A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKIN UNIVERSITY

ASSESSMENT OF THE DIFFERENT METHODS TO MEASURE HbA1c

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

MOHAMMED MAJID AZEEZ OBAID

ÇANKIRI

2023

ASSESSMENT OF THE DIFFERENT METHODS TO MEASURE HbA1c

By Mohammed Majid Azeez OBAID

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Assoc. Prof. Dr. Şevki ADEM
Co-Advisor : Asst. Prof. Dr. Riadh Abdulrassol HENWA

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Hakan ÇOLAK
Chemistry
Çankiri Karatekin University

Member : Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kirikkale University

Member : Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankiri Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Mohammed Majid Azeez OBAID

ABSTRACT

ASSESSMENT OF THE DIFFERENT METHODS TO MEASURE HbA1c

Mohammed Majid Azeez OBAID

Master of Science in Chemistry

Advisor: Assoc. Prof. Dr. Şevki ADEM

Co-Advisor: Asst. Prof. Dr. Riadh Abdulrassol HENWA

January 2023

The best method for measuring glucose and which is recommended is the cumulative glucose test method, which is considered a gold standard as recommended by the World Health Organization and ADA. Since HPLC is the reference method for HbA1c assay,we decided in our study to compare two routine methods: Boronate affinity correlation , enzymatic (Diazyme), with (HPLC), in order to find a compatible and interconnected way with (HPLC) to replace it in clinical laboratories, because the HPLC device is very expensive and there is difficulty in using it . Where we worked on 100 blood samples, 50 samples are healthy and the other 50 samples are already diabetic. work was done on three laboratory devices (LIFOTRONIC (HPLC) HbAIc H8 AUTOMETED ANALYZER & GP Immunoassay Flouroscence GETEIN 1100 Immunofluorescence Quantitative Analyzer &430 fully automated mindray chemistry analyzer BS). showed that the mean differences in HbA1c analysis . That there were significant differences in the mean levels of HbA1c, and the P-values were <0.001.

2023, 50 pages

Keywords: HbA1c, HPLC, Hb, Diabetes, Methods, Diazyme, Nycocard

ÖZET

HbA1c ÖLÇÜMÜNDE FARKLI YÖNTEMLERİN DEĞERLENDİRİLMESİ

Mohammed Majid Azeez OBAID

Kimya, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Şevki ADEM

Eş Danışman: Dr. Öğr. Üyesi Riadh Abdulrassol HENWA

Ocak 2023

Glikozu ölçmek için en iyi ve tavsiye edilen yöntem, Dünya Sağlık Örgütü ve ADA tarafından önerildiği gibi altın standart olarak kabul edilen kümülatif glikoz test yöntemidir. HPLC, HbA1c testi için referans yöntem olduğundan, çalışmamızda iki rutin yöntemi karşılaştırmaya karar verdik: Bor afinite korelasyonu, enzimatik (Diazyme), ile (HPLC), uyumlu ve bağlantılı bir yol bulmak için (HPLC) ile değiştirmek klinik laboratuvarlarda, çünkü HPLC cihazı çok pahalı ve kullanımında zorluk var. 100 kan örneği üzerinde çalıştığımız yerde 50 örnek sağlıklı ve diğer 50 örnek zaten şeker hastası. üç laboratuvar cihazı (LIFOTRONIC (HPLC) HbAIc H8 AUTOMETED ANALYZER & GP Immunoassay Flouroscence GETEIN 1100 Immunofluorescence Quantitative Analyzer & 430 tam otomatik mindray kimya analiz cihazı BS) üzerinde yapıldı. HbA1c analizinde ortalama farklılıkların olduğunu göstermiştir. Ortalama HbA1c düzeylerinde önemli farklılıklar olduğu ve P değerlerinin <0,001 olduğu.

2023, 50 sayfa

Anahtar Kelimeler: HbA1c, HPLC, Hb, Diyabet, Yöntemler, Diazyme, Nycocard

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Assoc. Prof. Dr. Şevki ADEM, for his patience, guidance and understanding.

Mohammed Majid Azeez OBAID

Çankırı-2023

CONTENTS

LIST OF SYMBOLS

%	percentage
N	Normal
SD	Standard deviation

LIST OF ABBREVIATIONS

DM	Diabetes Mellitus
EDTA	Ethylene Diamin Tetraacetic Acid
FPG	Fasting Plasma Glucose
GHB	Glycated Haemoglobin
Hb Variants	Hemoglobin Variants
HbA1c	Hemoglobin A1c
HPLC	High Performance Liquid Chromatography
OGTT	Oral Glucose Tolerance Test
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 1 Diabetes Mellitus

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Diabetes mellitus is a category of carbohydrate metabolic diseases in which glucose is both underused and overproduced, resulting in hyperglycemia (Mayfield 1998, American Diabetes Association 2014). The Diabetes of Control and Problems experiment and The United kingdom expected Diabetes research outcomes demonstrated that patients' glycemic status may be closely monitored to prevent diabetes problems' beginning and development (Mohammadi and Norozi 2016) The metabolic disorders associated with diabetes cause pathophysiological variables as a result of high blood sugar in various body systems(Syed 2011, Assadi and Nephropathol 2012, Bryśkiewicz and Majkowska 2011, Gheissari *et al.* 2012).Since complications of diabetes mellitus are assocated with blood sugar control, this makes the sugar level for most patients an appropriate target(Assadi and Nephropathol 2012, Bryśkiewicz and Majkowska 2011, Gheissari *et al.* 2012). Blood glucose and glycated haemoglobin are the two tests that are most frequently used to monitor glycemic status. (Mohammadi and Norozi 2016). Fasting blood glucose(FBG) measurements are used for short term to preview and have little relevance for evaluating glycemic management over the long run. (Mohammadi and Norozi 2016, Sudhakar *et al.* 2014). Additionally, it is impacted by the most recent meal as well as other factors including stress and drug use (Rifai 2017, McPherson and Pincus 2021). Therefore, measuring blood glucose levels to determine the degree of glucose control is insufficient. Measuring HbA1c, the primary glycated haemoglobin, is now frequently and widely utilised in Clinical laboratories for the long-term evaluation of glycemic status (Wan Mohd Zin *et al.* 2013, Hector *et al.* 2009). Glycated haemoglobin (GHB), It is widely used to diagnose diabetes, also called as haemoglobin A1c, is a biochemical test that tracks In the long run glycemic management and calculates the likelihood of developing problems (Diabetes Control and Complications Trial Research Group 1993, Pinzur 2011). The term "glycated hemoglobin" (GHb), also known as "HbA1c", refers to haemoglobin that has undergone an irreversible alteration as a result of the gradual, non enzymatic addition of glucose. Hemoglobin that has undergone irreversible glycation at either one or both of the N_terminal valines of the chains is known as HbA1c, as per the International Federation

for Clinical Chemistry (IFCC). And the ratio of GHb manufacturing is immediate related to natural glucose levels(Schnedl *et al.* 2005). Using HbA1c as a biological guide to identify and manage diabetes has been endorsed by both (ADA) and (WHO) (ADA 2014).which included members selected through the ADA, the International Diabetes Federation, and the European Association for the Study of Diabetes, Advised to diagnose diabetes by measuring hemoglobin (HbA1c), which represents long term blood glucose of concentrations (ADA 2010)Glycohemoglobin may be measured using several different techniques, (Syed 2011, Bryśkiewicz and Majkowska 2011). However, the disparity in the reported values by different methods is substantial, making it very challenging to compare these data (Bryśkiewicz and Majkowska 2011). Furthermore, a number of factors, including as the kind of anaemia, pregnancy, splenectomy, transfusion, and drug intake(salicylates), have an impact on various techniques (Syed 2011, Bryśkiewicz and Majkowska 2011). The economical method is considered to be an effective method as it is accurate, practical, convenient and cost-effective(Bodor *et al.* 1992, Turpeinen and Stenman 2020). High-performance liquid chromatography (HPLC) It is a reference method in unifying the rest of the other routine methods due to its accuracy, validity and long-term stability(Jeppsson 2002). In addition, the HPLS-based assay has been proven to support the possibility of comparison between different other methods(Bodor *et al.* 1992, Penttilä *et al.* 2016).

1.1 Objectives

The goal of this research is to compare two different ways using HPLC to ascertain which approach has a sufficient concordance and connection with the reference method. Many professionals are not entirely satisfied with the asymmetry of the HbA1c, which was measured in different ways, compared to the reference (HPLC) method. The HPLC apparatus, in contrast, is highly costly. and there is difficulty in using it as it takes a long time to work, and it also needs professional staff to work on it , which makes it difficult in contrast, diabetic patients also need frequent examinations from HbA1c, and most cannot afford the cost of (HPLC) so in our study we opted to examine two conventional methods: boronate affinity binding (Nycocard), enzymatic (Diazyme), and high

performance liquid chromatography (HPLC). To know the compatible and interconnected method with (HPLC) to replace it in clinical laboratories.

2. LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a group of turmoils that affect the metabolism of proteins, lipids, and carbohydrates. It is characterised by chronic hyperglycemia that can be caused by problems with insulin secretion or action of insulin or both inadequate secretion and ineffective action . According to recent epidemiological data, 9% of adults aged 18 and older had diabetes mellitus, and 1.5 million people died from the ailment in 2012. Diabetes will be the seventh leading cause of mortality by 2030, according to the World Health Organization (Mathers and Loncar 2006).

2.1.1 History

Diabetes is described in an Egyptian text dating from around 1500 B.C. where this manuscript spoke of “extremely large emptying of urine” (Poretsky 2010). When such a circumstance arises, The Ebers Papyrus includes writing an advice to drink anything. It is also likely that these cases are of the first type of diabetes (Poretsky 2010). Around the fifth century BC, the well-known Indian physician Sushruta published his treatise Samhita, determined through his work diabetes, using the name madhumeha (honey_ same urine) and it doesn't just refer to the sweet taste, but also referred to the viscosity in texture, as well as its ability On bringing ants, Sushruta also mentioned that diabetes is linked to Excessive intake of foods like grains and rice and sweets (U.K. Prospective Diabetes Study Group 1998, Mohammad *et al.* 2017). The first to use the term "diabetes" or "passage" was the Greek Apollonius of Memphis in the year 230 BC (Poretsky 2010). The work of Aretaeus of Cappadocia is the oldest surviving work on diabetes with detailed reference in the (The second or early third century AD). He described the disease and its symptoms, which he attributed to dampness and cold (Laios *et al.* 2012). The two types of diabetes were first identified in 400-500 AD by Indian doctors, Sushruta and Charaka, and that the two types are separate from each other, as one of them is related to youth and the other is related to being overweight (Poretsky 2010).

2.1.2 Types of diabetes mellitus

- **Type 1**

Diabetes is brought on as a result of autoimmune devastation of the pancreatic islet beta cells(Eisenbarth 2005). This type can be classified as immunologically mediated or of unknown etiology. Most of the type 1 diabetes has an immunological nature, as autoimmune attacks by T cells, as a result of which beta cells and insulin are lost. (DM) Type one diabetes mellitus is partially hereditary, With many genes, includ some HLA of genotypes,that affecting the risk of developing the disease for people who are more likely to have genetic factors, as the disease can occur due to one of the environmental variables, such as a viral illness or nutrition (Butalia *et al.* 2016). But there is no directory on that many viruses are involved (Serena *et al.* 2015). Among the nutritional factors, according to the data, Gliadin may play a role in the development of type 1 diabetes, however the mechanism is not entirely known(Visser *et al.* 2009, Laugesen *et al* 2015). Most persons with type one diabetes enjoy good health and a normal weight when the disease appears. Insulin sensitivity and Insulin responsiveness are frequently normal, Especially at the beginning of the condition. Dubbed Juvenile diabetes mellitus because to its prevalence in children, however the most of affected children have grown into adults (American Diabetes Association 2013).

- **Type 2**

Diabetes is brought on via lowering insulin output and impedance of insulin's effects (Eisenbarth 2005). Cardiovascular disease is still the main reason for death in people with type Two DM, Notwithstanding the morbidity and mortality linked to neuropathy, retinopathy, and nephropathy (Baynes 2015, Mahler and Adler 1999). Therefore, the influencing factors like obesity, hyperlipidemia and Hypertension must be addressed, as they are of great significance and must be accompanied by proper blood sugar control to reduce the number of deaths in type Two diabetes (United kingdom Prospective Diabetes Study Group 1998, Mohammad *et al.* 2017). People with low fasting blood sugar and blood glucose tolerance are more likely to development diabetes (Alberti

1996, Rezki *et al.* 2021). About Of these patients, one-third will later development diabetes, but exercise and Dietary adjustments can reduce the probability of progression from poor glucose tolerance to type 2 diabetes, as well as the development of glucose intolerance disorder in healthy individuals at high risk(Alberti 1996, Rezki *et al.* 2021). Type Two diabetes is the most widely popular (IDF Diabetes Atlas Committee 2019). It accounts for more than 90% of all cases and is the fifth highest cause of mortality in adults aged 50 to 74 years (Murray 2020). One of the reasons for the high blood glucose level is a decrease in secretion of insulin or a weak insulin's influence on its receptors. Type 2 diabetes mellitus is characterized via insulin resistance associated with a relative decrease in secretion of insulin (Shoback and Gardner 2018). There is evidence of prediabetes type two (imbalance of glucose during fasting - or impaired glucose tolerance) for many people with this disease before it is confirmed, as these people may delay the development of prediabetes and turn it into diabetes through lifestyle changes or Through medications that enhance insulin sensitivity or decrease hepatic glucose production (Edge *et al.* 1999, Natarajan *et al.* 2020). Type 2 diabetes is primarily due to lifestyle factors and genetics (Malik 2017).

- **Gestational diabetes**

There is a great similarity between Gestational Diabetes and type 2 DM in several things, the most important of which is a combination of relatively insufficient insulin secretion and response. It is estimated in about 2-10% of pregnancies, and it may improve or end completely after delivery (American Diabetes Association 2011). It is preferable for all pregnant women to take a blood sugar test from the twenty-fourth to the twenty-eighth week of pregnancy (Soldavini 2019). It is usually diagnosed in the second or third trimester of pregnancy, as a result of the increase in insulin-antagonist hormone levels that occurs at this time (Soldavini 2019). In addition, following pregnancy, Approximately 5-10% of women with gestational diabetes are found to have a different type of diabetes, often type 2 (American Diabetes Association 2011). It is possible to treat gestational diabetes completely under very careful medical supervision throughout pregnancy (Hansen and Scheier 2019). Undiagnosed gestational diabetes

may harm the health of the fetus's or the mother's health, as the child may be exposed to many risks, some of which threaten his life (Tarvonen *et al.* 2021).

- **Other types**

Mody is an uncommon autosomal dominantly, Diabetes that is passed on via families caused by one of numerous single gene of mutations that cause insulin production problems (Young 2020). It is far less prevalent than the other three primary kinds, accounting for only (1 - 2 %) of all cases. This is known as illness alludes to early speculations about its characteristics. Because it is caused by a faulty gene, this illness ages differ of onset and severity depending on the individual gene deficiency, There are about 13 types of MODY that people who suffer from it can control it without the need to use insulin (Thanabalasingham 2011).

Double diabetes is yet another type of diabetes that people might get. This occurs when a type 1 DM develops insulin resistance, a symptom of type Two DM, or if there is a familial history of type Two DM, Its first detected was in the year 1990 and it is also said in 1991 (Cleland *et al.* 2013).

2.1.3 Signs and symptoms

Type 2 diabetes constitutes about (85-95%) of all types of diabetes, as it is initially without symptoms from the subclinical stages and remains undiagnosed for a period of time that may extend to years (Atlas 2013). Unintentional weight loss, polyuria (excessive urination), polydipsia (excessive thirst), and polyphagia (increased appetite) are the hallmark signs of uncontrolled diabetes mellitus (Cooke and Plotnick 2008). Type One of diabetes mellitus symptoms could be immediate, but type Two diabetes mellitus significantly slower onset of symptoms and may be subtle or nonexistent (Zheng 2021). Several other signs and symptoms, while not specific to diabetes, can indicate the disease's beginning. In addition to the previously mentioned symptoms, they comprise impaired vision, headache, tiredness, itching skin and inadequate wound

healing. Prolonged high blood glucose levels can induce absorp of glucose in the eye's lens, causing changes in its shape and, as a result, visual alterations. It's possible for diabetic retinopathy to result in permanent vision loss. A set of skin rashes known as diabetic dermadromes can develop as a result of diabetes (Rockefeller 2015).

- **Diabetic emergencies**

Diabetics may be also develop DM ketoacidosis, a Metabolism disorder marked by stomach, vomiting, and nausea, Acetone odour on the breath, heavy breathing called breathing of Kussmaul, and in extreme instances, a lowered level of awareness. diabetic ketoacidosis necessitates hospitalisation for immediate treatment. Hyperosmolar hyperglycemic state, is the most widely frequent in type Two diabetic and is mostly caused by dehydration produced by high blood glucose, is an uncommon but more hazardous disease (Kitabchi *et al.* 2009).

Depending on the medicine used, low blood sugar caused by therapy (hypoglycemia) is prevalent in patients with type One diabetic and type Two diabetic. The majority of instances are minor and do not constitute medical emergency. In moderate situations, the consequences might range from sensations of discomfort, perspiration, shaking, and rise hunger to more significant effects like disorientation, behavioural modifications like aggression, convulsions, unconsciousness, and, in rare circumstances, irreversible brain damage or death (Kenny 2014).

- **Complications**

The major long-term complications are related to vascular damage. Diabetes doubles the risk of cardiovascular disease (Sarwar *et al.* 2010). As about 75% of deaths among diabetic patients are due to coronary artery disease (Yancy *et al.* 2013). Traditional diabetes mellitus complications include macrovascular disorders like coronary heart disease, stroke, and peripheral artery disease as well as microvascular problems like diabetic kidney disease, retinopathy, and peripheral neuropathy (MJ 2008). The

metabolic disorders associated with diabetes cause pathophysiological variables as a result of high blood sugar in various body systems (Syed 2011, Assadi and Nephropathol 2012, Bryśkiewicz and Majkowska 2011, Gheissari *et al.* 2012). Since complications of diabetes mellitus are assocated with blood sugar control, this makes the sugar level for most patients an appropriate target (Assadi and Nephropathol 2012, Bryśkiewicz and Majkowska 2011, Gheissari *et al.* 2012). The Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), two significant clinical trials, have shown that comprehensive treatment and monitoring of type 1 diabetes (T1DM) and type 2 diabetes (T2DM) can reduce complications of diabetes (Diabetes Control and Complications Trial Research Group 1993, Mohammad *et al.* 2017).

2.1.4 Pathophysiology

The body obtains glucose from 3 basic sources of food absorption in the intestine, glycogenolysis (the demolition of glycogen, the liver's storage form of glucose), and gluconeogenesis (the creation of glucose in the body from non-carbohydrate substrates). Insulin is required for the body's glucose levels to be controlled. Gluconeogenesis or the breakdown of glycogen can both be inhibited by insulin, speed up glucose transfer into cells of fat and muscle, and increase glycogen storage (Gardner and Shoback 2007).

In response to rising blood glucose levels, The circulation receives the release of insulin by β cells in the pancreatic islets of Langerhans. 2/3 of the cells in the body use insulin to take glucose from the blood, transform it into other molecules, store it, or utilise it as fuel. When the blood glucose level is low, beta cells generate less insulin and glycogen is broken down into glucose. This process is primarily driven via the glucagon hormone, which functions in the reverse way as insulin (Barrett *et al.* 2010). If there is insufficient insulin available, or if cells do not react well to insulin's effects (resistance to insulin), if the insulin is flawed, the body's cells that need glucose cannot adequately absorb it, because it is not properly stored in the liver or muscles. As a result, there is continuously excessive blood glucose, insufficient protein synthesis as well asother

metabolic abnormalities, as in situations of total insulin deficiency, metabolic acidosis (Gardner and Shoback 2007).

2.1.5 Diagnoses

Diabetes is diagnosed by detecting hyperglycemia. For a long time, The sole technique is Direct recommendation for diagnosis confirmation of hyperglycemia via measuring high glucose levels of glucose in the blood (National Diabetes Data Group 1979, WHO 1980, Szmuilowicz *et al.* 2019). To standardise the diagnosis, a set of measures according to the division of glucose focus at big danger groups was devised in 1979 (National Diabetes Data Group 1979, ADA 2015).The WHO has approved these proposals(WHO 1980, Szmuilowicz *et al.* 2019). The amended criteria included the following:

1) FPG value above or equals (seven mmol per liter)

2) The glucose concentration after a two-hour glucose tolerance test is 11.1 mmol per liter

3) Diabetes symptoms (That is, independent of the timing of the previous food) (Mayfield 1998, American Diabetes Association 2014).

Previously, FPG was employed as a criteria for diagnosing DM, either alone or in conjunction with an oral glucose tolerance test, which included included a 2 - hour level. This procedure is sensitive, but it is not reproducible, and patients must fast overnight before each test. Glycated haemoglobin (HbA1c), first identified by Rahbar et al in 1969 (Rahbar *et al.* 1969, Porta and Jörgens 2020). It was recommended as a technique for diabetes diagnosis and treatment monitoring. The International Expert Committee advised in 2009 that a HbA1c $\geq$ 6.5 % (48 mmol per mol) be employed in the diagnosis of diabetes (Gillett 2009). The American Diabetes Association has also backed this viewpoint (ADA 2010). The International Advisory Committee met in 2009

(Gillett 2009). Which included members selected through European Association for the Diabetes Study, the ADA, and the International Diabetes Federation, advised that diabetes be diagnosed by measuring haemoglobin (HbA1c), which represents long term blood glucose focus. The ADA (ADA 2010).

- **HbA1c**

Glycated haemoglobin (GHB), also known as haemoglobin A1c, is a biochemical test that is frequently used in the management of persons with DM to measure long_term glycemic management and hazard assessment of problems (Diabetes Control and Complications Trial Research Group 1993, UK Prospective Diabetes Study (UKPDS) Group 1998, Mohammad *et al.* 2017). Hb is one of the proteins that succumb non-enzymatic glucose and glycohemoglobin. GHBS was first described as glycated hemoglobin in 1968 by RaHBaR (Rahbar 1968, Porta and Jörgens 2020). include Potential glucose binding sites for haemoglobin Amolecule the N_terminal valine The four polypeptide chains' amino acids and as well as all of the lysine residue's free -amino. Whereas, the N_terminal valine remains in the chain is a glucose site and accounts for 60% of glucose that is bonded (Nuttall 1998, Arnold *et al.* 2020). Hemoglobin A1 (HbA1) is detected by cation exchange chromatography which has a negative charge greater than HbA. It consists of HbA1a, HbA1b, and HbA1c, which are referred to by their names in the order in which they emerged from the column, with HbA1c being the most frequent, defined as HbA that is irreversibly degraded by IFCC at one or both of the N_terminal values of the chains. (Shapiro *et al.* 1980, Hudson *et al.* 2018). A trustworthy and comprehensive determining the average blood sugar level during the lifetimes of circulating red blood cells is the Glycated hemoglobin concentration (ie, two to three months) (Nathan *et al.* 2007). Measurement of Glycated hemoglobin, whether HbA1c or total Glycated hemoglobin, is commonly used in clinical laboratories to monitor blood sugar. Current ADA recommendations advise HbA1c testing for diabetics once every six months, with a target of less than 7%. It also recommends that the goal of treatment is for a Glycated hemoglobin concentration to be less than 7% and that treatment should be changed or re-regulated if GHb values consistently reach >8%, as these values are based only on assay methods approved as

being traceable to the reference technique (Krishnamurti and Steffes 2001, Thiruppathi *et al.* 2021). The extensive Diabetes Control,Complications Trial (DCCT) has demonstrated that optimal glycemic control significantly decreases an increase in diabetes complications and delays their onset. Additionally, there is a relationship between HbA1c levels and mean plasma glucose, every 1% rise in HbA1c level equating to an increase of about 2 mmol/L in mean plasma glucose (Diabetes Control and Complications Trial Research Group 1993, Pinzur 2011). The results of DCCT and UKPDS revealed a strong association between diabetes mellitus long term problems and blood hemoglobin A1c levels, and Hb A1c assay has since to be the gold standard for measuring hyperglycemia. There are also several analytical issues related to the measurement of HbA1c, such as standardization of the assay, hemoglobin variants in patients with hemoglobinopathy, and the haemoglobin derivatives are seen in uremic individuals (Mohammadi and Norozi 2016). There are several methods for calculating blood HbA1c. The outcome of one patient's blood HbA1c tested using different techniques or kits may be significantly different. As a result, doctors are frequently concerned about the trustworthiness of hemoglobin A1c test findings, which may have an impact on patient therapy (Little 2009). These methods must be standardized to obtain the same HbA1c readings in total blood sample using others methods. The NGSP standardizing began standardizing hemoglobin A1c tests in 1996. The ADA also suggests using N.G.S.P -approved assays to measure hemoglobin A1c in whole blood (Little 2009).

There are four main methods that are most commonly used to measure HbA1c (immunoassay, HPLC, boronate affinity, enzymatic assays).

- **Immunoassay**

A biological test known as an immunoassay that determines utilising an antibody or an antigen (typically), the presence or concentration of a macromolecule or a small molecule in a solution (occasionally). The molecule identified by the immunoassay is generally referred to as a "analyte" and is often a protein, however it can be other sorts of molecules of various sizes and kinds so long as the appropriate antibodies with the

requisite characteristics for the test are created. Immunoassays are commonly used for medical and scientific purposes to evaluate analytes contained in biological fluids such as serum or urine (Yetisen *et al.* 2013). One of the most popular methods for measuring HbA1c at present is immunoassays that are used in laboratories of the clinical according to the laboratories of the clinical that follow (College of American Pathologists) HbA1c Aptitude Test plan (Molinaro 2008). Immunoassay techniques employ antibodies capable of detecting accumulating N-terminal glycated amino acids (Edison *et al.* 2012). When an antibody that identifies the variations is employed, the most prevalent variants (Hemoglobin S and Hemoglobin C) will produce analytical interference, leading to the creation of immunoassays. These antibodies are thought to bind Hemoglobin S similarly to Hemoglobin A since they provide correct Hemoglobin A1c values in individuals with Hemoglobin S and heterozygous Hemoglobin variable situations (Rhea *et al.* 2013).

- **HPLC**

It is an analytical chemical technique used to detach, quantify and identify all component within a admixture. It goes through a solid-filled column absorbent a solvent a pressurized liquid containing the sample mixture inside As each component in the sample reacts slightly differently with the adsorbent, It creates distinct flow rates for each component, and the components are separated as they exit the column. Because of the different degrees of interaction of each component of the sample with the absorbent molecules, the components of the sample mixture separateThere are different kinds of HPLC as in ion exchange, convergent chromatography, reverse the phase, partitioning, Exclusion based on size, and usual stage (Wikipedia website). High performance liquid chromatography is the manner of reference for standardizing other manner of reference due to its reliability, accuracy and stability in the results compared to other methods (Bodor *et al.* 1992, Turpeinen and Stenman 2020). The economical method is an accurate and very cost-effective method as well as a convenient operation (Bodor *et al.* 1992, Turpeinen and Stenman 2020). HbA1c measured by HPLC is susceptible to hemoglobinopathy (Xu 2016). Presently, ion-exchange HPLC is the more often used method for measuring HbA1c. also, in a patient with variable haemoglobin, HbA1c

determined by an HPLC test in standard mode (SM-HPLC) may not be reliable (Koga 2014) . Based on the charge difference, the HPLC separates the ion exchange between the types of hemoglobin as showing in (Figure 2.1). Models with sophistication of HPLC may be used to eliminate the problems of Hemoglobin products, as in: Hemoglobin A1a, Hemoglobin A1b, Hemoglobin F, labile Hemoglobin A1c, HemoglobinA1c, HemoglobinA0, and HemoglobinA2, Hemoglobin by-products or Hemoglobin variants (Rhea and Molinaro 2014). Affinity chromatography, immunoassays, and enzymatic tests have also been developed, and HbA1c levels are presently tested using a variety of techniques (Figure 2.1). While these tests are less precise than an HPLC assay, they offer the benefit of displaying correct HbA1c readings in the majority of individuals with variable haemoglobin (Koga 2014, Miyazaki *et al.* 2012).

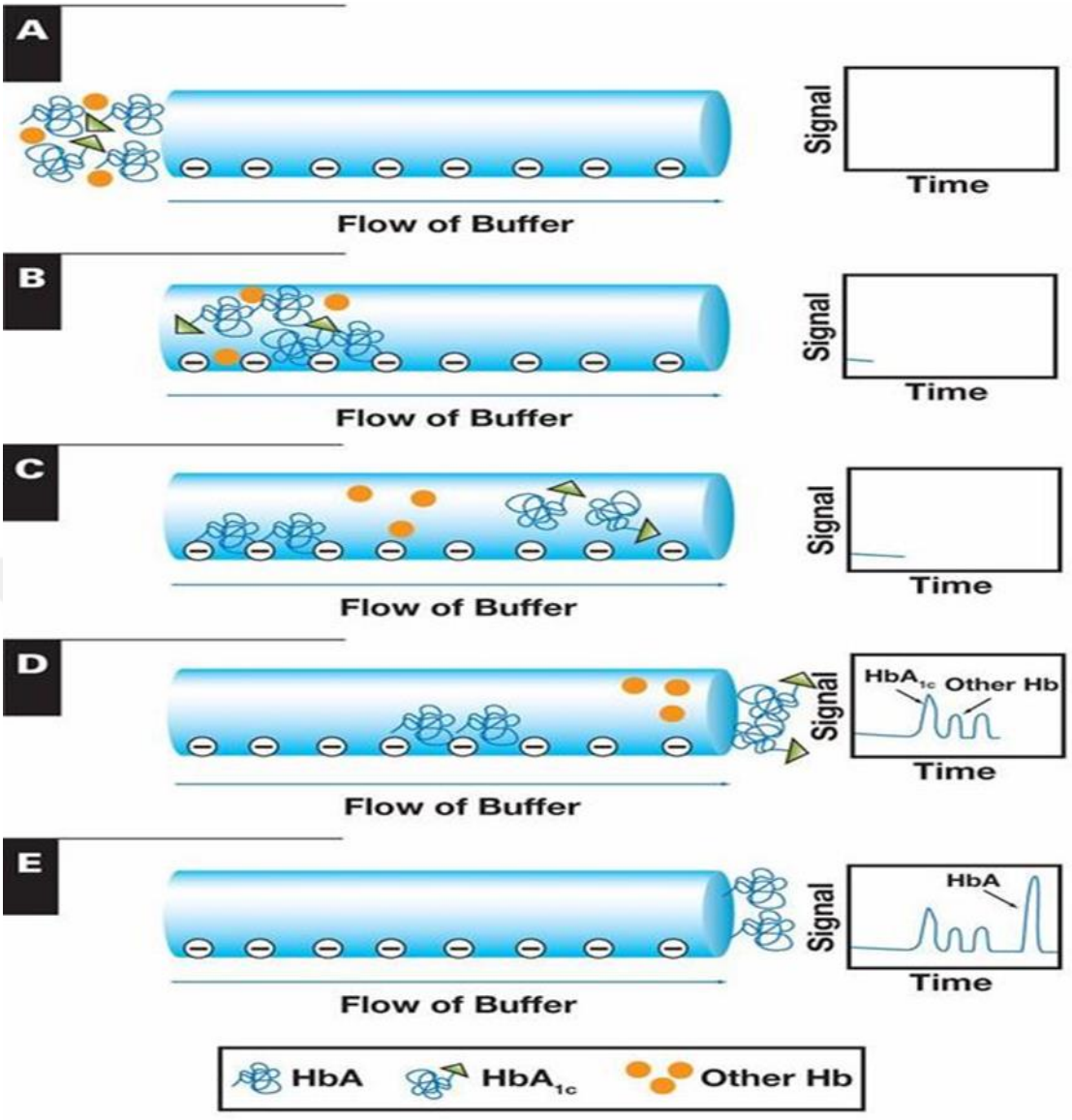

Figure 2.1 Hemoglobin A1c testing using ion-exchange HPLC (Rhea and Molinaro 2014)

Ion exchange divides Hb molecules according to their charge. The patient's RBC lysate is put into a negatively charged column. Positively charged Hb molecules move more slowly than negatively charged ones due to ionic interactions with the negatively charged resin. D, HbA1c elutes first because it is more negatively charged than HbA0. There is no scale representation of the HbA chromatogram trace (E). Ion-exchange HPLC may sometimes resolve and presumptively identify other Hb species and Hb variations. These would show up as new peaks on the chromatograph (Rhea and Molinaro 2014).

- **Boronate affinity chromatography**

Glycated haemoglobins (GHb), glycated plasma proteins (GPP) vary using a sugar moiety to link to non-glycated proteins (s) via a ketoamine bond at distinct binding sites. As a result, GHb and GPP possess 1,2-cis-diol groups not seen in non-glycated proteins, which may be used to separate glycated and non-glycated Contents using boronate affinity chromatography. A boronate, like phenylboronic acid, is attached to the column support's outside surface in this analytical procedure. Due to the complexing of its diol groups with the boronate when a protein solution passes through the column, the glycated contents is preserved. as shown (Figure 2.2). Following the elution of the column's unretained non-glycated component, the glycated component is eluted using a reagent that dislodge it from the boronate (Mallia *et al.* 1981, Gerber *et al.* 2015). A technique that identifies the glucose cis-diol groups coupled to haemoglobin is called boronate affinity (Little 2009). It usually shows the least amount of analytical overlap resulting from the presence of heterozygous haemoglobin variants(Bry 2001). Glycated haemoglobin affinity separation commonly employs m_aminophenylboronic acid and is based on a particular The interaction of glucose glycated hemoglobins and immobilised boronic acid. By the Glycated interact hemoglobin and boronic acid steady on agarose gel, also by the ionic and emergency forces of water, the incoherent haemoglobin and glycated haemoglobin products are removed separately from the column.The visible Separation of glycated and nonglycated substances using chromatography haemoglobin products is provided by this amethod. Although boron affinity assay can Measurement of total glycated hemoglobin, As a result, the data are given as adjusted haemoglobin A1c equivalents (Figure 2.2). It also occurs with enzymatic assays and immunoassay hemoglobin A1c ,The last user cannot distinguish the hypothetical presence of hemoglobin variables when using boron affinity, and it is very likely that it affects hemoglobin A1c clinical interpretation results in all instances where the age of red blood cells changes (Rhea *et al.* 2013).

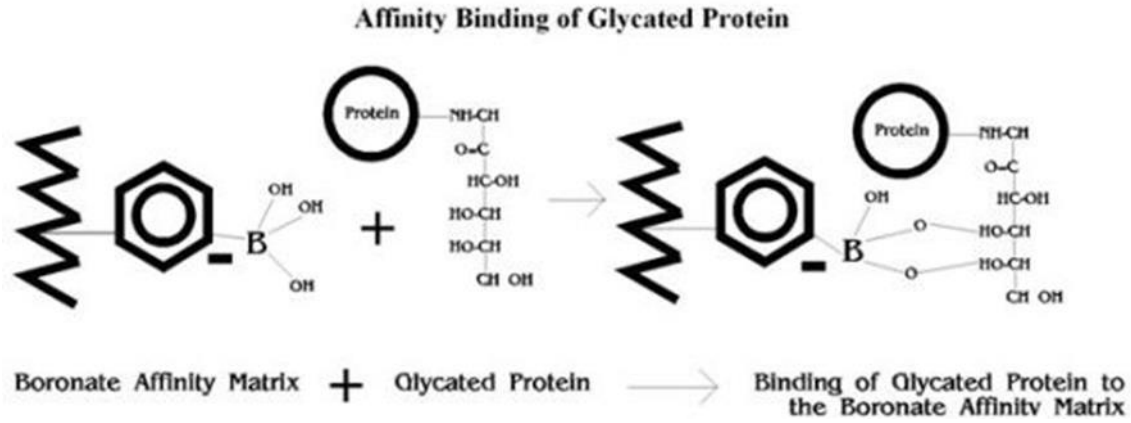

Figure 2.2 Affinty Binding Of Glycated Protein (Mallia *et al.* 1981)

- **Enzymtic methods**

Standard methods for HbA1c available are: high-performance liquid chromatography (HPLC), affinity chromatography, immunoassay, and enzyme assay (EA) (Lenters 2017). EA is one of the widely used methods for measuring HbA1c, as it has a lower cost and its ability to measure an increased quantity of samples and very quickly compared to different methods. As shown by Otabe *et al.* HbA1c results measured by EA (EA-HbA1c) are lower than HbA1c results measured by HPLC (HPLC-HbA1c) (Otabe *et al.* 2017). We also recently learned that HbA1c results measured by EA in clinical laboratories are lower than those measured by HPLC (Koga *et al.* 2020). Chromatographic assays and immunoassays for determining HbA1c concentration are the most common methods, and many methods have been developed to measure HbA1c due to productivity, cost, specificity and accuracy, some of these methods rely on the measurement of enzymes of HbA1c (Kim *et al.* 2012, Ferri *et al.* 2013, Shahbazmohammadi *et al.* 2019, Ogawa *et al.* 2019) To facilitate the enzymatic methods, this is done by the proteolytic digestion of Hb and the following particular cleavage of fructosamine by fructosyl oxidase - amino acids, which can be photometrically measured using the latest procedures, mostly depends on leuco stain (Kim *et al.* 2012, Ferri *et al.* 2013, Shahbazmohammadi *et al.* 2019). Whole blood samples are broken down into amino acids and tiny peptides using this approach (protein digestion). Hemoglobin A1c is a glycated Hb It contains glucose is selectively

linked to the Hemoglobin chain's N-terminal valine. N-terminal valine glycated from hemoglobin chains is released by proteolytic digestion. Glycated valine is a substrate for an enzyme that cleaves the N_terminal valine and creates hydrogen peroxide, which is then exposed to a chromogenic process. The resulting signal is proportional to the quantity of A1c in the sample. This approach, like immunoassay, offers just an A1c result and no information on the existence From Hb variants or convertible hemoglobin and carbamylated (Rhea and Molinaro 2014).

- **Capillary electrophoresis**

HbA1c reference techniques were accepted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 2002. High-performance liquid chromatography (HPLC) and mass spectrometry using electrospray ionisation, as well as a 2-dimensional technique employing HPLC and capillary electrophoresis (CE) with ultraviolet (UV) detection, were employed to separate and quantify HbA1c (Jeppsson *et al.* 2002). Sebia (Lisses, France) newly created a fluid- flow capillary electrophoresis automation system (CE) approach for measuring hemoglobin A1c using a Capillarys Two Flex Piercing equipment. This is the first capillary electrophoresis technique authorised by the HbA1c measurements are authorised according to the Food and Drug Administration of the United States of America. The equipment enables total automation for the rapid separation and measurement of hemoglobin A1c in free solution utilising the liquid-flow capillary electrophoresis principle. Electrophoretic mobility, in addition to electrolyte pH and electroosmotic flow, are used to separate charged molecules in a alkaline buffer in combination with a certain pH. Absorption spectroscopy is used to identify hemoglobins near the capillary's cathodic end (Tesija *et al.* 2018). Capillary electrophoresis divided it hemoglobin molecules based on of of hydrodynamic charge and size (Figure 2.3), and the separation depends on the electrolyte pH and electroosmotic flow. By this method, different Hb products can be identified, and it can also identify other Hb by-products or Hb variants. Studies have shown the most prevalent heterozygous hemoglobin variants (hemoglobin S, hemoglobin C, hemoglobin D, hemoglobin E) analytically overlaps with quantitative hemoglobin A1c. Using Capillary electrophoresis (Rhea and Molinaro 2014).

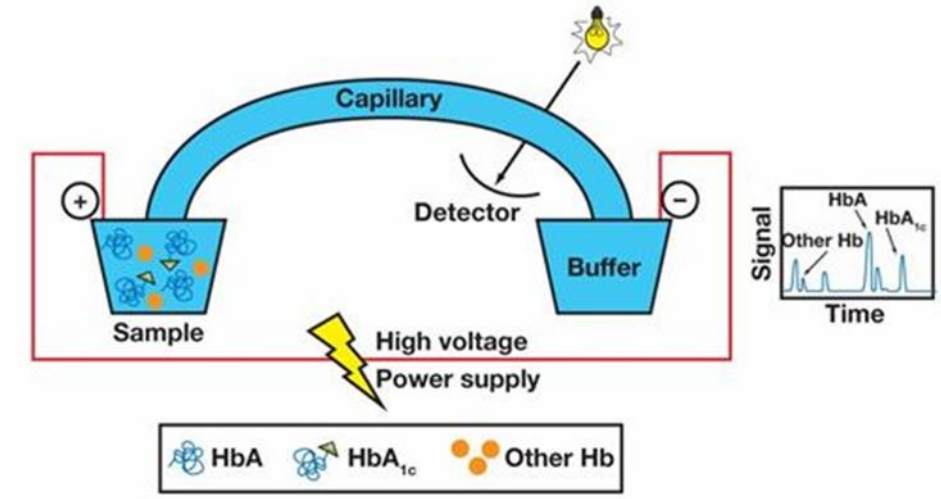

Figure 2.3 Capillary electrophoresis (CE) to measure hemoglobin (Hb) (Rhea and Molinaro 2014)

Capillary electrophoresis (CE) to measure hemoglobin (Hb) A1c. Hemoglobin types are separated by capillary electrophoresis (CE) based on their electrophoretic mobility, where a strong electric current source is utilised to produce a field of electricity that allows molecules to travel from the anode to the cathode through the capillary tube In the patient's RBC analyzer, the positively charged haemoglobin types are recognised first, in descending a list of the charge-to-mass ratios. Two elements that share a positive charge, for example, it will be decided by size, with the bigger particle travelling faster. Following these is neutral hemoglobins and, eventually, negatively charged hemoglobins. The example electrograph (not scaled) depicts the separation of HbA1c and HbA0 (Rhea and Molinaro 2014).

- **Hb Variants**

Hemoglobin variations are caused by genetic mutations or elimination in the genes that encode the α chain, resulting in differences in amino acids. HbS, haemoglobin E, haemoglobin C, and haemoglobin D are the four most frequent types (Rhea and Molinaro 2014). The HbA1c result may be inaccurate due to analytical interference or due to biological variables, which alter the lifespan of RBCs (Rhea *et al.* 2013).

- **Hemoglobin S**

People who carry one (heterozygous) copy The sickle cell trait is described as a result of the mutated gene. (hemoglobin AS), and people who have two defective copies (homozygous) of the gene suffer from sickle cell anaemia (hemoglobin SS). People in the United States have sickle cell trait, and about 90,000 - 100,000 people are influenced by sickle cell anaemia (Apanah and Rizzolo 2013).

What characterizes sickle cell anaemia is the development of long and inflexible chains of hemoglobin Which can facilitate erythrocytes from getting stuck in small capillaries, resulting in severe harm to organs. They have sickle cell disease and the lifespan of RBCs is usually shortened by approximately (10 to 20 days for hemoglobin SS vs 120 days for hemoglobin AA) (Marino 2013).

- **Hemoglobin C**

The HbC allele accounts for approximately 50% of people in West Africa, where this type of hemoglobin has spread to many other regions, and approximately 3% is transmitted through African Americans, that carriers of one allele of the HbC variant are healthy, while those with disease HbC also develop moderate hemolytic anaemia. The erythrocyte The average lifetime of patients with the haemoglobin C allele is 87 days (Rhea *et al.* 2012).

- **Hemoglobin E**

The Hemoglobin E allele is widespread in Southeast Asia and derives from a mutation in the Hemoglobin A chain where the lysine at position 26 replaces the glutamic acid. Through the activation of a cryptic messenger ribonucleic acid splice site, this decreases the synthesis of the β chain of haemoglobin E and results in a phenotype similar to thalassemia. A carrier rate of about 30 % exists among Southeast Asians residing in the

USA. Although Hemoglobin E is clinically asymptomatic, its effect on erythrocyte lifespan has not yet been established (Rhea *et al.* 2012).

- **Hemoglobin D**

It is found among North Indians and Pakistani and Afghan descendants residing in the USA at a rate of about 2%. It is formed as soon as the glutamic acid at site 121 on the HbA chain is converted to glutamine. The most common variant of HbD is HbD-Punjab / Los Angeles, while there are many variants of HbD. people heterozygous for HbD have no clinical symptoms. The RBC lifespan for HbD trait is 115 days (Rhea *et al.* 2012).

- **β-Thalassemia**

Around 1% - 5% of the world's people carries Beta-thalassemia as estimated by the World Health Organization (Talaulikar *et al.* 2017). That roughly 156,000 African Americans have Hemoglobin S Beta-thalassemia, which is known to result in artificially high Hemoglobin A1c values due to analytical interferences utilising specific methodologies (Zhu and Williams 2009).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Subjects

- **Patients**

This study was done in the department of biochemistry, college of (The Graduate School Of Natural And Applied Science,University Of ÇankIrI Karatekin. Collection of samples was conducted during the period from (October 2022) to (may 2022). 100 subjects were included in this study, they were admitted in the endocrinology unit in Imam Hussein medical city in Karbala.

Patient (diabetic patients) 50 patients . and age range from 37 to 65 years.

The patient was considered to have DM if he/she has one of the following inclusion criteria:

1- The patent already diagnosed as DM and attend the endocrinology unit for checking.

2- The patient have signs and symptom of DM and newly diagnosed as diabetic patients.

- **Control**

The control groups consist of 50 subjects. They were obtained from medical personnel and relatives Those who do not show signs and symptoms of diabetes. and their age ranges from 30 to 65 years.

3.1.2 Collection of Sample

6 milliliters of venous blood were drawn from each patient. Aspiration of the blood sample via the needle of syringe done was slowly to prevent hemolysis .blood was aspirated veins of cubital fossa by applying tourniquet apply 15 cm above cubital fossa and keep into 3 separated EDTA tubes for analysis (one for each type of analysis includ in this study).

Similarly the blood samples were taken from the control group.

The test were done for the patient and control HbA1c by different method.

3.1.3 Chemicals

The chemicals and kits used in this investigation were of the greatest quality, and their sources are indicated in the Table 3.1.

Table 3.1 Chemicals with their suppliers

Suppliers	Chemicals
LIFOTRONIC (HPLC) HbAIc	HbA1c kit
GP Immunoassay Flouroscence GETEIN 1100	HbA1c kit
mindray chemistry	HbA1c Kit

3.1.4 Instruments

The Table 3.2 below lists all of the instruments and tools utilised in this investigation.

Table 3.2 Instruments with their suppliers

Lifotronic (HPLC) Hbaic H8 Autometed Analyzer	Chania
GP Immunoassay Flouroscence GETEIN 1100 Immunofluorescence Quantitative Analyzer, For Laboratory	Chania
430 fully automated mindray chemistry analyzer BS	Chania

3.2 Methods

3.2.1 Enzymatic assay

Principle: Blood samples in their entirety are dissolved and displayed to intensive proteolytic treatment for enzymatic techniques. This process liberates amino acids from the Hb chains, especially the glycated N-terminal valine. HbA1c is calculated using the signal produced by glycated valines during an additional chromagenic. The existence of Hb variations has no analytical effect on these approaches. The end user, similar to the very precise 2nd- and 3rd generation HbA1c immunoassay methods, is incapable to detect the presumed existence of homozygous Hemoglobin mutations in patient specimens.

3.2.2 Boronate affinity chromatography

Principle: Boronate affinity, it is separation of glycated haemoglobin commonly employs m-aminophenylboronic acid and relies on a particular reaction between glycated and glucose haemoglobin and the immobilised boronic acid. It has a tendency to display less analytical influence from the existence of heterozygotes haemoglobin variations. The reaction of glycated haemoglobin and boronic acid immobilised on the agarose gel and ionic and hydrophobic haemoglobin forces, causes nonglycated haemoglobin and glycated haemoglobin products To get rid independently from the column.This approach uses visual chromatography to separate glycated and nonglycated haemoglobin products. Despite the fact that boronate affinity techniques test total glycated haemoglobin, the findings are provided as an A1c adjusted haemoglobin equivalent. However, like with haemoglobin A1c enzymatic method and immunoassay,

the last user is incapable to detect the existence of haemoglobin variations when utilising boronate affinity, which might be compromise clinical Explanation of haemoglobin A1c readings in circumstances when RBC lifetime is altered.

3.2.3 HPLC

Principle: Ion-exchange HPLC assays According to CAP proficiency testing programme statistics, are now the 2nd most prevalent kind of haemoglobin A1c technique used in clinical laboratories. Hb species are separated using ion-exchange HPLC based on charge differences. HPLC models with high sensitivity can be utilised to resolve a variety of haemoglobin products, including haemoglobin A1a, haemoglobin 1b, haemoglobin F, labile haemoglobin A1c, haemoglobin A1c, haemoglobin A0, and haemoglobin A2, as well as additional haemoglobin byproducts or haemoglobin variations. Although identifying the existence of some of these haemoglobin products is advantageous, it is crucial to remember that various HPLC techniques for HbA1c determination, even if they are from the same seller, have varying chromatographic resolution.The ability to quantify hemoglobin A1c utilising ion-exchange technologies rather than high-resolution chromatography (with longer runtimes) has the benefit of requiring less time in order to analyse samples. The problem with reduced chromatographic Accuracy is the raise likelihood of overlap from Schiff bases, carbamylated hemoglobin, or hemoglobin variations, And that may fade with peaks of interest and result in an incorrect hemoglobin A1c result. It is advantageous to be able to observe Analytical interference present in patient samples (for example, if these haemoglobin derivatives can't be separated from haemoglobin A or haemoglobin A1c) so that erroneous hemoglobin A1c readings are not reported. Some ion-exchange HPLC techniques, however, are analytically incorrect due to overlap from common haemoglobin variations.

3.3 Statistical Analysis

The questionnaire data and all test findings from the research groups' samples were recorded into a data sheet. The Social Sciences Statistical Package programme, version

28.0 (IBM, SPSS, Chicago, Illinois, USA), and the Real Statistics Resource Pack software for Mac (Release 7.2) of the Excel 2016 resource pack were used to create the data analysis for this study. Copyright (2013 – 2020) (2013 – 2020).

Each group's data was subjected to descriptive statistics. For categorical variables, values were represented by n (%), whereas scale variables were represented by mean ± standard deviation.

Analytical statistical tests revealed substantial differences in categorical variables across the parameters. All hypothesis tests with p-values < 0.05 (two-sided) were judged statistically significant.

4. RESULTS AND DISCUSSION

4.1 Demographic and Clinical Characteristics

The clinical demographic characteristics of patients group were summarized in Table 4.1, Figure 4.1, Figure 4.2 and Figure 4.3. Table illustrated the age range of analysed group which was within (30-60) Years, mostly of the participants were overweight and the gender distribution were equal in each group.

Table 4.1 Demographic and demographic characteristics of the studied population (n= 100)

Variable		Patient N(%)	Control N (%)
Age (Years)	30 - 40 Years	2(4%)	36(72%)
	41 - 50 Years	19(38%)	14(28%)
	51 - 60 Years	29(58%)	0(0%)
BMI Category	Normal weight	13(26%)	15(30.6%)
	Over weight	32(64%)	30(61.2%)
	Obesity	5(10%)	4(8.2%)
Gender	Male	21(42%)	21(42%)
	Female	29(58%)	29(58%)

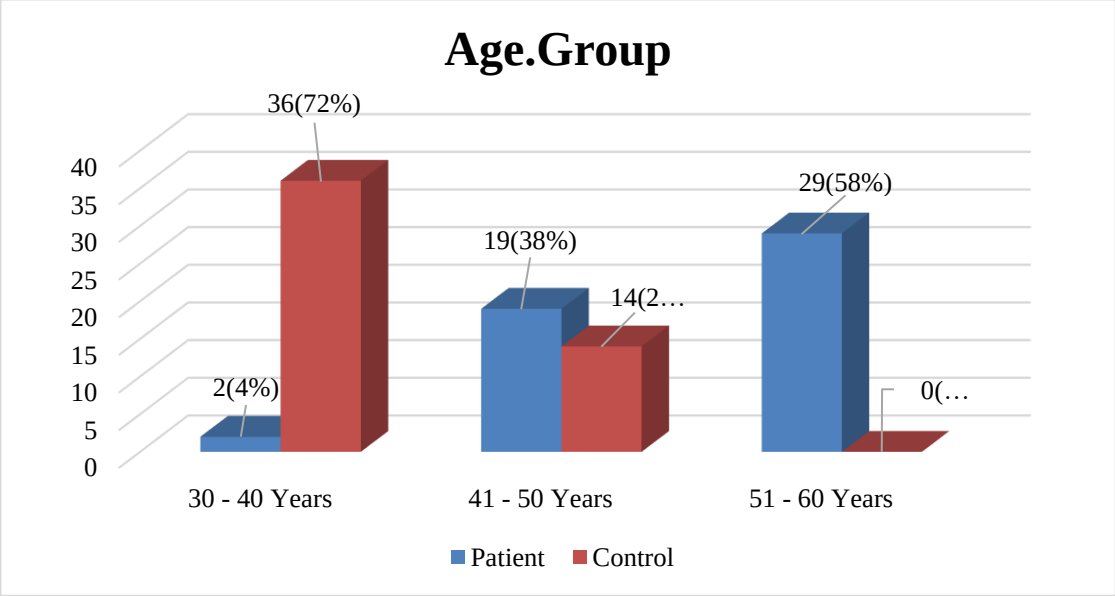

Figure 4.1 Distribution of types and frequency of Age group

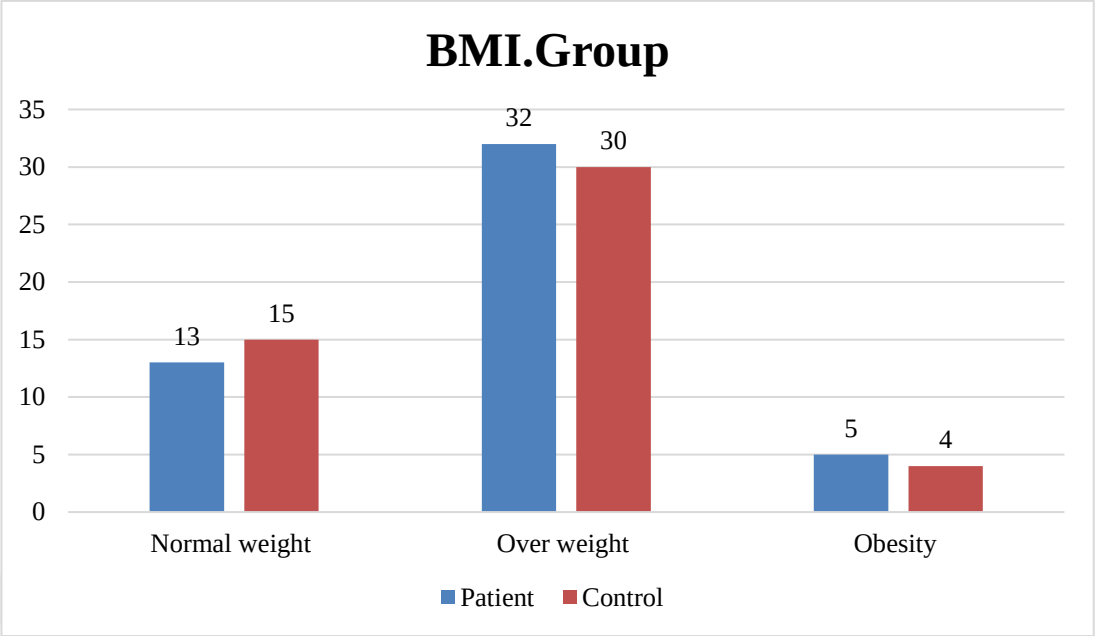

Figure 4.2 Distribution of BMI in study

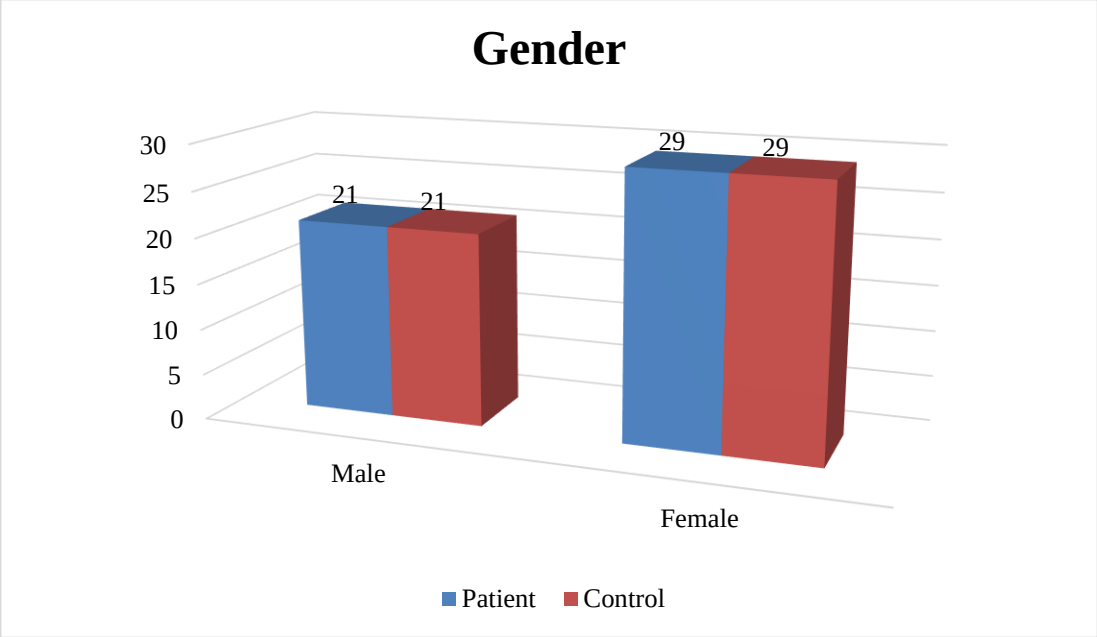

Figure 4.3 Distribution of gender in study

Table 4.2 demonstrated the mean differences of HbA1c analysis in Patients & control groups using three methods (HPLC, enzymetic, Boronate). Results were indicated that there was a significant differences in the mean levels of HbA1c , P values were < 0.001.

Table 4.2 Mean differences of HbA1c analysis in Patients & control groups using three methods

Variable	Patient (N=50) Mean± SD	Control (N=50) Mean ±SD	P value
HPLC	8.83±1.86	5.19±0.60	**<0.001[S]**
enzymetic	8.36±1.75	5.32±0.77	**<0.001[S]**
Boronate	8.68±2.04	5.62±0.77	**<0.001[S]**
Studded T-test was *: significant at p ≤ 0.05			

Also, In Table 4.3, analysis of HbA1c based on the gender groups were performed. Results were illustrated that gender groups were not effect the mean levels of HbA1c in patients & control groups using the three methods, p values were > 0.05.

Table 4.3 Mean differences of HbA1c analysis in patients & control groups based on gender groups

Variable	Male (N=50) Mean± SD	Female (N=50) Mean ±SD	P value
HPLC	7.16±2.55	6.90±2.09	0.572[NS]
Enzymetic	7.00±2.12	6.72±1.98	0.510[NS]
Boronate	7.35±2.38	7.01±2.02	0.441[NS]
Studded T-test was *: significant at p ≤ 0.05			

Furthermore, the analysis of HbA1c based on the age groups were also performed, as presented in Table 4.4. Results were indicated that age groups were affected no significantly the measurement of HbA1c in patients & control groups using the three methods, p values were >0.05, , as demonstrated in Table 4.4.

Table 4.4 Mean differences of HbA1c analysis in patients & control groups based on age groups

Parameter	HPLC	Enzymetic	Boronate	P Value
30 - 40 Years N=38	5.26±0.88	5.39±0.93	5.73±0.92	0.075[NS]
41 - 50 Years N=33	7.01±1.91	6.73±1.79	6.99±2.01	0.807[NS]
51 - 60 Years N=29	9.30±1.92	9.30±1.92	9.30±1.92	0.633[NS]
Studded ANOVA was *: significant at $p \leq 0.05$				

Results were indicated that the effect of body mass index groups were no significantly the measurement of HbA1c in patients & control groups using the three methods, p values were >0.05, as demonstrated in Table 4.5.

Table 4.5 Mean differences of HbA1c analysis in Patients & control groups based on BMI groups

Parameter	Normal Weight N=28	Over Weight N=62	Obesity N=6	P Value
HPLC	6.56±2.19	7.21±2.39	7.18±1.88	0.447[NS]
Enzymetic	6.54±1.87	6.88±2.09	7.58±2.22	0.413[NS]
Boronate	6.86±2.06	7.23±2.25	7.53±2.27	0.663[NS]

The coefficient of variation was calculated to measure the variability of a series of HbA1c levels independently of the unit of measurement in patients & control groups using three methods. The analysis of variance test showed significant differences in the mean levels of HbA1c, and the P-values were <0.001. The HPLC-Boronate technique had the lowest mean, indicating that the parallel values acquired by the Boronate method were nearer to HPLC than Diazyme method Table 4.6 and Table 4.7.

Table 4.6 The coefficient of variation calculated to measure the variability of a series of HbA1c levels

Study Groups	**Coefficient of variation %**		
	HPLC	**Enzymetic**	**Boronate**
Control group	11.55	14.45	13.76
Patients group	21.03	20.90	23.55

Table 4.7 HbA1c readings from the minimum, maximum, and mean, in addition the standard deviation

Measurement method	HbA1c value	
	Min – Max	Mean±SD
HPLC	3.80 - 13.70	7.01±2.29
Enzymetic	3.90 - 11.90	6.84±2.03
Boronate	3.50 - 12.30	7.15±2.17

Boronate's mean value was closest to HPLC among the administered techniques. The absolute value of the parallel mean difference produced by each approach was then computed by HPLC to arrive at its mean as follows:

HPLC- enzymetic : Mean 6.92±2.16.

HPLC- boronate : Mean 6.99±2.11.

The analysis of variance test revealed significant differences in the mean levels of HbA1c, and the P-values were <0.001. The HPLC-Boronate technique had the lowest mean, indicating that the parallel values acquired by the Boronate the gadget was closer to HPLC than Diazyme method.

Correlation coefficient was used for determining linear relationships between each two methods. According to the findings, there was a considerable differences in the marker measurements as shown in the figures below, ($p < 0.001$) Figure 4.4, Figure 4.5 and Figure 4.6.

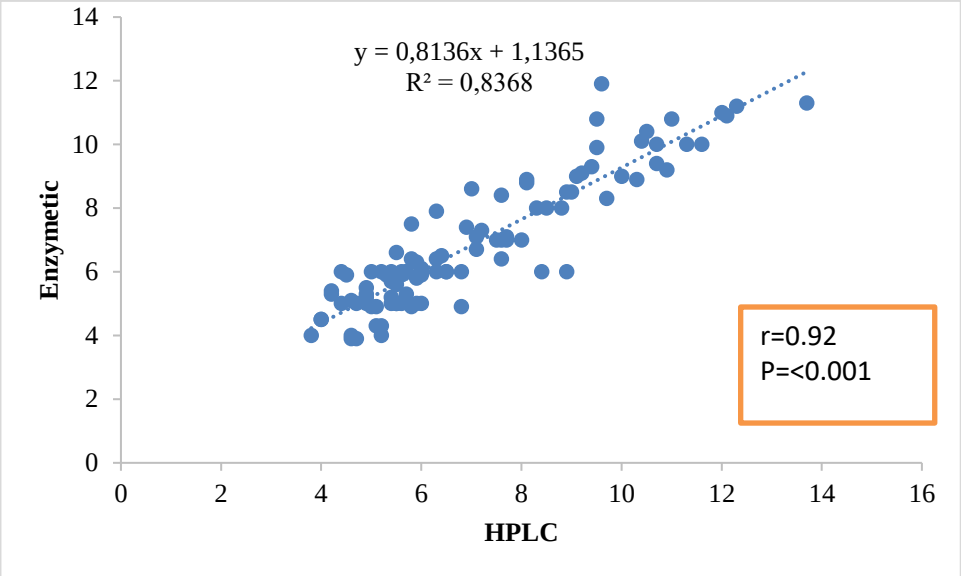

Figure 4.4 Simple linear regression Between HPLC & Enzymetic levels of HbA1c patients and control group

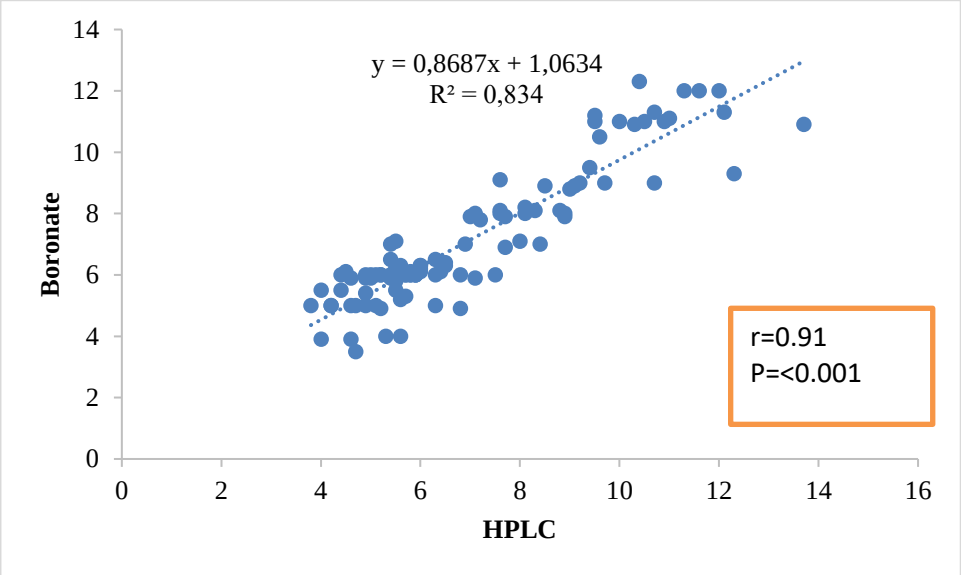

Figure 4.5 Simple linear regression Between HPLC & Boronate levels of HbA1c patients and control group

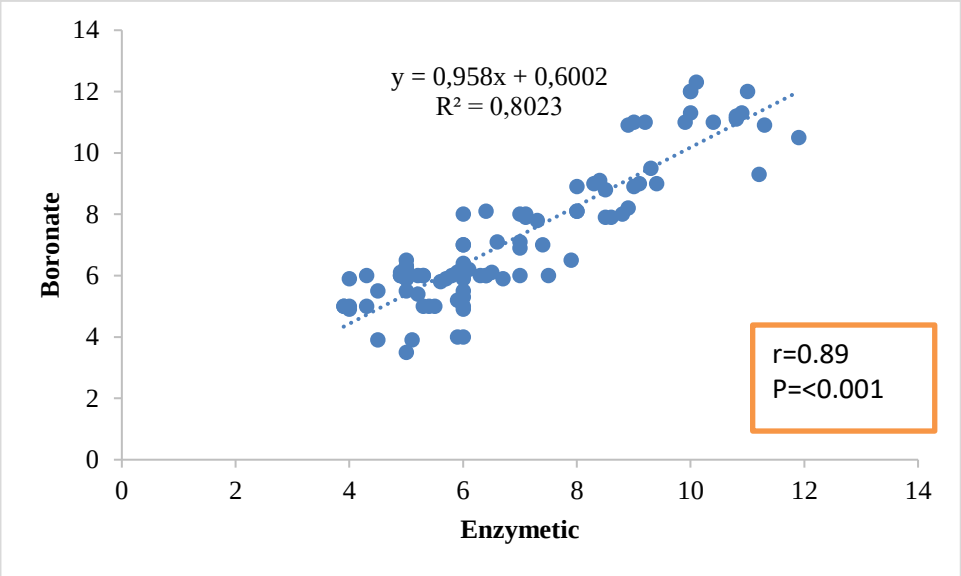

Figure 4.6 Simple linear regression Between Enzymetic & Boronate levels of HbA1c patients and control group

4.2 Discussion

Glycated haemoglobin is a common approach for assessing patients' long-term glycemic management. Regardless of the principles of hemoglobin A1c measurement, using N.G.S.P. approved assays is critical while taking a patient's blood pressure hemoglobin A1c, as is doing this test appropriately. As a result, mistakes must be minimised and technique performance must be clinically acceptable. To ensure the quality and clinical dependability of given findings, laboratories must follow an analytic performance control (CV less than 2%) programme applied to the technique used to assess HbA1c, which must be approved by the NGSP or the IFCC. Because the use of a reference technique (HPLC) isnt economical for many laboratories, the need for replacement assays whose results correspond with those of HPLC as nearly and strongly as feasible has been proven. Several studies have been conducted in this topic.

Karami and Baradaran compared three methods with HPLC , The average value period for each was technique compared to a reference method evaluated In the current investigation, the correlation coefficient of Boronate with HPLC and enzymetic with HPLC was 0.76 , 0.75, Straight, despite the fact that Diazyme had a better function and

key value that were closer to those of HPLC when compared to the other two techniques. When compared to the other 2 procedures, It also has the shortest value interval using HPLC (Karami Baradaran 2014).

Hawkins *et al.* Comparing 4 methods of attention with the Roche tinaqant, and obtaining a Pearson correlation index of greater than 0.9 for each of the four ways : D C A 2000, Boronate, Diastat and D 55. Diastat and D C A 2000 demonstrated the best functionality and linkage to the central laboratory. It is found that these two approaches may be combined a suitable alternative to each other, as well as to the Roche method (Hawkins 2003).

Turpeinen *et al.* Compare 3 devices. As a pearson correlation index involving poly CAT A (HPLC depending on column chromatography) , Diamat (autolysis based on ion exchange chromatography) 0.9 ± 0.3. Also, the correlation index between poly CAT A and IMX (depending on Boronat affinity correlation) was obtained as (0.85 ± 0.04). This study demonstrated the limitations of the Diamat approach as a reference method. It was also said that moving from one approach to another might cause major complications in clinical follow-ups (Turpeinen *et al.* 1995).

Thvarajah *et al.* campare 2 HPLC Techniques, 1 uses boronate affinity chromatography, another uses cation-exchange chromatography, and one uses immunoturbidimetry to measure HbA1c. They discovered a strong link between these strategies (Chakraborty 2008).

In the above research, the average value period for each method was evaluated using a reference method, and in our study, the correlation coefficient of diazmes with HPLC and Nycocard with HPLC was obtained as (0.92) and (0.91), respectively, as follows: Overall better functionality and average values closer to those of HPLC than Nycocard. Since the Pearson correlation coefficient was very close to 1 in both methods, these two methods may be ideal for HPLC replacement.

4.2.1 Demographic and demographic characteristics of the studied population (n= 100)

- **Age**

In our study this, the majority of the control ages 36 (72%) were included in the age group between 30-40 years and The majority of patients' ages 29(58%) in the age group between 51-60 years.

- **BMI category**

In the present study majority of the control BMI category 30 (61.2%) were included in the BMI group over weight,and majority of the patients' BMI Category 32 (64%) were included in the BMI group over weight.

- **Gender**

In the current study of control for gender, it was found (21.42% of male vs 29.58% of female), and patients were found (21.42% of male vs 29.58% of female).

4.2.2 Mean differences of HbA1c analysis in Patients & control groups using three methods

demonstrated the mean differences of HbA1c analysis in Patients & control groups using three methods (HPLC, enzymetic, boronate). Results were indicated that there was a significant differences in the mean levels of HbA1c , P values were < 0.001.

4.2.3 Mean differences of HbA1c analysis in patients & control groups based on gender groups

In the current study of control for gender, it was found, there were no significant differences in subject backgrounds, such as gender. Almost same findings were found in the investigation by (Koga *et al.* 2020).

4.2.4 Mean differences of HbA1c analysis in patients & control groups based on age groups.

In the current study of control for age, it was found, there were no significant differences in subject backgrounds, such as age. Almost same findings were found in the investigation by (Koga *et al.* 2020).

4.2.5 Mean differences of HbA1c analysis in Patients & control groups based on BMI groups.

In the current study of control for BMI, it was found, there were no significant differences in subject backgrounds, such as BMI. Almost same findings were found in the investigation by (Karami and Baradaran 2014, Mohammadi and Norozi 2016).

4.2.6 The coefficient of variation to measure the variability of a series of HbA1c levels independently of the unit of measurement in patients & control groups using three methods.

The coefficient of variation was studied to measure the variability of a series of HbA1c levels independently of the unit of measurement in patients & control groups using three methods. The analysis of variance test show up significant differences in the mean levels of HbA1c, and the P-values were <0.001. The HPLC-Boronate technique had the lowest mean, indicating that the equal values acquired by the boronate device were closer to HPLC than diazyme method.

Almost same findings were found in the investigation by (Karami and Baradaran 2014). The test for variance analysis using repeated observations revealed a statistically there is a considerable difference between the three derived means ($P < 0.001$). The HPLC-Diazyme technique had the lowest mean, indicating that the parallel values acquired by the Enzymetic method were nearer to HPLC than different 2 methods. The reasons for the difference in the results are that in his study he used the least number of samples, and the results were not examined on the same day, and he transferred the samples and examined them in another laboratory, and also because of the different devices used. While we used in this study the most number samples, where they were from patients (50 samples) and healthy people (50 samples), and we examined the samples on the same day and in the same laboratory.

4.2.7 The correlation coefficient (r)

The analysis of variance test show up significant differences in the mean levels of HbA1c, and the P-values were <0.001. The correlation coefficient (r) of diazmes with HPLC and Nycocard with HPLC was also obtained as (0.92) and (0.91), respectively, since the Pearson correlation coefficient was close Very out of 1 in both methods, these two methods may be ideal for HPLC replacement. Enzymetic had improved performance and increased agreement with HPLC than boronate. Almost same findings were found in the investigation by (Karami and Baradaran 2014). A Pearson correlation test revealed a substantial linear connection between HbA1c levels obtained by each technique and those obtained by HPLC ($P < 0.001$).A correlation coefficient of Boronate with HPLC and Enzymetic with HPLC were 0.76 and 0.75, respectively, despite the fact that Enzymetic Having higher mean and function values that were closer to those of HPLC when compared to the other 2 techniques. When compare to the other 2 procedures, it likewise had the smallest value interval with HPLC. However, as a result of the Pearson correlation index was 0.75 (far from 1 and hence not considered a full correlation), this approach cannot be considered a perfect replacement for HPLC. The reasons for the difference in the results are that in his study he used the least number of samples, and the results were not examined on the same day, and he transferred the samples and examined them in another laboratory, and also because of

the different devices used. While we used in this study the most number Samples, where they were from patients (50 samples) and healthy people (50 samples), and we examined the samples on the same day and in the same laboratory.

4.3 Summary

- In our study this, the majority of the control ages 36 (72%) were included in the age group between 30 to 40 years and The majority of patients' ages 29 (58%) in the age group between 51 to 60 years.

- In the present study majority of the control BMI category 30 (61.2%) were included in the BMI group over weight,and majority of the patients' BMI Category 32 (64%) were included in the BMI group over weight.

- In the current study of control for gender, it was found (21.42% of male vs 29.58% of female),And patients were found (21.42% of male vs 29.58% of female).

- demonstrated the mean differences of HbA1c analysis in patients & control groups using three methods (HPLC, enzymetic, boronate). Results were indicated that there was a significant differences in the mean levels of HbA1c, P values were < 0.001.

- In the current study of control for gender, it was found, there were no significant differences in subject backgrounds

- In the current study of control for age, it was found, there were no significant differences in subject backgrounds, such as Age.

- In the current study of control for BMI, it was found, there were no significant differences in subject backgrounds, such as BMI.

- Results were indicated that the HPLC methods were more precise in the analysis of HbA1c in only patients groups while the enzymatic method was shown the highest variation levels of measurements.

- The analysis of variance test showed significant differences in the mean levels of HbA1c, and the P-values were <0.001. The correlation coefficient of Enzymetic method with HPLC and boronat affinty with HPLC was also obtained as (0.92), (0.91), respectively, since the pearson correlation coefficient was close Very out of 1 in both methods, these two methods may be ideal for HPLC replacement. Diazyme outperformed the others in terms of performance and concordance with HPLC.

5. CONCLUSIONS AND RECOMMENDATION

Based on our study, we concluded that both of the two methods enzymatic (Diazyme) and boronate affinity binding (nycocard) can be trusted and measured.As two alternative methods to the HPLC reference method. Because they showed acceptable results and are very close to the reference method. From this, we took advantage of the fact that their results are reliable, since the HPLC reference method is expensive and not all laboratories work with it.

- Further studies with a larger sample size are recommended.

- It is recommended to work on additional standard clinical laboratory procedures and equipments

- It is recommended to conduct the study in other countries due to the difference in lifestyle.

REFERENCES

Alberti, K. G. M. M. 1996. The clinical implications of impaired glucose tolerance. Diabetic Medicine, 13(11): 927-937.

American Diabetes Association. 2010. Standards of medical care in diabetes—2010. Diabetes Care, 33(1): S11-S61.

American Diabetes Association. 2011. Diabetes statistics. 2011. Washington, DC, American Diabetes Association.

American Diabetes Association. 2013. Standards of medical care in diabetes—2013. Diabetes Care, 36(1): S11-S66.

American Diabetes Association. 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care, 37(1): S81-S90.

American Diabetes Association. 2015. 2. Classification and diagnosis of diabetes. Diabetes Care, 38(1): S8-S16.

Apanah, S. and Rizzolo, D. 2013. Sickle cell disease: taking a multidisciplinary approach. JAAPA, 26(8): 28-33.

Arnold, W. D., Kupfer, K., Hvidsten Swensen, M., Fortner, K. S., Bays, H. E., Davis, M. and San George, R. C. 2020. Fingerstick precision and total error of a point-of-care HbA1c test. Journal of Diabetes Science and Technology, 14(5): 890-895.

Assadi F. 2012. The epidemic of pediatric chronic kidney disease the danger of skepticism. Journal Nephropathol, 1: 61–64.

Atlas, I. D. 2013. International Diabetes Federation.–6th, ed. Brussels, Belgium-2013–160 p.

Baynes, H. W. 2015. Classification, pathophysiology, diagnosis and management of diabetes mellitus. Journal Diabetes Metab, 6(5): 1-9.

Bodor, G. S., Little, R. R., Garrett, N., Brown, W., Goldstein, D. E. and Nahm, M. H. 1992. Standardization of glycohemoglobin determinations in the clinical laboratory: three years of experience. Clinical Chemistry, 38(12): 2414-2418.

Bry, L., Chen, P. C. and Sacks, D. B. 2001. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clinical Chemistry, 47(2): 153-163.

Bryśkiewicz, M. E. and Majkowska, L. 2011. Glycated hemoglobin (HbA1c) as a standard diagnostic criterium for diabetes. Pol Merkur Lekarski, 30: 150–154.

Butalia, S., Kaplan, G. G., Khokhar, B. and Rabi, D. M. 2016. Environmental risk factors and type 1 diabetes: past, present, and future. Canadian Journal of Diabetes, 40(6): 586-593.

Chakraborty, S., Chakraborty, S., Ghosh, A. and Chakraborty, S. S. A. 2008. Comparative evaluation of estimation of glycated haemoglobin by ımmunoturbidimetry and high performance liquid chromatography in a tertiary care hospital of kolkata. Sciences, 2: 5-7.

Cleland, S. J., Fisher, B. M., Colhoun, H. M., Sattar, N. and Petrie, J. R. 2013. Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks. Diabetologia, 56(7): 1462-1470.

Cooke, D. W. and Plotnick, L. 2008. Type 1 diabetes mellitus in pediatrics. Pediatr Rev, 29(11): 374-84.

Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine, 329(14): 977-986.

Edge, J. A., Ford-Adams, M. E. and Dunger, D. B. 1999. Causes of death in children with insulin dependent diabetes 1990–96. Archives of Disease in Childhood, 81(4): 318-323.

Edison, E. S., Sathya, M., Rajkumar, S. V., Nair, S. C., Srivastava, A. and Shaji, R. V. 2012. A novel β-globin gene mutation HBB. c. 22 G> C produces a hemoglobin variant (Hb Vellore) mimicking HbS in HPLC. International Journal of Laboratory Hematology, 34(5): 556-558.

Eisenbarth, G. S., Ziegler, A. G. and Colman, P. A. 2005. Joslin's Diabetes Mellitus.

Ferri, S., Miyamoto, Y., Sakaguchi-Mikami, A., Tsugawa, W. and Sode, K. 2013. Engineering fructosyl peptide oxidase to improve activity toward the fructosyl hexapeptide standard for HbA1c measurement. Molecular Biotechnology, 54(3): 939-943.

Gerber, S. M., Stickle, D. F., Ahmed, I. and Jabbour, S. A. 2015. Glycated hemoglobin, serum proteins, and other markers as tools for monitoring. International Textbook of Diabetes Mellitus, 6(3): 853-871.

Gheissari, A., Mehrasa, P., Merrikhi, A. and Madihi, Y. 2012. Acute kidney injury: A pediatric experience over 10 years at a tertiary care center. Journal of Nephropathology, 1(2): 101.

Gillett, M. J. 2009. International expert committee report on the role of the A1c assay in the diagnosis of diabetes: diabetes care. The Clinical Biochemist Reviews, 30(4): 197.

Hansen, W. B. and Scheier, L. M. 2019. Specialized smartphone intervention apps: review of 2014 to 2018 NIH funded grants. JMIR mHealth and uHealth, 7(7): e14655.

Hawkins, R. C. 2003. Comparison of Four Point-of-Care HbA1c Analytical Systems against Central Central Laboratory Analysis. Singapore Med. J., 44(1): 8-11.

Hector, G. A., Alejandro, R. A. and Beatariz, C. C. 2009. Effect of the method to measure levels of glycated hemoglobin on individual clinical decisions. Journal Clinical Pathology, 1(32): 332-335.

Hudson, D. M., Archer, M., King, K. B. and Eyre, D. R. 2018. Glycation of type I collagen selectively targets the same helical domain lysine sites as lysyl oxidase–mediated cross-linking. Journal of Biological Chemistry, 293(40): 15620-15627.

IDF Diabetes Atlas Committee. 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Reserch Clinical Pract, 157: 107843.

Jeppsson, J. O., Kobold, U., Barr, J., Finke, A., Hoelzel, W., Hoshino, T. and Weykamp, C. 2002. Approved IFCC reference method for the measurement of HbA1c in human blood. The Clinical Biochemist Reviews, 4(3): 75-77.

Jeppsson, J. O., Kobold, U., Barr, J., Finke, A., Hoelzel, W., Hoshino, T. and Weykamp, C. 2002. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Approved IFCC reference method for the measurement of HbA1c in human blood. Clin. Chem. Lab. Med., 40(1): 78-89.

Karami, A. and Baradaran, A. 2014. Comparative evaluation of three different methods for HbA 1c measurement with High-performance liquid chromatography in diabetic patients. Advanced Biomedical Research, 3(1): 94.

Kenny, C. 2014. When hypoglycemia is not obvious: diagnosing and treating under-recognized and undisclosed hypoglycemia. Primary Care Diabetes, 8(1): 3-11.

Kim, S., Nibe, E., Tsugawa, W., Kojima, K., Ferri, S. and Sode, K. 2012. Construction of engineered fructosyl peptidyl oxidase for enzyme sensor applications under normal atmospheric conditions. Biotechnology Letters, 34(3): 491-497.

Kitabchi, A. E., Umpierrez, G. E., Miles, J. M. and Fisher, J. N. 2009. Hyperglycemic crises in adult patients with diabetes. Diabetes Care, 32(7): 1335-1343.

Koga, M. 2014. Glycated albumin, clinical usefulness. Clinica Chimica Acta, 433: 96-104.

Koga, M., Okuda, M., Inada, S., Ueda, S. I., Nakamura, Y., Okumiya, T. and Ishibashi, M. 2020. HbA1c levels measured by enzymatic assay during off-site health checkups are lower than those measured by on-site HPLC assay. Diabetology International, 11(1): 67-71.

Krishnamurti, U. and Steffes, M. W. 2001. Glycohemoglobin: a primary predictor of the development or reversal of complications of diabetes mellitus. Clinical Chemistry, 47(7): 1157-1165.

Laios, K., Karamanou, M., Saridaki, Z. and Androutsos, G. 2012. Aretaeus of Cappadocia and the first description of diabetes. Hormones, 11(1): 109-113.

Laugesen, E., Østergaard, J. A., Leslie, R. D. G. and Danish Diabetes Academy Workshop and Workshop Speakers. 2015. Latent autoimmune diabetes of the adult: current knowledge and uncertainty. Diabetic Medicine, 32(7): 843-852.

Lenters-Westra, E. and English, E. 2017. Evaluating new HbA1c methods for adoption by the IFCC and NGSP reference networks using international quality targets. Clinical Chemistry and Laboratory Medicine, 55(9): 1426-1434.

Little, R. R. and Roberts, W. L. 2009. A review of variant hemoglobins interfering with hemoglobin A1c measurement. Journal of Diabetes Science and Technology, 3(3): 446-451.

Mahler, R. J. and Adler, M. L. 1999. Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. The Journal of Clinical Endocrinology and Metabolism, 84(4): 1165-1171.

Malik, V. S. 2017. Sugar sweetened beverages and cardiometabolic health. Current Opinion in Cardiology, 32(5): 572-579.

Mallia, A. K., Hermanson, G. T., Krohn, R. I., Fujimoto, E. K. and Smith, P. K. 1981. Preparation and use of a boronic acid affinity support for separation and quantitation of glycosylated hemoglobins. Analytical Letters, Part B: Clinical and Biochemical Analysis, 14(8): 649-661.

Marino, B. S. and Fine, K. S. 2013. Blueprints pediatrics. Lippincott Williams and Wilkins, 6: 89.

Mathers, C. D. and Loncar, D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Medicine, 3(11): e442.

Mayfield, J. A. 1998. Diagnosis and classification of diabetes mellitus: new criteria. American Family Physician, 58(6): 1355.

Miyazaki, A., Kohzuma, T., Kasayama, S. and Koga, M. 2012. Classification of variant forms of haemoglobin according to the ratio of glycated haemoglobin to glycated albumin. Annals of Clinical Biochemistry, 49(5): 441-444.

MJ, F. 2008. Microvasculares e macrovasculares do diabetes. Cli. Dia., 26: 77-82.

Mohammad, A., Abujbara, M., Alshraideh, J., Jaddou, H. and Ajlouni, K. 2017. The Prevalence of Resistant Hypertension Among Type 2 Diabetic Patients Attending the National Center for Diabetes, Endocrinology, and Genetics. Journal of Endocrinology and Metabolism, 7(5): 153-158.

Mohammadi, R. and Norozi, V. 2016. HbA1c Measurement: Comparison of Results of Five Commonly Used Kits in Iran. Biomedical and Pharmacology Journal, 9(1): 125-131.

Molinaro, R. J. 2008. Targeting HbA1c: standardization and clinical laboratory measurement. MLO: Medical Laboratory Observer, 40(1): 10-4.

Murray, C. J., Aravkin, A. Y., Zheng, P., Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M. and Borzouei, S. 2020. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 396(10258): 1223-1249.

Natarajan, S., Kulkarni, R. and Tangri, A. 2020. Fatal cerebral edema in a young adult with diabetic ketoacidosis: blame the bicarbonate. Case Reports in Critical Care, 2: 15-20.

Nathan, D. M., Turgeon, H. and Regan, S. 2007. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia, 50(11): 2239-2244.

National Diabetes Data Group. 1979. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes, 28(12): 1039-1057.

Nuttall, F. Q. 1998. Comparison of percent total GHb with percent HbA1c in people with and without known diabetes. Diabetes Care, 21(9): 1475-1480.

Ogawa, N., Kimura, T., Umehara, F., Katayama, Y., Nagai, G., Suzuki, K. and Ichimura, M. 2019. Creation of haemoglobin A1c direct oxidase from fructosyl peptide oxidase by combined structure-based site specific mutagenesis and random mutagenesis. Scientific Reports, 9(1): 1-13.

Otabe, S., Nakayama, H., Ohki, T., Soejima, E., Tajiri, Y. and Yamada, K. 2017. Haemoglobin variants may cause significant differences in haemoglobin A1c as measured by high-performance liquid chromatography and enzymatic methods in diabetic patients: a cross-sectional study. Annals of Clinical Biochemistry, 54(4): 432-437.

Penttilä, I., Penttilä, K., Holm, P., Laitinen, H., Ranta, P., Törrönen, J. and Rauramaa, R. 2016. Methods, units and quality requirements for the analysis of haemoglobin A1c in diabetes mellitus. World Journal of Methodology, 6(2): 133.

Pinzur, M. S. 2011. Diabetic peripheral neuropathy. Foot and Ankle Clinics, 16(2): 345-349.

Poretsky, L. 2010. Principles of diabetes mellitus. Springer Science and Business Media, 2(1): 23-25.

Porta, M. and Jörgens, V. 2020. Three and a half thousand years of diabetes research. In Unveiling Diabetes-Historical Milestones in Diabetology. Karger Publishers, 29: 298-303.

Rahbar, S. 1968. An abnormal hemoglobin in red cells of diabetics. Clinica Chimica Acta, 22(2): 296-298.

Rezki, A., Fysekidis, M., Chiheb, S., Vicaut, E., Cosson, E. and Valensi, P. 2021. Acute and long-term effects of saxagliptin on post-prandial glycemic response in obese patients with impaired glucose tolerance. Nutrition, Metabolism and Cardiovascular Diseases, 31(4): 1257-1266.

Rhea, J. M. and Molinaro, R. 2014. Pathology consultation on HbA1c methods and interferences. American Journal of Clinical Pathology, 141(1): 5-16.

Rhea, J. M., Koch, D., Ritchie, J., Singh, H. V., Young, A. N., Burgess, T. and Molinaro, R. J. 2013. Unintended reporting of misleading Hb A1c values when using assays incapable of detecting hemoglobin variants. Archives of Pathology and Laboratory Medicine, 137(12): 1788-1791.

Rhea, J. M., Roberts-Wilson, T. K. and Molinaro, R. J. 2012. Impact of hemoglobin variants on Hb A1c interpretation: do we assume too much. MLO: Medical Laboratory Observer, 44(6): 8-10.

Rockefeller, J. D. 2015. Diabetes: symptoms, causes, treatment and prevention. JD Rockefeller.

SarwarN, G. 2010. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. Lancet, 375: 2215-2222.

Schnedl, W. J., Lahousen, T., Wallner, S. J., Krause, R. and Lipp, R. W. 2005. Silent hemoglobin variants and determination of HbA1c with the high-resolution program of the HPLC HA-8160 hemoglobin analyzer. Clinical Biochemistry, 38(1): 88-91.

Serena, G., Camhi, S., Sturgeon, C., Yan, S. and Fasano, A. 2015. The role of gluten in celiac disease and type 1 diabetes. Nutrients, 7(9): 7143-7162.

Shahbazmohammadi, H., Sardari, S., Lari, A. and Omidinia, E. 2019. Engineering an efficient mutant of Eupenicillium terrenum fructosyl peptide oxidase for the specific determination of hemoglobin A1c. Applied Microbiology and Biotechnology, 103(4): 1725-1735.

Shapiro, R., McManus, M. J., Zalut, C. and Bunn, H. F. 1980. Sites of nonenzymatic glycosylation of human hemoglobin A. Journal of Biological Chemistry, 255(7): 3120-3127.

Shoback, D. M. and Gardner, D. G. 2018. Greenspan's basic & clinical endocrinology. McGraw-Hill Education. Sciences, 2: 13-15.

Soldavini, J. 2019. Krause's food & the nutrition care process. Journal of Nutrition Education and Behavior, 51(10): 1225.

Sudhakar, B., Reddy, A. S. and Fallerio, J. 2014. Comparison of three methods for measurement of blood HbA1c as to reliability. Journal Bioassays, 3: 38-40.

Syed, I. 2011. Glycated haemoglobin, past, present, and future are we ready for the change. Diabetes, 1(1): 12.

Szmuilowicz, E. D., Josefson, J. L. and Metzger, B. E. 2019. Gestational diabetes mellitus. Endocrinology and Metabolism Clinics, 48(3): 479-493.

Talaulikar, V. S., Chatterjee, R. and Bajoria, R. 2017. Reversal of hypogonadotropic hypogonadism with spontaneous pregnancy in beta-thalassaemia major with transfusional haemosiderosis. European Journal of Obstetrics and Gynecology and Reproductive Biology, 216: 271-272.

Tarvonen, M., Hovi, P., Sainio, S., Vuorela, P., Andersson, S. and Teramo, K. 2021. Intrapartal cardiotocographic patterns and hypoxia-related perinatal outcomes in pregnancies complicated by gestational diabetes mellitus. Acta Diabetologica, 58(11): 1563-1573.

Tesija Kuna, A., Dukic, K., Nikolac Gabaj, N., Miler, M., Vukasovic, I., Langer, S. and Vrkic, N. 2018. Comparison of enzymatic assay for HbA1c measurement (Abbott Architect) with capillary electrophoresis (Sebia Minicap Flex piercing analyser). Laboratory Medicine, 49(3): 231-238.

Thanabalasingham, G. and Owen, K. R. 2011. Diagnosis and management of maturity onset diabetes of the young (MODY). Bmj, 3: 43.

Thiruppathi, M., Lee, J. F., Chen, C. C. and Ho, J. A. A. 2021. A disposable electrochemical sensor designed to estimate glycated hemoglobin (HbA1c) level in whole blood. Sensors and Actuators B: Chemical, 3(29): 119-129.

Turpeinen, U. and Stenman, U. H. 2020. Analysis of HbA1c and some Hb variants by HPLC. In Analytical and Preparative Separation Methods of Biomacromolecules, pp. 1-11, CRC Press.

Turpeinen, U., Karjalainen, U. and Stenman, U. H. 1995. Three assays for glycohemoglobin compared. Clinical Chemistry, 41(2): 191-195.

UK Prospective Diabetes Study Group. 1998. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. Bmj, 317(7160): 703-713.

Visser, J., Rozing, J., Sapone, A., Lammers, K. and Fasano, A. 2009. Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. Annals of the New York Academy of Sciences, 1165(1): 195-205.

Wan Mohd Zin, R. M., Ahmad Kamil, Z. I., Tuan Soh, T. R., Embong, M. and Wan Mohamud, W. N. 2013. Haemoglobin A1c: comparing performance of two point of care devices with laboratory analyser. BMC Research Notes, 6(1): 1-4.

Weykamp, C. 2013. HbA1c: a review of analytical and clinical aspects. Annals of Laboratory Medicine, 33(6): 393-400.

WHO Expert Committee. 1980. Diabetes mellitus second report. World Health Organ Tech Rep Ser., 6: 71-80.

Xu, A., Ji, L., Chen, W., Xia, Y. and Zhou, Y. 2016. Effects of α-thalassemia on HbA1c measurement. Journal of Clinical Laboratory Analysis, 30(6): 1078-1080.

Yancy, C. W., Jessup, M. and Bozkurt, B. 2013. 23747642: 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal Coll Cardiol, 62(16): e147-e239.

Yetisen, A. K., Akram, M. S. and Lowe, C. R. 2013. based microfluidic point-of-care diagnostic devices. Lab. on a Chip., 13(12): 2210-2251.

Young-Hyman, D. L. 2020. National institute of diabetes and digestive and kidney diseases. In Encyclopedia of behavioral medicine, pp. 1456-1457, Cham: Springer International Publishing.

Zheng, J. 2021. Diabetes, Metformin and Gastric Adenocarcinoma. Karolinska Institutet (Sweden).

Zhu, Y. and Williams, L. M. 2009. Falsely elevated hemoglobin A1c due to S-β+-thalassemia interference in Bio-Rad Variant II Turbo HbA1c assay. Clinica Chimica Acta, 409(1-2): 18-20.

CURRICULUM VITAE

Personal Information

Name and Surname : Mohammed Majid Azeez OBAID

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2020-Present
Undergraduate	Kerbala University Faculty of Sciences Department of Chemistry	2008-2015