Comparison of Two Methods for Measurement of HbA1c in Two University Hospitals of Mashhad

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**Abstract**

**Introduction:** The aim of this study was to compare the two analytical techniques for determination of Glycated hemoglobin (HbA1c), consisting immunoturbidimetric and enzymatic methods.

**Materials and Methods** A total of 140 out-patients were included in this study. Measurements of HbA1c were done in blood samples using immunoturbidimetric and enzymatic assay. The two methods were used by clinical laboratories of Ghaem and Emam Reza hospitals in Mashhad, respectively.

**Results:** Our results indicate that there was no significant difference between two methods, though; the average of HbA1c measured by enzymatic method was rather higher than the other method (7.38 and 7.34, respectively). The two methods correlated well with correlation coefficient of 0.967.

**Conclusion:** Both techniques were proved to be sufficiently reliable and the results of the two methods show strong correlation though, the enzymatic method has an additional advantage of simultaneous measuring total Hb which can omit the undesired effect of hemolysis occurring during sampling.

**Keywords:** HbA1c, Immunoturbidimetric, Enzymatic assay


**Introduction**

Glycated hemoglobin (HbA1c) is a glycoprotein formed as a result of the non-enzymatic addition of D-glucose to the β-chain of hemoglobin. The amount of HbA1c in the blood is dependent on mean glucose levels present during the 1 to 2 months preceding measurement, as HbA1c accumulates in red blood cells during their lifespan. The level of HbA1c is affected after 12h exposure to glucose, and is an indicator of glycemic levels on a long term basis. The concentration of HbA1c is associated with Glucose Tolerance Test (GTT) and Fasting Blood Sugar (FBS) (1). Since the standardization of HbA1c assays, it has been recommended as a tool in the diagnosis, follow up, and treatment of diabetes (2-4). But, as HbA1c testing is completely different, one may speculate whether the results may differ. Therefore, in the present study we aimed to compare and correlate the two analytical methods of measuring HbA1c.

**Materials and Methods**

**Study population**

One hundred and frothy patients, diagnosed as pre-diabetic or diabetic individuals were enrolled in the
study. The patients ranged 25 to 73 years old (average: 46.13 years) of which 62.2% of all cases were women and 37.8% were men. All outpatients were enrolled at the laboratory of the Ghaem Educational Hospital for checkup.

Whole blood was collected from all patients in EDTA vials. Before performing sample analysis, the test requires manual preparation of a sample hemolyzed. Samples were mixed with tetradecyl trimethyl ammoniun bromide containing hemolyzing reagent (10 μl whole blood and 1000 μl hemolyzing reagent supplemented in the kit) for 5 minutes according to the testing method. Samples are kept at 4°C in order to preserve the stability of the samples until following day that measurements were done with the other method at Emam Reza Educational Hospital.

Analytical procedures were conducted according to the immunoturbidimetric and enzymatic assay methods.

**Immunoturbidimetric Method**

The HbA1c values of hemolyzed samples were measured by Pars azmoon kit following the kit manufacturer's instructions. In this method total Hb and HbA1c in hemolyzed blood are attached to the latex particles with equal affinity. In the next step, monoclonal antibodies are used to detect HbA1c, next polyclonal antibodies against monoclonal antibodies can agglutinate the particles, and the resulted turbidity is measured spectrophotometrically. Trucal HbA1c calibrators were used for calibration and Trulab HbA1c serum control was used for control of quality.

**Enzymatic Assay Method**

The HbA1c values of hemolysed samples were measured by Pishtaz Teb kit according the kit manufacturer’s instructions. First the Hb concentration was measured spectrophotometrically. Simultaneously protease can produce fructosyl dipeptide from the amine end of β-chain of HbA1c. Next Fructosyl Dipeptide Oxidase (FDOX) can interact with fructosyl dipeptide and H2O2 is produced. Then the hydrogen peroxide can produce a chromogenic reaction with appropriate substrate in the presence of Peroxidase enzyme (POD). Thereafter the color development is measured by spectrophotometer.

**Statistical Analysis**

The data were analyzed using SPSS Version11. Mean (± SD) and frequency/ percentages were used to present the variables. Pearson correlation (r) was utilized for determining the strength of linear association between HbA1c measurements by the two mentioned methods.

Bland and Altman plots were used to calculate mean difference (Bias) and agreement between the two methodologies.

The measurements were compared using paired sample T-test and a P-value of less than 0.05 was considered statistically significant.

**Ethical consideration**

The study was performed in accordance with Helsinki declaration on medical research ethics. The leftover of the clinical samples admitted in Ghaem hospital for measuring HbA1c were used for experiments and no additional procedure was performed for sampling. The samples were coded and kept anonymous, and were rechecked at Emam Reza hospital with the other approach to evaluate the accuracy of the results.

**Results**

The mean HbA1c is slightly lower for immunoturbidimetric method than enzymatic assay.

Results depict that there isn’t significant difference between these two mean numbers (p=0.297). As shown in Fig. 1 two methods correlated well with correlation coefficient of 0.967.

![Figure1: Correlation of HbA1c levels measured by two methods.](image)

The results indicate that the mean range of HbA1c in females is higher than males in both measuring systems, though it was not significant (Table 1).

We also noted that there was a significant difference between measured values of two techniques in patients more than 60 years old (p=0.036).

<table>
<thead>
<tr>
<th>Mean of HbA1c</th>
<th>Immunoturbidimetric method (%)</th>
<th>Enzymatic method (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total study group</td>
<td>7.34</td>
<td>7.38</td>
</tr>
<tr>
<td>Females</td>
<td>7.53</td>
<td>7.60</td>
</tr>
<tr>
<td>Males</td>
<td>7.14</td>
<td>7.12</td>
</tr>
<tr>
<td>Patients 60&lt; years*</td>
<td>7.443</td>
<td>7.729</td>
</tr>
</tbody>
</table>

* considered as significant

**Discussion**

The availability of the hemoglobin A1c test has enhanced diabetic care and its measurement has become an integral part in the management of diabetes. Also the relationship between the improved glycemic...
control and risk of diabetic complications has been established (4, 6). The HbA1c levels of samples can be reliably measured by using various methods such as High-Performance Liquid Chromatography (HPLC), immunnoassay, boronate affinity chromatography, and enzymatic assay. Several studies have reported an observed difference between HbA1c measurements based on different techniques, since the methods were standardized using a widespread reference model and calibrated with the same calibrator. The two main routine techniques used in many countries are immunoturbidimetric and enzymatic assays (7, 8).

However, in some situations these two methods tend to yield results with undesirable differences; thus it is very important to compare the results from these methods which are used by different laboratories (9). In our study the comparison between the above mentioned methods was performed among 140 patients with HbA1c levels ranging from 4.9 % to 12 %.

In enzymatic assay the technique was based on digesting hemoglobin samples with a specific protease to generate fructosyl amino acid.

The measuring protocol was in line with the Diabetes Control and Complications Trial (DCCT) and National Glycohemoglobin Standardization Program standards (NGSP) (10).

Though some studies reported that the HPLC method can detect abnormal hemoglobin with favorable reproducibility and a CV < 1%, this technique needs a large dedicated devices and rather a time consuming procedure. In addition, many trained staffs are needed to maintain the instrumentation (9, 11, 12). The immunnoassay can be performed by an automated analyzer, thus this method does not take a long time for measuring a large number of samples.

However, in this method, the total hemoglobin needs to be assessed by an additional measurement.

On the other hand the enzymatic assay also provides an accurate, fast and uniform reaction and the error obtained from this method has been reported to be <1%

(9, 13). Enzymatic method is also fully automated system that requires no sample preparation and has a fast running time.

As indicated in other studies a relationship and concordance between these two methods support the reliability of both methods, if the assay protocol is properly standardized. Although the HbA1c measured values should be monitored periodically by Quality Control (QC) observations and each laboratory is responsible to determine the accurate reference values and correction equations for more reliable results. The American Diabetes Association (ADA) has suggested that one important remaining issue with HbA1c test is the lack of available and adequate assay to manage diabetes, especially in developing countries (14).

The turbidimetric immunoassay is easy to use and more available in most developing countries especially in considerable rural populations where limited accessibility to advanced devices and laboratories performing the proper assays is still an unsolved problem (7, 15). So far no significant superiority between various measurement methods has been reported and thus the immunoturbidimetric and enzymatic method which are both reliable and easy to perform can be used as alternative methods to HPLC measuring system with its known limitations.

Conclusion
Our results indicate no significant differences in HbA1C levels assessed by immunoturbidimetric and enzymatic methods. In addition, both methods have been shown to be accurate and the results of them where comparable in our study.

Acknowledgements
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References
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