation of data. Genotype data expressed as odds ratio (OR), confidence interval (CI) 95%. Statistical analyses were performed with SPSS (version 20). P-value less than 0.05 was considered to be statistically significant..

3. Results

Fasting glucose, insulin, HOMA and total cholesterol, TG, VLDL-cholesterol and LDL-cholesterol levels were found to be elevated significantly ($p < 0.001$) in type 2 diabetic patients when compared to those of the control group. However HDL-cholesterol was observed to be lowered significantly ($p < 0.05$) during comparable evaluation as shown in table 1.

Results indicated that 92 (89%) out of 103 type 2 diabetic patients were insulin resistant when they were evaluated by the HOMA method. However 5 (9%) out of 57 healthy individuals were observed to be insulin resistant when they were analyzed similarly, hence these patients were excluded from the current investigation.

The analysis of results indicated that the β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP genotype frequencies of wild genotype (TT) and heterozygous genotype (TC) were 80.43% and 19.57% in the insulin resistant type 2 diabetic patients and 94.23% and 5.77% in the control group respectively. The homozygous genotype (CC) were absent in insulin resistant type 2 diabetic patients and the control group as shown in table 2. The heterozygous genotype (TC) was significantly increase (OR=4.12, CI 95% 1.14-15.86, $P < 0.05$) the risk of type 2 DM by four folds with respect to those of the wild genotype (TT) after adjustment for age, sex and BMI. No significant variations were obtained when the analysis was carried out without adjustment.

The allele frequencies of T and C were 90.22% and 9.78% for the insulin resistant type 2 diabetic patients group and 97.12% and 2.88% for the control group respectively as shown in table 3. Finally, The results of present study reveal that significant increase ($P < 0.05$) in fasting insulin, HOMA, BMI and significant decrease ($P < 0.05$) in HDL-cholesterol of heterozygous genotype (TC) when compared with wild genotype (TT). Also there are no significant differences in other clinical characteristics between wild genotype (TT) and heterozygous genotype (TC) as shown in table 4.

4. Discussion

β3-adrenergic receptor is an essential component of the sympathetic adrenoceptor signaling systems. β3-adrenergic receptor is expressed in adipose tissues (white and brown adipose tissue). The receptor is activated during lipolysis in white adipose tissues, also it is activated during thermogenesis in brown adipose tissue. β3-adrenergic receptor mediates catecholamine induce lipolysis and thermogenesis of adipose tissue, these processes are important for the regulation of energy expenditure and body weight (10, 11). β3-adrenergic receptor polymorphisms have been suggested to contribute in the pathogenesis of obesity (12). In particular, a Trp 64 Arg in the β3-adrenergic gene i.e. substitution of tryptophan to arginine (TGG↔CGG) in codon 64 (Trp 64 Arg) (13). Trp 64 Arg polymorphism is located in the first intracellular loop of the β3-adrenergic receptor. This sit is thought to be important for binding of noradrenaline and G proteins with β3-adrenergic receptor in adipose cells (14). β3-adrenergic receptor polymorphism may lead to a decrease in thermogenesis and lipolysis in adipose tissue. Therefore, an impairment of β3-adrenergic receptor function may lead to obesity and insulin resistance through its effect on energy expenditure in adipose tissue (15). The current results are in consistence with the result of Siyan *et al.* (16) and Walston *et al.* (17) studies in which the β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP was found to be associated with type 2 DM. On the other hand it differ from those described by Oizumi *et al.* (18) who did not find an association between heterozygous genotype (TC) of β3-adrenergic receptor gene T↔C (Trp 64 Arg) SNP and type 2 DM.

The presence of C allele of β3-adrenergic receptor gene in this study show an association with obesity (BMI). It is well known that obesity is one of the most important risk factors for the development of insulin resistance and type 2 diabetes mellitus (19). The alteration in function of β3-adrenergic receptor may promote the development of obesity through enhance lipid accumulation in white adipose tissue and decrease biological energy expenditure from brown adipose tissue as a result of decrease lipolysis (20, 21). The result of present study agrees with the result of Mirrakhimov *et al.* (22) who show the C allele of the β3-adrenergic receptor gene in the studied group has an association with obesity and decreased HDL-cholesterol level, also the result agrees with the result of Naoki *et al.* (23) and Luis *et al.* (24) who show an association of the C allele of β3-adrenergic receptor gene with higher levels of insulin and HOMA.

5. Conclusions

β3-adrenergic receptor gene T↔C (Trp 64 Arg) NSP are associated and involved in the pathogenesis of insulin resistant type 2 diabetes mellitus in Iraqi patients. Insulin levels, insulin resistance and HDL-cholesterol are directed by the heterozygous genotype (TC) of the investigated patients.

References

1. Shaikh M., Devrajani B., Shaikh., *et al*.(2012) Plasma homocysteine level in patients with diabetes mellitus. World Applied Sciences Journal. 16 (9): 1269-1273.
2. Ian F. (2010) Insulin resistance and hyperinsulinaemia in the development and progression of cancer. Clinical Science. 118: 315-332.
3. Stunkard A., S0rensen T., Hanis C., *et al*. (1986) An adoption study of human obesity. N Engl J Med. 314: 193-198.
4. Ravussin E., Lillioja S., Knowler W., *et al*. (1988) Reduced rate of energy expenditure as a risk factor for body-weight gain. N Engl J Med. 318: 467-472.
5. Sorensen T., Price R., Stunkard A., *et al*. (1989) Genetics of obesity in adult adoptees and their biological siblings. Br Med J. 298: 87-90.
6. Lonnqvist F., Thorne A., Nilsell K., *et al*.(1995) Pathogenetic role of visceral fat β3-adrenoreceptors in obesity. J Clin Invest. 95: 1109-1116.
7. Colditz G., Willett W., Rotnitzky A., *et al*. (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med. 122 (7): 481-486.
8. Vogelstein B. and Gillepie D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA. 76 (2): 615-619.
9. Clement K., Vaisse C., Manning B., *et al.* (1995) Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med. 333: 352-354.
10. Tian J., Cheng Y., Xiao Y., *et al.* (2007) Association of β3 adrenergic receptor and peroxisome proliferator-activated receptor gamma 2 polymorphisms with insulin sensitivity: a twin study. Biomedical and Environmental science. 20: 99-105.
11. Abu-Amero K., Al-Boudari O., Mohamed G., *et al.* (2005) Beta 3 adrenergic receptor Trp64Arg polymorphism and manifestation of coronary artery disease in Arabs. Human Biology. 77 (6): 795-802.
12. Pierola J., Barcelo A., Barbe F., *et al.* (2007) b3-adrenergic receptor Trp64Arg polymorphism and increased body mass index in sleep apnoea. Eur Respir J. 30: 743-747.
13. Pasquale S., Roberto I., Alfonso S., *et al.* (2001) Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age. Cross-sectional and longitudinal findings of the olivetti prospective heart study. Journal of Hypertension. 19 (3): 399-406.
14. Shiwaku K., Nogi A., Anuurad E., *et al.* (2003) Difficulty in losing weight by behavioral intervention for women with Trp64Arg polymorphism of the b3-adrenergic receptor gene. International Journal of Obesity. 27: 1028-1036.
15. Emorine L., Blin N. and Strosberg A. (1994) The human beta 3-adrenoceptor: the search for a physiological function. Trends Pharmacol Sci. 15: 3-7.
16. Siyan Z. and Suzanne C.(2005) Meta-Analysis of the association of the Trp64Arg polymorphism in the β-3 adrenergic receptor with insulin resistance. Obesity Research. 13 (10): 1709-1719.
17. Walston J., Silver K., Bogardus C., *et al*.(1995) Time of onset of Non insulin dependent diabetes mellitus and genetic variation in the β3 adrenergic-receptor gene. N Engl J Med. 333: 343-347.
18. Oizumi T., Daimon M., Saitoh T., *et al.* (2001) Genotype Arg/Arg, but not Trp/Arg, of the Trp64Arg polymorphism of the beta(3)-adrenergic receptor is associated with type 2 diabetes and obesity in a large Japanese sample. Diabetes Care. 24 (9): 1579-1583.
19. Lorenzo C., Okoloise M., Williams K., *et al.*(2003) The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. Diabetes Care. 26: 3153-3159.
20. Kurabayashi T., Carey D. and Morrison N. (1996) The B3 adrenergic receptor gene Trp64Arg mutation is

over presented in obese women: effects on weight, BMI, abdominal fat, blood pressure and reproductive history in an elderly Australian population. Diabetes. 45: 1358-1363.
21. Proenza A., Poissonnet C., Ozata M., *et al.* (2000) Association of sets of alleles of genes encoding b3-adrenoreceptor, uncoupling protein 1 and lipoprotein lipase with increased risk of metabolic complications in obesity. Int J Obes Relat Metab Disord. 24: 93-100.
22. Mirrakhimov A.,, Kerimkulova A., Lunegova O., *et al.* (2011) An association between TRP64ARG polymorphism of the B3 adrenoreceptor gene and some metabolic disturbances. Cardiovascular Diabetology. 89 (10): 1-7.
23. Naoki S.,Toshihide Y., Tsunekazu U., *et al.* (1997) Effect of Trp64Arg mutation in the β3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. Diabetes Care. 20 (12):1887-1889.
24. Luis D., Ballesteros M., Ruiz E., *et al.* (2010) Polymorphism Trp64Arg of beta 3 adrenoreceptor gene: allelic frequencies and influence on insulin resistance in a multicenter study of Castilla-Leon. Nutr Hosp. 25 (2): 299-303.

Table 1: Mean Fasting Serum Glucose, insulin, HOMA and Lipid Profile Concentration in Type 2 Diabetic Patients and the Control Group

Parameter	Subjects	Mean ± SD	Range	P-value
Glucose (mmol/L)	Control	4.83 ± 0.68	3.2-5.9	< 0.001
	patient	9.94 ± 2.95	7.1-20.2	
Insulin (μIU/mL)	Control	8.24 ± 2.98	1.56-15.27	
	patient	15.82 ± 7.79	5.47-44.36	< 0.001
HOMA	Control	1.80 ± 0.68	0.33 - 3.37	
	patient	7.12 ± 4.29	2.16 - 28.45	< 0.001
Total Cholesterol (mmol/L)	Control	4.20 ± 0.72	2.66-5.39	
	Patient	5.04 ± 0.92	3.81-6.91	< 0.001
HDL Cholesterol (mmol/L)	Control	1.04 ± 0.26	0.58-1.79	
	Patient	0.96 ± 0.22	0.52-1.54	< 0.05
Triglycerides (mmol/L)	Control	1.25 ± 0.52	0.49-2.27	
	Patient	2.05 ± 0.94	0.56-3.97	< 0.001
VLDL Cholesterol (mmol/L)	Control	0.56±0.23	0.22-1.02	
	Patient	0.92 ± 0.42	0.25-1.78	< 0.001
LDL Cholesterol (mmol/L)	Control	2.59 ± 0.77	1.16-4.19	
	Patient	3.15 ± 1.01	1.15-5.39	< 0.001

Table 2: Genotypes Distribution of β3-adrenergic Receptor Gene T↔C (Trp 64 Arg) SNP in Insulin Resistance Type 2 Diabetic Patients and Control Group

Genotype		Type 2 DM	Control	Unadjusted OR (95% CI) P-value	Adjusted OR (95% CI) P-value
TT	No.	74	49	Reference	Reference
	%	80.43%	94.23%		
TC	No.	18	3	3.97 (1.11-14.21) P < 0.05	4.12 (1.14-15.86) P < 0.05
	%	19.57%	5.77%		
CC	No.	-	-	-	-
	%	-	-		
Total	No.	92	52	-	-
	%	100%	100%		

Table 3: Allele Frequency of β3-adrenergic Receptor Gene T↔C (Trp 64 Arg) SNP in Insulin Resistance Type 2 Diabetic Patient and Control Group

Allele		Type 2 DM	Control	OR (95% CI)	P-value
T Allele	No.	166	101	Reference	Reference
	%	90.22%	97.12%		
C Allele	No.	18	3	3.65 (1.04-12.7)	< 0.01
	%	9.78%	2.88%		
Total Allele	No.	184	104	-	-
	%	100%	100%		

Table 4: Genotypes Correlation of β3-adrenergic Receptor Gene T↔C (Trp 64 Arg) SNP with Clinical Characteristics in Insulin Resistance Type 2 Diabetic Patient Group

Clinical Characteristic	Genotype	Mean ± SD	Range	P value
Glucose (mmol/L)	TT	10.2 ± 3.00	7.2 - 18.6	NS
	TC	10.1 ± 3.20	7.6 - 20.2	
Insulin (µIU/mL)	TT	15.94 ± 6.58	7.1 - 36.21	< 0.05
	TC	20.85 ± 9.95	7.68 - 44.36	
HOMA	TT	7.22 ± 3.55	2.51 - 26.36	< 0.05
	TC	9.58 ± 5.96	5.53 - 28.45	
Total Cholesterol (mmol/L)	TT	5.10 ± 0.93	3.81 - 6.89	NS
	TC	4.81 ± 0.92	3.81 - 6.91	
HDL Cholesterol (mmol/L)	TT	0.96 ± 0.19	0.54 - 1.49	< 0.05
	TC	0.85 ± 0.23	0.52 - 1.44	
Triglycerides (mmol/L)	TT	2.03 ± 0.91	0.56 - 3.97	NS
	TC	1.94 ± 1.10	0.74 - 3.97	
VLDL Cholesterol (mmol/L)	TT	0.91 ± 0.41	0.25 - 1.78	NS
	TC	0.87 ± 0.49	0.33 - 1.78	
LDL Cholesterol (mmol/L)	TT	3.22 ± 1.07	1.15 - 5.39	NS
	TC	3.08 ± 0.92	1.50 - 5.13	
BMI	TT	29.94 ± 3.09	23.87 - 37.29	< 0.05
	TC	31.46 ± 3.77	25.68 - 38.07	

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