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Original Research Paper

Pathology

INCIDENCE OF THALASSEMIA AND OTHER HEMOGLOBINOPATHIES IN ANEMIC PATIENTS OF GREATER GWALIOR REGION ATTENDING OUR HOSPITAL OPD - A PROSPECTIVE STUDY.

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ABSTRACT

INTRODUCTION:- Thalassemia and other haemoglobinopathies are the most common monogenic disorders in the world. Incidence of Thalassemia could be reduced by thorough screening and counseling.

AIMS AND OBJECTIVES:- The purpose of this study was to fnd out the incidence of thalassemia in greater Gwalior region.

MATERIAL & METHODS: - This is a prospective study on 988 patients, aged <18 years arrived at JA hospital of GR Medical College with anemia.

CONCLUSION:- The incidence of thalassemia was found to be 9.6 % and that of other hemoglobinopathies to be 0.96%.

KEYWORDS :

INTRODUCTION

Thalassemia and other haemoglobinopathies (production of structurally defective genes) are the most common monogenic disorders in the world. Thalassemia has an autosomal recessive pattern of inheritance. It arises from a mutation or deletion in one or more globin gene(s), which leads to a reduction or absence in the production of hemoglobin [Hb] and an abnormal Hb ratio (α: non-α). Ultimately it causes varying degrees of microcytic anemia that can range from insignificant to life threatening.

Thalassemia is classified according to the affected globin, i.e. Alpha thalassemia and Beta thalassemia. Alpha thalassemia is characterized by deletion or mutation of one or more α-globin genes located in the short arm of chromosome 16. Whereas beta thalassemia is caused by mutation of β-globin gene on chromosome 11 affecting all aspects of transcription, translation and the stability of β-globin gene product.

Some mutant genes lead to the production of abnormal globin chain with a different amino acid sequence as compared to the normal globin chain. These defects are called hemoglobinopathies and are designated by alphabet i.e. D, E, H, J, K, L, M etc. or according to the place where first discovered.

This is a special importance in developing countries like India, where it increases the burden of health care delivery system. Every year 10,000 children with thalassemia major are born in India, which constitutes 10% of the total number in the world, and one out of every 8 carriers of thalassemia worldwide lives in India.

Approximately 250 million people (4.5% of world population) carry a potential pathological hemoglobinopathy gene. Annually 300,000 infants are born with major hemoglobin disorder.

Carrier frequency of thalassemia in India is about 3% and estimated frequency of β thalassemia at birth is 1:2700 that would mean that 9000 babies are born every year with thalassemia. They suffer from severe anemia and need repeated blood transfusion. Treatment is very expensive and maximum life span of affected child is 20 years. About 5200 are born annually with sickle cell disease

Figure is higher in some groups, in Gujrat 10-15%, sindh 10%, and northern 2/3 of Pakistan 6.5%. Population movement in 1947 brought substantial number of thalassemia carrier from sindh to Mumbai area and from northern Pakistan to Punjab. An Hb S trait occurs about 1% in many other groups and interacts with beta thalassemia trait to produce HbS/beta thalassemia. Hb E trait is found mainly in Bengal (3.5%) and interacts with beta thalassemia trait to produce Hb E-beta thalassemia

All hemoglobinopathy traits are harmless in themselves.

Madhyapradesh as a whole and Gwalior area in particular is situated in the central part of the country and constitute the population drown from almost all part of country, thus it become interesting to know the incidence as well as the pattern of incidence of thalassemia and other hemoglobinopathies, in different communities.

Wide variation in the distribution of hemoglobinopathies through out the world and paucity of studies from India, specially in Madhya Pradesh , prompted us to know the incidence of thalassemia and other hemoglobinopathies in the blood samples collected in the Central Pathology lab, Department of Pathology, G R Medical College Gwalior.

The present study is carried out in the special hematology section of Central Pathology lab. In the department of Pathology, G R Medical College Gwalior from October 2007 to October 2008, to know the incidence of thalassemia and other haemoglobinopathies (Hospital based study). This study is restricted mostly to the infant, children and adolescence population (Age ≤18 years) who attend the central pathology for anemia typing, complete haemogram along with red cell indices were obtained by automatic electronic counter, those individuals who have MCH ≤27pg and MCV ≤80fl were selected for Hb electrophoresis on cellulose acetate strip to see the types of haemoglobins present in their blood.

MATERIAL AND METHOD

The present study is carried out in the special hematology section of Central Pathology lab. in the department of Pathology, G R Medical College Gwalior from October 2007 to October 2008

The subject taking part in the study chosen from the person attending Central Pathology lab of Department of Pathology, G R Medical College Gwalior.

Criteria for selection of subjects :

This study is restricted mostly to the infant, children and adolescence population (Age ≤18 years) who are attending Central Pathology lab of Department of Pathology, G R Medical College Gwalior for anemia typing.

Background data of each individual like age, sex, caste, place of origin, consanguinity, etc is recorded.

Obtaining the complete haemogram with the help of electronic counter:

For this Medonic CA 530 Oden (manufactured by boule medical AB)

is used. Complete haemogram with red cell indices (MCH, MCV,MCHC, RDW etc.) were obtained by this automated electronic cell counter.

Examination of stained peripheral blood film:
The peripheral blood film stained with leishman's stain was examined and following red cell abnormalities were noted:

1. Change in size (anisocytosis) specially for microcytosis, Change in shape (poikilocytosis) such as Dacryocyte (tear drop cells), Codocyte (Targetcells), Schizocyte/schistocyte, Drepanocyte (sickle cells), Leptocyte (Thin flat cells)
- Haemoglobin distribution in the red cell, Hypochromasia (less haemoglobinisation),Normochromasia (normal haemoglobin isation)
- Erythrocytic inclusions, Diffuse basophilia,Polychromatophilia
- Punctate basophilia/basophilic stippling
2. Normoblasts
3. Platelets and WBCs.

Sickling Test
Principle: RBCs which contain HbS sickle when oxygen tension is lowered.

HAEMOGLOBIN ELECTROPHORESTS:
A. Preparation of haemolysate
B. Cellulose acetate Electrophoresis of Haemoglobin at alkaline pH

Principle:
Detection of abnormal haemoglobin with electrophoresis techniques is based on differences of their migration within the electric field. These differences in migration velocities are the result of electric charges of each haemoglobin variant brought about by various amino acid substitutions in the polypeptides of the molecule.

REMARKS:
Two minor cathodal fractions CAI and CA II are observed with these techniques which are non haemoglobin components.

The relative position of the various haemoglobins after electrophoresis can be seen.

OBSERVATIONS
The present study comprises of analysis of hematological specimen received for anemia typing in department of pathology G R Medical College Gwalior from October 2007 to October 2008.

During this period total Number of samples received for anemia typing were 6800 and number of cases having age ≤18 years were 988. These samples were selected for haemogram by electronic counter.

Table No. 1:Cases having predominantly Hb F on cellulose acetate Hb electrophoresis and peripheral smear suggestive of thalassemia major

S.N.	Case No.	Hb	PCV	RBC count	MCV	MCH	MCHC	RDW	Peripheral RBC picture
1	6	6.9	27	3.3	51.2	16.4	31.9	20.2	MC,HC, TG +++
2	13	7.3	22	2.6	64	21.2	33.7	14.3	MC,HC, TG ++
3	19	5.2	16	2.5	64	16.8	31.9	18.3	MC,HC, TG ++
4	28	8.9	24	3.0	78.6	28.8	36.6	17.5	MC,HC, TG +++
5	34	5.2	20.7	4.6	44.4	11.3	25.5	18.6	MC,HC, TG ++
6	39	5	18.8	3.8	49.1	13.2	26.9	19.5	MC,HC, TG ++
7	42	6.5	22	3.9	73	21.6	29.5	23.2	MC,HC, TG +++
8	50	4.5	13	2.1	61	21	34	14.2	MC,HC, TG ++
9	53	6.8	22	3.0	75.3	22.9	30.9	13.8	MC,HC, TG ++
10	64	5	13	1.8	72	26	36	16.9	MC,HC, TG ++
11	74	8	27	2.8	78	26	29.9	15.8	MC,HC,SC+ TG ++

MC,HC: Microcytic hypochomic
TG : Target cell
SC : Sickle cell

Table No. 6 shows various hematological parameters along with RBC picture of the cases diagnosed as thalassemia major, one case (case No. 74) was diagnosed as sickle cell beta thalassemia whose electrophoresis showed Hb F and Hb S both.

Table No. 2: Cases showing minor amount of haemoglobin F on cellulose acetate paper on Hb electrophoresis

S.N.	Case No.	Hb	PCV	RBC count	MCV	MCH	MCHC	RDW	Peripheral RBC picture
1	16	5	16	2.2	70	24	30	18.6	MC,HC, TG +
2	24	11.4	35	3.4	79	21	33	20.1	MC,HC,
3	25	9.28	28	3.8	73	24.4	33	14.2	MC,HC, TG +
4	30	4.8	13.9	2.1	65.1	22.7	34.9	13.9	MC,HC,
5	40	6	19	2.4	79	25	31.5	17.3	MC,HC, TG
6	48	9.5	28	3.7	75.3	25.5	33.9	18.8	MC,HC, TG +
7	58	4.2	14	1.8	77.7	29.3	30.9	19.2	MC,HC, TG +
8	69	7.2	25	3.3	75	22	29	21.1	MC,HC, TG +
9	72	4.3	13	1.9	72	22	33	22.8	MC,HC, TG +
10	86	6.8	22	3	75.3	22.9	30.9	13.8	MC,HC, TG

MC,HC: Microcytic hypochomic
TG : Target cell

Table No. 7 depicts various hematological parameters of 10 cases revealing microcytic hypochromic RBC picture on peripheral smear examination and these 10 cases also showed minor amount of Hb F on Hb electrophoresis.

Table No. 3: Cases showing minor amount of fast moving Hb (i.e. Hb H/ Hb Bart/Hb J) on cellulose acetate paper on electrophoresis

S.N.	Case No.	Hb	PCV	RBC count	MCV	MCH	MCHC	RDW	Peripheral RBC picture
1	8	4.93	15	1.9	76	25.9	35.6	17.5	MC,HC, TG +
2	10	7.3	22	2.6	64	21	23.1	13.8	MC,HC,
3	12	6.2	19	2.7	70	25	32.6	16.2	MC,HC, TG +
4	27	11.4	35	3.4	79	21	33	19.8	MC,HC,
5	77	6	20	2.6	74	23	30	14.5	MC,HC,

Table No. 8 depicts various hematological parameters of 5 cases revealing microcytic hypochromic RBC picture on peripheral smear examination and these 5 cases also showed minor amount of fast moving Hb (i.e. Hb H/ HbBart/ Hb J) on cellulose acetate paper electrophoresis.

Table No. 4: Incidence of thalassemia and other hemoglobin opathies in greater Gwalior region (out of 104 screened patients)

Incidence of thalassemia	*10 (9.61%) (6M & 4F)*
Incidence of other hemoglobinopathies	*1(0.96%)*

Table no. 9 shows incidence of thalassemia and other hemoglobinopathies in 104 screened patients. Thalassemia major was dedected in 10(9.61%) cases while other hemoglobinopathy was detected in 1 (0.96%) case.

Out of 10 positive cases of thalassemia major 6 (60%) were male and

4 (40%) were females.

DISCUSSION

Anemia patients coming to the hematology section of Pathology department of G R Medical College, Gwalior were selected for screening of thalassemia and other hemoglobinopathies. All anemia patient below 18 years of age having MCV below 80 fl and MCH below 27pg were selected for further hemoglobin electrophoresis. During this period of study (October 2007-October 2008) total 988 anemia patients were screened, out of these total 104 patients had MCV below 80 fl and MCH below27 pg, and these were subjected for hemoglobin electrophoresis.

The results of present study with relation to incidence of thalassemia and other hemoglobinopathies and their pattern of incidence in different communities in greater Gwalior region is discussed and compared with the findings of other workers.

Table No. 5: Incidence of positive cases of thalassemia major in suspected patients after electrophoresis

S.N.	Name of the author	Total suspected cases selected for electrophoresis	Positive cases of thalassemia major
1	Udani P M et al. (1961)	212	16 (7.5%)
2	Mathur K S et al. (1962)	512	7 (1.3%)
3	Gupta S C et al (1970)	1217	17 (1.3%)
4	Pawan Agrawal (1990)	33	06 (18.1%)
5	J. Ahmed et al (2003)	45	10 (22.2%)
6	Balgir R S (2003)	1015	54 (5.3%)
7	Chhotray G P et al. (2004)	520	46(8.84%)
8	Dr Lal A (2007)	12120	582 (4.7%)
9	Shivshankara A R et al. (2008)	150	15 (10%)
10	Present study (2008)	104	10 (9.6%)

Table No 12 depicts the results of incidence of thalassemia major as described by various workers. The incidence varies from 1.3% (Mathur K S et al.1962, Gupta et al 1970) to 22.2% (J Ahmed et al 2003). The incidence of present study is 9.6% and in concordance with results of Chhotray G P et al.(2004) 8.4% and Shivshankara et al.(2008)' 10% .

The general incidence of thalassemia trait and sickle cell hemoglobinopathies in India varies between 3-17% and 1-44% respectively (Balgir R S 2000, Balgir R S 2002, Balgir R S 2005).

Thalassemia major can cause life threatening situation and chronic ill health. It poses economic and psychological burden on the affected individual and their family and society as a whole. Hence it is justifies the regular and wide scale screening program for thalassemia detection.

Table No. 6: Shows percentage of cases suspected of thalassemia minor or trait.

S.N.	Name of the author	Total suspected cases selected for electrophoresis	cases suspected of thalassemia minor or trait
1	J. Ahmed et al (2003)	45	19 (42.22%)
2	Balgir R S (2003)	1015	185 (18.2%)
3	Chhotray G P et al. (2004)	520	103(19.80%)
4	Dr Lal A (2007)	12120	1738(14.34%)
5	Mulchandani D V et al (2008)	446	75 (16.18%)
6	Munshi A et al. (2008)	1592	347 (21.79%)
7	Shivshankara et al A R et al. (2008)	50	20 (40%)
8	Present study (2008)	104	15 (14.42%)

Table No.16 depicts results of cases of thalassemia minor or trait as reported by various workers. While J. Ahmed et al (2003) and Shivshankara et al A R et al. (2008) reported high incidence (42.22% and 40% respectively) of thalassemia minor or trait, other have varied incidence of 14.34% (Dr Lal A 2005) to 21.79% (Munshi A et al. (2008). Present study (2008) reported 14.42% thalassemia minor or trait and results of our study matches with the results of Dr Lal A (2007) 14.34%.

The variation of results as reported by different workers are possibly due to geographical variation of thalassemia minor or carrier rate and due to different sample size of studies and using different testing procedures with varied sensitivity and results.

In present study 15 cases suspected to be thalassemia minor or trait, out of which 10 showed variable amount of raised HbF on the cellulose acetate paper on electrophoresis and 5 showed fast Hb variants (HbH or Hb Bart), none of the cases showed raised Hb A2. These cases presented diagnostic difficulties, as it could not be possible for us to arrive at accurate and specific diagnosis in our setup. Electrophoresis is slow and labor intensive and inaccurate in the quantification of low concentration Hb variants (e.g. Hb A2) or in the detection of fast Hb variants (HbH or Hb Bart) (Gwendolyn M. Clarke et al 2000). Further studies like HPCL, globin chain electrophoresis, PCR and other molecular technique are required to confirm the diagnosis of those cases. Because of cost constraints these cases can not be delegated to other laboratories. Where funds and facilities exist, interpretation of such cases can provide very useful information. In present study (2008) Hemoglobinopathies other than thalassemia was found only in one case, which was belongs to 6-10 years age group. And showed HbF and HbS on cellulose acetate strip and showed positive sickling tests and was diagnosed as sickle cell beta thalassemia.

SUMMARY AND CONCLUSION

Present study is a prospective hospital based study of one year (October 2007-Octobe2008) with aims and object to find incidence of thalassemia and other hemoglobinopathies in the greater Gwalior region for this a screening criteria was formulated for selecting the patient which was as follows. Blood samples of the patients having age <18 years and MCH < 27 pg and MCV < 80 fl obtained by automatic hematology analyser were selected for Hb electrophoresis.

1. Total No of patients screened by complete blood count were 988 by haematology counter.
2. Out of which 512 (51.82 %) were males and 476 (48.17) were females.
3. Out of 988 patients only 104 were having MCH < 27 and MCV < 80 and were selected to do electrophoresis. Out of 104 individuals 58 (55.76 %) were males and 46 (44.23 %) were females.
4. Out of these 104 cases, total 10 cases were found positive for thalassemia major on electrophoresis. Out of 10, 6 were males and 4 were females.
5. Out of 6 positive males 4 (66.6%) were from age group 1-5 years, 1 (16.6%) was from age group 6-10 years and 1(16.66%) from age group 6-10 years.
6. Out of 4 positive females 2 (50%) belong to age group 1-5 years and 2 (50%) belongs to age group 6-10 years.
7. Out of 104 cases 10 (9.61%) were showing minor amount of Hb F and 5 (4.80%) cases showed minor amount of fast moving hemoglobins (i.e. Hb H/Hb Bart/Hb J) on cellulose acetate strip at alkaline PH. These total 15 (14.42%) cases were suspected to be heterozygous thalassemia or thalassemia trait or carrier.

8. Hemoglobinopathies other than thalassemia was found only in one case (0.96%), which belonged to male group, and was diagnosed as sickle cell beta thalassemia on electrophoresis.
9. Complete blood counting by automated hematology analyser is the useful screening procedure for detection of thalassemias and other hemoglobinopathies.
10. Screening of the communities having high incidence of thalassemia and other hemoglobinopathies should be done to prevent birth of new thalassemic child.
11. Apart from this extensive screening of population for haemoglobin disorders, regular newborn screening and prenatal diagnosis of haemoglobinopathies in high risk & carrier couples. Refraining from consanguineous marriages, pre-marriage genetic counseling and finally medical termination of pregnancy with consented parents will prevent haemoglobinopathies and decrease its prevalence to acceptable levels in society.

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