Anemia Induced by Hemoglobinopathies: A Nested Case Control study from Gujarat, India

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Abstract: Hemoglobinopathy is a condition in which an individual carries an abnormal variant of hemoglobin gene and common genetic abnormalities in India. In heterozygous conditions particularly thalassemia trait is reported to be associated adverse phenotypes such as anemia, kidney problem, etc. The aim of the present study is to understand the status of anemia with special reference to β-thalassemia carriers. A total of 2969 individuals of both sexes in the age group of 13-30 years were recruited and screened for hemoglobinopathies, where 199 cases and same recruited cohort 199 age and sex matched controls. The hematological parameter levels were estimated by CBC and HPLC to confirm and distinguish between different hemoglobinopathies and thalassemia. The frequency of anemia was higher (74.4%) among cases as compared to that of the controls (31.2%). Microcytic anemia is significantly higher among cases (87.8%) whereas normocytic anemia is significantly higher among controls (66.1%). β-thalassemia carriers are found to be affected by anemia specifically microcytic anemia. The indices, RBC count and hematological parameters provided high consistencies for differentiating β-thalassemia carriers and anemia.

Key Words: Hemoglobinopathies, Thalassemia carriers, Microcytic anemia, Mentzer Index, Srivastava Index

Introduction:
Thalassemia is one of the most common hereditary hemoglobinopathy. Its burden in population in general is in the form of thalassemia diseases i.e. homozygous condition of the thalassemia alleles. Till recently it was believed that thalassemia carriers are not at risk for any sort of morbidity or mortality.[1] However, recent literature hints towards the susceptibility of thalassemia carriers to various diseases through their anemic status. Apart from thalassemia, anemia can also be caused by nutritional deficiencies, though the type of anemia caused by nutritional deficiency may be different from that caused by thalassemia carriers status.[2] The World Health Organization[3] describes iron-deficiency anemia as the most common and widespread nutritional deficiency in the world. Nutritional deficiency specifically in terms of iron and Vitamin B₁₂ are the major causes for anemia. The most common cause of anemia worldwide is iron deficiency which is caused by loss of blood and inadequate intake of iron. World Health Organization defines three types of morphological classification of anemia as: normocytic anemia occurring due to chronic diseases (ACD), hemolytic anemia due to acute hemorrhage; and microcytic anemia due to iron deficiency anemia (IDA), thalassemia trait or a combination of both. Macrocytic anemia occurs when a person has both lack of Vit B₁₂ and folate deficiency.[4] The differentiation between IDA and β-thalassemia trait is important because of two main reasons. First, hemoglobin level will not improve in β-thalassemia trait if it is misdiagnosed as IDA and unnecessary iron being prescribed by the attending physician. The second essential reason is that misdiagnosed β-thalassemia as IDA may get married to a β-thalassemia trait, resulting in homozygous or thalassemia major condition that can affect the children.[5] Thus the present study attempts to understand the difference between individuals with abnormal hemoglobins, thalassemia carriers and normal hemoglobin with respect to anemia status. This study also focuses on the extent of contribution of nutritional deficiencies, thalassemia carrier status and abnormal hemoglobin status to anemia. Finally, the study also significantly validates Mentzer and Srivastava Indices among anemic individuals to identify thalassemia carriers.

Materials and Methods
In the present case-control study, 2969 individuals of both sexes in the age group of 13-30 years residing in Jamnagar district, Gujarat, were recruited by organizing multiple awareness base camp and screening programs from March to September 2017 to collect the samples for identify frequencies of hemoglobinopathies. Social and demographic data were also collected from all the participating individuals through pre-tested and modified interview schedule. Intravenous blood
(5ml) was collected in EDTA coated vacutainer using disposable syringes by a trained laboratory technician after obtaining prior informed written consent from the recruited individuals. Complete blood count (CBC) was performed as a preliminary test, and high performance liquid chromatography (HPLC) was done as confirmatory test for identifying hemoglobinopathies and thalassemia carrier detection. Of 199 carrier individuals, 76 were found to be having hemoglobinopathies which includes Hb-D Punjab, HbE, Hbs, HPH, Delta-Beta-thalassemia, Hb Ty Gard and Hb J-Meerut and the rest 123 were β-thalassemia carriers, all of which were considered as cases. From the same recruited cohort, 199 age and sex, matched healthy unaffected individuals were selected as controls with no abnormal Hb variants. A number of red blood cell (RBC) parameters and discriminant indices have been used as simple and inexpensive tools to determine whether a blood sample is more suggestive of β-thalassemia carriers or iron deficient anemia. Mentzer [6] and Srivastava Indices [7] were used to distinguish iron deficiency anemia from β-thalassemia carriers among the studied population. The Mentzer Index is obtained when the MCV is divided by the red blood count (RBC) value. If the value is less than 13, thalassemia carrier is confirmed, and if greater than 13 non-thalassemic anemia is indicated [8]. The Srivastava Index is obtained when the mean corpuscular hemoglobin (MCH) is divided by a red blood count (RBC) value. If the value is less than 3.8, it is considered as thalassemia carrier, and if greater than 3.8 then it is considered iron deficient anemia.

### Case definition WHO, 2011 cut off values for assessing anemia
- Normal: female >12 gm/dl : male >13gm/dl
- Mild anemia: female - 11-11.9 gm/dl : male - 11-12.9 gm/dl
- Moderate anemia: 8-10.9 gm/dl (both male and female)
- Severe anemia: lower than 8gm/dl (both male and female)

### MCV cut off value
- ≤ 80: Microcytosis
- 80 – 99: Normocytosis
- ≥ 99: Macrocytosis

Statistical software SPSS version 20.0 was used for data analysis and calculations of frequency and chi-square test.

### Results
In Table1, the distribution of anemic individuals among cases and controls reveal that anemia is significantly higher among the cases, be in thalassemia carrier, or other Hb variants or both combined as compared to the control group with no abnormal Hb variants (Table-1). Further β-thalassemia carriers are found to be more anemic compared to those individuals with other Hb variants. Moderate anemia is found to be higher among the β-thalassemia carriers whereas mild anemia is found to be higher among the individuals with other Hb variants and also control group. These observed differences between β-thalassemia carrier, Hb variant group and control were found to be statistically significant.

<table>
<thead>
<tr>
<th>β-thalassemia carriers</th>
<th>Hb variants</th>
<th>Total</th>
<th>Control</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>N %</td>
<td>N %</td>
<td>N %</td>
<td>N %</td>
<td></td>
</tr>
<tr>
<td>Anemic</td>
<td>112 91.1</td>
<td>36 47.4</td>
<td>74.4</td>
<td>62 31.2</td>
</tr>
<tr>
<td>Non-Anemic</td>
<td>11 8.9</td>
<td>40 52.6</td>
<td>25.6</td>
<td>137 68.8</td>
</tr>
<tr>
<td>Total</td>
<td>123 100</td>
<td>76 100</td>
<td>100 100</td>
<td>199 100</td>
</tr>
</tbody>
</table>

As per Table 2, distribution of different types of anemia among the β-thalassemia carriers along with Hb variant and controls reveal that microcytic anemia is highest among β-thalassemia carriers followed by Hb variants group and control group. Normocytic anemia has the highest prevalence among the control group followed by Hb variant group and thalassemia carriers. However, prevalence of macrocytic anemia is found to be highest among the Hb variant group, followed by control group and is absent among the β-thalassemia carriers. These observed differences are also found to be statistically significant (Table-2).

<table>
<thead>
<tr>
<th>β-Thalassemia carriers</th>
<th>Hb variants</th>
<th>Control</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>N %</td>
<td>N %</td>
<td>N %</td>
<td></td>
</tr>
<tr>
<td>Microcytic anemia</td>
<td>111 99.1</td>
<td>19 52.8</td>
<td>20 32.3</td>
</tr>
<tr>
<td>Normocytic anemia</td>
<td>1 0.9</td>
<td>8 22.2</td>
<td>41 66.1</td>
</tr>
<tr>
<td>Macrocytic anemia</td>
<td>0 0</td>
<td>9 25.0</td>
<td>1 1.6</td>
</tr>
<tr>
<td>Total</td>
<td>112 100</td>
<td>36 100</td>
<td>62 100</td>
</tr>
</tbody>
</table>

In Table 3, the prevalence of β-thalassemia carriers as per Mentzer Index and Srivastava Index are 89.9% and 90.8% respectively. Both the indices are evaluated for validation of our hematological parameters for discrimination of anemia, β-thalassemia carriers and other Hb variant individuals. It was observed that the anemic status between β-thalassemia carriers and other Hb variant individuals were clearly distinct by these two indices. Analysis of β-thalassemia carriers (both anemic and non-anemic groups) as per Mentzer Index and Srivastava Index showed that the mean index falls below 13 and 3.8, respectively whereas among the control group both the mean values of both the indices fall above the given cut offs in both anemic and non-anemic groups.
Table 3: Distribution of Mentzer index and Srivastava index among anemic individuals

<table>
<thead>
<tr>
<th></th>
<th>Anemic Mentzer Index</th>
<th>Anemic Srivastava Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 13</td>
<td>&gt; 13</td>
</tr>
<tr>
<td>β-thalassemia carriers</td>
<td>89 (89.9%)</td>
<td>23 (20.7%)</td>
</tr>
<tr>
<td>Hb variants</td>
<td>9 (9.1%)</td>
<td>27 (24.3%)</td>
</tr>
<tr>
<td>No hemoglobinopathies (Control)</td>
<td>1 (1.0%)</td>
<td>61 (55.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>111</td>
</tr>
</tbody>
</table>

Discussion
High prevalence of anemia observed among β-thalassemia carriers in the present study in concordance with previous studies [5,9,10]. Further, in the present study anemia is found to be significantly high among β-thalassemia carriers as compared to those individuals with other Hb variants. This indicates that individuals with β-thalassemia carrier status have to more carefully diagnose than individuals with other Hb variants. In the present studies, that almost all β-thalassemia carriers are found to be having microcytic anemia as also reported in previous studies [11] where thalassemia is reported to be identified as microcytic anemia. Iron deficiency anemia is the leading cause of microcytic anemia [12] and previous studies also suggested that β-thalassemia carriers are found to be associated with microcytic anemia [13]. However, the pathways leading to microcytic anemia through nutrition and β-thalassemia mutations are likely to be different. As a result, treatment of anemia, specifically microcytic anemia by iron supplementation among β-thalassemia carriers may not be effective [14]. This in fact can be supported by a recent literature where iron supplementation among β-thalassemia carriers lead to iron overload which intern leads to other clinical complications like kidney disorders and mood disorder [15]. In developing countries like India where diversity is huge leading to Mendelian populations with common gene pools, the burden of thalassemia carriers are reported to be increasing in recent years. This can be evidenced through differential distribution of beta-thalassemia carrier gene frequency in different endogamous populations in India ranging from as low as 3% to as high as 17% [16]. Now, it is high time to deal with thalassemic carriers with microcytic anemia with a different strategy other than iron supplementation [17].

The absence of macrocytic anemia among β-thalassemia carriers suggests the survival disadvantage of β-thalassemia carriers with macrocytic anemia. However, this observation needs to be validated with a larger sample size. Going by the categorization of β-thalassemia carrier both anemic and non-anemic as per aspects of Mentzer Index and Srivastava Index seems to be quite applicable in Indian context. Of the total β-thalassemia carriers identified through CBC and HPLC, almost 90% fall under β-thalassemia carrier with Mentzer Index and Srivastava Index. Suggesting that both indices can be used for identification of β-thalassemia carriers with hematological CBC parameters, which would be cost effective.

Conclusion
Individuals with microcytic anemia need to be dealt, managed and treated cautiously, considering the evaluation of their β-thalassemia carrier status first through these two indices followed by HPLC. Individuals with β-thalassemia carrier status should be dealt and managed cautiously, specifically in terms of iron supplementation.

Conflict of Interest: There is no conflict of interest

Ethical Clearance and Consent
The study was approved by the Institutional Ethical Committee, Department of Anthropology, University of Delhi. Intravenous blood (5ml) was collected by trained personnel after obtaining duly signed prior informed written consent from the participants.

Acknowledgements
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Abbreviations
RBC- Red blood count
CBC- Complete blood count
HPLC- High performance liquid chromatography
MCV- Mean corpuscular volume
MCH- Mean corpuscular haemoglobin
Hb- Hemoglobin

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