Study of Level of Glycated Hemoglobin (Hba1c) In Patients of Type 2 Diabetes Mellitus Associated With Iron Deficiency Anemia and Without Iron Deficiency Anemia in Rural Population in Jharkhand

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Abstract
Introduction: Glycated Hemoglobin (Hba1c) is the predominant hemoglobin found in HbA1 fractions. It is used as a gold standard for measuring glycemic control. Iron deficiency anemia (IDA) is one of the most prevalent form of malnutrition in rural population. Any condition that alters red cell turnover like, iron deficiency anemia, will lead to changes in Hba1c levels. The present study was aimed at determining the effect of IDA on Hba1c in type 2 diabetes mellitus (DM) patients in rural population. Methods: The present study was an cross sectional study conducted on 100 type 2 diabetic patients (50 with IDA and 50 without IDA) at RIMS Ranchi, Jharkhand. Hematological investigations, Hba1c, Serum Ferritin and FPG were analyzed. Result: The mean Hba1c level (7.48±0.580) in patients with IDA and type 2 DM was higher than that in the patients without IDA (5.98±0.141). Conclusion: Present study sparsuously elevates Hba1c levels. It is therefore very important to rule out IDA especially in rural population before making a therapeutic decision solely based on Hba1c level. Keywords: Hba1c, Iron Deficiency Anemia (IDA), Type 2 Diabetes Mellitus (DM)

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I. Introduction:
Glycated Hemoglobin (Hba1c) is the predominant hemoglobin found in HbA1 fractions and it is formed by the glycation of terminal valine at the β-chain of hemoglobin. It is used as a gold standard for measuring glycemic control over the previous three months and American Diabetes Association has recently endorsed Hba1c ≥ 6.5% as a diagnostic criterion for diabetes mellitus. However Hba1C levels can be influenced by a variety of other factors affecting erythrocyte turnover and glucose homeostasis. One such condition affecting erythrocyte turnover is anemia. Anemia may be associated with rapid erythrocyte turnover conditions like acute or chronic blood loss, hemolytic anemia, sickle cell anemia, vitamin B12 deficiency, pregnancy that lower Hba1C levels or with slower erythrocyte turnover conditions like Iron deficiency anemia (IDA), alcoholism that increases Hba1C levels. Iron deficiency anemia (IDA) is one of the most prevalent form of malnutrition in rural population. Any condition that alters red cell turnover such as, iron deficiency anemia, will lead to changes in Hba1c levels. Most epidemiologic studies suggest that iron-deficiency anemia (IDA) can result in spuriously high Hba1c values, though some suggest there is lower Hba1c among individuals with IDA or anemia. The present study was aimed at determining the effect of IDA on Hba1c in type 2 diabetes mellitus (DM) patients in rural population.

Due to the variation in the results of multiple studies, we decided to investigate and conducted a study in iron-deficient individuals with type 2 DM patients with normal fasting blood sugar (FBS) and iron-sufficient individuals to assess whether anemia has any effect on Hba1c levels, and anemia can be considered before making any therapeutic decisions based solely on Hba1c levels.

II. Material & Methods:
The present study was an observational study conducted on 100 type 2 diabetic patients (50 with IDA and 50 without IDA) at RIMS Ranchi, Jharkhand over a period of one year (2018-19). Both groups after taking their consent were subjected to a questionnaire. After exclusion of individuals with prefixed exclusion criteria such as Type 1 DM, patients with uncontrolled blood sugar, severe systemic illness, any hemoglobinopathies, a total of 100 patients were finalized for our study. There after sample of venous blood was taken for analysis and biochemical investigation was done. The instrument used for biochemical analysis was Beckman coulter AU480 Biochemical auto analyzer. Serum Ferritin was estimated on ARCHITECT i1000SR, fully automated Immunoassay analyzer. Hba1c was estimated on Bio-Rad D 10 analyzer. Red cell indices was estimated on...
The present study showed that the mean ±SD of haemoglobin in patients of type2 diabetes mellitus associated with iron deficiency anaemia among females is : 7.19 ± 1.14 gm/dl and among males is 7.50 ± 0.60 gm/dl which shows that females are more anemic than males in rural population. Also the mean ±SD of HbA1c in patients of type2 diabetes mellitus associated with iron deficiency anaemia among females is : 7.50 ± 0.60 % and among males is 7.43 ± 0.51 . The above data indicates that as level of haemoglobin decreases the level of HbA1c increases. [Table IV]

Inpatients of type 2 diabetes mellitus associated with iron deficiency anaemia ,Hb&HbA1c showedPearson’s correlationwhich was statistically significant (p<0.001). It signifies that both the variables i.e.Hb and HbA1c ,patients of type2 diabetes mellitus associated with iron deficiency anemia,patients statistically highly significant difference. It signifies that if one variable decreases the other one increases.

### Table No : I

**DISTRIBUTION OF STUDY POPULATION ACCORDING TO AGE IN BOTH GROUPS**

<table>
<thead>
<tr>
<th>AGE(in years)</th>
<th>GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM + IDA</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>51.58± 9.967</td>
</tr>
</tbody>
</table>

### Table No : II

**SEX DISTRIBUTION AMONG BOTH GROUPS**

<table>
<thead>
<tr>
<th>GENDER</th>
<th>GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM + IDA</td>
</tr>
<tr>
<td>MALE</td>
<td>14</td>
</tr>
<tr>
<td>FEMALE</td>
<td>36</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
</tr>
</tbody>
</table>

### Table No : III

**Mean , SD of Hemoglobin and HbA1C Both Groups**

<table>
<thead>
<tr>
<th>MEAN±SD</th>
<th>GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM+IDA</td>
</tr>
<tr>
<td>Hb(gm/dl)</td>
<td>7.90 ± 1.66</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.48 ± 0.580</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

### Table No : IV

**Distribution of Hb and HbA1cin patients of type2 diabetes mellitus associated with iron deficiency anemia(DM + IDA) on the basis of Gender**

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>9.71±1.43</td>
<td>7.19±1.14</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.43± 0.514</td>
<td>7.50 ± 0.609</td>
</tr>
</tbody>
</table>

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IV. Discussion:

Our result suggested that patients with Type 2 diabetes mellitus associated with iron deficiency anemia was associated with higher concentration of HbA1c. The earliest study to investigate the effect of iron deficiency anemia on HbA1c levels was conducted by Brooksetal. [14] who assessed HbA1c levels in 35 nondiabetic patients having iron deficiency anemia both before and after treatment with iron. They observed that HbA1c levels were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. The mechanisms leading to increased HbA1c levels were not clear. It was proposed that, iron deficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycation of the globin chain occurred more readily in the absence of iron. [14]

The results of this study show that HbA1c levels are spuriously elevated in the presence of IDA irrespective of controlled plasma glucose levels. Our finding confirms the study results of Tarim et al, who reported HbA1c level is elevated in diabetics with IDA than with iron-sufficient controls. This may be explained by iron deficiency related changes in the quaternary structure of hemoglobin molecule increasing the glycation of globin chain. [15]

Similar to this study, a study done by Alap L. Christy et al. [16] concluded that iron deficiency anemia elevates HbA1c levels in diabetic individuals with controlled plasma glucose levels. They postulated that iron deficiency anemia has a positive correlation with increased HbA1c levels.

A study done by Catherine Kim et al. [17] concluded that iron deficiency shifted the HbA1c slightly upwards independent of fasting glucose level.

Our study results are also consistent with the study done by El-Agouza et al in non-diabetics who reported that a decline in the Hb level might lead to increase in the glycated fraction at a fixed glucose level, because HbA1c is measured as a percentage of total Hb. [18]

Our results were also in concordance with the study results of Shanthi et al. [19], Coban et al. [20], and Silva et al. [21]. Coban et al. showed a very large difference between HbA1c levels in non-diabetic patients with and without IDA. Shanthi et al. conducted study in non-diabetics and reported that iron deficiency was associated with higher proportions of HbA1c and suggested that iron status must be considered during the interpretation of the HbA1c concentrations in Diabetes mellitus. Silva et al. reported that IDA affects HbA1c levels and causes spuriously increase in their results. Although these upward changes in HbA1c values are statistically significant, they may not be clinically relevant when the overall variability of the HbA1c test is considered. This effect is dependent on anaemia degree and the presence of mild anaemia is likely to have a minor effect on HbA1c levels.

Sluijter et al. [22] tried to provide an explanation for the above findings. They proposed that the formation of HbA1c in reversible processes and hence, the concentration of HbA1c in erythrocytes will increase linearly with the cell's age. For example, they found that in patients with normal blood glucose levels, but with very young red cells, as would be found after treatment of iron deficiency anemia, HbA1c concentration was reduced. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, leading not only to anemia but also to higher-than-normal average age of circulating erythrocytes, and therefore, increased HbA1c levels.

Studies by Ford ES et al. reported no significant difference in mean HbA1c concentration according to the IDA status as well as before and after iron treatment. [23] Sinha et al. [24] and Kalaskar et al. [25] contradicts with our results reporting that HbA1c levels are lowered in IDA.

Also Saudek et al. suggested that red cell age was unlikely to be a significant factor in explaining the changes in HbA1c levels during the treatment of IDA and believed that the reported differences in HbA1c concentrations before and after iron supplementation were due to differences in the laboratory methods used for measuring HbA1c. [26] Ferritin is a storage form of iron, and it reflects the true iron status. In our study, serum ferritin level was indirectly proportional to HbA1c. As explained previously, in IDA, ferritin is decreased with increase in the red cell life span which is associated with increased HbA1c. This goes in hand with other study results of Shanthi et al. [19] and Raj et al. [27]

Our results contradict with the study results of Sharifi et al. [28], who reported that there was no correlation between serum iron, serum ferritin and HbA1c in diabetic patients of either sex.

In these studies, the probable explanation of elevated HbA1c in iron deficiency-anaemia at baseline is that, if serum glucose is accepted to remain constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction but the exact mechanism still remains elusive.

Different studies have been carried out in both diabetic and non-diabetic groups; however, its distribution in well-controlled diabetics who are on regular therapy is inadequately studied. Although diabetes itselfcanelevatethe HbA1c levels, it has been proven that controlled plasma glucose levels for 3 months correlate very well with controlled HbA1c. Hence, patients with controlled plasma glucose levels are expected to have HbA1c below 6.5%
Asshown in the results, there was a significant elevation in HbA1c levels in iron-deficient anemic individuals with FPG less than 126. Therefore, westudied HbA1c distribution in these individuals accordingly. In this study group, Hb & HbA1c showed positive correlation in a negative direction meaning which was also statistically very significant (p < 0.001).

That is, in patients with Type 2 DM when the hemoglobin decreases the HbA1c will increase and vice versa.

V. Conclusion:

HbA1c was higher in patients with Type 2 Diabetes Mellitus associated with iron deficiency anemia compared to patients with Type 2 Diabetes Mellitus not associated with iron deficiency anemia. This study shows that whenever HbA1c is escalated to detect the glycemic status of a patient, factors other than glucoseosallopoyl apartinits calculated value, which should be kept in mind before therapeutic treatment is given. If we cannot rule out the different influences for HbA1c then it may result in unnecessary hypoglycemia in patients as they are over-treated to try to bring down the HbA1c to within set targets. Also, we must be cautious while treating any diabetic patients from a rural population especially females, keeping in mind that IDA is very common in female population of rural origin.

References: