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Evaluation of HFE Gene *(C282Y)* Mutation and Its Association Relation with Coronary Artery Diseases in Indian Population

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Abstract

Background: The gene associated with the CAD disease was found out to be the HFE (High Iron Fe) gene and the disease is described with the two, missense mutations of the gene– *C282Y* and *H63D*. The focus of this study was to assess the *C282Y* mutation in CAD patients that results from transition of guanine to adenine (G-A) at the nucleotide position 845, which in turn changes cysteine to tyrosine at the 282nd position.

Material and methods: *C282Y* mutation was analyzed by allele specific PCR (AS-PCR) in 100 samples which includes 96 males and 4 females. Data was collected from each patient. The blood from each patient was collected and analyzed for their cholesterol level, diabetes, RBS (Random blood sugar), LDL (low-density lipoprotein), HDL (high-density lipoproteins), TGL (Triglycerides), smoking, etc.

Results: Out of 100 patients, 12 were found to be C282 and 88 were found to be *C282Y*. The frequency of *C282Y* in CAD patients with respect to age was found to be 84 in males. Out of 88 *C282Y* patients 49 was found to have ≤ 140 mg RBS level and 39 patients was found to have >140 mg RBS level. The frequency of *C282Y* with respect to cholesterol level was found to be ≤ 200 mg in 82 patients and >200 mg in 6 patients. The frequency of *C282Y* with respect to HDL was found to be ≤ 40 mg in 79 patients and >40 mg in 9 patients. The frequency of *C282Y* with respect to LDL was found to be ≤ 100 mg in 70 patients and >100 mg in 18 patients. The frequency of *C282Y* with respect to TGL was found to be ≤ 150 mg in 45 patients and >150 mg in 43 patients. Out of *C282Y* patients 4 patients had CHD, 13 were hypertensive, 21 were diabetic, 55 were smokers and 40 were alcoholic.

Conclusion: There association between the *C282Y* mutation in the haemochromatosis gene and prevalence of CAD was not evident. Besides this mutation is not a threat for advanced disease or depressed ejection fraction in CAD.

Keywords: CAD; *C282Y* mutation; AS-PCR; CAD patients

Introduction

Atherosclerosis is a cause of Coronary artery disease (CAD) which is a multifactorial chronic disease. The initiation and development of atherosclerosis depends largely on genetic factors and life style, but the underlying cellular and molecular mechanism remains unclear [1,2]. The World Health Organization (WHO) estimated 20 million cardio vascular disease (CVD) deaths in 2015, accounting for 30 percent of all deaths worldwide [3-5]. The common autosomal recessive disorder studied in the patients of CAD is the Hereditary Hemochromatosis (HHC), which reduces iron absorption and results in excessive iron deposition in various organs of the body like the liver, pancreas, heart, joints and pituitary gland [3-6]. The age of onset of the disease varies with men and women. It is noted generally that the disease manifests later in women. This time delay in women is due to their frequent blood loss in the form of the menstrual blood losses and pregnancies [5]. The gene associated with this disease was found out to be the *HFE* gene and the disease has been described with the two, missense mutations of the gene– *C282Y* and *H63D*. The *HFE* gene is present at the 6p21.3, approximately 4.6 megabase telomeric from HLA-A. The protein HFE is a 343 residue type I transmembrane protein that links with the class I light chain β2-microglobulin association, disrupting their cellular transport and the presentation on their surface [4]. The phenotypic expression of HHC is variable; it is more of interplay between the genetic factors, environmental factors, the dietary consumptions if iron, etc. [5]. Early complaints of the disease may include fatigue, weakness, joint pains, palpitations and abdominal pain. These symptoms are nonspecific so the disease of often not diagnosed at this stage. The major endocrine disorder associated with HHC is diabetes mellitus. This is because it has been postulated that the iron deposition damages the pancreatic beta cells and provides insulin resistance [4,5]. The two allelic variants of HFE are missense mutations. The cause of *C282Y* mutation is the transition of guanine to adenine (G to A) transition at the nucleotide position 845, which in turn changes cysteine to tyrosine at the 282 position [7-9]. The focus of the present study was to examine the frequency of *C282Y* mutation in Coronary Artery Diseases in Indian population.

Materials and Methods

Study population and clinical data

A total of 100 CAD blood samples were included in this study. A cohort of 96 males and 4 female patients. Corresponding data from each patient was collected and analyzed for their cholesterol level, diabetes, RBS, LDL, HDL, TGL, smoking, etc. This population included patients with angina like chest pain and non-invasive tests suggesting ischemia, patients with depressed left ventricular ejection fraction of unknown origin and patients with valvular heart diseases. The exclusion criteria involved the patients with previously performed coronary bypass surgery or percutaneous transluminal coronary angioplasty (PTCA) because of their treated coronary status. The patients follow up was maintained regularly and samples were collected on regular basis. 5 ml peripheral blood was collected in EDTA (Ethylene Diamine Tetra Acetic Acid) Vials after obtaining informed consent from the patients.

The study was approved by concerned Institutional Ethics Committee. The distribution of the selected characteristics for CAD patients are summarized in Table 1.

HFE gene (*C282Y*) mutation analysis

DNA was extracted by DNA extraction kit (GENEAID) from the whole blood according to the manufacturer protocol. The mean OD of DNA samples was found to be 1.8 nm by using spectrophotometer. The mutation of *C282Y* gene in CAD patients was confirmed using primers specific for *C282Y* (Table 2). The reaction conditions for *C282Y* are as follows: 96ºC for 10 min, 96ºC for 30 s, 58ºC for 45 s and 72ºC for 45 s.

Variables	Number of Patients CAD Patients
	100
Gender	
Males	96
Females	4
Age	
≤ 50	47
>50	53
RBS	
≤ 140 mg	57
>140 mg	43
Cholesterol	
≤200 mg	93
>200 mg	7
HDL	
≤ 40 mg	91
>40 mg	9
LDL	
≤ 100 mg	79
>100 mg	21
TGL	
≤ 150 mg	51
> 150 mg	49
CHD	
Yes	8
No	92
Hypertension	
Yes	14
No	86
Diabetes	
Yes	23
No	77
Smoking	
Yes	63
No	37
Alcohol	
Yes	40
No	60
Pan Masala	
Yes	2
No	98

Table 1: Distribution of selected characteristics among the study population.

Results

Iron deposits in the arterial wall activate the low density lipoprotein cholesterol peroxidation and therefore contribute the formation of atherosclerotic lesions and to the inflammation and are the foremost cause to cardiovascular disease. *C282Y* carriers had an increased risk of myocardial infarction and coronary heart disease (CHD). Our results showed smoking has also been related to cardiovascular disease through increased thrombosis, inflammation in vascular epithelium, and oxidation of low-density lipoprotein (cholesterol). Therefore, to properly disentangle the association between *HFE* mutations and cardiovascular disease requires taking into account all the biochemical parameters and lifestyle status (Table 3). The aim of the present study was to examine the effect of *HFE C282Y* mutation in CAD patients. The *C282Y* mutation in the gene for haemochromatosis (HFE) has been associated with CAD at middle age. In the current study 47 patients were ≤ 50 years and 53 patients were >50 years. We reconnoitred the association between the *C282Y* mutations in CAD patients.

There is an enduring discussion of the validity of subgroup testing by sex in genetic studies; there are reasons to separate men and women for *HFE*. First, phlebotomy is the key approach for prevention of hemochromatosis and women are therefore naturally protected due to their menstruation. In our study an increased risk was found in Male carriers rather than in Female carriers. This may be related to the study design as we have studied elderly people and consequently male carriers may have been selected out from the population due to early mortality related to *HFE*. It was also found from our study that the major hazardous factors for causing CHD are smoking; body mass index, diabetes and lipid profile gets reinforced while adjusting HR for CHD in women and men. The extent of CHD risk conferred by these factors is of such magnitude that the genetic predisposition associated with *HFE* carrier status is rapidly outweighed as shown in Table 3. It is found from our study that greater CHD risk is associated with smoking status rather than with *HFE* mutations. The higher rate of smoking (current and former) among men compared to women at reference line, it is likely that the uncertain effect of *HFE* mutations on the risk of CHD is only apparent among women.

Frequency and correlation of *C282Y* mutation in coronary artery diseases with respect to age

The sample consisted of 96 males and 4 females (Figure 1a). Among 100 patients, 12 were found to be C282 and 88 were found to be *C282Y* (Figure 1b). While there is an on-going discussion of the validity of subgroup testing by sex in genetic studies, there are reasons to separate men and women for *HFE*. First, phlebotomy is the key strategy for prevention of hemochromatosis and women are therefore naturally protected due to their menstruation.

The frequency of *C282Y* in CAD patients with respect to age was found to be 84 in males. The frequency of *C282Y* mutation in CAD patients with respect to age was found to be 38 in ≤ 50 age group and 50 in >50 age group (Figure 1c). The current study had identified older age as strongest predictors for CAD.

Frequency and correlation of *C282Y* mutation in CAD with respect to hypocholesteremia and RBS

In addition to the assessment of the C28Y mutation, the cholesterol

F-C282Y	5′-CATGAAGTGGTGAAGGATAAG-3′	C282Ycommon forward	
R-C282	5′-CTGGGTGCTCCACCTGGC-3′	Reverse ASP for C282 allele	155
R-Y282	5′-TACCCGTGATGGCTGGGTGCTCCACCTAGT-3′	Reverse ASP for Y282 allele	167

Table 2: AS-PCR primers.

and RBS level were also evaluated. The frequency of *C282Y* with respect to cholesterol level was found to be ≤ 200 mg in 82 patients and >200 mg in 6 patients (Table 3). Out of 88 *C282Y* patients 49 was found to have ≤ 140 mg RBS level and 39 patients was found to have >140 mg RBS level (Figure 2).

Frequency and correlation of *C282Y* mutation in coronary artery diseases with respect to alcohol drinkers and pan masala eaters

The frequency of *C282Y* with respect to alcohol drinkers was found to be 40 in alcohol drinkers and 48 in non-alcohol drinkers (Figure 3). The frequency of *C282Y* with respect to pan masala eaters was found to be 2 in pan masala eaters and 86 in non-pan masala eaters (Figure 4). The frequency of *C282Y* with respect to smokers was found to be 55 in smokers and 33 in non-smokers (Figure 5).

Frequency and correlation of *C282Y* mutation in Coronary Artery Diseases with respect to different parameters of biochemical analysis

The frequency of *C282Y* with respect to diabetes was found to be 21 in diabetes patients and 67 in non- diabetes patients (Figure 6). The frequency of *C282Y* with respect to HDL was found to be ≤ 40 mg in
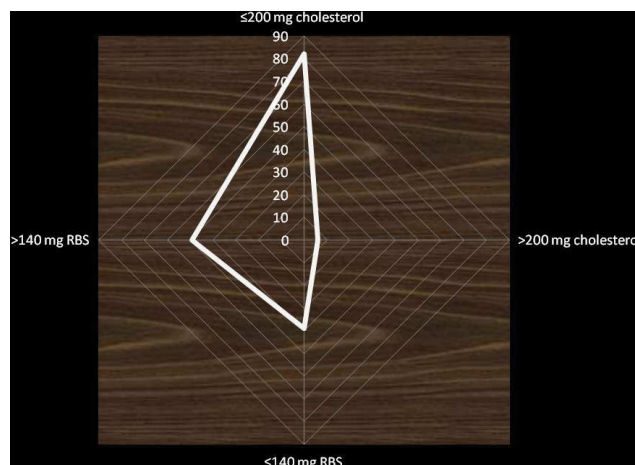

Figure 2: Frequency and correlation of C282Y mutation in CAD with respect to hypocholesteremia and RBS.
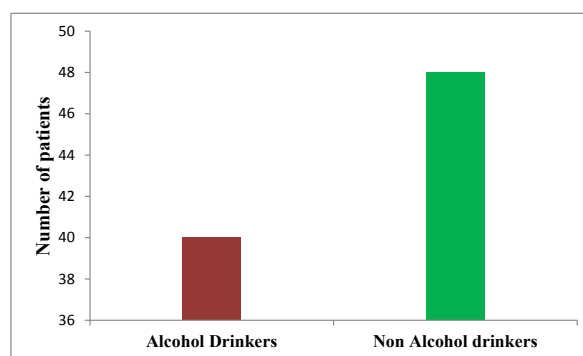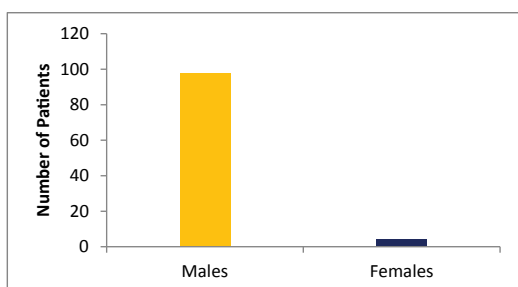

1a: No. of CAD patients.

1b: Genotype frequency.

1c: C282Y mutation in different age groups.

Figure 1: Frequency and correlation of C282Y mutation in CAD with respect to age.

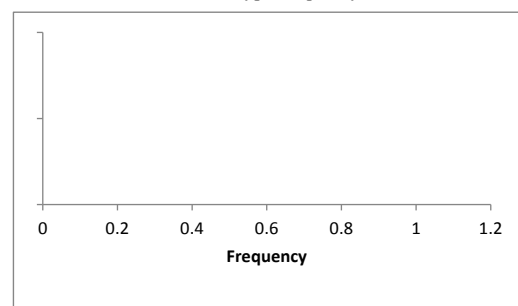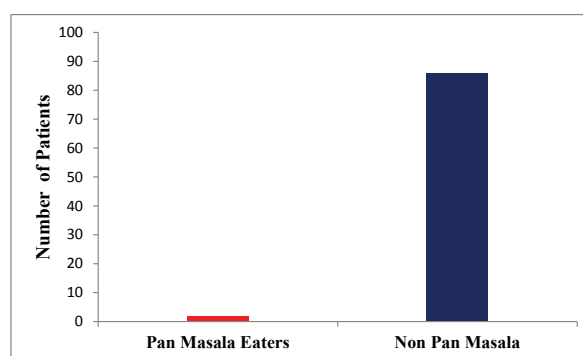**Figure 3:** Frequency of C282Y mutation in CAD with respect to alcohol drinkers.

Figure 4: Frequency of C282Y mutation in CAD with respect pan masala eaters.

79 patients and >40 mg in 9 patients (Table 3). The frequency of *C282Y* with respect to LDL was found to be ≤ 100 mg in 70 patients and >100 mg in 18 patients (Table 3). The frequency of *C282Y* with respect to CHD was found to be 4 in CHD and 84 in non-CHD (Table 3). The frequency of *C282Y* with respect to hypertension was found to be 13 in hypertension and 75 in non- hypertension (Figure 6). The frequency of *C282Y* with respect to TGL was found to be ≤ 150 mg in 45 patients and >150 mg in 43 patients (Table 3).
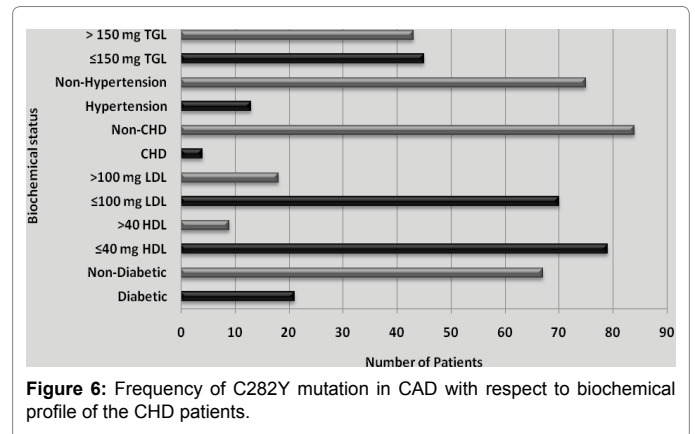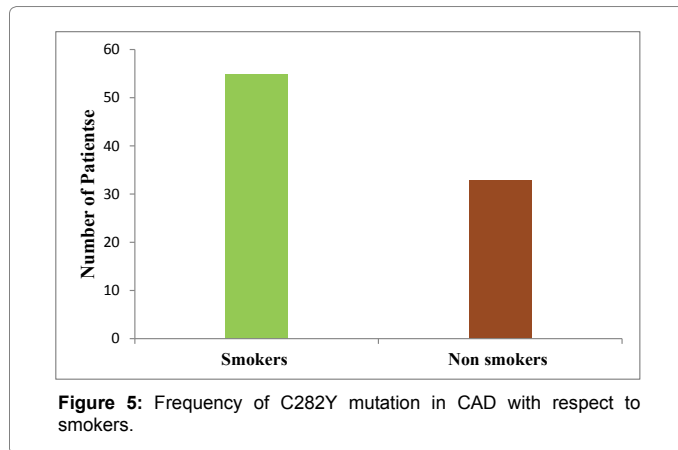
Figure 5: Frequency of C282Y mutation in CAD with respect to smokers.

Figure 6: Frequency of C282Y mutation in CAD with respect to biochemical profile of the CHD patients.

Table 3: Various biochemical parameters, lifestyle status and C282Y mutation Analysis in CAD patients.

Variables	CAD Patients	C282	C282Y	P Value
Number of Patients	100	12	88	
Gender				
Males	96	12	84	0.5947
Females	4	0	4	
Age				
≤ 50	47	9	38	0.0381
>50	53	3	50	
RBS				
≤ 140 mg	57	8	49	0.3452
>140 mg	43	4	39	
Cholesterol				
≤ 200 mg	93	11	82	0.8028
>200 mg	7	1	6	
HDL				
≤ 40 mg	91	12	79	0.3003
>40 mg	9	0	9	
LDL				
≤ 100 mg	79	9	70	0.4826
>100 mg	21	3	18	
TGL				
≤ 150 mg	51	6	45	0.8231
>150 mg	49	6	43	
CHD				
Yes	8	4	4	0.0067
No	92	8	84	
Hypertension				
Yes	14	1	13	0.4716
No	86	11	75	
Diabetes				
Yes	23	2	21	0.4455
No	77	10	67	
Smoking				
Yes	63	8	55	0.5238
No	37	4	33	
Alcohol				
Yes	40	0	40	0.0013
No	60	12	48	
Pan Masala				
Yes	2	0	2	0.7733
No	98	12	86	

Discussion

In Indian population, the *C282Y* missense mutation in *HFE* gene is the major cause of hereditary haemochromatosis [10-13]. In this study, the incidence of the *C282Y* mutation in *HFE* gene was evaluated in 100 people whose coronary anatomy was defined by means of coronary angiography. Out of 100 patients, 12% patients were homozygous and 88% were heterozygous for *C282Y*. The frequency of heterozygosity for *C282Y* mutation was consistent with the frequency predicted with Hardy-Weinberg equation. In our study, the deposition of excess iron in the atrial wall seems to contribute towards the formation of atherosclerotic lesions and inflammation eventually leading to CAD [14]. Experimental findings indicated that the excess in iron deposits triggered lipoprotein cholesterol peroxidation. In an animal model for atherosclerosis, iron deficient diets lead to the reduction in atherosclerotic lesion formation. Epidemiological studies have shown an association between the development of CAD and *C282Y* mutation. In an angiographically controlled population, the concentration of ferritin and transferrin showed no association with CAD. In a study, where 174 patients with angiographically confirmed CAD were compared to healthy subjects, no relation between the *C282Y* mutation and CAD was found [15-20]. In an evaluation of 265 patients with proven premature CAD, a lower frequency of the *C282Y* mutation was found as compared to healthy controls [19]. A post-mortem study conducted in 41 cases showed no association of the occurrence of CAD with iron overload and multiorgan haemosiderosis [19]. Coronary angiography is considered as the gold standard for detecting CAD and the magnitude of coronary atherosclerosis [15-18]. Recent data from a large angiographically controlled population, showed no evidence of any association between *C282Y* mutation and increase in incidence of CAD or MI. In contrast to the prediction, the Gensini score was found to be even lower in patients with CAD carrying the *C282Y* mutation. Reduced left ventricular ejection fraction was established only by the presence of CAD or MI and not by the heterozygosity of the *C282Y* mutation. These data do not support increased myocardial damage owing to iron overload in heterozygotes, in contrast to the development of cardiomyopathy in several homozygotes suffering from hereditary haemochromatosis [21]. Our findings in a large cohort of patients do not invalidate epidemiological studies inquiring the association between heterozygosity and cardiovascular mortality. Since we examined a population undergoing coronary angiography, therefore, be biased towards progression of the disease related to haemochromatosis. The only established risk factor in our study population, not associated with CAD, was arterial hypertension. This can be explained by angina-like chest pain in hypertensive patients accompanied by false positive stress testing due to left ventricular hypertrophy. This leads to diagnostic

coronary angiography and subsequently to an over representation of hypertensive patients in the group with exclusion of CAD.

Conclusion

Our population of 100 Indian people with angiographically confirmed coronary status, there is no evidence of an association between the *C282Y* mutation in the haemochromatosis gene and prevalence of CAD or previous MI. Moreover, this mutation is not a risk factor for advanced disease or depressed ejection fraction in CAD.

Authors' Contributions

All authors read and approved the final manuscript. Thanks to all authors for their support and help in this study.

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