A prospective study of hidden cases of beta thalassemia trait in voluntary blood donors at tertiary care hospital of south Gujarat

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Abstract

Background: Hemoglobinopathies represent a significant national health burden in India. The two most common causes of microcytic (MCV less than 80 fl) anemia are iron deficiency anemia and beta-thalassemia trait. In most of these cases a trial of iron treatment done but in populations where Thalassemia is common, like in India, this approach leads to unnecessary iron therapy / iron overload. Therefore the study has been done to detect hidden cases of beta thalassemia in voluntary blood donors with microcytosis. Materials and Methods: Total 393 donor samples were analyzed for complete blood count, ESR and serum ferritin levels. Mentzer index was calculated by MCV/RBC count formula. HPLC test was done on 12samples with Mentzer index <13 for quantification of HbA2. Results: Out of 393 blood donors, 384 were males and 9 were females. Low MCV detected in 58 samples [14.75%]. Out of 12 samples tested by HPLC, 6 cases were Beta thalassemia trait [1.53%], 5 were normal and one was? sickle cell trait/ ? Hb D trait. Out of 6 BTT cases, 3 cases show normal ferritin value, 2 with low ferritin level and one with high ferritin level. Conclusions: The study revealed a high prevalence of IDA and BTT in blood donors and a higher probability of finding these in the microcytic samples. Screening for thalassemia trait by routine blood counts in all the blood donor samples and if required by HPLC, should be included in the standard blood testing policy after blood donation.

Keywords: Beta-Thalassemia trait, screening, Ferritin, HPLC, Iron Deficiency Anemia

Introduction

Hemoglobinopathies represent a significant national health burden in India. Among all the hemoglobinopathies beta-thalassemia are major health problems in India but have received little attention because of other health priorities, such as malnutrition and communicable diseases.

The beta-thalassemia trait (BTT) or beta-thalassemia minor is a heterozygous condition in which only a single beta-globin gene is affected. The beta thalassemia carrier state in India ranges from 1% to 17% with an average rate of 3.2% [1].

Various studies are being done for evaluating the prevalence of thalassemia trait in different study groups [1,2]. Patients homozygous for beta-thalassemia usually present with the symptoms of the disease whereas carriers for beta thalassemia are usually found during examination of the relatives of more severely affected patients as part of screening programs or during the investigation of mild iron refractory hypochromic anaemia. Detection of hidden cases of beta-thalassemia (-thal) trait in the healthy population is important to reduce the incidence of thalassemia major birth rate substantially [1]. Voluntary blood donors represent an important population group who are easily accessible and can be screened for this purpose. However, the usefulness of this population in beta-thalassemia trait detection programs has not been much studied in India.

Beta--thalassemia trait (BTT) is the heterozygous form. Most patients are asymptomatic, and some patients have only mild anemia. BTT often shows microcytosis, a normal or an increased red blood cell (RBC) count, and an elevated level of HbA2, which provide the basis for laboratory screening. The two most common causes of microcytic (MCV less than 80 fl) anemia are iron deficiency anemia and beta-thalassemia trait [3]. A traditional approach followed by most general practitioners and blood bank physicians is a trial of iron treatment whenever anaemia and / or microcytosis are
encountered [2]. However, populations where Thalassemia is common, like in India, this approach leads to unnecessary iron therapy / iron overload [4]. Therefore it is important to diagnose these two most common causes of microcytosis in blood donors. Hence in the present study on 393 voluntary blood donors, various test for the measurement of Iron status were performed on all microcytic samples along with HPLC test to determine HbA2 level in few cases of microcytic samples after applying Mentzer index to detect the prevalence of beta thalassemia trait in voluntary blood donors.

Material and Method

Study setting, duration and type: A prospective study of 393 samples obtained from apparently healthy voluntary blood donors presenting to the blood bank at the tertiary care hospital of south Gujarat (either at the blood bank or at the outdoor blood donation camps) during the period from June 2012 to February 2014, were included in this study. The donors were selected randomly.

Inclusion criteria: Voluntary blood donors, regular as well as first time donors of any sex who are fitted according to the donor selection criteria laid down in the Technical Manual of the Directorate General of Health Services, Ministry of Health, Government of India for blood donation [5].

Exclusion criteria: Any person with any disease or on drug therapy which is sufficient for donor deferral. Along with that any voluntary donors who found to have increased ESR level as a marker for any hidden chronic infection which may affect the results of iron study parameter are not included in the study population.

Data collection procedure: The sample size was determined based on the Krejcie and Morgan criteria, for determining the sample size at 95 % confidence levels with a margin of error of 5% [6]. In last few years, every year around seven thousand five hundred blood donors between 18 and 60 years have been volunteering at the blood bank for blood donation in our Institute. Krejcie and Morgan in their criteria of sample selection have suggested a minimum sample of 365 against the 7500 population at 95% confidence level (p <0.05).The present study includes total 393 samples.

Ethical permission: The present study was duly approved by Human Research ethics committee of Government, Medical College, Surat.

Analysis: After blood donation of whole blood, an additional sample was taken via the bleed line into a 2.5 mL EDTA tube for assays of complete blood count and ESR estimation and into 4.0 mL plain tubes for measurement of serum ferritin. Serum was separated from plain samples collected in the pilot tubes from each donor and stored at -40 °C. Complete blood count was performed on three part hematology analyzer and ESR was performed by modified Westergren’s method.

That will rule out the presence of any chronic inflammatory disorder. Serum ferritin was measured by standard enzyme immunoassay technique. In all donor samples the Mentzer index is applied on count values. The Mentzer index is defined as mean corpuscular volume per red cell count. An index of less than 13 suggests that the patient has the thalassemia trait, and an index of ≥ 13 suggests that the patient has iron deficiency. In all samples with the Mentzer index value < 13, a further diagnostic test HPLC is performed to differentiate between iron deficiency anemia and beta thalassemia trait as a diagnosis. HbA2 quantitation was done by HPLC on an automated system (Bio Rad Variant II, Bio-Rad Laboratories, CA).

Diagnosis of IDA was made based on serum ferritin values lower than 15 ng/ ml. Diagnosis of BTT was made based on HbA2 levels more than 3.5%.

Results

A total of 393 blood donors were screened, out of which 384 were males and 9 were female donors. The donors were between 18 and 58 years of age. Details are as per table 1.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 – 20</td>
<td>31</td>
<td>01</td>
<td>32[8.14%]</td>
</tr>
<tr>
<td>21 – 30</td>
<td>169</td>
<td>03</td>
<td>172[43.77%]</td>
</tr>
<tr>
<td>31-40</td>
<td>109</td>
<td>02</td>
<td>111[28.24%]</td>
</tr>
<tr>
<td>41-50</td>
<td>66</td>
<td>03</td>
<td>69[17.56%]</td>
</tr>
<tr>
<td>51-60</td>
<td>09</td>
<td>00</td>
<td>9[2.29%]</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>09</td>
<td>393</td>
</tr>
</tbody>
</table>
Out of total 393 samples, low MCV (<80 fl) were detected in 58 samples (14.75%). In the present study total 12 samples with Mentzer index favoring for thalassemia were selected and screened by the High Performance Liquid Chromatography for quantization to know the cause of low MCV. Out of which 6 cases were turn out to be Beta thalassemia trait (1.53% of total number of donors) and 5 cases were normal and one case was in diagnostic dilemma situation with? sickle cell anemia trait/ Hb D trait which requires further family screening and repeat testing for confirmation.

In six diagnosed beta thalassemia trait cases 3 cases show normal ferritin value, 2 cases show low ferritin level that is associated with iron deficiency and one case of high ferritin level.

**Discussion**

The present study includes 393 donors, out of which majority were male donors and only 9 (2.2%) female donors. Low number of female donors could be because of local social factors and physical health like anemia barring them from blood donation. Majority of blood donors under study were in 21-30 years age group.

Red blood cells are described as being microcytic when the mean corpuscular volume is less than 80 fl [7]. IDA and beta-thalassemia minor are recognized as the most important causes of microcytosis. Microcytosis is important indicator of anemia, which can be detected during a routine complete blood count testing on cell counter. Anemia, to date, remains the most important cause of deferral in blood donors, and therefore, investigating donors by complete blood count can help in identifying the cause of anemia and also guides to the right treatment and counseling to the anemic donor. This is important in maintaining the steady pool of dedicated voluntary blood donors.

MCV measurement by cell counter is direct, rapid, inexpensive, and automated. To avoid much more expensive, time-consuming, and complicated procedures for discrimination between these disorders, researchers attempt to use either RBC indices such as MCV, MCH, and RDW, or formulas derived from these indices. This process helps to select appropriate individuals for more detailed examination.

Out of total 393 samples, microcytosis were detected in 58 samples (14.75%). Incidence of microcytosis was 10.5% in healthy Egyptian adult blood donors in study by Soliman A.R. et al, 5.4% in the study by Aseem K. Tiwari. 8.3%, in the high school students of Hong Kong in a study by Yu-Lung Lau et al who also followed the same criteria for MCV (< 80 fl) [8, 9, 10]. This difference could be because of the different sets of population and different mean age of subjects in this study.

Plasma ferritin is considered to be the single, most powerful test for diagnosis of IDA [11]. Even though plasma ferritin is an acute phase reactant that can be elevated in various inflammatory conditions, as this study group comprised of healthy donor population, the probability of inflammation was negligible and moreover all samples were previously screened by ESR to rule out the remote possibility of underlying chronic diseases. For diagnosis of IDA, plasma ferritin threshold of 15 ng/ml was used in this study, as suggested by Susan F Clark (2008) [12].

Out of total 58 donors with low MCV that is < 80 fl, only the donors with high RBC count and Mentzer index favoring for thalassemia were selected and were also screened by the High Performance Liquid Chromatography for quantization of because of the simplicity of sample preparation, superior resolution, and accuracy, combined with complete automation of the method [13]. Diagnosis of BTT was based on levels of HbA2 greater than 3.5% [14]. Reduction of HbA2 because of coincident iron deficiency did not preclude detection of BTT [15].

In the present study total 12 samples were tested by HPLC to know the cause of low MCV, out of which 6 cases were turn out to be Beta thalassemia trait (1.53%) which was comparable with another study by V K Meena (1.58%) and by Lisot CL (1.81%) and 5 cases were normal and one case was in diagnostic dilemma situation with? sickle cell trait/ Hb D trait which requires further family screening and repeat testing for confirmation [16, 17].

In six diagnosed beta thalassemia trait cases 3 cases show normal ferritin value, 2 cases show low ferritin level that is associated with iron deficiency and one case of high ferritin level. Both low ferritin level cases were frequent donors with one case donated 20 times and the other donated 3 times.

The case of other haemoglobinopathy that is Hb D/ sickle cell trait was also with low ferritin level. Out of five cases of normal HPLC report, 4 cases show markedly low level of ferritin and one case show ferritin normal level just near to lower limit of normal range suggestive of iron deficiency as a cause of low MCV.
The prevalence of BTT in blood donors in India is being reported for the first time by Aseem K Tiwari and based on their findings and the findings of current study have suggested an algorithm which recommends conducting a hemogram on all donor samples, routinely [9]. Plasma ferritin could be done only in microcytic samples.

Those with ferritin levels less than 15 ng/ml are diagnosed as IDA. HPLC is performed only for non-IDA samples, with ferritin levels higher than 15 ng/ml as BTT is more likely in samples with higher ferritin levels [9].

The same recommendation has been put forward by Loria and Hershko [18,19]. But in the present study, out of six cases of beta thalassemia trait two cases (33.33%) showed low ferritin value that is <12 ng/ml almost zero storage iron level. So this study suggests some practical correction in the algorithm suggested by Aseem Tiwari and that should be implemented after thorough study with large sample size data [9].

Limitation of study: This study has included only blood donors (need to be adult as per law), age distribution may not be true representation of prevalence of thalassemia in the population. But surely this study concludes the importance of hemoglobinopathy among the so called healthy blood donors.

HPLC could have been done on more number of samples but due to cost constraint it has limited to the few samples with high suspicion on the basis of application of Mentzer index on data.

Conclusion

Present study illustrates two important aspects; one, the prevalence of IDA and BTT was high among blood donors and second, the probability of both IDA and BTT in microcytic samples was significantly high.

What the study adds to the existing knowledge?

Even regular complete blood count testing by the automated cell counter will help to diagnose many hidden diseases in blood donors like iron deficiency anemia and thalassemia. So the study suggests establishing it as a routine protocol for the blood sample analysis in the blood donor samples.

Adequate utilization of these parameters can facilitate identification of the majority of BTT cases at no additional cost to the healthcare system.

Author’s contribution:

Concept and study design both done by Dr. Hemali J. Tailor and Dr. Prashant R. Patel. Data collection, data interpretation and manuscript writing was done by both Dr. Hemali J. Tailor. Review of articles, references and proof reading was done by Dr. Prashant R. Patel.

Findings: Nil; Conflict of Interest: None initiated

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Reference


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