DISTRIBUTION OF HAEMOGLOBINOPATHIES IN THE ETHNIC GROUPS OF EASTERN TERAI, NEPAL.

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ABSTRACT

Thalassemia and haemoglobinopathies are genetic disorders of the blood. Beta-thalassemia and HbE disorders are an emerging global health problem. Mutation leads to reduced or absence of globin chain synthesis and reduction in the production of haemoglobin. Any population based data on the prevalence of beta-thalassemia and HbE disorders in Nepal is lacking. Therefore, this study was done with the aim to find out the prevalence of beta-thalassemia and HbE disorders in some ethnic groups of Nepal. The study sites included three districts, Jhapa, Morang and Sunsari of eastern Nepal. Study population comprised of four ethnic groups, Koch Rajbanshi, Kochila Tharu, Musahar and Santhal. In this cross-sectional study haematological parameters of 1200 blood samples of all age groups (7 – 75 years) were estimated by an automated haematological cell counter. Peripheral blood smear was studied microscopically for target cells. Cases with Hb, MCV and MCH less than 25gm/dl. 80fl and 27pg were further analysed for haemoglobin pattern. Haemoglobin quantification was done by capillary electrophoresis (Sebia minicap flex piercing). Out of 1200 cases, 2.83% HbE homozygous, 2% HbE heterozygous and 4.083% beta thalassemia was diagnosed. This study showed that beta-thalassemia and haemoglobinopathies prevalent in the study population could develop into serious health problems.
Keywords: Beta-thalassemia, HbE, ethnic groups, capillary electrophoresis.

Introduction

Thalassemia and other haemoglobinopathies are genetic disorders of the blood. The defect in the genes produces diminished rate of synthesis of one or more structurally normal haemoglobin chains and, consequently, reduced rate of production of haemoglobin or haemoglobins. The failure to synthesize the α or β globin chains of haemoglobin in balanced amounts results to haemolysis, anaemia, and splenomegaly. Beta thalassemia is an autosomal recessive inheritance characterized by reduced or absent beta globin chain synthesis. It manifests as haemolytic disorders of varying degrees in homogenous condition but traits are often asymptomatic (Bernard S.S. 2009). Beta thalassemia are of three types- minor, intermediate and major. In beta thalassemia minor/trait or carrier state, the affected person carries one normal and one mutated beta globin chain. Thalassemia minor may be present in anyone without the awareness of being a carrier. There are 25% chances of producing homozygous beta thalassemia off springs for beta thalassemia carrier couple (Weatherall D.J. 2010). An estimated 300,000 to 400,000 children are born every year with severe haemoglobin disorders. 80% of these births are in developing countries. Approximately 1.5% of the world population are heterozygotes of β-thalassemia (WHO 2007). Prevalence of β-thalassemia in Americans- 0.3%, Southeast Asia- 11%, Sub-Saharan Africa - 0-12%, Western Pacific 0-13%, Europe 0-19%, (Suresh, 2016), American Blacks 1.5% (Pierce et al. 1977), Pakistan 1-5% (Ahmed,S. 2002), Turkey 2% (Cavdar A. 1971), Taiwan 1-3% (Ko TM. 1989), Iran 7% (Karimi M. 2008). The highest incidence was reported from Southeast Asia, Sardinia 12% and Cyprus 14% [13]. The defects producing beta thalassemia are heterogenous, and each ethnic group possesses its own specific set of mutations. Nepal is a multicultural and multi-ethnic country with a population of 29, 624, 035 and 125 ethnic groups. Consanguineous marriage still remains the choice of an estimated 10.4% of the global population (Bittles AH. 2010). Any population based data on the prevalence of β-thalassemia and HbE disorders in Nepal is lacking. The disease requires lifelong treatment, therefore prevention of births of homozygotes constitutes a major armament in the management

of thalassemia. In Nepal, most of the educated people are not aware of thalassemia. Therefore more efforts are required from responsible persons to bring an awareness of Thalassemia among the people. This study was an attempt to address thalassemia in Nepal.

Materials and Methods

Study sites: Jhapa, Morang and Sunsari Districts of East Terai Nepal.

Study population: Koch Rajbanshi, Kochila Tharu, Santhal and Musahar.

Fig. 1. Map of Nepal showing study sites

The following three basic methods were adopted for Beta thalassemia screening.

Complete Blood Count. CBC or Complete blood count evaluated the overall health and detected anaemia. Twelve hundred samples comprising of 503 females and 697 males were screened for beta thalassemia. About 3-5 ml intravenous blood was collected in EDTA (ethylene diamine tetra acetic acid) vials and analysed with Sysmex, United States of America (USA) automated cell counter for complete blood counts. The blood samples were stored at 4^0c for further investigations.

Peripheral blood smear

Peripheral blood smear of each sample was prepared on spot and stained with Leishman's stain. The slides were observed microscopically for red cell morphology for supporting diagnosis of haemoglobinopathies.

Haemoglobin electrophoresis

HbA2 quantification by capillary electrophoresis (Sebia Minicap Flex piercing) of cases with MCV (mean cell volume) less than 27 picograms and target cells in peripheral blood smear were performed.

Results

A total of 1200 cases (697 males and 503 females) were included in the present study. The age group of the patients ranged from 5-80 years. On the basis of red cell indices, microcytic hypochromic anaemia was present in 145 (12.083%) cases. The pattern of Hb distribution observed is depicted in (Table -1)

Table 1: Haematological Parameters of the ethnic groups.

ETHNIC GROUP	Hb conc (gm/dl)	MCV (FL)	MCH (pg)	MCHC (g/dL)	RDW-SD%
KOCH RAJBANSHI	10.561 ±1.0180	70.474 ±11.6988	23.23 ±6.8636	31.667 ± 3.3452	36.696 ± 5.8120
KOCHILA THARU	11.975 ± 1.5104	70.146 ±4.7516	23.098 ±2.0470	32.756 ± 9.429	33.450 ± 1.6332
MUSAHAR	11690 ±8.690	69.517 ± 3.5216	23.172 ±1.6918	38.143 ± 9.705	34.913 ± 3.9533
SANTHAL	11.000 ±7.670	69.444 ± 4.8291	23.056 ±1.7648	33.222 ± 9.428	36.22 ± 3.5512

Out of the 145 cases with microcytic hypochromic anaemia; target cells were present in 107 (8.916%) cases. Presence of target cells in the peripheral blood smear supported the diagnosis of beta thalassemia, which was confirmed on haemoglobin quantification by capillary electrophoresis. The overall prevalence of beta thalassemia trait, HbE homozygous and HbE heterozygous was 49 (4.083%), 33 (2.83%), and 24 (2%) respectively (Table: 2).

Table 2: Distribution of Haemoglobinopathies in the Ethnic Groups (n= 300)

S N	ETHNIC GROUP	BTT	HbEE	HbEA	Total No
1	Koch Rajbanshi	0	33	24	300
2	Kochila Tharu	13	0	1	300
3	Musahar	17	1	0	300
4	Santhal	36	0	0	300

BTT- Beta-thalassemia heterozygote/ trait, HbEE- Haemoglobin E homozygote, HbEA- haemoglobin E heterozygote.

Based on the levels of HbA2, HbA, HbE and persistent HbF; Beta thalassemia trait, HbE homozygote and HbE heterozygote was identified. In beta-thalassemia trait, the Hemoglobin A2 (Hb A2) values range between 3.5 to 9% , low haemoglobin, reduced MCV, MCH and raised RBC count suggested beta thalassaemia trait (Table 3). For diagnosing beta–thalassaemia major the HbF values of equal to or more than 90% of the total Hb was considered. Increased HbE (>90%), persistent HbF, increased HbA2 (>3.2) and absence of HbA confirmed HbE homozygote/disease (Table 3/Fig: 2) and HbE heterozygote/trait was confirmed by decreased HbA (<71%), HbE 24% - 25%, normal HbA2 (Fig: 3).

Table 3: Haemoglobin Interpretation in the Ethnic Groups

ETHNIC GROUP	HAEMOGLOBINOPATHY	NUMBER	HbA (%)	HbA2 (%)	HbF (%)	HbE (%)
Koch Rajbanshi	HbE homozygote	33	Absent	>3.3	0.7- 5.5	>90.83
Koch Rajbanshi	HbE heterozygote	27	70-72	3.0 - 4.0	0.3- 0.8	23-25
Kochila Tharu	Beta-thalassemia	13	94.4	5- 6	Absent	Absent
Kochila Tharu	HbE heterozygote	1	70-72.6	3.0- 4.0	0.3- 0.8	23- 25
Musahar	Beta-thalassemia	17	94.4	5- 6	Absent	Absent
Musahar	HbE homozygote	1	2.4	5.5	2.3	89.8
Santhal	Beta-thalassemia	36	94.3	5.0	0.7	Absent

The abnormal cases comprised of Koch Rajbanshi 57 (19%)Kochila Tharu 14 (4.66%) Musahar 18 (6%) and Santhal 36 (12%). (Table: 2) In the Koch Rajbanshi ethnic group HbE homozygote 33(11%) and HbE heterozygote/trait 24 (8%), Kochila Tharu Beta thalassemia heterozygote/trait 13 (4.33%), Musahar Beta thalassemia heterozygote/trait 17 (5.66%) and HbE homozygote 1 (0.083%), and Santhal 18 (12%) was confirmed. (Table: 3)

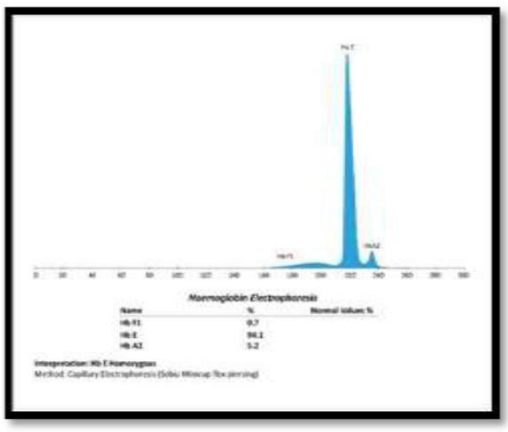

Fig. 2: HbE homozygous

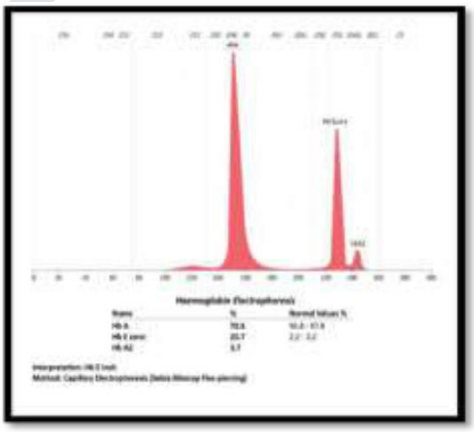

Fig. 3: HbE Heterozygous

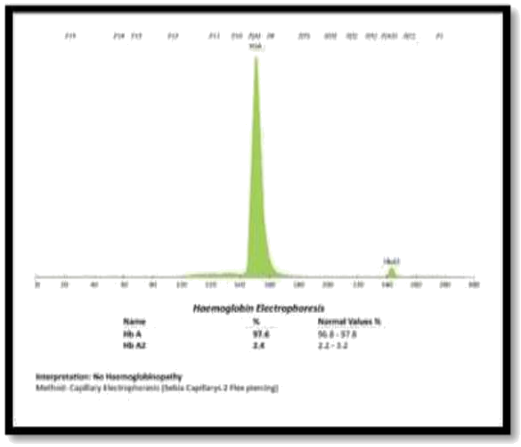

Fig. 4 No Haemoglobinopathy

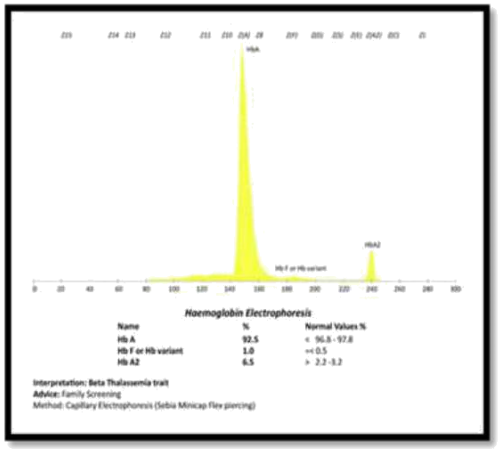

Fig. 5 Beta-thalassemia

DISCUSSION

Anaemia or low Hb concentration could be secondary to many factors including malnutrition, chronic blood loss or hereditary disorders such as hemoglobinopathies. Disorders of hemoglobin and thalassemia are autosomal recessive genetic disorders, mainly affecting the globin moiety of the Hb molecule (Balgir. 1996). Alpha and beta thalassemia are the most common gene linked hemoglobin diseases in the World (Modell. 2008. Weatherall. 2010). Thalassaemias and other hemoglobin variants were restricted to some particular geographical areas, caste, tribes and religion especially where marriages were confined to the same community and regions. However, now they are prevalent throughout the globe. The probable explanation to this is following increased migration of people from one place to other. Intercaste marriages are another reason for increase in the incidence and prevalence rates of haemoglobinopathies (Singh J. et al. 2016). The Nepali population is composed of various castes and tribal groups, each with different genetic traits. There are numerous Hb variants in the Nepali population many of which remain undetected due to lack of available infrastructure (Jha. 2015). Depending on the area of distribution, different hemoglobinopathies have been detected. β- heterozygous Thalassaemia/ trait is the commonest Hb abnormality in the Indian subcontinent (Sachdev et al. 2010).

Nepal is included in the world thalassemia belt, but there is no population based data in the country. In this present study, we attempted to observe the current situation of the occurrence and socio-demographic profile of thalassemia in some of the ethnic groups of eastern Nepal. The prevalence of haemoglobinopathies in four ethnic groups of eastern Nepal was determined. Beta-thalassemia is prevalent in the malarious regions of the world, including Nepal (Fuchareon and Winichagoon, 1997). With the control of communicable diseases and malnutrition, haemoglobinopathies have emerged as major global health issues (Weatherall & Clegg, 2001).Microcytic anaemia and haemoglobinopathies are serious health problems faced by the ethnic groups of Nepal.

Community based studies are representative of the actual situation prevailing in the area, as the test and interviews are carried out in their natural settings. In the present study a total of 1200 cases, 697 males and 503 females were included. On the basis of red cell indices, microcytic hypochromic anaemia was present in 145 (12.083%) cases, making this a significant finding. The cause of anaemia could be nutritional deficiency or underlying hemoglobinopathy. In the study by Mehdi et al. mean cell volume (MCV) and mean cell haemoglobin (MCH) were significantly low ($p < 0.001$) in cases of thalassemia presenting microcytic hypochromic picture of peripheral blood smear. Similar data was obtained from later studies, he therefore concluded that moderate degree of microcytosis (MCV < 78fl) and hypochromia (MCH < 27pg) was a feature of β-thalassemia. Likewise in our study (Table: 1) the values of haemoglobin concentration, MCV and MCH was significantly low. The lowest MCV (65.56 +/- 3.4) in β-thlassemia heterozygote/trait followed by HbE homozygote/disease and HbE heterozygote/trait was found. However the present study lacked the provision to rule out nutritional anemias in all cases by serum iron, vitamin B12 and folate levels.

Out of theses 145 cases, target cells in the peripheral blood smear was present in 107 (8.916%) cases. Presence of these target cells, supported the diagnosis of beta thalassemia. HbA2 quantification by Capillary Electrophoresis is considered the gold standard for identification of beta thalassemia (Palaeri and Musca. 2018). In the present study based on the levels of HbA2, HbA, HbE and persistent HbF; Beta thalassemia trait, HbE homozygote and HbE heterozygote was identified. In beta-thalassemia trait, the Hemoglobin A2 (Hb A2) values range between 3.5 to 9%. Low Hemoglobin, reduced MCV, MCH and raised RBC count suggest beta thalassaemia trait. For diagnosing beta–thalassaemia major the HbF values of equal to or more than 90% of the total Hb was considered.

In the surveyed sample 107 (8.91%) cases showed hemoglobinopathy. Among these, 65 were females and 42 were males. The most common hemoglobin disorder was beta thalassemia trait (4.08%) which is in accordance with study of Chandrashekhar V, Rao S and Hosseini S., however much less in percentage as compared to these studies. The present study identified no beta thalassemia major cases, whereas Rao S, Chandrashekhar V and Hosseini S reported a high incidence of beta thalassaemia major. 2.83% cases of HbE was found in the present study which is in low when compared to Rao S et al., Chandrashekhar V and Hosseini (Rao. 2012, Chandrashekhar 2011, and Hosseini 2014). The probable reason for this difference in the rates as well as the vast number of hemoglobin variants could be attributed to the fact that their study was conducted in a referral haematology centre receiving not only patients from all over India but also from abroad, ours on the other hand was a field study restricted to four ethnic groups in which beta thalassemia is known to have high prevalance. Therefore this study is only limited to the detection of beta thalassemia; Alpha thalassemia and sickle cell disease were not investigated for in the present study. A comparative analysis with the aforementioned studies is shown in Table: 4.

Table 4: Comparison with previous studies.

Haemoglobinopathy	Rao S et al n=800	Chandrashekar etal n=543	Hosseni et al n= 1932	Present study N= 1200
Beta thaalassemia Major	2.9%	2.3%	12.87%	----------
Beta thalassemia minor/heterozygote	18.1%	37.9%	27.66%	5.5%
Haemoglobin E homozygote	2.55	18.9%	4.6%	2.833%
Haemoglobin E heterozygote	---------- --	----------	=----------	2.083%

Among the four ethnic groups studied, haemoglobinopathy was most prevalent in the Koch Rajbanshi at 4.75% and the least in the Santhals (1.58%). Although prevalence of haemoglobinopathies was maximum in the Koch Rajbanshis, there were no beta thalassemia cases detected, instead, a significant association between Rajbanshis and HbE hemoglobinopathies was found (P< 0) prevalence of HbE trait was found to be highest amongst all haemoglobinopathies in them. Increased HbE in this area is similar to Assam and Tripura (Piplani. 2000). Ghosh et al. (2018) also reported a significant association between Rajbanshi with HbE haemoglobinopathy in his HPLC based study of haemoglobinopathies among antenatal women in Darjeeling district. This area is in geographic proximity to East Nepal suggesting a possibility of migration.

Previous study of Tharu patients of west Nepal, presenting for electrophoresis at a tertiary health care centre, reported high prevalence of haemoglobinopathies (26.8%) followed by Sickle cell disease (21.6%) (Jha. 2015). Likewise in the present study done in the Kochila Tharu of east Nepal beta thalassemia trait occurred in (4.66%) and a single cases of HbE heterozygous was identified. Amongst the Musahars prevalence of Beta thalassemia trait was 5.66%, HbeE homozygote only one was detected. Whilst in the Santhals Beta thalassemia trait was 12%. Prior to our study, there have been no reports of haemoglobinopathy in the Musahar Dalit and Santhal ethnic groups, making this the first study done

exclusively in this community, although it only pertains to population in East Nepal. HbE-beta thalassemia and Beta thalassemia major was not identified in our study. Illiteracy, ignorance and false beliefs regarding screening test attributed to non-responders in the study. Nevertheless it calls for a special emphasis to avoid their contribution to fatal disease. An additional factor contributing to non-responders could be because our research was community based field study and not hospital based among referred patients.

This indicates that hemoglobinopathies are a common problem in the ethnic groups in east Nepal today. The variation in the pattern of hemoglobinopathies can be attributed to the geographical area as well as increasing awareness among the patients and their relatives. All in all it is important to formulate a strategy to prevent hemoglobinopathies. In Nepal, screenings before marriages are still considered a taboo. The best approach would therefore be to target those patients attending the medical or Haematology OPD, the antenatal population and extended family members of known thalassemics/other hemoglobinopathies. Persons with a carrier state should be counselled regarding the disease nature (implications of being carrier which help in preventing birth of child with homozygous inheritance of hemoglobinopathies). The couple at risk should be counselled regarding the nature of the disease and the implications of being carriers. Options concerning birth control, including prenatal diagnosis and medical termination of pregnancy of the affected children should be informed. The couple should be informed of 25% recurrence risk and also advised to limit their family size. Analysis of global data reveals that effective screening methods in countries with high prevalence rates of thalassaemia significantly reduced the prevalence rates of hemoglobinopathies. Conducting a screening programme would be much cheaper to the exchequer rather than providing treatment to the affected.

CONCLUSION

The study showed that haemoglobinopathies are not uncommon in eastern Terai of Nepal. The data summarized confirmed that screening and genetic counselling for haemoglobin disorders must be an intrinsic part of health care in Nepal, as recommended by WHO.

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