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Gene Frequency Distribution in Abnormal Haemoglobin Varients and Blood Groups in North India Population

Physiology

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ABSTRACT

Abnormal haemoglobin variants are most common monogenic mutant forms of haemoglobin anamoly of erythrocytes in a population, occurred due to variants in genetics. The alteration of different amino acid sequences takes place when there are genetic changes in specific genes or globins present in the haemoglobin alpha and beta chains. Earlier study to this subject depicts around 3-17% prevalence of β-thalassemia cases. Within one population to another the different abnormal haemoglobin varients, ABO and Rhesus blood groups are frequently variable. With this effect there arise a necessity to rule out the prevalence of these indices in North India population, particularly Uttarakhand, India. In addition to this a timely diagnosis and treatment to this condition with parental councelling became mandatory. The real frame of reference of the study is to know the ubiquity of most recurrent haemoglobinopathies in the state of Uttarakhand and to explore the substitute deliberate plans that could be inflicted for the efficacious regulation and controlling of these altered haemoglobinopathies. A sum total of 933 subjects aged between 01-30 yrs were screened and analysed that 627 (six hundred twenty seven) were males and 306 (three hundred six) were females. Result of present study showed 4.2% prevalence of haemoglobinopathies. Out of total haemoglobinopathies screened subjects (41), β-thalassemia trait (32) was screened as highest followed by β-thalassemia intermedia (6), β-thalassemia major (1), E& -thalassemia (1) and S-D disease (1). The frequencies with respect to ABO and Rh blood group systems is shown as B > O > AB > A. The distribution of blood groups with 97.6% Rhesus positive (Rh+) out of which B+ is 17(14.4%), O+ is 13(31.7%), AB+ is 6(14.6%) and A+ is 4(9.7%). Only 1(2.4%) case of B- is seen. The maximum mutation detected was IVS 1-5 (GC) M with 21(51.2%).

KEYWORDS:

INTRODUCTION

There are various mutant forms of haemoglobin in a population. The occurrences of these haemoglobinopathies are the results of genetic variation in specific genes or globin that cause changes or alterations in their amino acid sequences, which leads to represent a clinical representation from asymptomatic findings on blood film to death in utero. It is wide spread distribution throughout the world with increased prevalence in multicultural states. Altered haemoglobin varients, Blood group (ABO) & Rhesus blood groups (Rh) are known to vary from one population to another. Thus there is need to elucidate the various types of gene frequency prevalent in North India population particularly Uttarakhand, India. Such genetic studies results as a background for initializing genetic counseling services with a view to reduce haemoglobinopathies.

As a rule that haemoglobinopathies are moreover autosomal recessive disorders and are inherited through one or both parents who themselves are the sufferers of this disorder or may be in the form of carriers. So, this study helps in distinguishing and investigating out the illness persons who are likelihood to threat of haemoglobinopathies. Furthermore, the genetic professional assistance became immenent to the parents who test positive for thelassemia and sickle cell disease.

The real frame of reference of the study is to know the ubiquity of most recurrent haemoglobinopathies in the state of Uttarakhand located in the northern part of India and also to explore the substitute deliberate plans that could be inflict for the efficacious regulation and controlling of these altered haemoglobin anamolies.

AIMS AND OBJECTIVES

OBJECTIVES:-

a. To rule out the prevalence of Haemoglobinopathies /abnormal Haemoglobin patterns in north India population, particularly Uttarakhand.

b. To assess the relation ship between blood groups and haemoglobin patterns.

REVIEW OF LITERATURE

CURRENTLY KNOWN ABOUT THE TOPIC

Different haemoglobinopathies are a number of monogenic anomalies of haemoglobin (Hb). Haemoglobin is a heterocompound molecule found inside erythrocytes (RBC) that loosely attaches to and transports blood gases (oxygen and carbondyoxide) in the body. Genetic disarray of haemoglobin can fabricate atypical haemoglobins and blood deficiency, which leads to the state, termed as "Haemoglobinopathies". Unconventional haemoglobins materialize in any one of these two cardinal situations: diminish creation of one of the globin chain e.g. thalassaemia, or an aberrant globin chain e.g. sickle cell disease [1,2,3].

During haemoglobin synthesis, because of inherited disorder β-thalassaemia occurs which is designated by a depletion of (β^{+}) or non-appearance (β^{0}) of synthesis of beta globin chains of haemoglobin. This overall culminates in an weakened on demaged chain synthesis, which decides the intensity of the disease [4].

Such inheritance disarray of haemoglobin constitute a prodigious health well being trouble in various parts of the world and in India too[5]. The dispensation of inherited mess alter world wide and in mixed communities [6]. WHO statistic evaluate that 5 % of the globe populance is bearer for haemoglobin disorganization[7]. They source average to drastic hemolytic anemia guiding to lofty grade of anaemia mortality and morbidity. The occurences of β-thalassaemia in India extent from 3.5 to 15 % in widespread community. In one year 10,000 children with thalassaemia major, minor and intermedia are existing as a result of birth in India, which represents 10 % of the composite arithmetical value in the globe[8], [105.] The gross and comprehensive α gene obliteration rate is 0.05 to 0.98 % which is very demorkating in India [9] . In western central part of Gujarat, α gene

obliteration is as towering as 95 % 6. The prevalence for β-thalassemia is 4.2% and 12000 infants born per year with abnormal haemoglobinopathies[91]. By the help of chromatogram of patients with abnormal haematogram in different casts and communities was reported 1-17% with mean of 3.3%[93, 94, 95]. It was observed that the preponderance of females is more then males in haemoglobi nopathies this is traced because of their antenatal visits[97]. In another general clinical study 11% cases were recorded as haemglobin opathies using HPLC technique[98].

MATERIAL AND METHODS

SUBJECT SELECTION

The present study was carried out in the Department of Physiology at Dolphin (PG) Institute, D.Dun during the period of April 2014 to April 2017. A sum total of 933 study participants were selected by random sampling after obtaining the consent from the participants. The age were between 1 year to 30 years.

BLOOD SAMPLE COLLECTION AND PREPARATION

Total 6 ml venous blood was collected in EDTA vials and few drops of fresh whole blood was placed on slides for blood group investigation. The anticoagulant blood was used for performing CBC/Red cell indices, haemoglobin electrophoresis HPLC. Rest of the 2 ml of blood will be retained in EDTA vial and stored at -200C for genotype DNA analysis.

COMPLETE BLOOD COUNT

This is measure by using complete blood cell counts (CBC) by full automated blood cell counters, which was calibrated with commercially available controls and using blood films.

FOR THE ESTIMATION OF RED BLOOD CELL INDICES:

Red blood cell indices determine is the most common laboratory test and is usually carried out by automated electronic cell counters.

HAEMOGLOBIN ELECTROPHORESIS

Different type of haemoglobin testing system provides an integrated method for separation and determination of relative percent of specific haemoglobins (eg A_2, F, Ao) in whole blood.

GENE POLYMORPHISM

Polymorphism studies will be carried out using Amplification Refractory Mutation System (ARMS) method.

DNA EXTRACTION FROM WHOLE BLOOD

Methods: Genomic DNA is extracted from the peripheral blood leucocytes by Commercial Kit (QIAamp DNA Blood Midi kit 100 samples)

Table – 1: Sex wise distribution of patients with different Haemoglobion opathies (n=933)

Gender	HbAA	E&βTh	S-D	βT Intermedia	βT trait	βT Major	Total
Male	598	1	0	5	22	1	627
Female	294	0	1	1	10	0	306
Total	892	1	1	6	32	1	933

Table – 3: Distribution of different haemoglobinopathies in accordance with Blood group in study population (n=41)

Blood Group	Haemoglobinopathies (n=41)					
	Male	%	Female	%	Total	%
A+	0	0	4	9.7	4	9.7
B+	13	31.7	4	9.7	17	41.4
AB+	5	12.4	1	2.4	6	14.6
O+	10	24.3	3	7.3	13	31.7
B-	1	2.4	0	0	1	2.4

Table – 5: Rhesus (Rh) D distribution among abnormal Haemoglobin varients in ABO blood groups (n=41)

Blood Group	Rh (D) Positive (%)	Rh (D) Negative (%)
A	4 (9.7)	
B	17 (41.4)	1 (2.4)
AB	6 (14.6)	
O	13 (31.7)	

Table – 6: Frequency of mutation detected in Abnormal Haemoglobin variants (n=37/41)

Mutation Detected	No. of Patients Detected with Mutation	Frequency (%)	Amplified Product size (bp)
IVS 1-5 (GC)M	21	51.2	285
619 bp deletion	06	14.6	242
Fr 8/9 (+G)M	05	12.1	215
Codon 41/42 N (TCTT)	05	12.1	439

RESULT

Table 1 indicates different patterns of haemoglobin variants according to the gender. Out of 933 subjects, I found 892 with normal (HBAA) and 41 subjects with abnormal haemoglobin variants on haemoglobinopathies viz. β-Thalessemia Major, Minor, Intermedia, E & β-Thalessemia and S-D disease.

In this study, total observed abnormal haemoglobinopathy (41) in which the most frequent haemoglobinopathy was β-Thalessemia trait (32) β-Thalessemia Intermedia (6) while less frequent in β-T major (1), E & β-Thalessemia (1) and S-D(1).

Table 3 shows the prevalence of blood group with observed haemoglobinopathies. The present study show that haemog lobinopathy was most frequent in Rh positive B (41.4%) followed by Rh positive O (31.7%), Rh positive AB (14.6%), Rh positive A (9.7%), Rh negative B (2.4%).

The study of ABO blood group is an important factor in determining the direction of recruitment of voluntary donars as required for that region of the country. In my observation the ABO blood group and Rh positive in male & female in observed haemoglobinopathies (41) depicts that the blood group B and O positive was most prevalent in male followed by AB & A. On analysis individually in males the sequence of ABO and Rh positive falls in decreasing sequences as B and O proceeding ahead by blood group AB and A. Blood group B and A positive female followed by blood group O & AB.

Table 5 indicates the Rh (D) distribution among abnormal haemoglobin varients in ABO blood group. It is revealed that B+ was found more with 17(41.4%), followed by O 13(31.7%) then AB 6(14.6%) & A 4(9.7%). In Rh (D) negative only B- is found in 1(2.4%) of case.

Table 6 depicts the spectrum of β-Thalalssemia mutations in north india population (uttarakhand) within this study a total of 41 β-Thalalssemia alleles have been deciphered out of 933 individuals in North India population out of these 41 β-Thalassemia alleles, 32 β-Thalassemia trait, 6 β-Thalassemia Intermedia, 1 β-Thalassemia Major, 1 E & B Thalassemia and 1 S-D after screening ARMS PCR, the amplicons were subjected for gel electrophoresis with 1.6% as agarose. The product were visualized under UV transmitter for the DNA bands. Screening for 04 different types of β-Thalassemia mutations were observed i.e. IVS 1 – 5 (G-C)M, as most common, followed by 619 bp deletion, Fr 8/9 (+G)M and codon 41/42 N (TCTT), at 285 bp, 242bp, 215 bp and 439 bp respectively. (As shown in gel pictures) this accounts for 51.2%, 14.6%, 12.1% and 42.1% respectively. 9.71% were uncharacterized.

SUMMARY & CONCLUSION

This different type of haemoglobinopathies and particularly thalassemia are the largest number of genetic disorder which causes great public health disorder and are of clinical importance since birth. During the synthesis of haemoglobin this inherited disorder is of public health importance in Uttarakhand region. In this study of

haemoglobinopathies indicates the genetic heterogenecity of the population of this region. In the passage of time several ethanic group and communities might have been mixed into the mainstream population which has led to varied genetic consequences. This is to ideally stated that the prevalence of varied haemoglobinopathies in this region are in agreement with the population admixture in Uttarakhand.

DISCUSSION

I had observed varied pattern of haemoglobin varients according to the gender. On screening the total subjects which were 933, I observed 41 subjects with abnormal haemoglobin varients on haemoglobinopathies like wise β-thalassemia major, β-thalassemia minor, β-thalassemia intermedia, E& β Thalassemia and S-D disease.

The study of ABO blood group is an important factor in determining the direction of recruitment of voluntary donars as required for that region of the country. In my observation between ABO blood group and Rh positive in both genders in observed haemoglobinopathies depicts that in males the blood group B and O positive was most prevalent and followed by AB and A. on analysis individually in males the sequence of ABO and Rh positive falls in decreasing sequences as B and O proceeding ahead by blood group AB and A. Blood group B and A positive females followed by blood group O and AB.

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