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Polymorphism of HFE Gene in Ischemic Heart Diseases and Its Relation with Iron Overload

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ABSTRACT

Background: Ischemic heart disease (IHD) burden consists of years of life lost in worldwide. Diabetes mellitus is the major risk factor for coronary artery diseases; C282Y allele of HFE gene is significantly associated with iron overload (IO). To which extent HFE gene mutations and metabolic alterations contribute to the presence of atherogenic lipoprotein modifications in primary IO remains undetermined. To determine the association between C282Y and H63D mutations in the *HFE* gene with increased risk of Ischemia heart diseases (IHD) in obese patients.**Aim:** To detect whether or not the mutations in the *HFE* gene might be associated with increased risk of ischemic heart diseases in obese patients and then to see its correlation with iron overload. **Materials and Methods:** This cross-sectional study was conducted in cardiac care unit at Al-Zahra Teaching Hospital / Karbala and Najaf center for heart diseases between Dec. 2015 to Sep., 2016, it included100 subjects (50 obese IHD patients and another 50 obese individuals as control group. Iron, total iron binding capacity (TIBC), ferritin, hs-Troponin I, lipid profile were determined. DNA was extracted from fresh blood by using Geneaid kit. PCR amplification by using specified primers, and then polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and electrophoresis by using agarose gel (2%) with ethidium bromide were applied to detect the specific fragments. **Results**: The mean age of patient group was 58.06 ± 10.22 (ranged 38 – 79) years (30 male and 20 female). There is a significant difference between the allele frequencies of C282Y mutations in obese IHD patient groups as compared with obese controls ($P < 0.05$).The relationships between the GA and GG genotypes in C282Y mutation in parameters: hs-Troponin I, lipid profile (total cholesterol, TG, LDL-C, HDL-C and VLDL-C), BMI, Iron, TIBC, and serum ferritin. The data observed no significant differences between the two groups ($P > 0.05$) with all parameters except iron overload and hs-troponin I with C282Y mutation in patient groups. **Conclusion:** 1. HFE gene polymorphism C282Y is associated with development of ischemic heart diseases. 2. Iron, TIBC, and ferritin are related with allele polymorphism C282Y mutation. 3. The HFE mutations may act as genetic markers of IHD risk in Iraq population. 4. There were significant elevations in serum lipid profile (total cholesterol, and LDL-cholesterol) in patients as compared with control group due to effect of IHD. 5. Iron overload was elevated in IHD patients as compared with obese control group.

INTRODUCTION

Ischemic heart disease and cerebrovascular disease account for >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 and parameters of this aggregation is questioned and genetics could. The impact of risk factor confluence on ischemic heart disease (IHD) risk by testing whether genetic risk scores (GRSs) associated with these factors **(3).** The familial aggregation of coronary heart disease is not entirely explained by the familial clustering of currently known coronary risk factors. Such familial aggregation could be caused by yet undefined genetic factors, by environmental factors common to family members, or by interaction of genetic factors with environmental agents **(4).** Polymorphisms in the HFE gene may influence the risk of ischemic heart disease (IHD) **(5).** The genetic background of hereditary hemochromatosis (*HH*) is homozygosity for a cysteine-to-tyrosine transition at position 282 in the *HFE* gene. Heterozygosity for *HH* is associated with moderately increased iron levels and could be a risk factor for heart diseases **(6).** Iron may promote coronary atherosclerotic disease (CAD) by increasing lipid peroxidation. Studies on biochemical or genetic markers of body iron stores as risk factors for CAD have yielded conflicting results **(7).** Several studies **(8), (9)** have reported the association between higher iron storage and an increased risk of T2D mellitus. Many studies indicate that genes associated with increased risk of T2D mellitus, such as genes involved in iron metabolism **(10), (11)** *HFE* gene variants were observed to be associated with iron overload **(12).** *HFE* gene variants cause hereditary hemochromatosis (HH) that often results in an increased risk of coronary artery disease (CAD) **(13).** It has been known for a long time that T2D mellitus accounts for 50 and 80% of patients with hemochromatosis **(14).** Which causes an increased risk of CAD **(15).** Iron overload causes iron depots in some organs including the heart, which has been associated with CAD and reduced life expectancy **(16).** Some studies also reported an increased risk of myocardial infarction **(17),** and CAD in C282Y and Cys282Tyr in carriers **(16)**. Most of published studies have been cross-sectional, with a restricted number of potentially explanatory variables **(18).** Thus, considering the relationship between *HFE* mutations, and the clinical features and complications of diabetes considering the variables such as lipid profile, body mass index among others is very important **(19).**

MATERIALS AND METHODS:

A cross–sectional study concerned with 100 subjects (50 patients and 50 controls).

All of them were detected from two common mutations, 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. It should be noted that **Karbala** and **Najaf** are of the largest cities in Iraq, and there is no much difference in genotyping distribution from providence to another, therefore our study population could represent the Iraqi population.

The exclusion criteria of patients include: Diagnosed T1DM, chronic renal failure, chronic liver diseases, flow up tong drugs, 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, No appear mutations in Molecular analysis.

Inclusion criteria: patients were diagnosed by physicians as having type II diabetes according to the criteria of diagnosis of diabetes were based on WHO guidelines**,** ischemic heart disease (MI and angina).Obese.

The control group included 50 subjects (25 male and 25 female) selected from advisory diabetes clinics, the general population who attend the hospital for checkup also from relatives and colleagues. **Exclusion criteria:** acute or chronic diseases like hypertension and ischemic heart disease. **Inclusion criteria:** Fasting plasma glucose <100 mg/dl. Past medical history of type 2 diabetes. Family history of diabetes in first-degree relations matched to patients with regard to age, sex, and geographical distribution. Age at examination > 35 years. 26 kg/m^2<BMI < 35 kg/m^2.

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 (PCR), Primers and Polymerase chain reaction –restriction fragment length polymorphism (PCR-RFLP) is used for genotyping depending on restriction endonuclease cleavage. Present of SNPs that alter the restriction sequence can be genotyping by this method.

RESULTS:

1- Demographic characteristic in both groups

The study included 100 subjects (50 T2DM, IHD, and Obeses 50 control T2DM, Obeses without IHD individuals). The clinical and biochemical characteristics of the recuritued individuals were presented in table. It shows significant differences in Age, Systolic BP. Diastolic BP and BMI in the group patients when compared with those of the control group.

Table 1: Demographic characteristic in both groups

	Group	No.	Mean ± SD	*P*-Value
Age (yrs.)	Patients	50	58.06 ± 10.22	**0.002**
	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	

Lipids characteristic in both groups

The study result obtained showed that serum TG, HDL-C, LDL-C, total cholesterol and VLDL-C are significantly elevated in ischemic heart diseases (MI & Angina) patients ($p>0.05$) as compared with control group as shown in table **2.**

Table-2 Lipids characteristic in both groups

Parameters	Group	No.	Mean ± SD	*P*-Value
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	

Table3: Parameters characteristic in both groups

Parameters	Group	No.	Mean ± SD	*P*-Value
F-B-S (mg/dL)	Patients	50	206.70 ± 70.70	**0.342**
	Control		193.52 ± 67.14	
Iron (mg/dL)	Patients	50	97.96 ± 49.75	**0.510**
	Control		91.57 ± 33.51	
TIBC (mg/dL)	Patients	50	295.88 ± 149.26	**0.510**
	Control		272.72 ± 100.53	
Ferritin (mg/dL)	Patients	50	130.28 ± 154.67	**0.092**
	Control		90.44 ± 59.73	
hs-Troponin I (pg/ml)	Patients	50	295.44 ± 646.46	**0.002**
	Control		7.49 ± 1.39	

2- **Molecular Analysis**

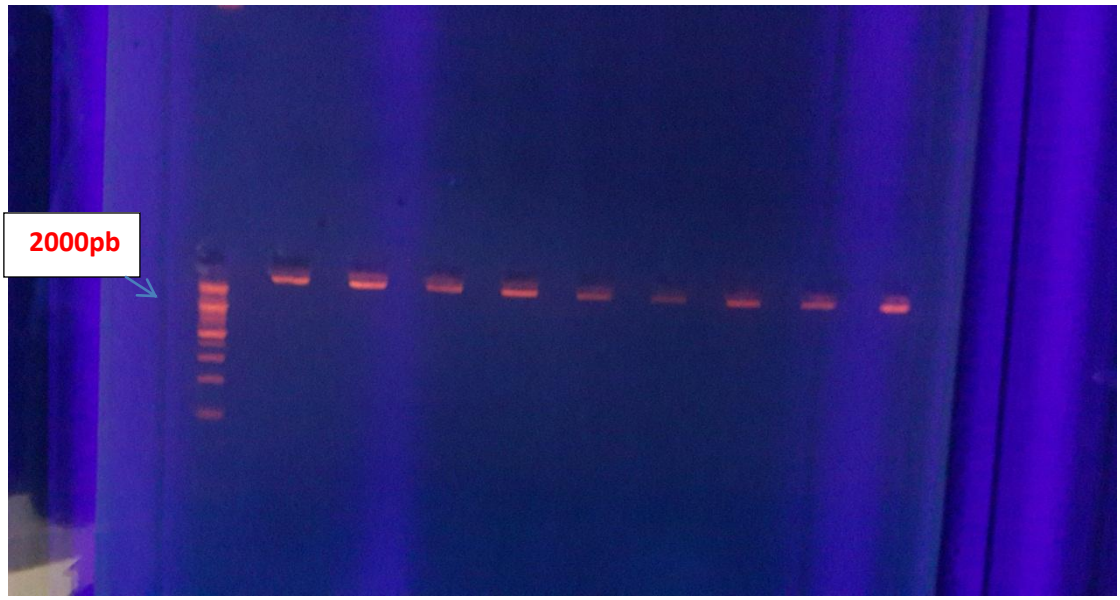

Figure-1. Electrophoresis band for DNA Extraction

Results of amplification reactions of **Cys282Tyr mutation**

Amplification the PCR product of HFE gene polymorphism C282Y,

The amplicon is 489 base pair. This study pointed out that amplicon size is 489 base pair as shown in figure 2.

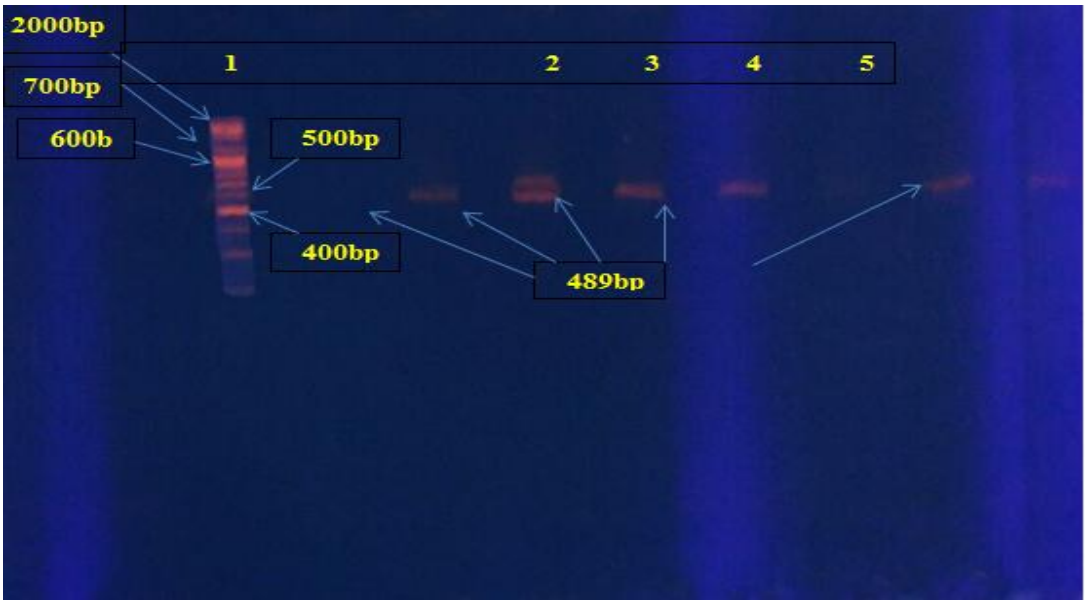

Figure-2 Electrophoretic pattern of amplification products of C282Y (282) polymorphic region of HFE gene. Amplified products were electrophoresed in 2% agarose gel 47V, 100mA for 60 minute and direct visualization with Ethidium Bromide under UV light.

RFLP analysis of C282Y mutation

PCR product of HFE gene polymorphism (C282Y) was digested by *Ras-I* restriction enzyme. The products of digestion were analyzed by 2% electrophoresis agarose gel, products of digestion were (100 V and 100 min) and then stained with ethidium bromide for 15 min, later visualized under UV light. Results discovered one band (253bp) for GG wild type, two bands of three (196, 29 bp) because the 3rd band is too small about 22 67 bp and could not be captured by (2%) agarose gel indicates to CA heterozygous genotype. Two heterozygote genotypes detected included GG and GA, but no homozygote for the C282Y mutation (AA) was found, as shown in figure (3):

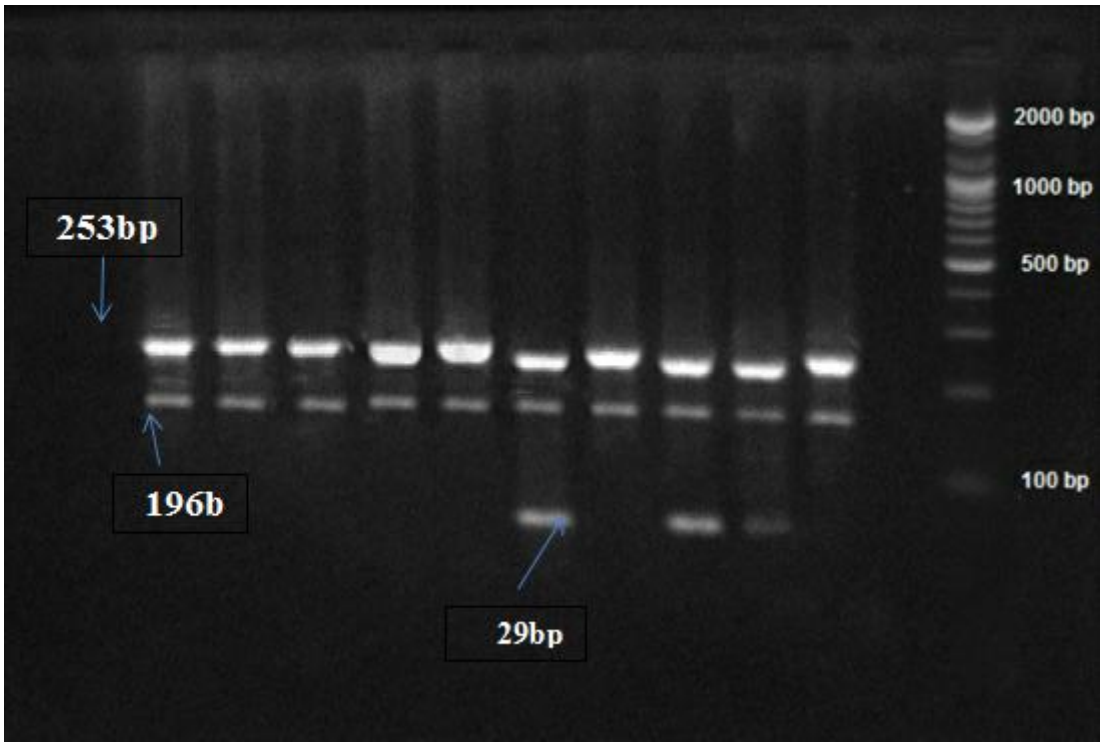

Figure-3. RFLP pattern of C282Y mutation for HFE gene.

Ladder DNA marker (100bp), line1 GG (253bp), line2 GA (196bp).

DISCUSSION:

From our knowledge, no Iraqi studies have been conducted of HFE gene polymorphisms in respect ischemic heart disease, obsess and suffer type 2 DM.

Molecular of HFE gene

In Karbala and Najaf populations (50 patients) with myocardial infarction and angina, a lower frequency of the C282Y mutation comparing to controls was found. Therefore, this result leads us to longitudinal studies 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 mutation in the Iraq population, we precede a nested cross-sectional study of 100 subjects' include 50cases of IHD with a history of T2D and obese. 50 matching control subjects. We then detection the prevalence of the two mutations in patient and control subjects. A significant difference between the allele frequencies of H63D and C282Y mutations among cases and controls was observed. This genotype distribution suggests a significant association between HFE mutations and IHD**.**

Studies performed by several authors in Brazil also demonstrated a higher frequency of H63D than other polymorphic variants in the healthy control group, whereas C282Y was more frequent in the patient group. The prevalence of the *HFE* gene alleles C282Y, H63D and S65C in the general healthy population in Brazil ranges from 1.15-2.19%, 9.54-14.57 and 0.31-1%, respectively. At the same time, for the patient group, the alleles range from 7.36-50% for C282Y, 6.25-26.59% for H63D and 0-2.23% for S65C (Bueno *et al.*, 2006; Oliveira *et al*., 2006; Cançado *et al.*, 2006; Cançado *et al*., 2007; Santos *et al.*, 2010; Santos *et al*., 2011; Leão *et al*., 2014; Dionísio Tavares Niewiadonski *et al.*, 2015).

The incidence of diabetes is globally increasing and though considered as an epidemic. It shows the importance of investigations in this study due to macrovascular complications that individuals with this condition may experience and consequently cardiovascular diseases. On the other hand, CVDs are the most prevalent causes of mortality and morbidity among people with diabetes (**Gomes, 2013**). Diabetic patients aggregate other comorbidities such as obesity, dyslipidemia, and hypertension, which also contribute to increasing the risk for CVDs. It has been stated that diabetes acts as an independent risk factor for CVD in both men and women (**Gomes, M.D.B., 2013**). Several forms of CVDs are listed as the cause of death in 65% of patients with diabetes. It could be expected that the frequency of *HFE* gene mutations could be elevated among T2D patients and as a result, among CAD cases (**Gomes, M.D.B., 2013)**, (Oliva**, Rafael, *et al*.2004**). The HFE protein is a type I transmembrane protein bound to β2-microglobulin, and mutated HFE protein at position 63 or position 282 can result in the lack of association with β2-microglobulin thereby disrupting the association

of *HFE* with transferrin receptor, which leads to increased iron absorption (**Zhang, Dongfeng, *et al*.2013).**

The different or small number of samples and the region of each study can explain the variations in the frequency of the HFE gene polymorphisms in Brazil. Moreover, it is necessary to take into consideration a large amount of miscegenation existing in Brazil. Nevertheless, we found a significant correlation between the genotype distribution in patients and the general population for the C282Y variants in our study. Therefore, we have highlighted the need to characterize specific populations for SNPs associated with disease.

CONCLUSION:

1. HFE gene polymorphism C282Y is associated with development of ischemic heart diseases.

2. Iron, TIBC, and ferritin are related with allele polymorphism C282Y mutation

3. The HFE mutations may act as genetic markers of IHD risk in Iraq population

4. There were significant elevations in serum lipid profile (total cholesterol, and LDL-cholesterol) in patients as compared with control group due to effect of IHD

5. Iron overload was elevated in IHD patients as compared with obese control group

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