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Correlation between c282y mutation of (hfe) gene with high sensitive troponin i in patients of ischemic heart diseases

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Objective Ischemic heart diseases are the most screen prevalence disease in the world and also diabetic mellitus increases all over the world, both have related to atherogenic lipoprotein modifications. Found the most common C282Y mutation of high ferritin (HFE) gene-related myocardial infarction and unstable angina. The aim of the presented work is to determine whether or not the C282Y mutation in the HFE gene might be correlated with the increased levels of hs-troponin I like a risk index of ischemic heart diseases in diabetic obese patients.
Methods A total of 100 individuals were used in this study, which were divided into (ischemic heart disease, diabetic and obese) and control (diabetic and obese) groups. Conducted in the cardiac care unit at Al-Zahra Teaching Hospital / Karbala and Najaf center for heart diseases between Nov. 2015 to Nov. 2016, after genomic DNA of blood extracted, the HFE gene mutation was detected using the PCR–RFLP technique, hs-troponin I, was determined by ELAIS technique.
Results The data analysis revealed a significant allele frequency of C282Y mutation among the case groups and controls ($P < 0.05$). The relationships between the GA and GG genotypes in C282Y mutation in hs-troponin I show a significant difference between the two groups ($P = 0.002$), lipid profile (TC, TG, LDL-C, HDL-C), Blood Pressure, Body Mass Index (BMI), didn't show a significant difference between the two groups ($P > 0.05$). Lipid profile and BMI level were significant difference with independent predictors of IHD ($P < 0.05$).
Conclusion The results revealed a significant correlation between C282Y mutation and development of IHD in obese T2D patients with increasing levels of hs-troponin I.
Keywords IHD, C282Y, lipid profile, T2M, hs-troponin I

Introduction

Ischemic heart disease 20% of worldwide mortality is the two leading causes of death on a global basis.[1] Ischemic heart disease (IHD) is a large public health problem and is associated with a number of modifiable risk factors.[2] The biological parameters of this aggregation is questioned and genetics could. The impact of risk factor confluence on IHD risk by testing whether genetic risk scores (GRSs) associated with these factors.[3]

Familial could be caused by environmental factors common to family members.[4] Polymorphisms in the HFE gene may influence the risk of ischemic heart disease (IHD).[5] High ferritin (HFE) is a protein of 343 amino acids that includes a signal peptide, an extracellular transferrin receptor-binding region (α1 and α2), an immunoglobulin-like domain (α3), a trans membrane region, and a short cytoplasmic tail.[6] HFE is glycosylated at asparagine residues 110, 130 and 234 during transport to the cell membrane.[7] Homozygosity for a C845G→A mutation (cysteine® tyrosine at amino acid 282, p.C282Y) was found.[6]

The troponin complex regulates the concentration of striated muscles and consists of three subunits (troponin C, troponin T, and troponin I). Troponin C is an 18 ku protein that binds to calcium ions. Troponin T is a 37 ku protein that binds to tropomyosin, thereby attaching the troponin complex to the thin filament. Troponin I is 24 ku proteins that binds to actin and decreases troponin C affinity for calcium, thus inhibiting actin-myosin interaction.[8] Troponin T and troponin I are present in cardiac and skeletal muscles, but are encoded by different genes in the two types of muscle, yielding proteins that are immunologically distinct.[9] Assays that are based on high-affinity antibodies and are specific for cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are available. Because the amino acid sequence of cardiac troponin C and skeletal troponin C is identical, no such assays have been developed for the troponin C component.[10] Cardiac troponin is the preferred biomarker for the diagnosis of acute myocardial infarction (AMI). The recent development of a high-sensitive cardiac troponin I (hs-cTnI) assay permits the detection of very low levels of cTnI. Using the hs-cTnI assay improves the overall diagnostic accuracy in patients with suspected AMI.[11] It has been known for a long time that T2D mellitus accounts for 50 and 80% of patients with hemochromatosis.[12] This causes an increased risk of CAD.[13] Iron overload causes iron depots in some organs including the heart, which have been associated with CAD and reduced life expectancy.[14] Some studies also reported an increased risk of myocardial infarction, and CAD in C282Y and Cys282Tyr in carriers, most of published studies have been cross-sectional, with a restricted number of potentially explanatory variables.[15] Correlated between HFE mutations, diabetic considering the variables such as lipid profile, body mass index among others is very important.[16]

The aim of the presented work is to determine whether or not the C282Y mutation in the HFE gene might be associated with increased levels of hs-troponin I as a risk index of ischemic heart diseases in diabetic obese patients.

Materials and Methods

A cross-sectional study concerned with 100 subjects (50 patients and 50 controls) which were divided into (ischemic heart disease, diabetic and obese) control (diabetic and obese) groups. Conducted in the cardiac care unit at Al-Zahra Teaching Hospital / Karbala and Najaf center for heart diseases between Nov. 2015 and Nov. 2016, after genomic DNA of peripheral venous blood cells extracted, the HFE gene mutation was analyzed using the

PCR–RFLP technique, hs-troponin I, was determined by ELAIS technique. All of them were detected from most common mutation is C282Y HFE gene. Information that has been taken from study subjects included age, sex, family history, degree of relatives of both parents; drug history, medical history and other relevant information, for all subjects' weight, height and BMI had measured. Karbala and Najaf are cities in Iraq, and there is no much difference in genotyping distribution from providence to another, so could represent the Iraqi population.

The exclusion criteria of patients include diagnosed T1DM, chronic renal failure, chronic liver diseases, congestive heart failure, inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematous, contraindication to thrombolysis, patients with malignancy, complicated STEMI at presentation such as heart failure or arrhythmia, malabsorption diseases, patients consume alcohol. Patients were taking hormonal replacement therapy.

Extraction of DNA was done by using whole-blood samples of patient and control groups after collection in EDTA tubes, using Genomic DNA Mini Kit (Blood / cultured cell) (Geneaid), and using Polymerase Chain Reaction (shown Table 2), primer and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) is used for genotyping depending on the restriction endonuclease cleavage. Presence of SNPs that alter the restriction sequence can be genotyped by this method using Ras*I* as restricted enzyme.

Results

Anthropometric and biochemical characterization of studied individuals

A total of 50 type 2 diabetic, obese with ischemic heart disease patients were recruited for the current investigation as well as to 50 (type 2 diabetic and obese) individuals were used as a control group. Characteristics of the enrolled persons are summarized in Table 3. Comparison of the anthropometric and biochemical properties revealed significant variations for all parameters except the age and BMI of patients.

Results of Amplification Reactions

The amplification product of C282Y of HFE gene polymorphism as obtained to have a size of 489 bp. The PCR product was electrophoresed on 2% agarose and directly visualized with ethidium bromide under UV light. The amplification and the size of the resulted amplicons were confirmed by agarose gel electrophoresis analysis (Fig. 1).

Table 1. **Sequence of primer used for PCR amplification of HFE gene polymorphisms**

Gene (HFE)	PCR primer		T_m	PCR product
845G⟶A	Forward	5′-TCCTCTTTCCTGTCAAGTGC-3′	51.4 C	489 bp
	Reverse	5′-GATGACTCCAATGACTAGGG-3′	47.9 C	

Tm, PCR melting temperature; bp, base pairs.

Table 2. **PCR reaction program protocol for C282Y mutation of HFE gene**

Stage	Cycle	Step	Temp.	Time
1	1	1	95	5:00
2	30	1	94	0:30
		2	54	0:30
		3	72	0:30
3	1	1	72	5:00
		2	4.0	HOLD

RFLP Analysis

The amplification product of C282Y of HFE gene polymorphism was digested by Ras*I* restriction enzyme. The product of digestion was analyzed by agarose gel electrophoresis. The results demonstrated three bands, one (253 bp) for GG wild type, two other bands (196 bp, GA) , 29 bp). The 3rd band (29 bp.) is too small that cannot be visualized by (2%) agarose gel electrophoresis with ethidium bromide which indicates to GAH eterozygous genotype, as shown in Fig. 2.

Biochemical Characteristics of Ischemic Heart Disease Patients in Relevance to the Distribution of the Genotypes of C282Y of HFE Gene Polymorphism

To verify the involvement of the investigated SNPs in directing the changes of the pathophysiology in IHD patients, the data were analyzed with respect to the distribution of the genotypes. Genotypes of the C282Y of HFE gene were considered only due to the significant changes of this SNP with respect to the occurrence of troponin I that was obtained.

When the data were analyzed under the dominant model, approximately comparable results were obtained. There are no significant blood pressure ($P = 0.003$), total cholesterol ($P = 0.001$), triglycerides ($P = 0.003$) and LDL-cholesterol ($P = 0.002$) in the group of patients with the CG genotype when they were compared with those of the GA genotype (Table 4).

Table 3. **Anthropometric and biochemical characteristics of the patient and control groups**

	Group	No	Mean ± SD	*P*-Value
Age (years)	Patients	50	58.06 ± 10.22	0.012
	Control	50	51.58 ± 9.64	
BMI (kg/m^2)	Patients	50	1.08 ± 0.404	0.069
	Control	50	1.64 ± 0.485	
Systolic BP (mmHg)	Patients	50	147.80 ± 20.80	< 0.001
	Control	50	133.30 ± 12.48	
Diastolic BP (mmHg)	Patients	50	86.60 ± 17.45	< 0.001
	Control	50	84.22 ± 7.34	
TG (mg/dL)	Patients	50	297.19 ± 136.38	< 0.001
	Control		206.37 ± 67.05	
HDL-C (mg/dL)	Patients	50	41.06 ± 10.67	< 0.001
	Control		51.68 ± 9.74	
LDL-C (mg/dL)	Patients	50	73.06 ± 30.09	0.001
	Control		53.48 ± 27.26	
TC (mg/dL)	Patients	50	243.68 ± 58.57	< 0.001
	Control		199.28 ± 34.11	
VLDL-C (mg/dL)	Patients	50	59.68 ± 27.20	< 0.001
	Control		41.50 ± 13.60	
hs-troponin I	Patients	50	295.44 ± 46.46	<0.001
	Control		7.49 ± 1.39	

Discussion

Various studies concerned with the relationship between genetic variants and the changes in some cardiac biomarkers have been done on different heart diseases including myocardial infarction, coronary atheroseclerosis, and hypertension of Iraqi patients.[17–20]

In Karbala and Najaf populations (50 patients) with IHD a lower frequency of the C282Y mutation comparing to controls was found. Therefore, this result leads us to longitudinal studies

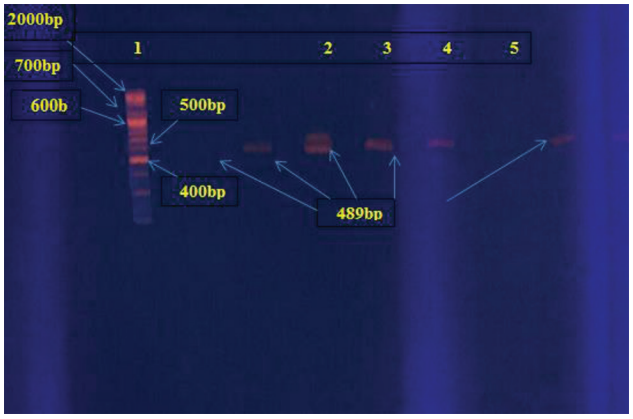

Fig 1. **Electrophoretic pattern of amplification products of C282Y (282) polymorphic region of HFE gene. Amplified products were electrophoresed in 2% agarose gel 47 V, 100 mA for 60 minutes and direct visualization with ethidium bromide under UV light.**

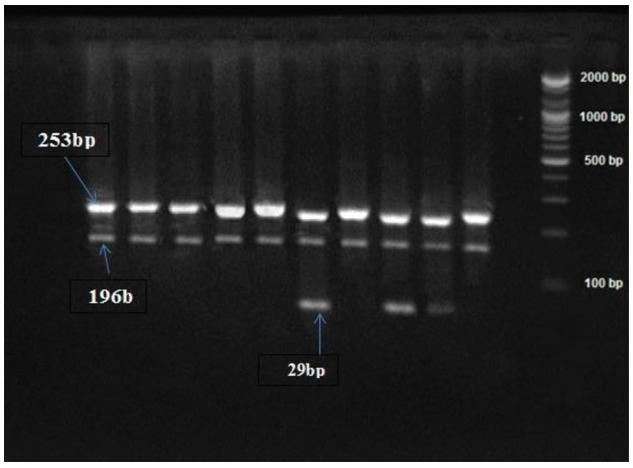

Fig 2. **RFLP pattern of C282Y mutation for HFE gene by using DNA. Ladder DNA (1000-100bp), Lane1-5 ; 7 and 10: GG (253bp and 196 bp.) Lane 6; 8 and 9: GA (196 bp. and 29 bp.)**

Table 4. **Biochemical characteristics of IHD patients in relevance to the genotypes of C282Y mutation**

Parameters	IHD Patients		
	GG	GA	*P*-value
N	26	24	
SBP(mmHg)	132.39 ± 11.85	132.29 ± 12.51	0.017
DBP(mmHg)	84.61 ± 7.74	83.33 ± 7.02	0.513
TG mg/dL	202.76 ± 85.88	212.55 ± 46.59	0.08
HDL-C mg/dL	52.44 ± 9.5	51.44 ± 10.56	0.067
LDL-C mg/dL	48.94 ± 24.59	54.92 ± 28.5	0.242
Total Cholesterol mg/dL	197.12 ± 38.77	200.24 ± 28.76	0.721
Hs-Trop I	314.57 ± 688.83	280.46 ± 11.81	0.002

Table 5. **Biochemical characteristics of control in relevance to the genotypes of C282Y mutation**

Parameters	Controls		
	GG	GA	*P*-Value
N	30	20	
SBP (mmHg)	149.31 ± 20.3	145.00 ± 22.12	0.24
DBP (mmHg)	91.72 ± 13.9	79.5 ± 20.12	0.31
TG mg/dL	324.89 ± 150.94	255.31 ± 106.34	0.15
HDL-C mg/dL	39.89 ± 8.4	43.08 ± 13.44	0.21
LDL-C mg/dL	66.54 ± 31.2	82.57 ± 27.22	0.19
Total Cholesterol mg/dL	249.41 ± 63	234.7 ± 53.40	0.188
Hs-trop I ng/dL	7.35 ± 1.35	7.5 ± 1.84	0.645

with high statistical population at these mutations in association with ischemic heart disease in type 2 diabetic patients. To obtain the prevalence of the C282Y in the Iraq population, we precede a nested cross-sectional study of 100 subjects' include 50 cases of IHD with a history of T2D and obese. 50 matching control subjects. Then detection the prevalence of mutation in patient and control subject. A significant difference between the allele frequencies C282Y mutation among cases and controls was observed. This genotype distribution suggests a significant association between C282Y mutation and IHD. The incidence of diabetes is globally increasing though considered as an epidemic. It shows the importance of investigations in this study due to macro vascular complications that individuals with this condition may experience and consequently cardiovascular diseases. On the other hand, CVDs are the most epidemic causes of mortality and morbidity among people with diabetes.[21] Diabetic patients aggregate other comorbidities such as obesity, dyslipidemia, and hypertension, which also contribute to increase the risk for CVDs. It has been declared that diabetes acts as an independent risk factor for CVD in both men and women.[22] Various forms of CVDs are patented as the cause of death in 65% of patients with diabetes. It could be expected that the hesitancy of HFE gene mutations could be elevated among T2D patients and as a result, among CAD cases.[22] In the study observation, elevated triglyceride, decreased high density lipoprotein (HLD) and increased low density lipoprotein (LDL), study subjects found having controls appeared with abnormal lipid profile. Such result can be explained through the fact that type II diabetics have an increased prevalence of lipid profile abnormalities which contributes to higher IHD.

Conclusion

C282Y mutation is associated with the development of ischemic heart; there were significant elevations in serum lipid profile (total cholesterol, and LDL-cholesterol) in patients as compared with the control group.

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Conflict of Interest

None.

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