Heterozygosity of H63D and C282Y HFE Genotypes: A Genetic Modifier of Iron Deficiency Anaemia

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Abstract

Introduction: **HFE geneis a key regulator of iron hemostasis. In mutant condition it leads to iron overload, but in heterozygous condition it absorbs iron more efficiently than wild type conditions. A balanced polymorphism is one in which the beneficial effect of the heterozygous state balances the deleterious effect of the homozygous state. Thus our objective was to determine the gene frequency of the three HFE mutations and to relate genotypes to various clinical and laboratory variables.**

Materials & Method: **Study subjects were 435 iron deficiency anaemia patients and 560 age and sexmatched healthy controls. The complete blood count and red cell indices were measured by an automated cell analyzer (SYSMEX K-4500, Kobe Japan). Serum ferritin level was used to estimate the iron status of patients. Serum ferritin and CRP were measured by ELISA technique while ESR analysis was done according to Wintrobes's method**

Results: **Among the IDA patients, 19 were heterozygous for H63D mutation. Six IDA patients were heterozygous for C282Y mutation, while S63C mutation was not identified in subjects. Controls were presenting 0.71% H63D homozygous frequency while neither patients nor control were presenting C282Y homozygous mutation. Heterozygous were identified with frequency of 4.46 and 1.6% in H63D and C282Y mutations in controls respectively.**

Conclusion: **Heterozygotes for HFE mutations had a lower prevalence of iron deficiency anaemia, thereby conferring protection against iron deficiency and iron deficiency anemia. Screening for HFE mutations may be useful tool in grading of IDA.**

Conflict of Interest: **Authors declare no conflict of interest.**

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Introduction

Human hemochromatosis (HFE) gene was identified in year 1996 on the short arm of chromosome 6 which is a candidate gene for hereditary haemochromatosis.[1] C282Y (Leads to substitution of cysteine at amino acid position 282 by tyrosine) mutation has the majority of patients with haemochromatosis (85%–90%) in European countries. This mutation causes G to A substitution on exon 4 of the HFE gene.[2,3] H63D, HFE gene mutation from histidine to aspartic acid at amino acid position 63 has also been implicated; however, this mutation causes mild phenotype of haemochromatosis.[3-5]

A rare mutation in the HFE gene, S65C, has been identified in eight French Hereditary Hemochromatosis (HH) patients.[6] However; its role on iron homeostasis remains controversial as the S65C variant is associated with increased percent transferrin saturation in healthy Canadian blood donors. The allelic frequency of S65C is 0.6%-1.95% in Caucasian population.[7] Two splice site mutations IVS5+1 G/A and IVS3+1 G/T have been identified and also shown to be associated with hereditary haemochromatosis; these mutations affect expression of the HFE protein through an alteration in splicing of the HFE gene mRNA.[8,9] Heterozygotes of HFE genotype have slightly higher Hb along with significantly raised serum iron, transferrin saturation (TS) and even serum ferritin. Mutation in the HFE gene is an example of balanced polymorphism and C282Y & H63D heterozygotes absorb iron much more efficiently than the wild types hence are protected from Iron deficiency.[7,10,11] The genetic forms of iron deficiency anaemia are also associated with iron overload and a non responsive phenotype. Based on this background, it was hypothesized that presence of any of these may influence the iron absorption and render protection from severity of iron deficiency. Subjects with these mutations would be expected to have better hemoglobin (Hb) and iron profile than those of the wild types. Also their response to iron supplementation would be inadequate or absent.[12] C282Y homozygosity has been reported in 67–100% of patients with genetic hemochromatosis (GH) and 3.2–13% of Caucasians have been found to be heterozygous for this gene alteration. Such a high prevalence of a single mutation is unusual for a disorder with an autosomal-recessive mode of inheritance.[1,13-17] Microsatellite analysis of HFE gene locus revealed that the 282 mutation occurs on chromosomes with the same haplotype in different populations. This observation is a strong argument for a founder effect of the mutation arising on a single chromosome. The high frequency of this particular genetic defect may therefore be the result of a selection advantage for heterozygous carriers of this mutation. The metabolic consequences of the heterozygous state on iron metabolism may confer such a biological advantage.[18,19] In the present study, the hypothesis have been tested that the H63D and C282Y mutation in the HFE gene represents a protective factor against iron deficiency anaemia patients. No previous genetic studies have been reported among patients with iron deficiency anaemia from India. This is the preliminary report from Asian Indian population with objective to determine the gene frequency of the HFE mutations in relation of genotypes to phenotypes of iron deficiency anaemia.

Materials And Method

Subject: Study subjects were 435 iron deficiency anaemia patients and 560 age and sex-matched healthy controls. Tribal subjects were predominantly recruited for the study. Ten millilitres of venous blood was collected from the subjects after obtaining the signed informed consent. Study was approved by the institutional ethic committee. Patients were diagnosed on basis of hemogram and iron profile studies especially serum ferritin level. Controls were age and sex matched healthy population of the same region. Patients were recruited from tertiary care hospital of Vindhyan region, Madhya Pradesh who came for screening of iron deficiency anaemia. Study does not cover the consecutive patients. IDA patients with other chronic disease were excluded from the study.

All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Evaluation of Haematological and Iron Status: The complete blood count and the red cell indices were measured by an automated cell analyzer (SYSMEX K-4500, Kobe Japan). Serum ferritin level was used to estimate the iron status of patients. Serum ferritin and C-reactive protein (CRP) were measured by Enzyme Linked Immuno Sorbent Assay (ELISA) technique while erythrocyte sedimentation rate (ESR) analysis was done according to Wintrobes's method.

Molecular study of HFE Gene Mutations: Total genomic DNA was isolated from peripheral blood leukocytes by the kit (Bio-Serve) method. HFE gene mutations; C282Y, H63D and S56C were screened by specific polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) according to published literatures.[1,20,21]

Statistical analysis: Statistical analysis was performed on GraphPad (version 3.06) software. Student's t test was applied to compare the means of two groups. P-value <0.05 was considered statistically significant.

Results

A total 435 Iron deficiency anaemia patients were evaluated (260 male and 175 female with a mean age of 22.5±3.1 and 20.2±3.1 years respectively). Out of 175 female, 80 were in gestation and 95 were in non gestation period. A compleet blood count, Iron profile, CRP and ESR were performed in all subjects as well as in age – sex matched controls (360 male and 200 female with mean age 23.3±2.4 and 19.6±3.2 years repectively) whereas 90 female were pregnant. Patients were recruited between age group of 6 to 40 years. Frequencies of HFE were estimated in patients as well as control and data analyzed according to presence of HFE and absence of HFE in IDA patents. Thus the research was a retrospective case control study. Three HFE gene mutations, namely the C282Y, H63D and S63C were evaluated. Among the IDA patients, 19 were heterozygous for H63D mutation. Six IDA patients were heterozygous for C282Y mutation, and S63C mutation was not identified in the subjects. Controls were presenting 0.71% H63D homozygous frequency while neither patients nor control were presenting C282Y homozygous mutation. Heterozygous were identified with frequency of 4.46 and 1.6% in H63D and C282Y mutations respectively in controls. Comparative Iron profile of heterozygous and wild genotype of HFE in IDA patient is given in table-1. Frequency of clinical symptom is given in Fig.1.

Discussion

Iron deficiency anemia is one of the most prevalent diseases globally, especially in women and children that leads to decreased fitness. Mutation that protects against it would be subject to positive selection. A recent survey of 499 women for the C282Y mutation showed that heterozygotes had slightly higher haemoglobin levels than did controls, [10] but another study of similar size found no protection against iron deficiency.[11] A study suggests that HFE serves as a receptor for an infectious agent. If HFE mutations protects against iron deficiency, it might be presumed that one or more of the HFE mutations would have reached fixation in areas in which iron deficiency was common [22]. We report 25 heterozygous of H63D genotype in controls while 1.6% controls were presenting C282Y heterozygous conditions. Amongst IDA patients, frequency of H63D heterozygous was 4.36% while 1.3% patients were presenting C282Y heterozygosity. Neither control nor patients showed S65C

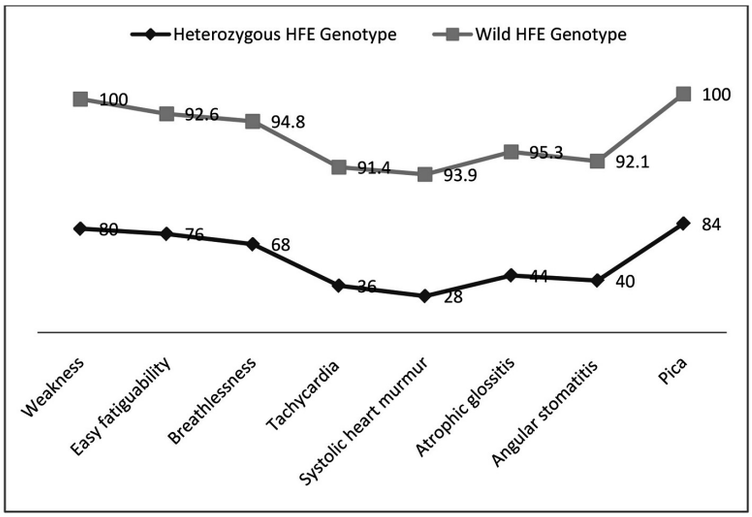

Fig.1: Comparative clinical frequency of HFE (H36D & C282Y) genotype

Table.1: Comparative parameters of HFE (H36D & C282Y) genotype

IDA Patients (N=435) Mean ±SD					
Iron/biochemical profile (normal range with unit)	**HFE genotype (H63D & C282Y) (+/-& +/+) N= 25 N=410**	**HFE genotype (H63D & C282Y) (-/-)**	**SE**	**95% CI**	**P value**
Serum Ferritin (15-300 μg/L)	11. 4±2.3	10.3±1.4	0.302	0.507 to 1.693	<0.001
Total iron binding capacity (TIBC)300-400 μg/dl	460.6±52.9	487.6±71.3	14.504	-65.507 to -8.493	0.011
Transferrin saturation (20-25 %)	15.5±3.7	13.4±1.9	0.421	1.273 to 2.927	<0.001
Erythrocyte Sedimentation Rate(ESR) 0-29 mm/hr	18±4	25±3	0.631	-8.24 to -5.76	<0.001
Creactive protein (CRP) 0-3 mg/Lmg/L	0.9±0.5	1.3±0.4	0.084	-0.564 to -0.236	<0.001
Haemoglobin (HGB) 12-17 g/dl	7.3±1.3	6.2±1.2	0.248	0.612 to 1.588	<0.001

SE = Standard Error, CI = Confidence interval

genotype. Homozygous conditions was absent in studied subjects. This observation suggested that frequencies of these mutations are slightly higher in controls than patients. Healthy Indian population showed lower prevalence of C282Y and H63D mutation than that in the healthy populations of northern European origin (4%–10%), though data available on the study is limited. [23] A study did not find C282Y mutation in any of 134 healthy subjects and 249 patients with chronic liver disease of various aetiologies from India.[24] Another study from India, found heterozygous C282Y mutation in only 1 of 112 healthy persons and 3 of the 75 patients with thalassaemia major.[25] H63D is prevalent and C282Y is rare in North Indians and the presence of H63D mutation does not increase body iron as measured by Serum ferritin in beta thalassemia traits. The low prevalence of haemochromatosis in India may be the result of the low prevalence of C282Y mutation in the Indian population.[26] One study did not found any individual with C282Y or H65C either in the cases or in the controls while 12% H63D heterozygosity in normal individuals and 14.8% in 236 patients were reported.[27] The data is in consonance with a report in which C282Y mutation was found to be absent in other Asians including those from China, Japan, Micronesia and Nepal.[28] The gene frequency of the C282Y mutation in Northern European populations is extremely high. For example, in Ireland, a gene frequency of 0.123 has been documented, such that 20% of the population is heterozygous.[29] H63D has an established role in iron homeostasis and heterozygotes for H63D showed a significant increase in transferring saturation and serum ferritin levels as compared to HFE wild type controls. [30-33] Our study reported significant elevation of serum ferritin and haemoglobin level in HFE (H63D & C282Y) heterozygous genotype while decreasing levels of CRP and ESR were observed. Value of these parameters are statistically significant. TIBC Level was elevated while transferrin saturation level was decreased in HFE (H63D & C282Y) wild type genotype and p-values were statistically significant. H63D mutation frequency shows high variability worldwide.[34] It ranges from 23.0% in Bulgaria to 3.5% in Ecuador.[35,36] Allelic frequency of H63D in Jordan is 11.25%.[34] The carrier frequency of the H63D mutation is 21.6% in Europe, 5.4% in Africa/Middle-East, 2.8% in Asia and 22.8% in America. The frequency of C282Y heterozygosity is 1–3 percent in southern and eastern Europe and as high as 24.8 percent in Ireland.[37] A study in Italy with untreated celiac disease revealed HFE mutations do not constitute a protective factor against the development of iron deficiency [38] while a study in Brazilian female blood donors reported the protective effect of the heterozygous genotype for HFE C282Y mutation against ID and IDA.[39] Another study found no difference in HFE expression in hemochromatosis patients who were iron overloaded when compared to hemochromatosis patients who were iron depleted.[40] A Turkish study did not find any C282Y mutation among the women. Case-control study showed that H63D had a positive association with breast cancer.[41] Another study provided evidence for a protective role of the C282Y mutation in the HFE gene against iron deficiency in young women and suggests that a more efficient utilization of nutritional iron may have contributed to the high prevalence of the mutation in Caucasian populations.[10] Data from this research showed that HFE heterozygosity play a key role as predators of IDA and need diagnosis of HFE genotype in iron deficiency anemia. It can be used in base line investigation and treatment decision.

Conclusion

In conclusion, our interpretation from the present study strongly suggests the role of the H63D and C282Y heterozygosity in the HFE gene in prevention of iron deficiency in Vindhyan population. Protection against iron deficiency and its morbid consequences have conferred a selection advantage for heterozygous carriers of the mutation. Further genetic studies are needed to identify other gene mutations responsible for the phenotypic influence in patients of iron deficiency anaemia. Study was restricted to regional population of Vindhya region of Central India. Further it is suggested that, cohort study with various iron haemostasis gene effect may be designed and promoted for future relevant outcomes in all over the country.

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