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Research Article

FREQUENCY OF HMOX1, UGT1A1 AND ALPHA GLOBIN GENE VARIANTS IN SICKLERS.

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ABSTRACT

The study was planned to optimize the phenotypic and genotypic variability of SCD patient due to interaction of different modulating factors and to investigate effect of modulating factors on the phenotype of sicklers. The aim of the study was to correlate the effect of different genotypic factors with the sickle cell patient's phenotype. Study concluded these factors associated with hematological and clinical profile.

INTRODUCTION:

Sickle cell anemia is the consequence of homozygocity for a single base pair substitution in position 6 in the open reading frame of the beta globin gene that specified a beta S – globin chain. This mutation endows the hemoglobin S (HbS) molecules with a new property: the capacity to polymerize when deoxygenated. The intra erythrocytic polymerizations of HbS induce the increased rigidity of the sickle red cell and produce a cascade of pleiotropic effect that determines the clinical picture. Sickling is the direct consequence of the presence of HbS, but pleiotropic effect follows. Example include hemolysis and increase in reticulocyte, increase in the size of the spleen followed by decreased of its activity and finally the almost disappearance of the organ, and decrease in density of some of the sickle cell among many others. While sickle cell anemia patient are homozygous at position 6 of the beta globin locus, Phenotypicaly this disease is characteristically heterogeneous. Another source of genetic variation comes from the multicentricity in origins of sickle gene. Since the sickle gene arose more than once in the world, it had the opportunity to interact with different linked gene known to ameliorate the disease. The genetic factor like α-thalassemia, β-globin gene and fetal hemoglobin haplotype are some of the factors responsible for these variations.

Methodology: Sicklers were recruited from the various OPD of hospitals in Vindhyan region. This study was approved by the institutional ethical committee. About 5 ml of venous blood was drawn after obtaining signed consent from the patient. DNA was extracted by the phenol-chloroform method. Hemogram was taken from 3 part hematoanalyzer and Sicklers were screened by Hb electrophoresis. Confirmation of Sickling was done according to published literature [1]. Identification of alpha genotype was done according to Baysal et al [2], Smetanina et al. [3], Shaji et al. [4], and Chang et al. [5]. The UGT1A1 (TA) n promoter length variability was genotyped according to the method of Eden et al., [6] and HMOX1 polymorphism was identified according to Vasda et al. [7]. Mean values, standard deviations, and frequency distributions were used to evaluate the hematological and clinical data. Student's t-test was used to compare the means of groups using Graph-Pad software (version 3.06). $P < 0.05$ was considered statistically significant.

Result & Discussion: In this study, total 60 patients were screened from SGMH, OPD as well as from other OPD that came for hemoglobinopathies screening. Out of them, 25 were Sickle β-thalassemia and 15 patients were Sickle traits while 20 were Sickle homozygous.

1. Co-existence of alpha thalassemia with sickle cell anemia and effect on phenotype of patients

A total of 60 sickle cell anemia patient's blood sample collected and characterized. After identification of mutation, subjects were categorized in three groups according to the presence of alpha thalassemia genotype. Eighteen patient had alpha deletions with a mean age 10.4±6.7 (10 male and 8 female) and 3 patient had anti α-3.7kb (alpha triplication) with mean age group 18±6.08 (2 male and 1 female) while 39 patients were without any alpha deletions with mean age of 11.41±8.07 (24 male and 16 female).

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Patient with presence of alpha deletions had higher hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) levels and mean corpuscular hemoglobin concentration (MCHC). Mean difference of hematological variables amongst patient with alpha deletions and without alpha deletions were statistically significant (p-value <0.05). Details are given in the **table-1.** Patient without the presence of alpha deletions had severe and higher frequency of clinical manifestations. Comparative clinical frequency details are given in **fig.1** Screening of single (–α3.7, 4.2kb) deletions, double (--SA,--SEA) deletions and alpha triplication (anti 3.7kb) were performed by Gap-PCR in all subjects. Highest frequency of alpha 3.7 heterozygous (50%), followed by alpha 3.7 homozygous (38.89%) and 4.2 heterozygous (11.11%) were found in sickle cell anemia patients while alpha triplication found in three (5%) patients. None of the patient were identified with double (–SA,--SEA) deletions.

Our cases of sickle cell anemia present only single α chain deletions (-3.7 and 4.2 kb) and alpha triplication. Alpha 4.2 present only in heterozygous condition. Overall 30% patient had alpha thalassemia genotype. Patient of sickle homozygous with alpha deletion showed great significant differences in hematological as well as clinical presentation.

We report the higher frequency of splenomegaly (19.04%), acute chest pain (16.66%) and painful crisis (21.42%) in SCD patients without presence of alpha deletions while SCD patients with co-existence of alpha thalassemia had less frequency of splenomegaly, chest pain and painful crisis.

The cases of sickle cell homozygous patients with co-existence of alpha deletion and without presence of alpha thalassemia genotype showed great variability of clinical and hematological parameters. Our sickle cell anemia patients, without alpha genotype had lower mean hemoglobin, MCV, MCH, HCT and MCHC while these parameters were improved with the presence of alpha deletions. Red cell indices were statistically significant an entire group of patients. Clinical symptoms were in higher frequency and severe in patients without alpha deletions in compression to patient who had alpha deletions. Due to lesser number of alpha triplicated patients, we did not evaluated separately and included in without alpha deletion group of patients. It was observed, the presence of alpha deletions in sickle cell anemia present mild symptom, improve hematological parameters and less dependency of blood transfusion in compression to without alpha deletion sickle cell patients. It suggested the presence of α-thalassemia genotype influenced clinical manifestations of Indian sickle cell anemia patients.

2. HMOX1 -polymorphism and effect on sickle cell anemia patients phenotype

Study subject was 60 sickle homozygous (35-male and 25-female with mean age 11.32±7.61). Among the sickle homozygous 27 were heterozygous and 19 were homozygous. Haematological parameters i.e. Reticulocytes, haemoglobin and red cell indices were improved in xmn-1 (+/+) carrier. Frequency of clinical condition was severe in xmn-1(+/-) heterozygous in sickle homozygous patients. All the details of haematological parameters and frequency given in the **table- 2 and fig.2**. We conclude that the phenotype of Indian sickle cell patients influenced by XmnI polymorphism.

Table 1: Comparative hematological parameters with and without co-existence of alpha thalassemia genotype

	Mean ±SD		
Parameters	**SS with α del. (N=18)**	**SS without α del. (N=42)**	**P-value**
RBC **millions/µl**	3.4±2.4	3.1±0.8	0.033
HGB **g/dl**	9.01±2.5	7.2±2.4	0.012
HCT **%**	27.4±4.8	25.2±3.1	0.031
MCV **fl**	66.1±6.4	58.3±3.4	<0.001
MCH **pg**	28.8±6.7	24.1±4.2	0.001
MCHC **g/dl**	32.03±2.9	30.2±1.8	0.004

In sickle cell anemia patients.

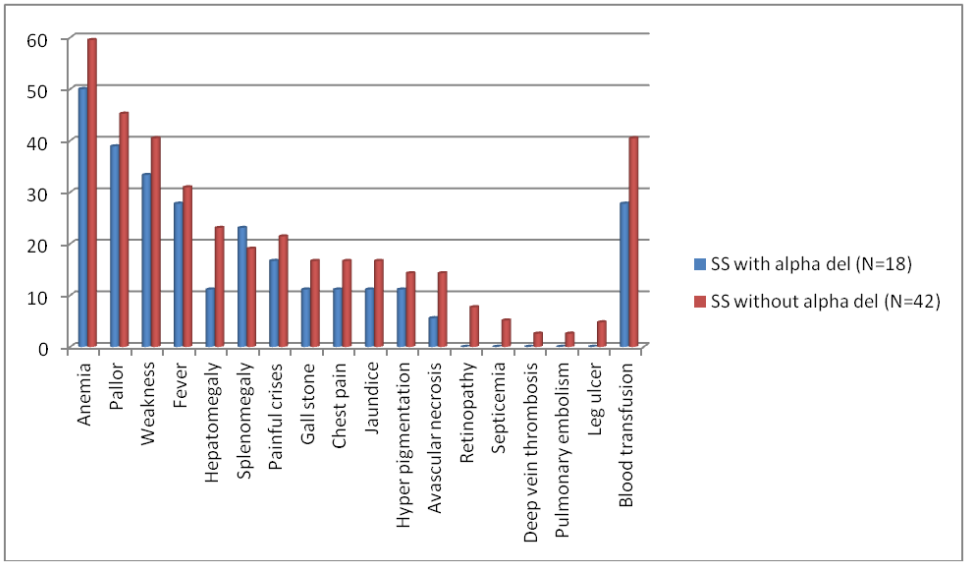

Figure 1: Comparative clinical parameter with and without alpha thalassemia genotype in sickle cell anemia patients.

Table 2: Comparative Hematological Parameters of Carrier and Non Carrier Hmox1 in HbSS.

Mean±SD				
Hematological Parameters	HMOX1 (+/+) N=19	HMOX1 (+/-) N=27	HMOX1 (-/-) N=14	P-value
RBC **millions/µl**	3.8±2.3	3.2±1.7	3.5±1.2	0.001
HGB **g/dl**	10.3±1.7	9.3±1.5	9.2±1.2	<0.001
HCT **%**	20.6±3.4	20.2±1.3	19.8±2.1	<0.001
MCV **fl**	73.1±5.2	71.3±4.3	71.5±4.7	<0.001
MCH **pg**	31.6±7.4	30.2±3.2	29.6±3.4	<0.001
MCHC **g/dl**	32.7±5.2	32.4±3.5	32.6±4.2	<0.001
HbF **%**	25.2±4.3	20.18±3.7	13.2±4.5	<0.001

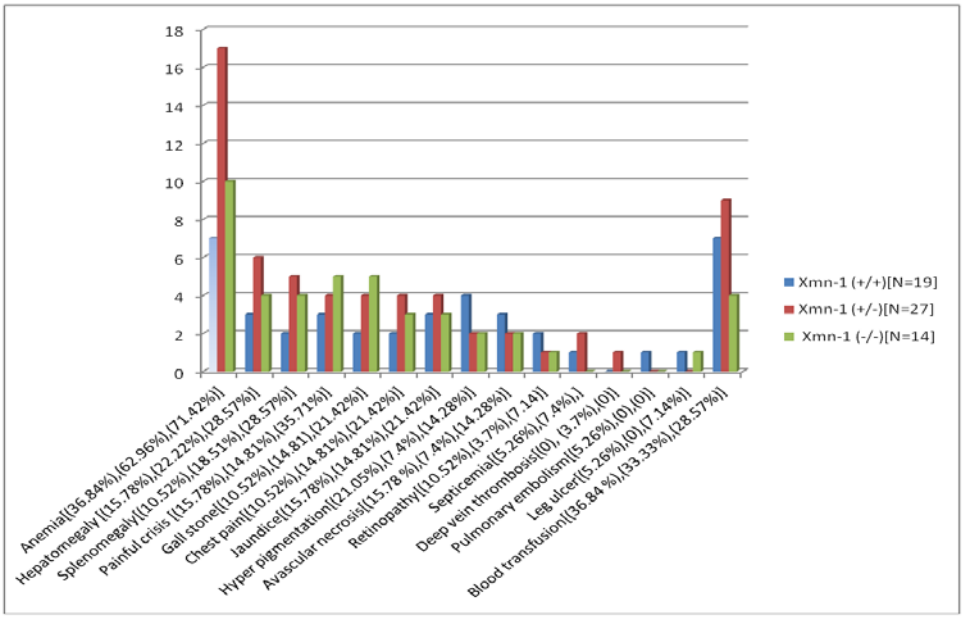

Figure 2: Comparative clinical parameter with carrier and non carrier HMOX1in HbSS

3. UGT1A1 polymorphism and effect on sickle cell anemia phenotype

The total bilirubin was 3.2±1.3 mg% in the sickle cell homozygous patients and it was 2.5±1.4 mg% in the sickle beta thalassamia patients. The total bilirubin value was higher in the sickle cell anaemia patients than in the sickle beta thalassamia patients and it was statistically significant (p-value 0.006), while the unconjugated bilirubin concentration was 2.1±0.45 mg% in the sickle homozygous patients and it was 1.9±0.38 mg% in the sickle beta thalassemia patients (p-value 0.009). The TA repeats, 6/6, 6/7 and 7/7 of the UGT1A1 genotype promoter polymorphism were identified in our cases. The allele 7/7(TA) repeats were more frequent in sickle beta thalassemia (54.28%) as well as in sickle homozygous (52%) patients, while the 6/6 TA repeats were 18% in the sickle homozygous patients and they were 15.71% in the sickle beta thalassemia patients. The 6/7 TA repeats were 30% in the sickle homozygous as well as in the sickle beta thalassemia patients. The allele frequencies of the 6/6, 6/7 and the 7/7 (TA) repeats in the sickle homozygous patients was 0.104, 0.436 and 0.456 respectively, while they were 0.094, 0.424 and 0.478 respectively in the sickle β-thalassemia patients. The bilirubin levels were higher in the 7/7 (TA) genotype in the sickle cell anaemia patients as well as in the sickle beta thalassemia patients and all the values were statistically significant (p-value <0.05). The bilirubin levels in the sickle homozygous and in the sickle β-thalassemia patients with and without the presence of gall stones had a great variability with significant differences (p-value <0.05).

References:

1. Christy M Waterfall. Single tube genotyping of sickle cell anemia using PCR-based SNP analysis. Nucleic Acid Research. 2001- vol 29. no.23 e119
2. E. Baysal and THJ Huisman. Detection of common deletional alpha thalassemia -2 determinants by GAP-PCR. American Journal of Hematology. 1994; 46:208-213
3. RV Shah. Determination of the breakpoint and molecular diagnosis of a common alpha Thalassemia -1deletion in the Indian population. British Journal of Hematology. 2003;123:942-947
4. Arnold SC Tan et al- A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha thalassemia. Blood; 2001-98:250-251
5. JG Chang. Rapid diagnosis of alpha –thalassemia -1 of Southeast Asia type and hydrops fetailis by polymerase chain reaction. Blood .1991-78: 853-854.
6. Haverfield E V. The UGT1A1 variation and gallstone formation in sickle cell disease. Blood. 2005; 105:968-72.
7. Vasavda N. The linear effect of alpha thalassemia the UGT1A1 and HMOX1 polymorphism on cholelithiasis in sickle cell disease. British Journal of Haematology. 2007 Jul;138(2):263-70