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Original Research Article

Gene prevalence in abnormal haemoglobin divergent and blood groups in Uttarakhand

Anil Kumar Malik[1,*]

[1]*Dept. of Physiology, Dolphin (PG) Institute, Dehra Dun, Uttarakhand, India*

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ABSTRACT

Context: Abnormal haemoglobinopathis are generally observed deviant disarray happen due to genetic inequality within the alpha and beta chains amino acids sequences alters.
Aims and objectives: a total of 933 cases were included in the study for detection of Thalassemic cases. Out of these positive thalassemic cases were further analysed for gene frequency.
Materials and Methods : Starting with complete Blood Cell counts which has led to suspected cases for further analysis by haemoglobin electrophoresis by HPLC (high performance liquid chromatography) for confirmation of Thalassemic cases. Finally gene frequency detection by ARMS (Amplification Refractory Mutation System) by using PCR (Polymerase Chain Reaction).
Isolation of DNA from whole blood by commercially available kit (QIA amp DNA blood midi kit 100 samples).
Result: A sum total of 933 subjects, aged between 01-30 yrs were studied and it depicts 4.1% prevalence. β-thalassemia trait was screened as highest and S-D disease as lowest. The frequencies with respect to ABO is shown as B>0>AB>A. The amplicons which were analysed after gel electrophoresis were screened for ARMS, PCR, which IVS1-5 (G-C) Maccounts for almost 50.0% and lowest is Fr 8/9 (+G) M is 12.1%.
Conclusion: As such patients requires repeated blood transfusion so availability of maximum type of affected Blood group is the time of need for availability to with blood banks. Further genetic studies will definitely help in effective for further pharmaceutical companies.

1. Introduction

The beginning elaborations started in 1930 on Tunisians people accumulating date of relationship of blood groups (ABO) system with altered haemoglobinopatheis.[1–4] This cumulative work with multiple speculations along with altered haemoglobinopatheis was initial of its kind.[4]

Further many observers has developed more precise method for the detection of β-thalassemia mutations in South East Asia, based on PCR generated restriction sites which are very synonymous to β-thalassmia mutations and later on practised in ruling out pathological mutations in mitochondrial DNA.[5] These modifications are observed at positions IVS1-5, IVS1-1 of β-globin gene.[6–8]

2. Materials and Methods

These participants were children, married or pre-married candidates, patients with familiar, unfamiliar, doubtful or having family history, clinically suspicious or low haemoglobin drop patients which were mention by the physician and some may be self-participants.

2.1. Number of cases

A total of 933 cases were included in the study for detection of Thalasemic cases. Out of these only positive thalasemic cases were further analyzed for gene frequency]

* *Corresponding author*.
E-mail address: anuk429@gmail.com (A. K. Malik).

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2.2. Diagnostic criterion

By performing complete Blood Cell Counts; By HPLC (high performance liquid chromatography) for the detection and confirmation of Thalassemic cases; by (Bio-Rad D-10) haemoglobin testing system. By analysis by ARMS (Amplification Refractory Mutation System); PCR (Polymerase Chain Reaction) for gene frequency distribution. Isolation of DNA from whole blood which was preserved in anticoagulant (EDTA) by using the already commercially available kit (QIA amp DNA blood midi kit 100 samples).

3. Results

That the total number of 933 cases included in the study population we found blood group B (313) was observed to be more frequent and least is O (148). Among Rh positive male, blood group B (192), and in female, blood group A (87) was most prevalent blood group (Table 1).

While observing the frequency of ABO Rh blood group in abnormal haemoglobin variants, which were accounted as 40 cases. Within these blood group B positive (42.5%) is observed maximally and blood group A positive (9.7%) is least (Table 2).

The frequency of mutation detected in abnormal haemoglobin variants are 36, out of total 40 cases included in the study. The mutation IVS 1-5(G→C)M(50.0%) is maximum and frequently observed and the least in Fr 8/9 (+G) M (12.1%) sharing with codon 41/42 N (TC TT). While 04 cases were uncertain in which study were insufficient for analysis (Table 3).

If we segregate the abnormal haemoglobionopathies according to gender then we observe the males (27) are more sufferer then females (13) (Table 4).

As far as we study abnormal haemoglobinopathies distribution according to age & sex wise then we observe that more cases in males were observed during 0 to 10 yrs and then subsequently decreases but in females the least between the age 11 to 15 yrs, lesser between 0 to 5yrs but maximum cases were observed between the age 6 to 10 yrs (Table 5).

4. Discussion

The sequel of abnormal haemoglobinopathies are moreover autosomal recessive disorders and are genetically inherited through one or both parents who might be the carrier or suffering in another presentable form with the disease.[9,10]

An aggregate of 933 patients were analysed for abnormal haemoglobin variants, ABO & rhesus blood groups ranging from 01 to 30 years. Out of the total 933 subjects 629 were males and 304 were females.

Table 1 depicts the dispersal of the blood group (ABO) & Rhesus (D) between study subjects. Blood group B was observed as the majority frequent 313 while blood group O was least frequent 148. Here we observe the same frequency in earlier studies.[11,12]

In Rh (D) blood typing 667 was Rh positive and 266 was Rh negative. Amongst Rh positive male blood group B (192) was observed as the most prevalent blood group proceeding ahead by blood group A (130), AB (73), O (66).

Between Rh positive female, blood group A (87) was most common followed by blood group B (58), AB (48) & O (13).

The frequencies pattern with respect to ABO can be shown by B>A>AB>O in males and A > B > AB > O in females. This study is almost synonymous to earlier studies.[13,14]

Table 2 shows that blood group B is observed maximally with 42.5% followed by blood group O (35%), AB (12.5%) & A (9.7%). Out of these Rh + were 39 (98.0%) & Rh- is 1 (2.4%).[13,14]

Table 3 depicts the spectrum of β-Thalalssemia mutations in north india population (Uttarakhand) within this study a total of 40 β-Thalalssemia alleles have been observed out of 933 individuals in North India population out of these 40 β-Thalassemia alleles, 34 β-Thalassemia trait, 4 β-Thalassemia Intermedia and 2 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,[15] 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.[16,17] The amplicons which were subjected for gel electrophoresis after screening by ARMS PCR were IVS 1-5 (G-C)M (50.0%), 619 bp depletion (14.6%), Fr 8/9 (+G)M (12.1%) and codon 41/42 N(TCTT) (42.1%) respectively.[16,17] 9.32% were uncertain. The earlier studies to their connection of its frequency in β-thalassemia trait reported in Gujrat (10-15%), followed by Sindh (10%), Punjab (6.5%), Tamil Nadu (2.4%).[18–20]

Table 4 indicates the gender wise distribution ofhaemglobinopahics. Here we can easily observe that the number of males are more, 27 (67.5%) then females 13 (32.5%) who were found as positive thalassemic cases.

This shows that such mutations in thalassemic patients are more prevalent in males then females.

Table 5 depicts that males shows more features and incidence during the age between 0 to 5 and 6 to 10 yearsparallarly, but decreases on increasing age. This may be because of less life survival rate after the age of 10 years. In comparison to males, females thalassmemic cases are more observed during the age between 6 to 10 years, then 0 to 5 years. This may be because of negligence and laid back attitude towards females in male dominance society.

Table 1: Dissemination of ABO and Rh blood group in the study population (n=933)

Blood Group	Male		Female		Total		Total
	Rh $^+$	Rh $^-$	Rh $^+$	Rh $^-$	Rh $^+$	Rh $^-$	
A	130	34	87	37	217	71	288
B	192	36	58	27	250	63	313
AB	73	44	48	19	121	63	184
O	66	54	13	15	79	69	148
Total	461	168	206	98	667	266	933

Table 2: Frequency of ABO Rh blood group in Abnormal Haemoglobin varients (n=40)

Variables Blood Groups	No. Observed	Prevalence (%)
A	4	9.7
B	17	42.5
AB	5	12.5
O	14	35
Rhesus (Rh)		
D+	39	98
D-	1	2.4

Table 3: Frequency of mutation detected in Abnormal Haemoglobin variants (n=36/40)

Mutation Detected	No. of Patients Detected with Mutation	Frequency (%)	Amplified Product size (bp)
IVS 1-5 (G→C)M	20	50.0	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

Table 4: Gender Wise Distribution of Heamoglobin pathies.

Gender	No. of cases
Males	27
Females	13

Table 5: Age and Sex Wise Distribution of Hemoglobin pathies.

Males		Females	
Year	No.	Year	No.
0-5	11	0-5	4
6 -10	11	6 -10	7
11-15	4	11-15	1
16-20	0	16-20	0
21-25	0	21-25	0
26-30	2	26-30	0

5. Conclusion

These varied type of abnormal haemoglobinopathies with particular to thalassamia are the greatest factor of genetic mutational anamoly, which has eventually lead to wide spread public health disorder, therefore because clinical importance after birth.

Elaborate study of varied haemoglobinopathies and their screening will definitely be a center stone while observed their occurrences with every region of the state along with the data shoring with regional research center – precise by formulating a data of blood groups in relation to abnormal haemoglobinopathies may furnish details about the available of human blood during emergencies, and also enlightens the possibility of future burden of disease.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Anil Kumar Malik Assistant Professor

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