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Artículo original

Metabolismo del hierro, mutaciones H63D y hemocromatosis hereditaria en pacientes con anemia drepanocítica en la India

H63D hereditary hemochromatosis and iron metabolism in Indian sickle cell patients

Sanjay Pandey,* Sweta Pandey,** Vineet Shah,** Rahasya Mani Mishra,*** Tulika Seath,* Renu Saxena*

RESUMEN

Antecedentes: la hemocromatosis hereditaria es un trastorno del metabolismo del hierro caracterizado por aumento en su absorción y almacenamiento. La mutación H63D del gen HFE puede estar asociada con sobrecarga de hierro en pacientes hindúes con anemia de células falciformes (drepanocítica).
Objetivo: determinar la prevalencia de mutaciones H63D y su efecto en pacientes hindúes con anemia drepanocítica.
Material y método: los sujetos de estudio fueron pacientes con drepanocitosis (50 con hemoglobinopatía S homocigota y 67 con hemoglobina S / β-talasemia). Se incluyeroon también 178 controles sanos apareados por sexo y edad. Se realizó conteo celular completo mediante un analizador celular automatizado y medición cuantitativa de hemoglobina por medio de cromatografía líquida de alta resolución (HPLC). Se extrajo ADN de leucocitos de sangre periférica con el método fenol-cloroformo. La mutación de H63D fue determinada por PCR-RFLP y los productos del PCR fueron digeridos por restricción de la enzima Bcl-1. Los estudios del hierro fueron realizados por un método estándar de laboratorio. El análisis estadístico se realizó con el software Epi-Info. Se utilizó la prueba de la x^2 de Yates para determinar si existen diferencias significativas entre los grupos de estudio y la t de Student para comparar las medias aritméticas, mediante el software GraphPad.
Resultados: la prevalencia de H63D fue significativamente mayor en el grupo de enfermos (28%) que en los controles (10%) (valor de p ± 0.004 y ± 0.057). El hierro sérico fue mayor en el grupo con mutación H63D que en el que no la padecía (valor de p<0.001).
Conclusión: la mutación H63D se relaciona significativamente con aumento de la absorción y metabolismo bajo del hierro en pacientes hindúes con células falciformes.
Palabras clave: HFE, SCD, H63D, hemocromatosis

ABSTRACT

Background: Hereditary hemochromatosis is an iron metabolism disorder characterized by increased iron absorption and storage. H63D mutation of HFE gene may be associated with iron overload in Indian sickle cell patients.
Objective: determine the prevalence of the H63D mutations and their effect on iron metabolism in Indian sickle cell patients.
Material and Method: Study subjects were sickle cell patients (50 sickle cell anemia and 67 sickle β-thalassemia). One hundred seventy, age and sex matched healthy controls were recruited to compare the frequency of H63D mutation. Complete blood count was measured by automated cell analyzer and quantitative assessment of hemoglobin was performed by high performance liquid chromatography. DNA was extracted from the peripheral blood leucocytes by phenol-chloroform method. HFE gene mutations H63D was determined by PCR-RFLP and PCR products were digested with restriction enzymes Bcl-1. Iron studies were done by standard laboratory method. Statistical analysis was performed on EpiInfo statistics software. Yates' chi-square test was used to assess inter-group significance and t- test used to compares the means of two groups on GraphPad software.
Result: The prevalence of HFE mutation H63D was investigated among 50 sickle cell anemia and 67 sickle β-thalassemia patients. The prevalence of H63D was statistically significant in the group (p-value, ± 0.004 and ± 0.057). Serum iron in the H63D group were higher in comparison to without H63D group of patients (p-value <001).
Conclusion: H63D mutation present significantly and associated with increased iron absorption and low iron metabolism in Indian sickle cell patients.
Key words: HFE, SCD, H63D, Hemochromatosis

* Department of Hematology, All India Institute of Medical Science, New Delhi, India.
** Department of Cardiac Biochemistry, All India Institute of Medical Science, New Delhi, India.
*** Department of Environmental biology, Awadhesh Pratap Singh University, Rewa, India.

Correspondence: Sanjay Pandey. Department of Hematology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India. E-mail: pandeysanjaybt@rediffmail.com
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Hereditary hemochromatosis is a common autosomal recessive disorder of iron metabolism in Caucasians, with a prevalence of one in 300-500 individuals. Iron homeostasis is maintained by regulating iron absorption. Unlike for other essential minerals, the human body does not have a regulatory mechanism for excreting excess iron. In Mediterranean countries the most common cause of iron overload is homozygous β-thalassemia.[1] Hereditary hemochromatosis is an iron metabolism disorder characterized by increased iron absorption and storage, resulting in progressive and multisystemic oxidative organ damage. In 1996, the HFE gene was identified on candidate for the gene bearing the primary defect responsible for hemochromatosis.[2-4] Genetic factors and acquired conditions are likely to modulate the expression of HFE hemochromatosis.

Two missense mutations (C282Y, H63D) have been described on the HFE gene in patients suffering from hereditary hemochromatosis on the basis of phenotypic data. H63D is a C-G transition at nucleotide 187 of the HFE gene which results in a histidine to aspartic acid substitution. It has been found to be present with a frequency of 3.3%-15.2% in the general population across the world.[5-7] The H63D mutation has been found to be highly prevalent among Brazilians (carrier frequency: 27.5%), with a frequency similar to what is observed among white Europeans, particularly among Italians.[8, 9] There are only a few studies from India on the frequency of the known HFE gene mutations.[10-13] Thus the aim of this study was to determine the prevalence of the H63D mutations and their effect on iron metabolism in Indian sickle cell patients.

MATERIAL AND METHODS

Study subject were sickle cell patient (50 sickle cell anemia and 67 sickle β-thalassemia) who were attending the outpatient department of hematology. Age –sex matched 170 controls were recruited to compare the frequency of H63D mutation. Screening of the patients was done by cation exchange high performance liquid chromatography (HPLC). About 5 ml venous blood was taken for DNA extraction and iron study; after informed consent from all the patients and controls. Study was approved by institutional ethical committee. Complete blood count and red cell indices were measured by automated cell analyzer (SYSMEX K-4500, Kobe Japan). Quantitative assessment of hemoglobin Hb F, Hb A, Hb A2 and Hb S was performed by HPLC (Bio-Rad-Variant™Bio Rad, CA, USA). DNA was extracted from the peripheral blood leucocytes by phenol-chloroform method. HFE gene mutations H63D was determined by specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to Feder[14] (1996) et.al.. The PCR products were digested with restriction enzymes Bcl-1 (New England Bio labs, Beverly, MA) to identify the H63D variants. Serum iron, total iron binding capacity (TIBC) and % transferrin saturation estimation was done by standard laboratory method. Statistical analysis was performed on EpiInfo statistics software (Version 3.5.1). Yates' chi-square test was used to assess inter-group significance and p-value <0.05 was considered statistically significant._ t test was applied to compare the means of two group on GraphPad (version 3.06) software.

RESULTS

Sickle cell patients were divided in two groups. First group was had fifty sickle cell anemia and second group was had 67 sickle β-thalassemia patients. One hundred seventy, age and sex matched healthy controls were recruited to compare the frequency of H63D mutation. In group-1; male were 30 (60.0%) and female were 20 (40.0%) with mean age of 10.72±7.68 years. Forty nine (73.1%) male and 18(26.9%) female with mean age group of 11.91±8.30 years, patients were in group-2. In controls; 102 (60.0%) were male and 68(40.0%) were female with mean age of 10.99±7.61 years. Out of the 67 sickle β-thalassemia patients, 13(19.4%) were heterozygous and 7(10.45%) were homozygous for the H63D mutation. In the group of sickle homozygous, nine (18%) were heterozygous and 5(10%) were homozygous. One Hundred seventy controls were had 12 (7.05%) heterozygous and 4(2.35%) homozygous. Serum iron with H63D mutation was high (113.7±20.5µg/dl) in comparison to without H63D patients (94.6±25.3µg/dl). Total iron binding capacity (TIBC) was 315.9±28.3µg/dl in H63D mutant while 327.4±15.7µg/dl was without H63D mutant. Transferrin saturation % was 32.6±7.3% in H63D mutant group and 29.7±5.3% in without H63D mutant group. The frequencies of H63D and iron parameters are given in **table 1 and 2** respectively.

Table 1. Frequency of H63D Mutation

Mutation	*Genotype*	*Patients*		*Control N=170*	*p-value*	*OR*	*95%CI*
		HbSS N=50	HbSβthal. N=67				
H63D	-/-	36(72%)	47(70.15%)	154(90.58%)	0.00003	0.25	0.13-0.51
	±	9(18%)	13(19.4%)	12 (7.05%)	0.004	3.05	1.37-6.89
	+/+	5(10%)	7(10.45%)	4(2.35%)	0.057	3.56	0.97-14.12

Table 2. Iron, TIBC and Transferrin saturation% levels

Parameters	*Sickle patients with H63D N=34*	*Sickle patients without H63D N=83*	*P-value*
Serum Iron µg/dL	113.7±20.5	94.6±25.3	0.0001
TIBC µg/dl	315.9±28.3	327.4±15.7	0.005
Transferrin saturation %	32.6±7.3	29.7±5.3	0.018

DISCUSSION

Iron overload can be associated with various pathological conditions.[15] Hereditary hemochromatosis is considered to be the most common inherited disorder in Caucasians and presents a variable prevalence among different ethnic groups.[3,16] Primary iron overloads is uncommonly encountered in Indians and happens to be common in the Caucasians of North Europe. In the west, the C282Y mutation of the HFE gene is associated with Hereditary hemochromatosis in majority of cases. Variations in prevalence of the HFE gene mutations (C282Y and H63D) have been established in many European populations and descent (United States, Canada, Australia and South Africa). Few studies are available from India on the prevalence of these mutations in the general population.[10-13] The prevalence of *hereditary haemochromatosis* seems to be low in people of Asian origin. In our study, sickle homozygous was had 18% H63D heterozygous. The sickle β-thalassemia was had 19.4% heterozygous and controls was had 7.05% heterozygous. Homozygous H63D were 10%, 10.45% and 2.35% in sickle homozygous, sickle β-thalassemia patients and controls respectively. Prevalence of heterozygous and homozygous were statistically significant (p-value+/-0.004 and +/+ 0.057). In Brazil, H63D screening in 4 specific populations (Caucasians, African descendants, Parakana Indians, and a racially mixed group) indicated the H63D mutation varied from 0% in Parakana Indians to 16.3% in Caucasian descendants.[8] Screening for hemochromatosis mutations in beta-thalassemia minor patients from Iran indicated significant differences in the frequencies of C282Y and H63D mutants in relation to control individuals[17] but these differences were not observed in Portugal and India.[18,19] In a study carried out in Hong Kong, iron overload in alpha-thalassemia was not related to hemochromatosis mutations.[20] Most European studies have reported that 60-90% of typical hemochromatosis patients are homozygous for the C282Y mutation of the HFE gene (C282Y/C282Y). Compound heterozygotes (C282Y/H63D) and, less commonly, H63D homozygotes usually have normal iron tests, but it has been described that in some cases these genotypes may resemble C282Y homozygotes, with mild-to-moderate iron overload.21-24 In our cases the 56 sickle cell patients were transfusion dependent where 23 patient were H63D mutant either heterozygous or homozygous condition. Serum iron was higher in H63D positive sickle patients group and statistically significant (p-value-0.0001). In conclusion H63D mutation present significantly and associated with increased iron absorption and low iron metabolism in Indian sickle cell patients.

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