Correlation of serum fructosamine, erythrocyte Na\(^+\)-K\(^+\) ATPase and glutathione peroxidase with HbA1c levels

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Glycated hemoglobin is the frequently used test in the diagnosis of diabetes mellitus. However, because this test is affected by various factors and may not be accurate for patients of certain health conditions, the present study aims to explore the association between potential and cost-effective markers like serum Fructosamine, Erythrocyte Na\(^+\)-K\(^+\) ATPase, and Glutathione peroxidase with altering levels of established marker HbA1c (Glycated haemoglobin). The study showed that serum Fructosamine has a statistically significant \((P<0.0001)\) association with increasing levels of HbA1c as well as blood glucose. There was a 100% sensitivity and specificity for serum fructosamine test against HbA1c in ROC analysis, however, the erythrocyte glutathione peroxidase and erythrocyte membrane Na\(^+\)K\(^+\) ATPase activity was not affected by increasing HbA1c levels.

Keywords: Diabetes mellitus, Fructosamine, Glycated hemoglobin, Sodium potassium ATPase
studies have found out that uncontrolled hyperglycemia is associated with oxidative stress as it causes an increase in production of superoxide anion and other reactive oxygen species as well as impairs the activity of antioxidant enzymes including glutathione peroxidase.

The current study was designed to evaluate the association between the glycated protein Fructosamine, erythrocyte enzymes Na\(^+\)-K\(^+\) ATPase, and Glutathione peroxidase with altered levels of HbA1c. This will help to further investigate whether an increase in the level of HbA1c causes a decrease in the activity of Erythrocyte Na\(^+\)-K\(^+\) ATPase and Glutathione peroxidase. There have been studies conducted previously that have found a significant association between HbA1c and fructosamine, HbA1c, and Glutathione peroxidase activity in erythrocytes and slightly lesser significant association between HbA1c and Na\(^+\)-K\(^+\) ATPase. But there has been no attempts made to study the simultaneous association of the three parameters (serum fructosamine, erythrocyte Na\(^+\)-K\(^+\) ATPase and glutathione peroxidase) with different levels of HbA1c.

Material and Methods

The study protocol was approved by the Institutional Ethics Committee, Kasturba Medical College, Manipal, India. A total of 191 whole blood samples that were referred for the HbA1c test and blood glucose test were collected from the Clinical Laboratory of Biochemistry, KMC, Manipal after proper anonymization. The samples were collected based on their HbA1c levels and age (ranging from 35-65 years old) of the patient. Based on HbA1c levels the samples were categorized into 5 groups — 4 cases (ranging from 6.5%-14%) and 1 control (4.7%-5.7%). The control group was defined based on their HbA1c levels and age (ranging from 35-65 years old) of the patient. Based on HbA1c levels and age (ranging from 35-65 years old) of the patient. Based on HbA1c levels and age (ranging from 35-65 years old) of the patient.

Sample distribution into groups based on HbA1c levels is illustrated in (Table 1).

A steady increase in serum fructosamine levels was observed in each group with an increase in HbA1c levels (Fig. 1). A significant correlation observed with \(P < 0.0001\), between HbA1c levels and serum fructosamine levels when all cases and controls were combined. (Fig. 2) A significant correlation with \(P < 0.001\) was observed between serum fructosamine levels and blood glucose levels when all cases and controls were combined. (Fig. 3).

The largest AUC in ROC analysis was obtained for Group 4 with specificity and sensitivity at 100%. (Fig. 4). Whereas, for Group 3 sensitivity was 98.6% and specificity was 100% under ROC analysis (Fig. 5).

Figure 6 represents an association of erythrocyte membrane Na\(^+\)-K\(^+\) ATPase activity with HbA1c levels between the groups. The activity of the enzyme was found to be lower in groups 2 & 3 when compared to control and a sudden increase in group 4 was observed.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1c(%)</th>
<th>HbA1c(mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5-5.7</td>
<td>27.9-35.34</td>
</tr>
<tr>
<td>Group 1</td>
<td>6.5-7.5</td>
<td>40.3-46.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>7.6-8.5</td>
<td>47.12-52.7</td>
</tr>
<tr>
<td>Group 3</td>
<td>8.6-9.5</td>
<td>53.32-58.9</td>
</tr>
<tr>
<td>Group 4</td>
<td>9.6-14</td>
<td>59.52-86.8</td>
</tr>
</tbody>
</table>

![Image](image-url)
We will further look for the reason behind this sudden increase.

There was no significant change observed in the activity of Glutathione peroxidase enzyme with an increase in the HbA1c levels.

**Discussion**

Previous studies\(^3\) have shown a significant association between serum fructosamine and HbA1c, similar to the results obtained in the current study, where a steady increase in serum fructosamine was...
observed in each group. Also, when the groups were dissolved, serum fructosamine levels were observed to have a significant ($P < 0.001$) association with HbA1c levels. Similarly, blood glucose levels showed a significant ($P < 0.001$) association with serum fructosamine levels as well. The ROC curve obtained show that serum fructosamine test has an excellent sensitivity and specificity to the HbA1c levels. These results all-in-all indicate an increase in the formation of serum fructosamine as there is an increase in blood glucose levels and that it can be considered for its use as an alternative marker to HbA1c, as it is cost-effective, requires lesser time and can detect rapid changes in blood glucose levels compared to HbA1c.

As we know, Na$^+$-K$^+$ ATPase is a membrane protein, which has a vital role in maintaining the resting membrane potential. Based on the previous studies, activity of erythrocyte Na$^+$-K$^+$ ATPase is affected in conditions like diabetes due to the increase in blood glucose levels. But, the current study showed no association between the erythrocyte Na$^+$-K$^+$ ATPase activity and the HbA1c levels when compared between control and the four diabetic groups (Group 1-4). There are only scarce data on the correlation between erythrocyte Na$^+$-K$^+$ ATPase and HbA1c levels. A study, who obtained similar results as ours, stated that in diabetic condition an increase in blood glucose level does not cause the impairment in the activity of erythrocyte Na$^+$-K$^+$ ATPase. Contradictory to this, a Japanese study on type II diabetic patients revealed a slight reduction in the activity of erythrocyte Na$^+$-K$^+$ ATPase, but only in those with microalbuminuria. Further, an elaborate study needs to be done to clarify the differences in the results obtained by us and previous studies and to find out the reason behind the sudden decrease in the activity of erythrocyte membrane Na$^+$-K$^+$ ATPase in group 2 and group 3 (Fig. 6)

Diabetes has been suggested to produce abnormally high levels of reactive oxygen species via mitochondrial electron transport chain and glucose autoxidation. The increased levels of free radicals formed during such conditions together with products of non-enzymatic glycation, glucose oxidation, and lipid peroxidation cause damage to enzymes, cell functioning, and insulin resistance due to oxidative stress. Antioxidant enzymes on the other hand, such as erythrocyte glutathione peroxidase counteract against free radicals formed during oxidative stress. In our attempt to evaluate its association with HbA1c levels, we observed that there was no association between erythrocyte glutathione peroxidase with increasing levels of HbA1c. There was no difference in the activity of erythrocyte glutathione peroxidase when compared between the control and diabetic groups (Group 1-4). Different studies have reported variations in the activity of antioxidant enzymes in different tissues like liver, kidney, muscle, erythrocytes etc., in normal and diabetic conditions and an increase in the glutathione peroxidase activity was observed in erythrocytes. Whereas, there are studies imply there was a significant decrease in the activity of erythrocyte glutathione peroxidase when compared between diabetic and non-diabetic groups. At the same time, there are studies that found no significant difference in activity of glutathione peroxidase in erythrocytes between the two groups, similar to the current study. There is discrepancy among the results from previous studies and the present study. There could be various reasons for this which is yet to be cleared. Although, a recent study has shown that erythrocyte glutathione peroxidase activity begins to improve as insulin treatment is started. This could be one of the reasons for variations in the results.

Conclusion

The findings from the present study suggest that serum fructosamine has similar susceptibility to an increase in blood glucose levels as HbA1c. We observed that as there is an increase in HbA1c level and blood glucose level there is an increase in serum fructosamine levels. The fructosamine test is a cost-effective, time-saving, and more sensitive to changes in blood glucose levels. On the other hand, erythrocyte glutathione peroxidase and erythrocyte membrane Na$^+$-K$^+$ ATPase activity were found to be not affected by increasing HbA1c levels (a non-enzymatic glycated protein).

Conflict of interest

All authors declare no conflict of interest.

References


