

## Research Article

# Reference range of glycated hemoglobin in the diagnosis of diabetes mellitus

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### ABSTRACT

**Background:** Diabetes mellitus is now affecting many in the workforce; it has major and deleterious impact on both individual and national productivity. Glycated hemoglobin (HbA<sub>1C</sub>) can be used as diagnostic test for diabetes subject to stringent quality assurance tests, while assays are standardized to a criteria aligned to the international reference values. HbA<sub>1C</sub> has now been recommended by American Diabetic Association (ADA) as a tool to diagnose diabetes. The main objective of the present research is to establish the reference range for glycated hemoglobin in healthy non-diabetic subjects in our hospital laboratory and compare it with the values reported by standard laboratories.

**Methods:** The study was conducted in the Department of Biochemistry, Super Specialty Hospital, Government Medical College, Jammu. Total number of subjects was 50 (25 males, 25 females), aged between 30-70 years. 2ml of venous blood was collected from antecubital vein under aseptic conditions from each individual and put in EDTA vials and the samples were estimated in fully auto-analyzer.

**Results:** This study has delivered different results for males and females. In males, the normal levels were  $6.12 \pm 0.76\%$  while in females; the levels were  $6.30 \pm 0.62\%$ . The overall range in males was 4.3- 7.18% while in females it was 4.7 - 7.30%.

**Conclusions:** The values were comparable ( $p > 0.05$ ) with those reported by standard laboratories, e.g. Dr. Lal Path Labs ( $< 6\%$ ) and SRL Lab ( $\leq 5.7\%$ ). Hence, our values are suitable to be used as cut-off while interpreting the results of patients with diabetes mellitus.

**Keywords:** Diabetes mellitus, Glycated hemoglobin, Reference range

### INTRODUCTION

The socioeconomic consequences of diabetes and its complications could have a seriously negative impact on the economies of developed and developing nations. Recent estimates indicate that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.<sup>1</sup> Type 2 diabetic patients, diagnosed before 70 years of age, have only 70% of the life expectancy of non-diabetic people. The long term specific effects of diabetes include

development of macrovascular complications like diabetic retinopathy, diabetic nephropathy, and neuropathy.<sup>2</sup> People with diabetes are also at increased risk of cardiac, peripheral, arterial and cerebrovascular disease.<sup>3</sup>

Hemoglobin A<sub>1C</sub> (glycated hemoglobin, HbA<sub>1C</sub>)<sup>4</sup> is defined by the International Federation of Clinical Chemistry (IFCC) as hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains. Formation of HbA<sub>1C</sub> is irreversible and the level

in the red blood cell depends on the blood glucose concentration.<sup>5</sup> Thus, measurement of HbA<sub>1c</sub>, which was first introduced in the 1970's, provides a measurement of glycemic control over time, which has been proven to evoke changes in diabetes treatment resulting in improved metabolic control. It is now accepted as a unique and important index in diabetes management reflecting the degree of metabolic control and was a major determinant of the landmark Diabetes Control and Complications Trial (DCCT).<sup>6</sup> Amongst the various markers of glycemic control, glycated haemoglobin (GHb)<sup>4</sup> has now been established as the most reliable, though many other proteins are also glycated in the diabetic and non-diabetic states.<sup>7</sup> The prognostic role of HbA<sub>1c</sub> is well established and accepted. The long lifespan of erythrocytes (mean 120 days) enables HbA<sub>1c</sub> to be used as an index of glycemic control over the preceding two to three months and as the adequacy of treatment in diabetic patients. As the average amount of plasma glucose increases, the fraction of glycated haemoglobin<sup>4</sup> increases in a predictable way.<sup>8</sup> In diabetes mellitus, higher amounts of glycated haemoglobin,<sup>4</sup> indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy and retinopathy.<sup>9</sup> Glycated haemoglobin<sup>4</sup> can be estimated at any time of the day and does not require any special preparation such as fasting.<sup>10</sup> These properties have made it the preferred test for assessing glycemic control in people with diabetes and as a screening test for persons at high risk of diabetes.<sup>11</sup> The types of glycated haemoglobin<sup>4</sup> are shown in Table 1.

In 2010, ADA proposed diagnostic criteria for diabetes and prediabetes based on HbA<sub>1c</sub> levels. These are HbA<sub>1c</sub> > 6.5% (> 48 mmol/ mol) to diagnose diabetes mellitus and for pre-diabetes 5.6-6.4% (39-46 mmol/ mol). The use of HbA<sub>1c</sub> can avoid the problem of day to day variability of glucose values and importantly it avoids the need of a person to fast.<sup>12</sup> Due to the inconsistencies in the reported reference range of glycated hemoglobin from different labs, the present study was planned to establish the reference range for

glycated hemoglobin (HbA<sub>1c</sub>) in healthy non-diabetics in our hospital laboratory and compare it with values reported by standard laboratories. Thus, the study had two aims, i.e. to obtain the reference range in the local population *visa-a-vis* ascertaining the quality performance of our laboratory compared to other reputed laboratories. Methods of glycated hemoglobin assays have primarily evolved around three basic methodologies:

1. Based on difference in ionic charge.
2. Based on structural characteristics.
3. Based on chemical reactivity.

Each method has its own advantages and disadvantages (Table 2).<sup>4</sup>

## METHODS

The study was conducted in the Department of Biochemistry, Super Speciality Hospital, Government Medical College, Jammu. Total number of subjects was 50 (25 males, 25 females), aged between 30-70 years. 2ml of venous blood was collected from antecubital vein under aseptic conditions from each individual and put in EDTA vials. The anti-coagulated whole blood specimen was lysed automatically in the system for the whole blood application or it may be lysed manually using the HbA<sub>1c</sub> diluent and analysed in fully auto-analyzer (Architect c4000 nad c8000 systems). In either gender, the lowest and the highest values obtained were considered to be the limits of the reference range. The mean and standard deviation were calculated and compared with the values reported by standard laboratories by employing one way analysis of variance (ANOVA).

## RESULTS

The present study delivered different results for males and females. In males, the normal levels were  $6.12 \pm 0.76\%$  while in females; the levels were  $6.30 \pm 0.62\%$ .

**Table 1: Types of glycated hemoglobin.**

| Name                      | Composition  |
|---------------------------|--|
| HbA                       | Constitutes = 97% adult Hb   |
| HbA <sub>0</sub>          | Synonymous with HbA  |
| HbA <sub>1a1</sub>        | HbA with fructose-1,6-diphosphate attached to the N-terminal of the β-chain                            |
| HbA <sub>1a2</sub>        | HbA with glucose-6-phosphate attached to the N-terminal of the β-chain                                 |
| HbA <sub>1a</sub>         | Comprises HbA <sub>1a1</sub> and HbA <sub>1a2</sub>  |
| HbA <sub>1b</sub>         | HbA with pyruvic acid attached to the N-terminal of the β-chain  |
| HbA <sub>1c</sub>         | HbA with glucose attached to the N-terminal of the β-chain   |
| Pre-HbA <sub>1c</sub>     | Unstable Schiff base (aldimine); a labile intermediary component in the formation of HbA <sub>1c</sub> |
| HbA <sub>1</sub>          | Consists of HbA <sub>1a</sub> , HbA <sub>1b</sub> and HbA <sub>1c</sub> and other                      |
| Total glycated hemoglobin | Consists of HbA <sub>1c</sub> and other hemoglobin-carbohydrate adducts                                |

**Table 2: Advantages and disadvantages of various HbA<sub>1c</sub> assay methods.**

| Assay                       | Advantage   | Disadvantage   |
|-----------------------------|---|--|
| Ion Exchange Chromatography | Can inspect chromatograms for Hb variants. Measurements with great precision.                           | Variable interference from hemoglobinopathies, HbF and carbamylated Hb but the current ion exchange assays correct for HbF and carbamylated Hb does not interfere. |
| Boronate Affinity           | Minimal interference from hemoglobinopathies, HbF and carbamylated Hb.                                  | Measures not only glycation of N-terminal valine on $\beta$ chain, but also $\beta$ chains Glycated at other sites and Glycated $\alpha$ chains.                   |
| Immunoassays                | Not affected by HbE, HbD or carbamylated Hb. Relatively easy to implement under many different formats. | May be affected by hemoglobinopathies with altered amino acids on binding sites. Some interference with HbF.   |

The lowest and highest values in males were 4.3% and 7.18%, i.e. overall range in males was 4.3- 7.18%. On the other hand, the lowest and highest values in females were 4.7% and 7.30%, i.e. overall range in females was 4.7 - 7.30%. On applying ANOVA for multiple group comparison, the values were comparable ( $p>0.05$ ) with those reported by standard laboratories, e.g. Dr. Lal Path Labs (<6%) and SRL Lab ( $\leq 5.7\%$ ).

## DISCUSSION

The laboratory levels of HbA<sub>1c</sub> at Department of Biochemistry, Super Specialty Hospital, Government Medical College, Jammu, are comparable with the reference range of different reputed laboratories and hence suitable to be used as cut-offs while interpreting the results for patients with diabetes mellitus. Besides lack of assay standardization, the problems related to its measurement in particular patient groups with hemoglobinopathies, fetal hemoglobin, renal failure (who form hemoglobin derivatives) and hemolytic diseases, pose numerous analytical difficulties associated with glycated hemoglobin measurement and need to be borne in mind.

## CONCLUSION

It is recommended that each laboratory should establish its own reference range to represent its native population, prior to interpreting patients' values.

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