

RESEARCH ARTICLE

GLYCATED HEMOGLOBIN A1c: IMMUNOTURBIDIMETRY AND IMMUNOFLUORESCENCE METHODS

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Abstract

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Key words:-Glycated Hemoglobin, Immunoturbidimetry, Immunofluorescence, Madagascar

Objective: to compare the two immunological methods of HbA1c determination available at the two university hospital centers in the capital of Madagascar.

Methods:Prospective comparative study between two HbA1C assav techniquesat the University Hospital Center Joseph RavoahangyAndrianavalona and University Hospital Center Joseph RasetaBefelatanana both localized at Antananarivo Madagascar was conducted. The two techniques used are immunoturbidimetry and immunofluorescence. Fifty samples were run simultaneously on both techniques to assess the dispersion and correlation of HbA1C values. A history of haemoglobin abnormalities is previously researched in the medical records for the samples.

Results: The correlation coefficient for the 2 techniques is 0.97. The Bland –Altman plot shows a funnel-shaped appearance. The Interclass Correlation Coefficient is 0.851 CI [-0.036 - 0.962]. No hemoglobin abnormalities were documented in any of the 50 samples

Conclusion: It is essential to keep the same technique for monitoring HbA1c. Our study shows poor reproducibility for the two techniques compared. A strong relationship between the technique used and the HbA1c value was found for the extreme values. It is important to specify the technique used to support prescribers.

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Introduction:-

Glycated Hemoglobine A1c (HbA1c) is a glucose-modified hemoglobin created during the spontaneous reaction between glucose and N-terminal value residues on β chains of hemoglobin-creating β -N-1-deoxy fructosyl [1]. The determination of glycated haemoglobin and mainly its fraction HbA1C is a major tool in the management of diabetic patients [2].

Our objective was to compare the two immunological methods of HbA1c determination available at the two university hospital centers in Antananarivo, Madagascar.

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Methods:-

This is a prospective comparative study between two HbA1C assay techniques. The first technique is a FinecareTM immunofluorescence (IF) technique. The FinecareTM HbA1c Quantitative Test uses a sandwich immunodetection method to measure percentage of HbA1c in human blood. The complexes of detector antibody and HbA1c are captured to HbA1c antibody that has been immobilized on test strip. The fluorescence-labeled detector Hemoglobin (Hb) antibody binds to Hb in blood specimen; the complexes are captured to HbA1c and Hb in blood specimen. The second technique is the immunoturbidimetric technique (ITM) on BS 300 by MINDRAY[®]. This method directly determinates HbA1c level in whole blood, using an antigen and antibody reaction. Total hemoglobin and HbA1c compite for the unspecific absorption rate to the latex particles. Mouse anti-human HbA1c monoclonal antibody bonds to the coated particles with HbA1c. The presence of goat anti-nouse IgG polyclonal antibody causes the agglutination of the particles (complexe). The amount of agglutination is proportional to the concentration of the HbA1c in the sample and can be measured by turbidimetry.

Results of the 2 techniques are expressed in percent (%).

Fifty samples previously collected on EDTA tubes were run simultaneously through the 2 techniques.

A haemoglobin abnormality was looked for in the medical records corresponding to the samples.

The statistical comparison technique we used allowed us to identify the correlation coefficient of the two techniques, to draw the Bland and Altman plot and to calculate the interclass correlation coefficient.

Results:-

The correlation coefficient for the 2 techniques is 0.97. The scatter diagrams are shown in Figure 1.

A funnel effect can be seen on the Bland and Altman plot (Figure 2).

The Interclass Correlation Coefficient is 0.851 CI [-0.036 - 0.962].

No haemoglobin abnormalities were documented in any of the 50 samples.





Figure 2:- Bland-Altman plot.

Discussion:-

In 2009 the International Expert Committee recommended the use of HbA1c to diagnose diabetes mellitus with a threshold > 6.5% [3]. Methods of HbA1c analysis can be divided into 2 categories: methods based on molecular charge and those based on structure. The former category includes CE-HPLC and electrophoresis, and the latter includes immunoassays, boronate affinity chromatography, and mass spectrometry[4].

Only immunological methods based on immunoturbidimetric and immunofluorescence techniques are available and routinely used in the laboratories of the University Hospital Centers of the Malagasy capital.

In this study the correlation coefficient of the immunofluorescence and immunoturbidimetry technique is 0.97. This value, which is close to 1, attests to a strong positive linear relationship between the two techniques.

However, in the Bland and Altman plots, a funnel effect can be observed, showing the dependence of the variability on the level of the measurements. The difference between the methods increases when the values are extreme (increasing or decreasing).

The intra-class correlation coefficient is 0.851 with a 95% CI [-0.036 - 0.962]. This value < 0.9 indicates an unsatisfactory reproducibility of the two techniques.

None of the samples were documented to have a haemoglobin abnormality. Indeed, for immunochemical techniques, the impact of the presence of a variant on the determination of the HbA1c level varies according to the type of mutation. Indeed, the antibodies used react with the glycated N-terminal value. This is the case for HbS and HbC variants, but also for HbF [5,6]. Also for the immunochemical method, in case of the presence of Hb Raleigh that cannot be glycated, the ratio of haemoglobin to glycated haemoglobin was modified, leading to an abnormally low value [7].

On the other hand, various situations, mainly haemolysis, are responsible for an underestimation of HbA1c [8]. Conversely, iron deficiency may cause an overestimation of HbA1C [9,10]. Chronic alcohol consumption is also a situation that may increase the measured HbA1c level, due to suppression of erythropoiesis [11].

In total, the technique used and the analytical interferences are interdependent and have impacts on the HbA1c result. In this study the two techniques are not reproducible and the results differ for very high or very low HbA1c values.

When an aberrant HbA1c value is generated and/or the value does not match the clinical impression, the possibility of interference by Hb variants should be considered, and the interpretation of Hb A1c values should be based on the patient's medical history and other laboratory results [7].

Conclusion:-

The method of HbA1c testing can influence the result and should be taken into account in the interpretation of the result. The retention of the same testing technique as well as the obligation to mention the technique used should be adopted for a better monitoring of blood glucose control.

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Disclosure of conflict of interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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