The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/18.4462

ORIGINAL PROF-4462

HFE GENE MUTATIONS;

FREQUENCY OF TWO COMMON HFE GENE MUTATIONS (C282Y & H63D) IN AN IMMIGRANT POPULATION (BRITISH PAKISTANIS) IN UK.

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Article received on:
27/09/2017
Accepted for publication:
15/12/2017
Received after proof reading:
02/01/2018

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ABSTRACT… Objectives: To determine the frequency of two common HFE Gene Mutations (C282Y & H63D) in an immigrant population (British Pakistanis) in UK. **Study Design:** Cross sectional study. **Setting:** University of Lincoln UK. **Duration:** Duration of study was 12 months from 01/09/2012 to 31/08/2013. **Material and Methods:** Two hundred immigrant Pakistani (BP) chromosomes (100 samples; 50 male and 50 female) from major cities of UK and 200 ancestral origin Pakistani chromosomes (100 samples; 50 male and 50 female) were analysed by PCR-RFLP for the presence of the H63D and C282Y mutations. **Results:** Eight individuals were found to be heterozygous for the H63D mutation and one individual was found to be homozygous for the H63D mutation, therefore, the H63D mutation was observed to have a frequency of 8% in immigrant Pakistani (BP) population sample and similar results were observed in ancestral origin population from Pakistan. The C282Y mutation was not detected at all. **Conclusion:** We found that our results are close to Saudi-Arabian and Indian population (8.5% & 9.1% H63D mutation, respectively) and in accordance with the global spread of the H63D mutation.

Key words: HFE Gene Mutations (C282Y & H63D), Hereditary Hemochromatosis (HH)

Article Citation: Ali N, Ahmed B, Akram H, Akhtar J, Williams R, Dixon R. HFE gene mutations; frequency of two common HFE gene mutations (C282Y & H63D) in an immigrant population (British Pakistanis) in UK. Professional Med J 2018;25(1):129-134. **DOI:**10.29309/TPMJ/18.4462

INTRODUCTION

Hereditary hemochromatosis is mostly resulted by the mutation in the HFE gene, which supports the regulation of the iron amount absorbed from diet. The two known mutations of HFE are C282Y and H63D. As this is an autosomal recessive disorder, the person must have two mutated genes in case of appearance of disease. Hemochromatosis is the most common form of iron overload disease that is further divided into Primary and secondary Hemochromatosis depending their mode of onset. Primary Hemochromatosis is, an autosomal recessive blood disorder, characterized by an increase in iron absorption. It is also termed as Hereditary Hemochromatosis (HH). If it remains untreated, it can lead to massive iron depositions in organs and ultimately results in multi-organ failure.[1] Secondary Hemochromatosis instigated by the others abnormalities other than hereditary, such as anaemia, alcoholism and other disorders as well. Hemochromatosis induced too much absorption and storage of iron in the body. This massive iron deposited in the different organs of the human body and cause abnormalities there. Without proper treatment, patients may face failure of liver, heart, and pancreas.

Iron is a vital element found in a broad range of food

supplements. Iron fortified breads, cereals and red meat are the chief source of iron. In the human body, iron comes to be part of haemoglobin, an essential molecule in the human blood that serves as transporter of oxygen from lungs to peripheral tissues of body. About 10% of all the ingested iron is absorbed by the healthy people, which is up to the mark of normal nutritional requirements. People having hemochromatosis usually absorb up to 30% of the consumed iron. The human body has not the system to get rid itself from excess absorbed iron, then it eventually stored in different body tissues, more specially heart, liver and pancreas. Reports of clinical hemochromatosis in regions of Asia have been relatively rare, and the diagnostic conditions are less well defined.[2] In these particular regions, the association of HFE mutations with iron overload is minimal and varied.[3] There have been only a few brief reports of non-HFE hemochromatosis in Asia, which appear to be isolated mutations with most cases around Eastern Asia. [4-6] At present, a difficulty with recognizing hereditary hemochromatosis in some parts of Asia is the presence of haemoglobin disorders such as thalassemia and the resulting secondary iron overload.[7] Furthermore, the presentation of the disease may also be masked by iron deficiency in these developing countries.[8] In this research H63D and C282Y mutations is investigated to find out any signs of these two mutations in an immigrant population sample (British Pakistanis) in UK and their ancestral origin population from Pakistan. We intended to determine the frequencies of these two most common missense mutations in the HFE gene and contribute to knowledge concerning the incidence rate of these two mutations in an immigrant Pakistani population (BP) in UK. Incidence of the H63D and C282Y HFE mutations (common in Europe) in Pakistan and in South Asia was generally rare.

According to the 2010 census, there are more than 1.2 million British Pakistanis (BP) in the UK. A large number of Pakistani immigrants arrived in the UK in the 1950s and 60s, to study in British universities, as skilled medical doctors to fill medical professional shortage in NHS and as factory workers due to labour shortages in the UK.[9] These immigrants were originally from specific areas in Pakistan, mostly Mirpur (located within the Kashmir area of Pakistan) and Kashmir, and settled mostly in Yorkshire, Lancashire, and in cities such as Birmingham, Bradford, Manchester, Newcastle-on-Tyne and Glasgow in Scotland.[9,10] These individuals adopted the life and culture of the UK, but a characteristic of this ethnic group is that they usually restrict marriages to their own population. There is high rate of consanguinity in this population which has resulted in the highest rate of autosomal diseases as compared to other immigrant populations in the UK.[10,11]

MATERIAL AND METHODS

Informed consent was obtained from 100 unrelated immigrant individuals (BP) in UK from Pakistan, (50 males and 50 females) from different cities of UK (Figure 1). Further 100 unrelated samples were collected from their ancestral origin population from Pakistan and these were mostly from Punjab and Kashmir (Figure 2).

Figure-1. Percentages of samples collected from British Pakistanis in UK.

Figure-2. Ancestral origin places of British Pakistanis in Punjab & Kashmiri part of Pakistan.

DNA extraction

Blood stains collected on FTA® cards (Whatman, Kent, UK) were proceeded for DNA extraction using FTA® Purification Reagent (Whatman) according to the guidelines of manufacturer's protocol. DNA from buccal swabs was extracted using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany).

PCR amplification & restriction analysis

About 1.2 mm FTA® disc and 1 ng of DNA purified from buccal swabs were used. PCR is conducted separately for the two mutations with the pairs of primers which are described in already published article. PCR mixtures contained 0.5 μl of primers (forward and reverse), 12.5 μl Taq DNA polymerase Master Mix 2x (Qiagen), 3 μl of genomic DNA (1 ng) or 2 FTA discs (>5 ng DNA), and nuclease-free water to a final volume of 25 μl. In this PCR amplification the 9700 DNA thermal cycler (Applied Biosystems) was used and PCR amplification conditions were: 5 min at 95°C, followed by 30 cycles of 30s denaturation at 94°C, 30s annealing at 58°C and 1min extension at 72°C, followed by 5 min at 72°C and holding at 4°C.

Enzymatic digestion was carried out in the isolated PCR products encompassing potential C282Y and H63D mutation sites. A restriction endonuclease recognizes a specific target nucleotide sequence in the DNA and cleaves the polynucleotide chain at this target sequence. The C282Y in human DNA mutation creates a new RsaI restriction site. The 355 bp PCR reaction product digested with RsaI gives two digest fragments of 203 and 152 bp from normal DNA. However, DNA carrying the C282Y mutation generates three fragments (203, 123 and 29 bp). The H63D mutation destroys an MboI site present in the 294 bp PCR product giving products of 237 bp and 57 bp, while normal DNA generates three fragments of 138, 99 and 57 bp. (see Figure). A volume of 13 µl of the PCR product of 294 bp (H63D) and 355 bp (C282Y) was digested using 4U of MboI (NEB, R0147S) or 2U RsaI (NEB, R0167S), respectively, in 2.5 µl NEBuffer 4 in a final volume of 25 µl, and incubating at 37°C 6 hours or overnight. The digested samples were then run on a 2% agarose gel at 120V for 1 h, stained with ethidium bromide (Sigma) and photographed under UV light.

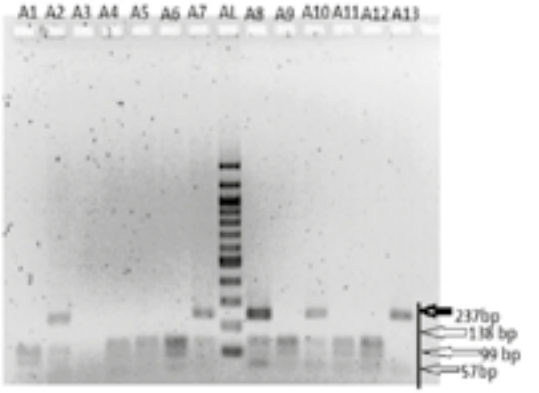

Figure-3. Genotype determination of the HFE gene codon 63 region. The normal genotype gives rise to 3 MboI fragments of the codon 63 region PCR product (138bp, 99bp and 57bp) (as seen for samples A1, A4, A5, A6, A9, H9 , A11 &H12). The H63D mutation abolishes one MboI site resulting in a 237bp band. Heterozygotes give all 4 bands (as seen for samples A2, A7, A8 andA10). Homozygotes give 2 bands, 237bp and 57bp only (as seen for sample A13) and AL=100 BP allelic ladder).

RESULTS

H63D mutation frequency was observed 8% both for 200 chromosome of immigrant Population (British Pakistani) and ancestral origin population

(Pakistani) each. The C282Y mutation was not detected at all for both populations. Eight individuals were found to be heterozygous for the H63D mutation and 1 individual was found to be homozygous for the H63D mutation in both populations (Figure 3).

DISCUSSION AND CONCLUSIONS

In the current study, 8 individuals were found to be heterozygous for the H63D mutation and 1 individual was found to be homozygous (BP samples) for the H63D mutation, therefore, the H63D mutation was observed to have a frequency of 8% in immigrant Pakistani (BP) population sample (Figure-3). Explained in more detail, a mutant homozygote was detected for one sample (BP) (A13 in Figure-3) (by observation of the 273 and 57bp bands in an agarose gel electrophoresis) and heterozygotes (237, 138, 99 and 57bp bands in an agarose gel electrophoresis)

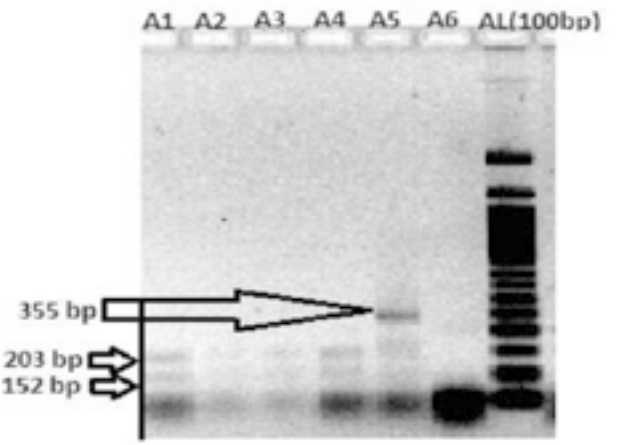

Figure-4. Genotype determination of the HFE gene codon 282 region. The normal genotype gives rise to 2 RsaI fragments of the codon 282 region PCR product (203bp, 152bp and 355bp PCR product) as seen for all samples tested on this gel (A1,A2,A3,A4). The C282Y mutation, if present, introduces a second RsaI site resulting in three bands (203bp, 123bp and 29bp).

Number of samples	H63D Allele Frequency		C282Y Frequency	Reference
Europe North				
North Ireland	404	14.10%	9.9%	12
Denmark	200	12.75%	6.75%	13
Norway	94	11.20%	6.4%	14
Sweden	117	12.39%	3.84%	15
South				
France	503	12.23%	1.07%	16
Germany	153	14.07%	2.96%	17
Greece	96	13.50%	1.3%	14
Spain	420	20.40%	3.09%	18
Turkey	70	13.57%	0%	14
Africa/Middle East Middle East				
Saudia Arabia	118	8.5%	0%	14
North				
North Africa	171	13.2%	9.0%	16
Benghazi-Libya	100	175	0%	19
Asia				
China	72	2.8%	0%	14
Indonesia	90	2.8%	0%	14
Taiwan	80	1.9%	0%	14
Pakistan	100	8%	0%	Current Study
Indian	100	9.1%	0%	20
British Pakistanis (UK)	100	8%	0%	Current Study
Australasia				
Australia (aboriginals)	93	05	0%	14
Australia (vanuatuans)	90	0.6%	0%	14
America				
Mexico	54	6.5%	0%	14
Jamaica	90	2.2%	1.1%	14
United States	5171	13.5%	5.4%	21

Table-I. Estimated prevalence of HFE gene (H63D&C282Y) mutation in the general population

were detected in 8 out of 100 samples (of which 4 examples are shown in Figure-1), giving a mutant allele frequency of 8/200 = 4%. So the expected genotype frequency of mutant homozygotes (p2) is 0.04×0.04 = 0.0016. The expected genotype frequency of normal homozygotes (q2) is 0.96×0.96 = 0.9216 giving a total expected homozygote frequency of 92.16%. The expected frequency of heterozygotes (2pq) is 0.04×0.96×2 = 0.0768 or 7.68 %. The observed frequencies were: homozygotes 92 % and heterozygotes 8%, which does not deviate significantly from the expected. There is no evidence in this data of an excess of homozygotes that might be expected from the results of STRs, although the sample is relatively small. The C282Y mutation was not detected at all (Figure-4).

The C282Y allele frequency observed in the Pakistani population of the current study is below the detection limit of 1%. The H63D allele frequency in Pakistani population described here (8%) is lowest than that in the Algerian Mzab population (9%) or Saudi Arabian population (8.5%).[14,22] The H63D frequency described here is much lower to that reported by Zorai (2003), the H63D and C282Y allele frequencies are, respectively, 17.5% and 0.5%, (North African population) and for European populations (British Irish) (18.9%).[14,23] However, it remains less than the highest European frequency found in Spain (Table-I). These results show that the mutation responsible for the His63Asp substitution is only found in the European and European derived population in United States.[22] It is suggested that the wider distribution of the H63D mutation around the world and the restriction of the C282Y to European or European associated populations indicate that the H63D variant appeared earlier.

ACKNOWLEDGEMENT

The author is grateful to all the individuals who participated in this research by donating their DNA samples and also thankful to Faculty Development Programme of Sargodha Medical College, University of Sargodha, Pakistan for providing funding support.

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2	Bashir Ahmed	Manuscript Setting	
3	Dr. Humaira Akram	Final proofreading	
4	Junaid Akhtar	Manuscript writing and editing	
5	Ross Williams	Research Supervisor	
6	Ron Dixon	Data analysis	