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Original Article

Screening for Thalassemia in Healthy Population at a Tertiary Care Hospital in Eastern Nepal

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Abstract

Background

Thalassemia is a type of congenital anemia, where there is deficient synthesis of one or more type of globin subunits of normal hemoglobin. This study was undertaken with aims & objective to study the prevalence of thalassemia by comparing red blood cell indices, peripheral blood smear and electrophoresis in adult volunteers.

Material and Methods

The study comprised of 518 cases attending hematological department, who were enrolled in our study after proper informed consent, of which 462 cases were further studied. All cases were subjected to blood sampling for estimation of Hemoglobin, red cell indices and peripheral blood smear. Those samples where peripheral blood smear and red cell indices were suggestive of thalassemia were subjected to Bio-Rad high performance liquid chromatography based electrophoresis to observe the presence of any abnormal hemoglobin.

Results

The mean age of screening sample was 42.91 ± 16.85 years with minimum age of 18 years and maximum age of 85 years. The highest number of cases was in between 21-30 years age groups (19.5%) followed by 41-50 years (17.7%). In the study group, 299 (64.7%) cases were male and 163 (35.3%) cases were female. The prevalence of anemia was found to be 48.16% in males and 68.71% in females with overall prevalence of 55.41%. On electrophoresis reports, 19 cases were diagnosed with thalassemia. The only thalassemia observed was thalassemia minor. The prevalence of thalassemia was found to be 4.11%.

Conclusion

Significantly high prevalence of thalassemia minor is found in healthy population.

Keywords: *Electrophoresis, Hemoglobinopathy, Thalassemia*

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Introduction

The hemoglobinopathy in general and thalassemia in particular is autosomal recessive, quantitative and qualitative disorder of red blood cells [1]. The prevalence is higher in certain populations and ethnic groups. The frequency of occurrence is higher in the tropic and subtropics, in belt extending from the Mediterranean basin through the Indian subcontinent, and Southeast Asiastretching through Southern China down the Malaysian peninsula to the Indonesian islands. But now it is a worldwide phenomenon due to easy migration. According to WHO report, prevalence of thalassemia and hemoglobino-pathies carriers worldwide is 5.2% [2].

The support and treatment of thalassemia like bone marrow transplantation and repeat blood transfusion is costly. Therefore, screening of population mostly prenatal and genetic counseling is a cost-effective preventive strategy [3]. Many countries have screening programs, the aim of which is to identify carriers of thalassemia to assess and prevent the risk of having a severely affected child. In Nepal, such screening programs do not exist. However, few studies have been conducted to evaluate the burden of thalassemia and other hemoglobinopathy in Nepal [4,5].

As per the recommendation of The International Committee for Standardization in Haematology, tests like Complete Blood Count (CBC), haemoglobin electrophoresis, with quantification of HbA_2 and HBF has been recommended for Thalassemia [6, 7]. The most common approach to screen would be CBC, particularly the red cell indices. A normal MCV and normal MCH can rule out most cases of thalassemia and may not need any further evaluation [8]. A low MCV and MCH would need further evaluation with HbA_2 and HbF Quantification. The level of MCH< 27 pg and MCH < 77 fl appears to be the most acceptable cutoff value [9]. RBC count > 5 million/cmm and RDW < 15.5% in complete blood count along with HbA_2 > 3.5% is strongly in favor of beta thalassemia trait. The present study was conducted to screen apparently healthy individuals, presenting in our hospital to evaluate the prevalence of thalassemia.

Material and Methods

This prospective study was conducted on adult volunteers, attending the hematological unit of department of Pathology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal for period of 15 months from February 2017 to May 2019. Written consent was obtained, and blood sample was collected by department of Pathology of Nobel medical college. Ethical clearance was obtained from Institutional ethics committee of Nobel Medical College and teaching hospital, Biratnagar, Nepal. The healthy individuals of either age or sex, who visited for premarital checkup voluntarily, patients with abnormal hemograms and Patients with positive family history of thalassemia, were included in the study. Patients, who had received blood transfusion within the last one month, were excluded from the study.

The sample size was calculated using formula, n= $[z^2xpxq/d^2]$, where *z* is confidence level at 95% (standard value of 1.96), d is margin of error which is taken as 5% and p is the expected prevalence of 4% [10]. The sample size was calculated as 59 but we enrolled all the cases. So, a total of 518 cases attending hematological department of pathology were enrolled in our study. Mass screening was done amongst apparently healthy adults including attendants of patientsand medical staff after proper informed written consent and their age, sex, religion and family history were recorded for demographic comparison. Out of 518 samples taken, 56 cases were discarded due to various reasons like lack of follow up, attrition and unsatisfactory sample collection and only 462cases were further studied. Written consent was obtained from all patients. All cases were subjected to blood sampling for estimation of Hemoglobin (Hb), Total red cell count (TRBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).Disposable syringes were used to collect 3ml of venous blood in EDTA containing tube using proper aseptic measures. Tubes were properly labeled following collection of blood and Peripheral Blood Smear (PBS) was performed to observe anisocytosis, poikilocytosis, microcyto-sis, hypochromia, target cells, basophilic stippling, anisocytosis and nucleated RBC's. Samples showing following characteristics were subjected for electrophoresis: 1) Normal or decreased hemoglobin 2) Deceased PCV 3) Decreased MCV 4) Decreased MCH 5) Decreased MCHC 6) Increased or normal RBC count7) Microcytosis 8) Hypochromia 9) Anisocytosis 10) Poikilocytosis 11) Target cell 12) Basophilic stippling 13) Nucleated RBC's.

RBC indices were analyzed by Sysmex 5-part differential hematology analyzer and electrophoreses was performed using Bio rad D10 HPLC analyzer using variant hemoglobin testing system.HbA2 level of >3.5% is used as a cutoff for diagnosis of beta- thalassemia carriers. Anemia

was defined as hemoglobin level less than 13 gm/dl in male and less than 12 gm/dl in female. Screening of parents was advised in all cases, but it could not be done in some cases as one or both parents could not be made available. The results of the study were statistically analyzed using SPSS version 25, using independent sample t test. Results on continuous measurements are presented as mean ± standard deviation (min-max) and result on categorical measurements are presented in percentage and frequency. A p-value of <0.05 was considered statistically significant.

Results

A total of 462 cases were studied after exclusion of 56 cases. The mean age of screening sample was 42.91 ± 16.85 years with minimum age of 18 years and maximum age of 85 years. The highest number of cases were in between 21-30 years age groups (19.5%) and lowest <20 years (11.7%) as shown on Table 1. In the study group, 299 (64.7%) cases were male and 163(35.3%) cases were female with male to female ratio of 1.83:1 as shown in Table 1.

Table 1: Age and sex distribution in study group

Age groups	Sex		Total
	Male	Female	
<20 years	38 (8.2%)	16 (3.5%)	54 (11.7%)
21-30 years	49 (10.6%)	41 (8.9%)	90 (19.5%)
31-40 years	48 (10.4%)	33 (7.1%)	81 (17.5%)
41-50 years	52 (11.3%)	30 (6.5%)	82 (17.7%)
51-60 years	62 (13.4%)	19 (4.1%)	81 (17.5%)
>60 years	50 (10.8%)	24 (5.2%)	74 (16.0%)
Total	**299 (64.7%)**	**163 (35.3%)**	**462 (100%)**

On further hematological study of cases, the prevalence of anemia was found to be 48.16% in males and 68.71% in females with overall prevalence of 55.41% in study group as shown in Table 2.

Table 2: Prevalence of Anemia in study group

Age groups	Sex		Total
	Male	Female	
<20 years	16 (6.3%)	9 (3.5%)	25 (9.8%)
21-30 years	15 (5.9%)	27 (10.5%)	42 (16.4%)
31-40 years	24 (9.4%)	25 (9.8%)	49 (19.1%)
41-50 years	20 (7.8%)	20 (7.8%)	40 (15.6%)
51-60 years	38 (14.8%)	14 (5.5%)	52 (20.3%)
>60 years	31 (12.1%)	17 (6.6%)	48 (18.8%)
Total cases with anemia	**144 (56.3%)**	**112 (43.7%)**	**256 (100%)**
Total cases	299	163	462
Prevalence	**48.16%**	**68.71%**	**55.41%**

Out of total 462 persons in study group, 101 persons were diagnosed with microcytic anemia. Out of those 101 persons, 58 persons had suggestive features of thalassemia, based on RBC count, various red cell indices and peripheral blood picture (microcytic hypochromic, anisocytosis, target cells, tear drop cells). Those 58 samples were subjected to electrophoresis to observe the presence of any abnormal hemoglobins.

On further analysis of electrophoresis reports, 19 cases were diagnosed with thalassemia. The only thalassemia observed was Beta-thalassemia minor. No case of Alpha-Thalassemia, Beta-thalassemia major or intermediate type was found as shown in Table 3.

Table 3: Distribution of Thalassemia patients diagnosed by electrophoresis

Types of Thalassemia	No. of patients	Percentage
Alpha-Thalassemia	0	0%
Beta-Thalassemia Major	0	0%
Beta-Thalassemia Minor	19	32.76%
Beta-Thalassemia Intermediate	0	0%

In present study, the prevalence of thalassemia was found to be 4.11%as shown in Table 4.

Table 4: Prevalence of Thalassemia in normal healthy population

Total number of samples	Number of Thalassemia patients	Prevalence (%)
462	19	4.11%

The level of mean corpuscular volume and mean corpuscular hemoglobin was found to be lower in patients with thalassemia as compared to other study groups, which is statistically significant (p<0.001 and p<0.001 respectively). Similarly, the mean RBC countis higher in patients with thalassemia as compared to other study groups, which is statistically significant (p<0.001). However, the mean hemoglobin, packed cell volume and mean corpuscular hemoglobin concentration was not significantly different in study groups (p=0.859, p=0.396 and p=0.463 respectively) as shown in Table 5.

Table 5: Comparison of various RBC indices of patients in study group using independent sample t-test

RBC indices	Patients with Thalassemia Mean ± S.D.	Patients without Thalassemia Mean ± S.D.	P value
Hb (g/dl)	11.56 ± 1.24	11.65 ± 2.34	0.859
RBC count (x10^6/ml)	4.85 ± 1.16	3.87± 0.69	**<0.001***
PCV (%)	33.05±3.69	34.29 ± 6.33	0.396
MCV (fl)	74.64± 7.37	87.25 ± 9.79	**<0.001***
MCH (pg)	24.83 ± 3.28	29.72 ± 4.69	**<0.001***
MCHC (g/dl)	32.82 ± 1.80	33.15 ± 1.92	0.463

*Statistically significant

On further analysis of electrophoresis reports, the mean HbA, $HbA_2$and HbF of patients with thalassemia was found to be 90.58 ± 1.43 %, 5.81 ± 0.65 % and 3.61 ± 1.42 % respectively, which showed statistically significant difference as compared to other study groups as shown in Table 6.

Table 6: Comparison of different types of hemoglobin in patients subjected to electrophoresis

Type of hemoglobin	Patients with Thalassemia Mean ± S.D.	Patients without Thalassemia Mean ± S.D.	P value
HbA (%)	90.58 ± 1.43	97.41 ± 1.43	**<0.001***
HbA_2 (%)	5.81±0.65	1.60±0.75	**<0.001***
HbF (%)	3.61±1.42	1.05 ± 1.33	**<0.001***

*Statistically significant

Discussion

Thalassemia is one of the commonest genetic disorders that get inherited to next generation owing to improper awareness amongst carrier parents. Awareness of carrier parents, screening, premarital counseling and prenatal diagnosis can lead to prevention of birth of a thalassemia major child. Premarital screening is a successful approach for thalassemia prevention but is difficult in Nepal owing to social and cultural issues and improper availability of screening and diagnostic services to people living in remote locations devoid of facilities for screening. Many studies have reported success of antenatal screening followed by prenatal diagnosis [11, 12]. In the study group, 299 (64.7%) cases were male and 163 (35.3%) cases were female with male to female ratio of 1.83:1, comparable to study conducted by Balgir et al. which showed 62.1% of male patients and 37.9% of female patients [13]. Similar data was noted in study conducted by Yagnik and Mannan which showed 56% and 47% of male respectively [14, 15].

The prevalence of thalassemia was found to be 4.11% in present study which includes all cases of beta thalassemia minor. The results were comparable with study conducted in eastern region of India which showed prevalence of thalassemia 4.6% [10]. However, the prevalence was lower in study conducted by Xu et. al. in Southern China and Lau et al. in Hongkong, which showed prevalence of 2.54% and 3.4% respectively [16.17]. According to Shalev and Yehzkel et. al., proper CBC and peripheral smear study can identify large number of thalassemia carrier, thus serving as effective screening test [18]. The level of mean corpuscular volume and mean corpuscular hemoglobin was found to be lower in patients with thalassemia as compared to other study groups, which is statistically significant ($p<0.001$ and $p<0.001$ respectively), which is in accordance to the study conducted by Mehdi SR et. al. [19].

Out of 256 anemic persons in study, 101 (39.45%) cases were diagnosed with microcytic anemia and 23 (8.98%) of cases were diagnosed with macrocytic anemia which is lower as compared to study conducted by Mishra et al. which showed 47% of microcytic anemia and 31% of macrocytic anemia [20]. The mean HbA, $HbA_2$and HbF of patients with thalassemia was found to be 90.58 ± 1.43 %, 5.81 ± 0.65 % and 3.61 ± 1.42% respectively, which showed statistically significant difference as compared to other study groups. Similar to present study, the mean level of HbA_2 in beta thalassemia minor was reported to be 5.1% (range 4.7 to 5.5) in study conducted by Jha R [21]. According to study carried out in Mediterranean countries and UK, the cost on prevention of thalassemia major child is equivalent to treatment cost for one year[22]. So, screening of thalassemia and genetic counselling should be effectively carried out in developing country like Nepal.

Conclusion

The prevalence of thalassemia is reported to be 4.11%, which includes all cases of beta- thalassemia minor in our study. Hence Screening of Thalassemia, should be carried out in healthy individual and premarital women so as to prevent the risk of having a severely affected thalassemic child

Conflicts of interests: None

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