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Research Article

To establish the reference range of glycated hemoglobin

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ABSTRACT

Background: Diabetes mellitus (DM) has emerged as a major healthcare problem in India. There were an estimated 40 million persons with DM in India in 2007 and this number is predicted to rise to almost 70 million by 2025. It is estimated that every fifth person with diabetes will be an Indian. The objective of the present investigation was to establish the reference range for glycated hemoglobin (HbA_{1C}) 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, MMIMSR, Mullana (Ambala, Haryana). Total number of subjects was 50 (25 males, 25 females), aged 30 to 70 years. 2 ml of blood was collected from antecubital vein under aseptic conditions from each subject and put in EDTA vials. Hemolysed blood was estimated by semiautoanalyzer for HbA_1C.
Results: In females, the levels were 6.50 ± 0.74 % while in males the levels were 6.27 ± 0.94 %. The overall range in females was 4.8 - 7.56 % while in males it was 4.2 to 7.56 %. The values were comparable ($p>0.05$) with those reported by standard laboratories, e.g. Dr. Lal PathLabs (<6%), Charak diagnostic (4.5-6.3%) and Mayo Clinic (6.5-7%).
Conclusion: Our laboratory levels of HbA_{1C} are comparable with the reference range of different laboratories and hence suitable to be used as cut-offs while interpreting the results of patients with DM.

Keywords: Diabetes mellitus, Glycated hemoglobin, HbA_{1C}, Reference range

INTRODUCTION

Diabetes Mellitus

Worldwide projections suggest that more than 220 million people will have diabetes mellitus by the year 2010, and the majority of these, approximately 213 million, will have type 2 diabetes.[1]Type 2 diabetes mellitus is associated with increased cardiovascular and overall mortality. In fact, type 2 diabetic patients diagnosed before 70 years of age, have only 70% of the life expectancy of non-diabetic people.[2, 3] The long–term relatively specific effects of diabetes include development of retinopathy, nephropathy and neuropathy.[4] People with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease.[5]

Glycated hemoglobin

Glycohaemoglobin is formed by a non-enzymatic interaction between glucose and the amino groups of the valine and lysine residues in haemoglobin. Formation of glycohaemoglobin is irreversible and the level in the red blood cell depends on the blood glucose concentration. Thus, measurement of glycohaemoglobin, 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).[6]

Epidemiological data suggest that classic cardiovascular risk factors, such as hypercholesterolemia, hypertension and smoking alone do not account for the excess risk of cardiovascular morbidity and mortality in type 2 diabetes mellitus. Rather, the excess morbidity and mortality is linked to the disease itself. Type-I diabetes is accompanied by long-term micro- and macrovascular complications, the primary causes of morbidity and mortality in these patients. Diabetic nephropathy, as the single most common cause of end-stage renal disease, accounts for more than one-third of all cases. Thus, understanding the pathogenesis and preventing and/or ameliorating these long-term complications have been major goals of research in diabetes mellitus.

The core of the issue is glycemic control. It has long been suspected that high blood glucose is harmful in a variety of ways and that all the complications whether microvascular or macrovascular, were to a larger or lesser extent linked with it. In recent times this has been well established. Amongst the various markers of glycemic control, glycated hemoglobin (GHb) has now been established as the most reliable, though many other proteins are also glycated in the diabetic and non-diabetic states[7].

The prognostic role of HbA1c is well established and accepted. In the normal 120-day lifespan of the red blood cell, glucose molecules react with hemoglobin, forming glycated hemoglobin. Glucose forms an aldimine linkage with NH_2- of valine in the β-chain, undergoing an Amadori rearrangement to form the more stable ketoamine linkage. Glycated hemoglobin has been used primarily to as a marker to identify the average plasma glucose concentration over prolonged periods of time. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy.[8]

HbA1c reflects average plasma glucose over the previous eight to 12 weeks.[9] It can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycemic control in people with diabetes. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes.[10] The types of glycated hemoglobin are shown in Table 1.[11]

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_{1C}) in healthy non-diabetic subjects in our hospital laboratory and compare it with the values reported by standard laboratories.

Table 1: Types of glycated hemoglobin.[11]

Name	Composition
HbA	Constitutes ≈97 % adult Hb
HbA0	Synonymous with HbA
HbA1a1	HbA with fructose-1,6-diphosphate attached to the N-terminal of the β-chain
HbA1a2	HbA with glucose-6-phosphate attached to the N-terminal of the β-chain
HbA1a	Comprises HbA1a1 and HbA1a2
HbA1b	HbA with pyruvic acid attached to the N-terminal of the β-chain
HbA1c	HbA with glucose attached to the N-terminal valine of the β-chain
Pre-HbA1c	Unstable Shiff base (aldimine); a labile intermediary component in the formation of HbA1c
HbA1	Consists of HbA1a, HbA1b, and HbA1c and other
Total glycated haemoglobin	Consist of HbA1c, and other hemoglobin-carbohydrate adducts

METHODS

The study was conducted in the Department of Biochemistry, MMIMSR, Mullana (Ambala, Haryana). Total number of subjects was 50 (25 males, 25 females), aged 30 to 70 years. 2 ml of blood was collected from antecubital vein under aseptic conditions from each subject and put in EDTA vials. Hemolysed blood was estimated for HbA_{1C} by semi-autoanalyzer.[12]

Table 2: Normal reference range for HbA_{1C}

Normal	≤5.97%
Good Control	5.97-6.81%
Fair Control	6.81-7.65%
Poor Control	≥7.65%

RESULTS

In females, the levels were 6.50 ± 0.74 % while in males the levels were 6.27 ± 0.94 %. The overall range in females was 4.8-7.56 % while in males it was 4.2 to 7.56 %. The values were comparable ($p>0.05$) with those reported by standard laboratories, e.g. Dr. Lal PathLabs (<6 %), Charak diagnostic (4.5-6.3 %) and Mayo Clinic (6.5-7 %).

DISCUSSION

There remain numerous analytical problems associated with glycated hemoglobin measurement, such as the lack of assay standardization and the problems related to its

measurement in particular patient groups with hemoglobinopathies, fetal hemoglobin, renal failure (who form hemoglobin derivatives) and hemolytic diseases. Each method has its own advantages and disadvantages (Table 3).[13]

Methods of GHb assays have primarily evolved around three basic methodologies:[8]

(1) Based on difference in ionic charge.

(2) Based on structural characteristics.

(3) Based on chemical reactivity.

Table 3: Advantages and disadvantages of various HbA1c assay methods.[13]

Assay	Principle	Advantage	Disadvantage
Ion Exchange Chromatography	HbA1c has lower Isoelectric point and migrates faster than other Hb components.	Can inspect chromograms 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	Glucose binds to m-aminophenylboronic acid.	Minimal interference from haemoglobinopathies, HbF and carbamylated Hb.	Measures not only glycation of N-terminal valine on β chain, but also β chains glycated at other sites and glycated α chains.
Immunoassays	Antibody binds to glucose and between 4-10 N-terminal amino Acids on β chain	Not affected by HbE, HbD or carbamylated Hb Relatively easy to implement under many different formats.	May be affected by Haemoglobinopathies with altered amino acids on binding sites. Some interference with HbF.

CONCLUSIONS

Our laboratory levels of HbA_{1C} are comparable with the reference range of different laboratories and hence suitable to be used as cut-offs while interpreting the results of patients with diabetes mellitus. It is recommended that each laboratory should establish its own normal range representing its patient population.

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