Original paper

Association between Resisten Gene Polymorphism (420C/G) and Lipid profile in Ischemic Heart Diseases

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Abstract

Background: (resistin is considered as a pro-inflammatory molecule and plays a role in the inflammatory response that lead to atherosclerosis)

Aim: To evaluate the risk of resisten gene polymorphism (420C/G) in the development of Ischemic Heart Disease. To verify the relationship of the investigated SNPs with the metabolic changes related to (IHD), in particular, serum lipid profile.

Methods: A case control study was performed at which 150 patients with IHD and 150 healthy individuals. Genotyping for SNP 420C>G in the resistin gene was performed by the polymerase chain reaction –restriction fragment length polymorphism method. Lipid profile were measured.

Results: The genotype and allele frequencies of resistIn gene polymorphism in IHD and control persons were examined under the co-dominant, dominant and recessive models with the use of multi nominal logistic regression analysis. Neither genotype distribution nor the minor allele frequency showed significant changes among the comparison of the of IHD patients with the control group. The frequency of the G allele of 420(C\G) polymorphism was significantly higher in ischemic heart diseases (IHD**)**. There are significant increases in the level of LDL, triglyceride, VLDL, BMI and a significant decrease in the level of cholesterol in the group of patients with the GG+CG genotypes when they were compared with those of the CC genotype. It shows significant differences in BMI, Age, Cholesterol, LDL, and HDL in the group of IHD, and no significant difference was seen in VLDL and sex.

***Conclusion*:** The −420C>G SNP of resistin gene is not associated with ischemic heart disease in the population of Kerbala and Najaf. The G allele is seemed to increase serum lipid concentrations so it could be considered as an atherosclerotic parameter.

Keywords: ischemic heart diseases (IHD), SNP single nucleotide polymorphism.

Introduction

Ischemic Heart Diseases (IHD) is the most common type of cardiovascular diseases [(1)], is a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls that develops slowly overtime. These bulges are called atherosclerotic plaques [(2)]. is a group of diseases that contain: unstable angina, stable angina and myocardial infarction. [(3)] estrogen hormones play a cardio-protective role in women so they have a lower risk and incidence of CAD compared to age-matched men. (4) There is many risk factor for (IHD) some can modified like Diabetes Mellitus, High blood pressure, Lipoprotein and Obesity.Some cannot be modified like Gender, Age and some can use as protective factors like HDL cholesterol [(5,6)]. Resistin, belongs to a family of cysteine-rich secretory proteins and is a polypeptide hormone secreted by adipocytes [(7)]. Its molecular weight is 12.5 kD and its length

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is 108 amino acids in humans [8]. It is considered as a pro-inflammatory molecule and plays a role in the inflammatory response [9]. in insulin resistance and diabetes in a variety of biological processes metabolic syndrome, cardiovascular disease and atherosclerosis. Resistin plays important regulatory function [10-12]. In healthy humans the normal plasma resistin concentration range between 2–40 ng/ml [13-17]. SNP-420C > G (rs1862513) in the promoter is one of the most studied SNPs of RETN gene [18-21]. It has been demonstrated an association of the-420C>G SNP with coronary artery disease, arteriosclerosis and cerebrovascular disease (22). Researchers have focused on the-420C > G polymorphism (rs1862513). Because it locates within the 5′ flanking region of the RETN gene [20,23].

Materials and Methods

Study subject

A case-control study of 300 subjects (150 IHD and 150 control) was conducted to study the association of 420C\G SNP in Resistin with Ischemic Heart Disease in patients from Kerbala and Najaf. The patient population included 150 subjects (90 men and 60 women) with Ischemic Heart Disease who attended the cardiology center in Najaf governorate and the AL-Hussen Medical City in Kerbala from January to April 2017.The Inclusion criteria were: (1) Those patients who were diagnosed by physicians as having (IHD); (2) Age of subjects was >40 years old. The exclusion criteria were: (1) Patient with renal dysfunction; (2) Patient with liver disease; (3) Patients with diabetes mellitus. The control group included 150 apparently healthy subjects (74 men and 76 women) randomly selected from the general population. The inclusion criteria were: (1) No past medical history of IHD; (2) No family history of IHD; (3) Matched to patients with regard to age, sex, and geographical Distribution. All cases complete a detailed questionnaire that included information about age, sex, family history, drug history, medical history and other relevant information, for all subject weight, height, BMIwere measured. Informed consent has been taken from all subject. Karbala Medical College Ethical Committee has approved the study protocol.

Sample Collection and processing. For chemical analysis venous blood samples were acquired from patients subject and healthy subject. Blood sample were allowed to clot at room temperature, then separate serum by centrifuge, and kept at -80 ^{o}C. Blood samples collected in EDTA tube to prevent coagulation for molecular study.

Phenotypic data

Biochemical analysis were performed. total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C), low-density lipoproteien cholesterol (LDL-C) was calculated using the formula of Friedewald et al .and BMI.

Genotypic data

In EDTA-anticogulant tube, blood samples of (IHD) and control group are collected, and DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction Kit (Promega, U.S. A). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K.). Genotyping of resistin gene (420C\G) was perphormed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using thermocycle (Biometra, Germany). From [24] The primer sequences were obtained. forward 5-TGT-CAT-TCT-CAC-CCA-GAG-ACA-3.and reverse 5-TGG-GCT-CAG-CTA-ACC-AAA-TC-3 .Amplification was performed in a total volume of 25 µl which contained 12.5 µl of Go Taq Green Master Mix, (Promega Corporation, Madison, WI), 1.5µl of each primer (One Alpha, U.S.A.), 3.5 µl of nuclease free water, and 6 µl of DNA template. Cycling conditions were 95^{o}**C** for 4 min followed by 30 cycles of 94^{o}**C** for 45 Sec, 60^{o}**C** for 45 Sec, 72^{o}**C** for 45Sec, and

a final extension of 72 **°C** for 7 min. Amplification product of a resistin gene was533bp.The product digested with 10 u of restriction enzyme (Bbs1) (Biolabs) and 2% agarose gels .To determine genotyping error rate, we performed random duplication in 20% of the samples.

Statistical analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium by **X2** test using online software web-Assotest [25]. Genetic power was calculated using the online software OSSE [26].Genotype and allele frequencies in IHD and control group were tested by multino-mial logistic regression analysis with and without adjustment for age, sex and (BMI) using SPSS.

Result

Result of digestion with restriction enzyme (Bbs1) for resistin gene (420C>G) including 533 bp band for the wild type (CC) genotype, for the heterozygous genotype (CG) three bands 533,323 and 210bp and for homozygous genotype (GG) two bands 323,210 bp. Genotype and allele frequencies of resistin gene are shown in Table 1. Genotype frequencies of 420C>G were consistent with Hardy-Weinberg equilibrium in both IHD subject (P=0.002) and control subject (P=0.004). The power of this study to detect a significant difference at level of 0.05 was 10%. The results shown that resistin gene polymorphism 420C>G (homozygous GG and heterozygous CG genotype) was insignificantly associated with IHD subject. The current study included 300 subjects (150 IHD and 150 control individuals). The clinical and biochemical characteristics of the recuritued individuals were presented in table 2. It shows significant differences in BMI, Age, Cholesterol, LDL, HDL in the group of IHD patients when compared with those of the control group. However, no significant difference was seen in VLDL and sex.

When the data were analyzed under the dominant model, differences being more obvious. There are significant increases in the level of Low density lipoproteins (P=0.029), triglyceride (P=0.001), VLDL(P=0.001), BMI(P=0.001) and a significant decrease in the level of cholesterol (P=0.007) in the group of patients with the GG+CG genotypes when they were compared with those of the CC genotype.

Table 1. Genotype and allele frequency of 420C>G polymorphism of resistin gene and association of this variant with IHD in the study subjects.

Resisten(420C\G)	Control n=150	IHD n=150	Unadjusted OR (95% CI)	Pvalue	Adjusted OR (95%CI)	P value
CC(Reference)	96	88				
CG	22	27	1.39 (0.71-2.52)	0.36	1.22 (0.63-1.92)	0.33
GG	32	35	1.19 (0.68-2.08)	0.53	1.32 (0.73-1.92)	0.48
MAF%	86(18%)	97(20.6%)	1.10 (0.62-1.93)	0.85		

Table 2. Clinical and biochemical characteristics of study subjects.

Parameter	**Controlsubjects**	**IHD subjects**	**P value**
No (M/F)	150(74/76)	150(90/60)	0.064
Age (y)	49.16±4.6	57.41±6.38	0.001
BMI (kg/m2)	22.76±2.91	28±3	0.001
Cholesterol(mg/dl)	168.71±23.86	186.12±25.89	0.001
Triglycerides(mg/dl)	116.78±34.73	124.39±27.82	0.033
VLDL (mg/dl)	24.00±7.54	25.00±6.00	0.231
LDL (mg/dl)	92.67±25.91	110.00±24.00	0.001
HDL (mg/dl)	56.94±15.17	51.72±9.55	0.001

Table 3. Clinical characteristics of IHD subjects according to resistin gene 420(C\G). Genotypes.

Clinical characteristic	CC (n=88)	CG(n=27)	GG (n=35)	P Value
Cholesterol(mg/dl)	181.41±20.42	189.15±19.34	193.65±37.77	0.001
Triglycerides(mg/d)	116.32±22.84	126.41±28.19	143.11±30.25	0.328
VLDL (mg/dl)	23.26±4.56	25.28±5.63	28.62±6.05	0.498
LDL(mg/dl)	105.91±1790.	109.98±18.51	118.26±37.16	0.075
HDL(mg/dl)	52.23±8.30	53.88±9.55	48.74±11.83	0.137

Discussion

Resistin gene polymorphism did not studied nationally and few studies were carried out globally [27]. We herein investigated whether the resistin gene (420C\G) could be associated with development of (IHD) in patients from Kerbala and Najaf. The SNP(420C\G) locates in the promoter region of the gene, so it can direct the changes of resistin action through its concentration or via other routes. It has been found that 2/3 of serum resistin concentration is related to ethnic variation [28]. Unfortunately, resistin levels could not be determined due to logistic difficulties. we found no significant difference in Resistin (420C\G) genotypes between IHD and control subject. the minor allele frequency G was found to be insignificantly altered in the patients and control subject. These results suggested the absence of the association of this SNP with the occurrence of the ischemic heart diseases in our population. However, it is very critical to consider the factors that may affect the results. Genetic power of the study one of the principal factors, it was found to be 10%. This is a low value take out significant difference and the subsequent decision making. The insignificant changes of the genotype frequencies of the G allele was evident among the two investigated groups. In addition, the, the minor allele frequencies did not significantly change during a comparable assessment. Thus, the little difference of the AMF among the two groups will lead to the low obtained genetic power. To solve this problem, it is reasonable to increase the sample size, but the increment is too high that could not be tolerated under the current logistic conditions. Anyhow, the present findings could be considered as a pilot data for further studies conducted on resistin gene polymorphisms in numerous pathological condition. Several studies, have demonstrated that resistin is insignificantly associated with adiposity or metabolic traits, at least in the absence of obesity or type 2 diabetes [29, 30]. Consistent with our results, a study of the −420 variant in Europeans found no correlation with carotid atherosclerosis in a cohort study and no association with myocardial infarction in a case–control study [27]. It seems that resistin polymorphism should need further studies with appropriate sample size to verify its relationship with the ischemic heart disease The analysis of the changes of serum lipid concentration in relevance to the genotype distribution under the co-dominant model exhibited significant elevation for cholesterol levels only, parallel with the presence of the G allele of −420C>G SNP in the resistin gene. Changes appeared to be more profound when the analysis was achieved under the dominant model. Thus, cholesterol, triglycerides, VLDL, and LDL were elevated in carriers of the G allele when they were compared with those of the reference allele. These results suggested that resistin is implicated in elevating serum lipid concentration and the G allele could be considered as a risk factor for the development of atherosclerosis and ischemic heart disease. It is not easily to explain why the G allele is enhancing the rise of serum lipid concentrations and further studies with appropriate sample size

is crucial to solve this problem. In the literatures, there were very little data regarding the changes of serum lipid concentrations or other metabolites in relevance to resistin polymorphisms. It has been found weak associations of the −420G allele with obesity, plasma triglycerides and the metabolic syndrome [27]. An association of the −420G allele with higher glucose levels were reported in Finnish population [22]. and in China [31], Europe and Quebec [32,33]. However, these results were not reported in Scandinavian population [32]. Differences in the current results from those reported previously may belong to the ethnic diversity or may relate to the mechanism by which resisten can modulate these metabolites.

Conclusions

The −420C>G SNP of resistin gene is not associated with ischemic heart disease in the population of Kerbala and Najaf .2- The findings are pilot for further studies try to assess −420C>G SNP of resistin gene in Iraq as well as the periphery.3-The G allele is seemed to i. ncrease serum lipid concentrations so it could be considered as an atherosclerotic parameter.

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