

FREQUENCY OF HFE GENE MUTATION IN IRON OVERLOAD PATIENTS

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ABSTRACT

Objective: To determine the frequency of HFE gene mutation in iron overload patients.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), from Feb 2017 to Jan 2018.

Patients and Methods: Sampling technique use was non-probability consecutive sampling, patients who reported in AFIP were selected. Data was collected from 196 participants, of 20 to 60 years of age and both genders. Therefore 43 participant individuals, were included who were presented with transferrin saturation >45% or either serum ferritin >1000 ng/mL. Serum ferritin was analyzed by Immulite 2000, serum iron and serum total iron binding capacity were analyzed by Random access ADVIA®1800 to calculate %TS by Total Iron/TIBC×100, C-Reactive Protein (CRP) was measured by BT® 1500. DNA was extracted by using Puregene® Blood Core kit.

Results: Forty-three patients sample was analyzed that showed increased serum ferritin, transferrin saturation (%TS). Mean age of study participants was 42.16 ± 11.18 years. Out of 43 patients there were 38 (88.4%) males and 5 (11.6%) were females. Frequency of serum ferritin >1000 ng/mL was 35 (74.5%), <1000ng/mL was 8 (17%) as well as transferrin saturation <45 was 11 (23.4%) and >45 was 32 (68.1%). Consanguinity was reported 34 (79.1%). Only a single 33 years old patient was reported with C282Y mutation in our study samples.

Conclusion: C282Y homozygosity should be suspected in patients showing biochemically elevated levels of %TS and serum Ferritin.

Keywords: C282Y mutation, Ferritin, Hemochromatosis, HFE gene, Iron overload.

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INTRODUCTION

Iron is an essential nutrient of our body which plays a vital role in daily life, majority of the iron is found in the hemoglobin and myoglobin where it functions as an oxygen transporter¹. It helps in different functions of body like boosting of immune system, cellular respiration and oxygen transport. Iron depletion may occur in body may be due to dietary deficiency, blood loss etc; it may result in anemia. Excessive iron concentration in the body is harmful for the cell and tissues that may lead to different pathological conditions².

Hereditary hemochromatosis is an autosomal recessive disorder which is characterized by increased intestinal absorption of dietary iron

more than twice that of normal³, due to excessive retention of iron in the circulatory system it may lead to various pathological changes in body including fatigue, arthralgia, abdominal pain, and loss of sex drive. With time, the symptoms develop into diabetic mellitus, cirrhosis, endocrine changes, sexual dysfunction etc^{4,5}.

In 1996, two gene mutation of HFE were found to be responsible for hemochromatosis i.e. C282Y mutation which is caused by substitution of tyrosine for cysteine at position 282 due to the substitution of adenosine for guanosine at nucleic acid position 845 (845G>A) and H63D mutation due to substitution of aspartate for histidine at position 63 of amino acid due to the substitution of guanosine for cytidine at nucleic position 187 (187C>G)⁶. Mutation in HFE gene is the most common cause of hereditary hemochromatosis⁷. It can be manifested by different mutations in HFE gene mainly due to C282Y and H63D allele.

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Mutation in C282Y allele enhances the absorption of iron due to down regulation of regulatory system and leads to hemochromatosis^{8,9}, where H63D has less effect but causes the defect in HFE ability in down regulation of iron¹⁰. Almost 80-92% of the time, cases are due to mutation at C282Y homozygosity¹¹. Whereas H63D has no role in producing homozygous hereditary hemochromatosis^{12,13}. HH carry a low risk for heterozygote mutation in C282Y and H63D¹⁴. The severity in symptoms is not same. It may depend upon diet, sex, age and race^{15,16}. In Asia C282Y mutation has been relatively rare, and poorly defined due to poor diagnostic conditions¹⁷. Many studies have been conducted all around the world showing the importance of HFE gene mutation in HH. The aim of the study was to find out the frequency of gene mutation of HFE in Pakistan in iron overload patients.

PATIENTS AND METHODS

This was a cross sectional study, conducted over a period of one year (February 2017 to January 2018) after approval from Institutional Review Board (IRB), Armed Forces Institute of Pathology (AFIP), Rawalpindi. Sample size was calculated by using the formula $(n=Z^2\alpha/2(SD)^2/(MOE)^2)$. A total of 196 individuals were enrolled in the study but 10(5%) of them did not provide consent to participate. Non-probability consecutive sampling was done from individuals who were presented with transferrin saturation >45% or serum ferritin >1000 ng/mL at AFIP. The research ethics committee of AFIP approved the study protocol. Therefore, 3ml blood venous blood was collected in plain gel tube for analysis of C reactive protein, serum iron, TIBC, ferritin and 3 ml in EDTA tube for genetic testing after taking informed consent.

Blood specimens were analyzed in the Department of Chemical Pathology & Endocrinology AFIP Rawalpindi. Inclusion criteria was adults between 20 to 60 years of age of both genders who understood informed consent. Percent TS >45% or serum ferritin >1000 ng/mL were included for mutation analysis of

C282Y for HFE genotype. All individual were asked about history of diabetes mellitus, arthritis, liver disease, congestive heart failure, impotence, infertility. Individuals who were diagnosed cases of hemochromatosis were excluded from the study.

Biochemical serum markers, serum iron and serum total iron binding capacity were measured by Ferrozine-no deproteinization method by random access ADVIA® 1800 Chemistry auto-analyzer. Percent TS was calculated by total Iron/TIBC×100, CRP was measured by Turbidimetry method by BT® 1500 biotechnica Instrument. Serum ferritin was measured by solid phase, two site chemiluminescence method by Immulite 2000® autoanalyzer. Analytical sensitivity of TIBC, iron was 1.8µmol/L and 0.9µmol/L respectively while recovery is 95% and 97% respectively. Patients results validated by Internal quality control.

Internal quality control (IQC) material used for TIBC and iron was lyophilized, which was reconstituted with 5ml of distilled water and results plotted on Levy Jening chart (LJ), IQC material used was Certified Reference Material (CRM) and also which was traceable to National Institute of Standardization and Technology (NIST). Linearity for serum ferritin assayed by dilution series (1/4-1/16) while analytical sensitivity was 0.4ng/ml with 94-97% recovery. Serum ferritin results validated by IQC, which were lyophilized, Randox multi cal control material used and Certified Reference Material and traceable to NIST. Analytical sensitivity of CRP was 0.6mg/L and end point method with 340nm wave length. CRP IQC material used was in liquid form. Patients' results were validated by plotting control values against LJ charts and the patient's results were analyzed after quality check. External quality control program was run on monthly basis for all analyte.

DNA was extracted by using Puregene® Blood Core kit B USA. Tetra ARMS-PCR primers were designed. Polyacrylamide gel electrophoresis was used to visualize results.

Statistical analysis was performed using SPSS version 24. Descriptive statistics like Mean ± SD was calculated for quantitative variables whereas qualitative variables were computed in the form of frequency and percentages.

RESULTS

A total of 43 patients' sample was analyzed which showed biochemical increased in serum ferritin, transferrin saturation (%TS) that fulfilled

Therefore 43 patients were analyzed for HFE genotype C282Y with high ferritin level >1000ng/ml or %TS >45%. Mean age of study participants was 42.16 ± 11.18 years and further divided into four categories. Age groups were 20-30 years, 31-40 years, 41-50 years and 51-60 years comprised of 8 (18.6%), 13 (30.2%), 9 (21%) and 13 (30.2%) individuals respectively. Out of 43 patients there were 38 (88.4%) males and 5 (11.6%) were females. Study participants that

Table-I: Biochemical profile of patients.

Variables	n (%)	Male (Mean ± SD)	n (%)	Female (Mean ± SD)
Age	(88.4%)	42.34 ± 11.044	(11.6%)	48.40 ± 11.371
Iron (µmol/L)	(100%)	28.50 ± 6.60	(100%)	30.20 ± 9.85
Ferritin (ng/mL)	(100%)	1355.03 ± 536.55	(100%)	2280 ± 64.51
TIBC (µmol/L)	(100%)	51.55 ± 6.50	(100%)	52.00 ± 13.89
Transferrin saturation (%)	(100%)	55.11 ± 11.97	(100%)	58.20 ± 15.33
CRP(mg/L)	(100%)	5.78 ± 2.42	(100%)	7.84 ± 1.73

Table-II: Tetra ARMS-PCR primers for C282Y.

Name	Primer sequence
Forward Inner	ACCCCTGGGGAAGAGCAGAGATATACTTG (G allele)
Reverse Inner	ATCCAGGCCTGGGTGCTCCACCTTGT (A allele)
Forward Outer	CTTCAGTGACCACTCTACGGTGTCTGGGC
Reverse Outer	CTCAGCCCACCCCTAACAAAGAGCAGA

the inclusion criteria. Data were collected from 196 participants out of whom 10 (5%) patients

belonged to urban area were 12 (27.9%) and from rural area were 31 (72.1%). Frequency of serum

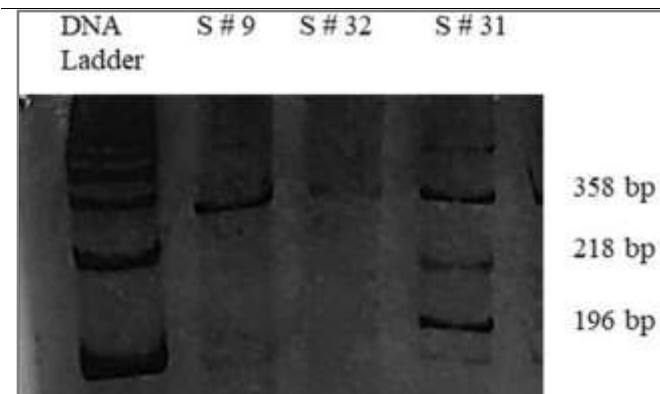


Figure-1: Polyacrylamide gel image showing C282Y mutation obtained from Tetra ARMS-PCR.

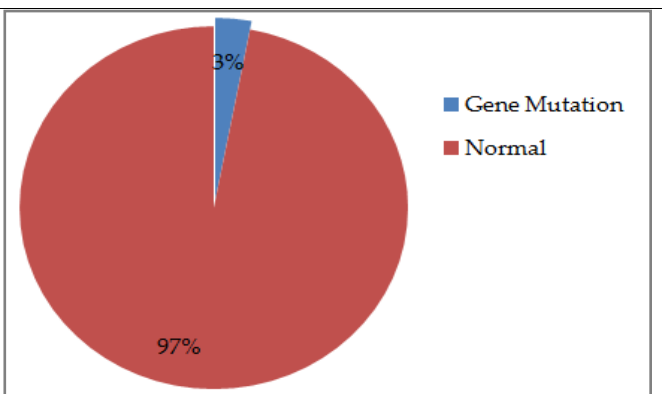


Figure-2: Frequency of gene mutation: C282Y (n=43).

refused to participate in current study and 143 patients were excluded because of iron deficiency on the basis of serum ferritin level less than 20 ng/ml and normal ferritin level 21-330ng/ml.

ferritin >1000 ng/mL was 35 (81.4%), <1000ng/mL was 8 (18.6%) as well as %TS <45 was 11 (25.6%) and >45 was 32 (74.41%). Consanguinity was reported in 34 (79.1%). Some patients were

found with co-morbidity like liver disease (4.7%, 2/43), DM (2.3%, 1/43) and arthritis (4.7%, 2/43). Mean \pm SD was reported for age, gender, Total iron binding capacity, total iron, serum ferritin, %TS, and C-reactive protein (table-I). Primers were designed using a method followed by Ye *et al*, available at <http://primer1.soton.ac.uk/primer1.html>¹⁸. Primer sequence is given in table-II. Outer primers served as template for inner primers. Therefore, outer primers were optimized first and then reaction was carried out by adding mutation specific inner primers. Polyacrylamide gel electrophoresis was used to visualize results. There was frequency computed for gene mutation among all participants (fig-1). Only a single 33 year old patient was reported with C282Y mutation (fig-2) in our study samples. He had serum Ferritin 2360 ng/mL (Reference Interval: 24-336), %TS: 73% and consanguinity.

DISCUSSION

This study was conducted to find out C282Y gene mutation for hereditary hemochromatosis (HH) which is an autosomal recessive disorder, damaging multiple organs due to excess deposition of iron and most commonly caused by mutation of HFE gene like C282Y and H63D¹⁹. Frequency of HFE gene mutation C282Y varies among populations. In our study 43 patients were analyzed for HFE genotype C282Y with high Ferritin level >1000ng/ml or iron saturation >45%. Our study showed 3% frequency of C282Y mutation.

A study performed by Ali *et al* in British immigrant Pakistani population in UK showed 1% frequency for the genotype C282Y and 8% frequency for the H63D allele, which is quite similar to results of our study²⁰.

Another study conducted by Zlocha *et al* in Slovakia showed the frequency of allele C282Y to be around 3%, which was comparable to our study but conflicted in the part that current study had small size while in large sample size and geographical location in published study²¹. In 2014 a study performed by Unal *et al* in Turkish population among the eighty-seven children and

young adults' beta-thalassemia patients and healthy controls showed concordant results with our study in which no HFE gene mutation was determined of both variants i.e. C282Y and H63D¹⁹.

One of the studies performed in Caucasians showed 6% mutation in allelic frequency of HFE gene of C282Y variant with or without symptoms of iron over load, results of this study also varied due to multi centric, long period and large number of participant²². Similar study was performed by Grosse *et al* which showed 8% patients to have HFE homozygote C282Y mutation²³.

Another study performed in Brazil by Alves *et al* showed the frequency of C282Y, H63D and S65C polymorphisms in the general population to be 1.67, 11.67 and 0.83%, respectively in the epidemiology of HFE gene, results of this study are different due to geographical location and ethnicity²⁴.

There are some limitations in our study. Firstly, our sample size was small. Secondly, ours was a single centered study. We suggest that there is need to conduct Multi-centric population-based studies with greater sample size.

CONCLUSION

C282Y homozygosity should be suspected in patients showing biochemically elevated levels of %TS and serum Ferritin.

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CONFLICT OF INTEREST

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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