Spectrum of hemoglobinopathies in a tertiary care centre

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ABSTRACT

Background: Thalassemia and other structural hemoglobinopathies are the most common single gene disorders throughout the world with the highest frequency in the tropics, subtropics, Mediterranean basin and Southeast Asia. This study aims to provide a better assessment on the spectrum of hemoglobinopathies in our context.

Materials and Methods: This descriptive study was done at our tertiary care center, from November 2014 to October 2015. Hematological indices were derived from coulter counter, haemoglobin electrophoresis was carried out by cellulose acetate medium at alkaline PH. Sickling test, Hb H inclusions demonstration and Hb F estimation were performed. Parental screening was done wherever feasible.

Results: Out of 350 cases referred from various out-patient departments of TUTH and different peripheral hospitals for suspected hemoglobinopathies, 97 cases (27.71%) had hemoglobinopathies. The most predominant hemoglobinopathy was thalassemia (57.73%) followed by sickle cell disorders (28.87%) and Hb D/Hb E hemoglobinopathies (13.40%). Most disorders were observed in the age group 21-30 years with many of the cases seen clustered in the families and a slight male preponderance. Terai region (71.13%) had a very high number. A significant high frequency of thalassemia (33.93%) and sickle cell disorders (75%) were seen in Tharu community.

Conclusion: Hemoglobinopathies were seen widespread in Nepal. An extensive screening of the population is important to assess the prevalence of hemoglobinopathies, which will help in identification of carriers and take adequate therapeutic and preventive measures.

INTRODUCTION

Hemoglobinopathies are characterized by the presence of structurally defective haemoglobin (Hb) due to abnormalities in the formation of globin moiety of the molecule.¹ Hemoglobinopathies fall into two main groups: thalassemia syndromes and structural hemoglobin variants (abnormal hemoglobin).²

α-thalassemia and β-thalassemia are characterized by absent or decreased production of α- and β-globin subunits respectively. Sickle cell disease is caused by a point mutation in the sixth position of β-globin.³
Sickle Cell Disorders 

| Thalassemia n(%) | Sickle Cell Disorders n(%) | Others n(%) | Total n(%) |
|------------------|---------------------------|-------------|------------|
| Male             | 34 (60.70)                | 16 (57.10)  | 7 (53.80)  | 57 (58.76) |
| Female           | 22 (39.30)                | 12 (42.90)  | 6 (46.20)  | 40 (41.24) |
| Total            | 56 (100)                  | 28 (100)    | 13 (100)   | 97 (100)   |

D (Hb D) disease is derived from a point mutation in the β-globin gene with the substitution of glutamine for glutamic acid at 121st position. Hemoglobin E (Hb E) is produced due to the substitution of lysine for glutamic acid at 26th position. This study was designed to provide a better assessment on the spectrum of hemoglobinopathies in Nepal. The frequencies of various hemoglobinopathies, age and sex distribution, geographical distribution, ethnic communities commonly affected were analysed and compared between different disorders.

**MATERIALS AND METHODS**

This descriptive study is a hospital based cross-sectional study carried out in the Department of Pathology, TUTH. The study duration was of one year (November 1, 2014 to October 31, 2015). Ethical approval was obtained from Institutional Review Board of the institute and consent was taken while filling the proforma. 350 patients were initially registered for the work up of hemoglobinopathies. Out of 350 cases, 97 were diagnosed to have hemoglobinopathies. The study included 97 samples for further analysis. Patients with inconclusive/normal Hb electrophoresis results, the repetition of the same case, those who received blood transfusion three months back were excluded from the study. EDTA anti-coagulated blood was used. Hematological indices were derived from Sysmex 500i 5-part coulter counter. Morphological alterations in red cells were studied. Hemoglobin electrophoresis was carried out by cellulose acetate medium at alkaline PH. Other tests as sickling test using sodium metabisulfite, HbH inclusions (Golf balls) demonstration using brilliant cresyl blue stain, and Hb F estimation by Singer’s method were performed. Detailed history (like age, sex, caste, ethnicity, and place of origin) was recorded. Parental screening was done to confirm the diagnosis wherever feasible. The data collection was done in pre-designed proforma and data entry was done in SPSS and results were computed using Statistical Package for the Social Sciences (SPSS) version 20.

**RESULTS**

A total number of 350 blood samples were received in the Department of Pathology for hemoglobin electrophoresis during the study period of one year, out of which 97 cases (27.71%) had hemoglobinopathies.

There were 56 cases (57.73%) of thalassemia constituting the most predominant one, followed by 28 cases (28.87%) of sickle cell disorders. Remaining 13 cases (13.40%) were comprised by Hb D and Hb E hemoglobinopathies with 3 cases (3.09%) of HbD hemoglobinopathies and 10 cases (10.31%) of HbE hemoglobinopathies. Out of 97 cases of hemoglobinopathies, 57 (58.76%) were males and 40 (41.25%) were females in the ratio of 1.4:1, thus showing a preponderance of males over females (Table 1). Thalassemia, sickle cell disorders, Hb D/Hb E hemoglobinopathies were more common in Terai region, the malaria endemic zone (71.13%), followed by Kathmandu (15.46%) and Hills (13.40%). Maximum cases of hemoglobinopathies were between 21-30 years of age group followed by patients under 10 years of age. Likewise, Terai Janajati had the highest frequency of hemoglobinopathies 42 (43.30%) followed by Hill Janajati 24 (24.74%) (Table 2).

Electrophoresis findings of 97 cases were noted based on the bands seen on Hemoglobin electrophoresis at alkaline pH. β-Thalassemia trait was the most common hemoglobinopathy (36, 37.11%), followed by sickle cell trait (16, 16.49%), β-Thalassemia Major (9, 9.28%) and Sickle β-Thalassemia (7, 7.22%) (Table 3).

Among the districts, Dang accounted for the majority with 26 cases (Fig. 1). Few hemoglobinopathies with their bands are depicted (Fig. 2).

**DISCUSSION**

Hemoglobinopathies affect people worldwide including Nepal. However, there is a limitation of scientific study which demonstrates the actual burden of the same. This study made evident that hemoglobinopathies were seen widespread in Nepal. Thalassemia were the most common constituting 57.73% of the total cases, the findings being broadly consistent with the studies done by Mondal et al in India and Waheed et al in Pakistan.

Modell et al reported that sickle cell disorders are the most common hemoglobinopathies. This means different hemoglobinopathies have diverse frequencies across the world. The present study that embodied 97 patients consisted of 57 (58.76 %) males and 41 (41.20 %) females in the ratio of 1.4:1 thus giving a preponderance of males over females. The findings were comparable to the study done in Orissa, India by Balgir et al. Similarly, Hussain et al came up with correspondent findings in a study done in Pakistan where 35 (56.50%) males and 27 (43.50%) females...
In our context, 49.48% of the hemoglobinopathies were seen in the age group 21-30 years, followed by 23.71% under 10 years of age. Whereas, Tiwari in her study in Nepal had drawn the conclusion of maximum cases in under-10 age group. This could be because in our context parental screening was done for almost all cases of homozygous hemoglobinopathies, and majority of the parents fell into that age range.

Table 2: Caste/Ethnicity distribution of patients (categorisation as per 2001 census)

| Ethnic Group           | No of Patients (Caste) | Male/Female |
|------------------------|------------------------|-------------|
| HILL BRAHMIN           | 10                     | 10.31       |
| Adhikari               | 3                      |             |
| Dhital                 | 1                      |             |
| Gautam                 | 3                      |             |
| Regmi                  | 3                      |             |
| HILL CHHETRI           | 10                     | 10.31       |
| Kunwar                 | 1                      |             |
| Thapa                  | 3                      |             |
| Achhami                | 3                      |             |
| Rokka                  | 3                      |             |
| HILL JANAJATI          | 24                     | 24.74       |
| Kumal                  | 3                      |             |
| Lama                   | 3                      |             |
| Magar                  | 3                      |             |
| Majhi                  | 6                      |             |
| Rai                    | 6                      |             |
| Tamang                 | 3                      |             |
| TERAI JANAJATI         | 42                     | 43.30       |
| Chaudhary              | 41                     |             |
| Dhimal                 | 1                      |             |
| TERAI/MADHESI BRAHMIN/CHHETRI | 11 | 11.34 |

Table 3: Final Diagnosis of the patients with various bands

| Disease                | Types of bands              | Frequency (n=97) | Percent |
|------------------------|-----------------------------|------------------|---------|
| Hb H Disease           | Fast Moving Band            | 5                | 5.15    |
| α-Thalassemia Trait    | Normal band                 | 6                | 6.19    |
| β-Thalassemia Major    | HBF                         | 9                | 9.28    |
| β-Thalassemia Trait    | HbA+HbF+HbA2                | 36               | 37.11   |
| Sickle Cell Disease    | HbS                         | 5                | 5.15    |
| Sickle Cell Trait      | HbS                         | 16               | 16.49   |
| Sickle β-Thalassemia   | HbS                         | 7                | 7.22    |
| Hb E Disease           | Hb A2, C, E, OArab region   | 1                | 1.03    |
| E β-Thalassemia        | Hb A2, C, E, OArab region   | 3                | 3.09    |
| E Thalassemia Trait    | Hb A2, C, E, OArab region+Hb A | 6 | 6.19 |
| Hb D Disease           | HbS                         | 1                | 1.03    |
| Hb D Trait             | HbS                         | 2                | 2.06    |

Figure 1: Map of Nepal showing various districts affected by hemoglobinopathies. Dang district, in Terai terrain accounted for the majority (26 cases)
Terai janajati (Tharu/Chaudhary and Dhimal communities) had the highest and a substantial risk of hemoglobinopathies with high prevalence of both thalassemia and sickle cell disorders comprising 42 cases (43.30%) similar to the conclusions drawn by Tiwari and Shrestha et al in their studies conducted in Nepal.\textsuperscript{11,12} Terai region is an endemic zone for malaria. The increased percentage of thalassemia and sickle cell disorders in Terai region in our research also supports several studies as Enevold et al in Tanzania population and Kuesap et al in Thailand.\textsuperscript{13,14} In addition, the practice of consanguineous marriages also contributes to higher rate of hemoglobinopathies in the Terai region.

The most predominant hemoglobinopathy was β-thalassemia trait (36 cases, 37.11%), the observations being congruent with the studies in Eastern India\textsuperscript{6,10} and in Pakistan.\textsuperscript{6,10} Maximum cases were the parents of children with homozygous diseases such as β-thalassemia major, Sickle β-thalassemia and E β-thalassemia. The HbS β-thalassemia patients had a more severe disease with lower Hb level, MCV (8.57 g/dl, 64.17fl) as compared to HbSS (8.86 g/dl, 67.08 fl) which was comparable to the study done in Central India.\textsuperscript{15} Our research also touched on a few rarer hemoglobinopathies as hemoglobin D hemoglobinopathies which are seen sporadically in Blacks and Europeans. Fast moving Hb H bands were seen in 5 cases (5.15%) of all the hemoglobinopathies embracing three children and two adults. Furthermore, numerous HbH inclusions were demonstrated with brilliant cresyl blue stain. Nadkarni et al in his paper “Molecular diversity of Hemoglobin H Disease in India” has unequivocally elucidated that Hb H disease is the most severe form of α-thalassemia compatible with life.\textsuperscript{16} This statement was consonant with our results as age at diagnosis varied from 11 months to 32 years. E-thalassemia trait and Hb-E disease in our context was 6.19% and 1.03% which was almost similar to the values found in Thailand where the prevalence was 5.2% and 0.9 % respectively.\textsuperscript{17} These findings are also akin to other studies that show hemoglobin E hemoglobinopathies to be common among Southeast Asians.\textsuperscript{18}

Hemoglobinopathies pose considerable economic and psychosocial burden on the affected individuals, families, society and the country, at large. This study through its findings aims to come up with conclusive results that will help to induce changes and amendments in the health sector for instance, screening of the population, identification of carriers and adoption of appropriate measures for prevention of hemoglobinopathies.

**LIMITATION**

The study being an institutional study, samples in our research may not be representative of the whole nation. Some of the disorders may require HPLC and capillary hemoglobin electrophoresis along with DNA testing for confirmation not available in our institute.

**CONCLUSIONS**

This study made evident the widespread prevalence of hemoglobinopathies in Nepal. The population of Nepal, essentially those from Terai territory were seen the most significant group affected by hemoglobinopathies. Thalassemia was seen to be the most prevailing hemoglobinopathies followed by sickle cell disorders and HbD/HbE hemoglobinopathies. Tharu/Chaudhary population was seen to be the predominant ethnic tribes affected by the same with many of the cases seen clustered in the families. Hemoglobinopathies pose considerable economic and psychosocial burden on the affected individuals, families, society and the country, at large.
It is very important that the population be screened so that carriers could be detected and informed about the various complications and reproductive risks. In addition, preventive measures could also be adopted in the form of genetic counseling, prenatal diagnosis and termination of the affected babies.

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