

**Original article:**

## **Glycosylated haemoglobin (HbA1c) assays for patients with diabetes mellitus have been developed and validated**

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### **ABSTRACT:**

**Introduction:** Disorders associated with a person's way of life are referred to as lifestyle diseases. Non-communicable diseases are another name for lifestyle diseases (NCDs). It is impossible for these diseases to transfer from one person to another through food, water, or air. These are frequently brought on by drug use, tobacco use, and alcohol misuse, as well as by a lack of exercise, stress, and bad eating patterns.

**Aim :** To Study on sensitive point of care diagnostic assay for detection of glycosylated haemoglobin (HbA1c) in whole blood samples

**Materials and Methods:** The present study was conducted on 120 patients of Diabetes admitted in Medical Wards of Fathima Institute of Medical Sciences and Hospital, Kadapa, Andhra Pradesh.India, with age Group of 40-60 years. During the Months of Jan-Feb 2016.In this 60 were controls (non diabetics) and 60 diabetic patients. Out of sixty (60) cases, 30 were men and 30 were women. A total of 60 whole blood samples were collected in vacutainer with EDTA from people attending the Medicine department at FIMS, Kadapa.

**Conclusion:** Summarises the entire investigation and comes to the conclusion that in-house ELISA kit are useful diagnostic tools to determine HbA1c without the use of a complex instrument. It was discovered that this test's sensitivity and specificity were comparable to those of existing techniques. Consequently, the possibility of false positive testing is reduced. HbA1c readings can be simply interpreted without using any complicated instruments based on the test line's signal intensity. That will allow for the earliest possible diagnosis and control of diabetes.

**Key words:** HbA1c,Diabetes Mellitus, ELISA assay.

### **INTRODUCTION:**

Disorders associated with a person's way of life are referred to as lifestyle diseases. Non-communicable diseases are another name for lifestyle diseases (NCDs). It is impossible for these diseases to transfer from one person to another through food, water, or air. These are frequently brought on by drug use, tobacco use, and alcohol misuse, as well as by a lack of exercise, stress, and bad eating patterns.[1-2] The probability of mortality and morbidity will considerably increase if these risk factors are clustered together. The main cause of death and disability worldwide is lifestyle disorders. According to the World Health Organisation report (The global burden of disease 2004)[3]; 36 million people died due to lifestyle diseases every year, principally due to diabetes mellitus (DM), and cardiovascular diseases (CVD) followed by cancer and chronic respiratory disease[3].

Diabetes develops when your body's cells are unable to absorb sugar (glucose) and use it as fuel. As a result, your bloodstream begins to accumulate additional sugar. Diabetes that is not properly managed can have catastrophic effects and harm a number of body organs and tissues, including the heart, kidneys, eyes, and nerves. Type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and steroid-induced diabetes are only a few of its many subclassifications. The two primary subtypes of DM are type 1 and type 2; each has a unique aetiology, presentation, and therapy, although both can result in hyperglycemia.[2]

422 million people worldwide have diabetes, according to a 2016 WHO report (World Health Organization report, 2016). Diabetes is becoming more and more common every day. The International Diabetes Federation (IDF) estimated that 381 million people worldwide have diabetes in 2013, and by 2030, this number is expected to double. Type 2 diabetes accounts for 85–90% of all instances of diabetes. Diabetes type 2 is more prevalent and occurs all around the world, although it is most prevalent in developing nations. However, the most notable incidence is rising in low- and middle-income nations, especially those in Asia and Africa [4]. When compared to developed nations, the incidence is rising quickly in developing nations due to the trend of changing lifestyles, including a decline in physical activity, an increase in sedentary behaviour, a change in global dietary patterns, an increase in the consumption of foods high in sugar and saturated fats, and rapid urbanisation [5]. According to research by Agardh et al. (2011),[6] lower socioeconomic characteristics are related and associated with a higher chance of developing diabetes, particularly type 2 DM.

In red blood cells, haemoglobin that has been glycosylated (HbA1c) is attached to glucose. In a two-step process, plasma glucose and the N-terminal valine of the chain of haemoglobin interact non-enzymatically to generate HbA1c. Aldimine complex formation, a reversible event, comes first, taking anywhere from a few minutes to several hours. Amadori rearrangement, an irreversible reaction, then produces the stable ketoamine HbA1c. Over the course of a red blood cell's life, this glycation reaction will proceed gradually and continually (120 days). The HbA1c percentage will reveal glycemic history and show the typical blood glucose level over the course of an erythrocyte's entire life [7]

Currently, a variety of detection techniques are employed for the estimation of HbA1c levels. These techniques include high-performance liquid chromatography (HPLC) (Eckerbom et al., 1994)[8], boronate affinity chromatography (Psotova et al., 1995; Frantzen et al., 1997),[9-10] turbidimetric immunoassay, colorimetric method, capillary isoelectric focusing, electrophoresis (ESI-MS). Among the diagnostic techniques discussed above, borate affinity chromatography and HPLC are frequently employed to measure HbA1c.

**Aim:** To Study on sensitive point of care diagnostic assay for detection of glycosylated haemoglobin (HbA1c) in whole blood samples.

#### **MATERIALS AND METHODS:**

The present study was conducted on 120 patients of Diabetes admitted in Medical Wards of Fathima Institute of Medical Sciences and Hospital, Kadapa, Andhra Pradesh, India, with age Group of 40-60 years. During the Months of Jan-Feb 2016. In this 60 were controls (non diabetics) and 60 diabetic patients. Out of sixty (60) cases, 30 were men and 30 were women. A total of 60 whole blood samples were collected in vacutainer with EDTA from people attending the Medicine department at FIMS, Kadapa. 6 mL of whole blood sample was collected from anti-cubital vein from each participant. The present study was approved by the Institutional Ethics

Committee (IEC) of Fathima Institute of Medical Sciences and Hospital, Kadapa, Andhra Pradesh, India.

All study participants provided their informed permission. All participants who attended the diabetes clinic provided their informed permission. All of the entire blood samples that were taken were handled carefully and kept at 40°C pending further usage. All samples in vacutainer tubes were put in red bin bags when the testing process was finished and taken to the biomedical waste department for proper disposal.

**Inclusion criteria:**

Patients clinically diagnosed to have Diabetes Mellitus were selected for the study.

**Exclusion criteria:**

Patients with previous Myocardial Infarction or present Myocardial Infarction and patients with recent infections, liver disease and renal failures are excluded.

**Preparation of reagents:**

All the reagents required for preparation of the coated ELISA plates and for carrying out the initial competitive ELISA Before the start of the following phases, tests were prepared. TMB substrate (3,3',5,5'-Tetramethylbenzidine), coating buffer, washing buffer, blocking buffer, phosphate-buffer saline (PBS), and stop solution were among these reagents (0.5M sulphuric acid). Weighing the appropriate reagents and washed away in the appropriate media.

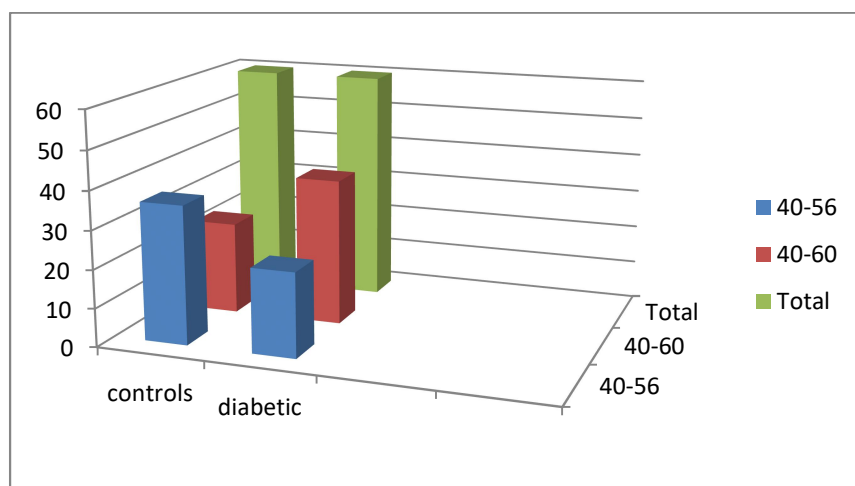
**RESULTS:**

The present study was conducted on 120 patients of Diabetes admitted in Medical Wards of Fathima Institute of Medical Sciences and Hospital, Kadapa, Andhra Pradesh, India, with age Group of 40-60 years. During the Months of Jan-Feb 2016. In this 60 were controls(non diabetics) and 60 diabetic patients. Out of sixty (60) cases, 30 were men and 30 were women.

A total of 120 specimens were involved in this study and their samples were analysed for validation of HbA1c ELISA kit. According to the recommendations made by American Diabetes Association (ADA report, 2009) the study specimens were categorized into controlled DM ( $\leq 6.5$  /HbA1c %) and uncontrolled DM ( $\geq 6.5$  HbA1c %). Based upon the ADA recommendations the 120 whole blood samples were divided into two groups, 60 controls (non-diabetic) and 60 uncontrolled (diabetic ) with the age range from 40- 60 years. Out of sixty (60) cases, 30 were men and 30 were women.

**Table 1: Total number of samples based on Age.**

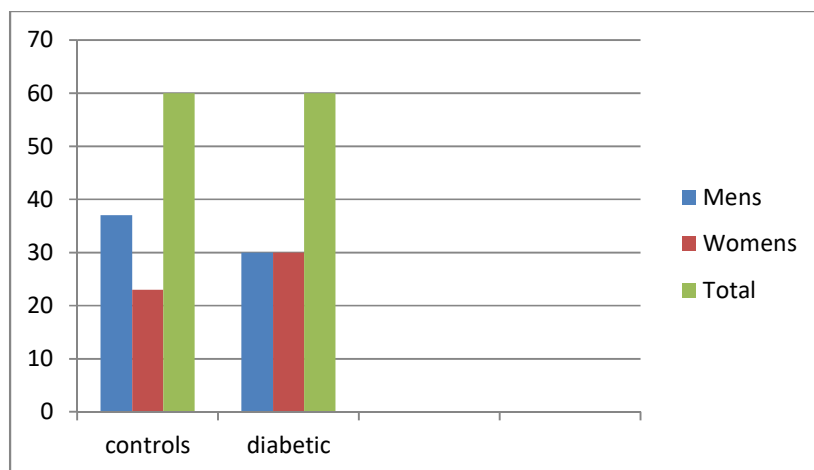
| S.NO                         | Total number of samples(40-56 years) | Total number of samples(40-60 years) | Total |
|------------------------------|--------------------------------------|--------------------------------------|-------|
| controls (non-diabetic)(60)  | 36                                   | 24                                   | 60    |
| uncontrolled (diabetic )(60) | 22                                   | 38                                   | 60    |
| Total                        |                                      |                                      | 120   |



**Graf 01: Total number of samples based on Age.**

**Table 2: Total number of samples based on sex.**

| S.NO                         | Total number of samples(mens) | Total number of samples(Womens) | Total |
|------------------------------|-------------------------------|---------------------------------|-------|
| controls (non-diabetic)(60)  | 37                            | 23                              | 60    |
| uncontrolled (diabetic )(60) | 30                            | 30                              | 60    |
| Total                        |                               |                                 | 120   |



**Graf 02: Total number of samples based on sex.**

**DISCUSSION:**

Diabetes has grown to be a serious health issue on a global scale. In Asia, particularly in South Asia, its incidence and harmful health impacts are rising quickly[11]. Diabetes is a metabolic illness that can cause a variety of health issues, including retinopathy, neuropathy, cardiovascular illness and kidney damage. Most persons suffering from nephropathy and cardiovascular disease. the majority of those who live with Diabetes was underdiagnosed in South

Asia, particularly India. Many people have diabetes for a long time before being recognised in the early stages since it is often asymptomatic or moderate [12].

The beta cells' function had already significantly deteriorated at the time of diagnosis. Screening tests were used to diagnose diabetes earlier in order to limit damage (American Diabetes Association, 2013). Those examinations could aid in delaying, preventing, and managing long-term consequences of diabetes, which lower quality of life and raise mortality and morbidity. American Diabetes Association published diagnostic criteria for diabetes and included haemoglobin A1c as a diagnostic marker for diabetes [13]. In the present study aimed to develop a ELISA test for HbA1c. It uses anti haemoglobin A1c and anti haemoglobin monoclonal antibodies, and the sensitivity and specificity of the assay were 98.40% and 100% respectively. Most diagnostic laboratories now don't meet the requirements for infrastructure, technical personnel and pricy equipment. Due to their high cost, the majority of laboratories rely on a single type of screening test and do not verify results using a uniform procedure. Particularly those living in impoverished nations like India do not receive effective diabetes diagnosis or care. Consequently, diabetes-related problems are spreading quickly, especially those caused by metabolic syndromes such cardiovascular disease and retinopathy. Therefore, it is essential to create a quick, affordable, point-of-care diagnostic approach. Consequently, a sensitive, affordable, and highly specific ELISA test was created in this study for the quick and accurate detection of HbA1c.

#### **CONCLUSION:**

Summarises the entire investigation and comes to the conclusion that in-house ELISA kit are useful diagnostic tools to determine HbA1c without the use of a complex instrument. It was discovered that this test's sensitivity and specificity were comparable to those of existing techniques. Consequently, the possibility of false positive testing is reduced. HbA1c readings can be simply interpreted without using any complicated instruments based on the test line's signal intensity. That will allow for the earliest possible diagnosis and control of diabetes.

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