Comparison of a Human Portable Blood Glucose Meter and Automated Chemistry Analyzer for Measurement of Blood Glucose Concentrations in Human Diabetic Patients

Author

Asad-Ur-Rehman

Regn Number

00000319123

Supervisor

Dr. Asim Waris

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY

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A thesis submitted in partial fulfillment of the requirements for the degree of

MS Biomedical Sciences

Thesis Supervisor:

Dr. Asim Waris

Thesis Supervisor's Signature:

_____

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2. Name: Dr. Syed Omer Gilani Signature:_____

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Dedication

Dedicated to my Parents for their endless love and support throughout my MS journey

Acknowledgments

My sincere thanks to my supervisor **Dr. Asim**, for his useful guidance needed in the completion of this research project.

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Abstract

Diabetes is the most prevalent chronic metabolic disorder in Pakistan which is characterized by an increased Blood Glucose level. An accurate diagnosis of diabetes is very essential for its early detection and treatment. Almost 90\% of the cases are of Type II Diabetes and the patients are required to continuously monitor their Blood Glucose level to maintain a near-normal range of Blood sugar. A practical way to monitor sugar levels is using a portable Glucometer at home which is faster and more convenient for diabetes patients. The purpose of this study is to compare Blood Glucose levels measured using Oxidase Method and a Portable Glucometer with reference Hexokinase Method in Type II Diabetes patients to determine if Glucometers can be used as a home-based self-monitoring device. A cross-sectional study was conducted with a total of 150 Type II Diabetes patients. Blood samples were collected from the capillary of fingers for a portable Glucometer and from veins for Hexokinase and Oxidase Plasma methods after overnight fasting of 8 hours. Independent t-test and Pearson Correlation were used to check the significance of our results. There was no statistically significant difference in Blood Glucose readings obtained from Oxidase Method (128.31 + 61.4, $p = 0.947$) and Glucometer (122.53 + 59.6, $p = 0.370$) with Reference Hexokinase Method (128.78 + 61.2). A statistically significant positive correlation was observed in both methods with reference Hexokinase Method. Glucometer readings positively correlate with Hexokinase, showing that Glucometer can be used as a home-based self-monitoring Blood Glucose Device.

Keywords: Automated Chemistry Analyzer; Glucometer; Type II Diabetes; Blood Glucose Monitoring

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Abbreviations

American Diabetes Association (ADA)

Diabetes Miletus (DM)

Blood Glucose (BG)

Red Blood Cells (RBC)

Hemoglobin A1c (HbA1c)

Chapter 1 Introduction

1.1 Diabetes Mellitus

Diabetes Mellitus (DM) is a metabolic disorder defined by chronic hyperglycemia (high level of blood sugar) caused by defects in the secretion of insulin, the activity of insulin, or both (HM, 2015). DM is a common disease that affects how the body converts food into energy. The food which humans eat is broken down and converted into glucose, which is released into the bloodstream (Mashige et al., 2016).

It is the most frequently occurring and serious chronic metabolic disease of the 21st century with a major impact on the health of individuals worldwide (Saeedi et al., 2019; Sun et al., 2022). The incidence of diabetes has doubled in the past 20 years s (Zimmet et al., 2014). The prevalence of diabetes was reported to be 463 million people in 2019. In 2021, the global prevalence of diabetes among people of the age group 20-79 was approximately 536.6 million people (Sun et al., 2022).

1.2 Types of Diabetes Mellitus

There are 3 types of DM, but it is broadly categorized into two main types, Type I, and Type II Diabetes, the Gestational type being the third type (HM, 2015, Alam et al., 2021).

1.2.1 Type I Diabetes

Type 1 DM also called "juvenile diabetes or insulin-dependent diabetes", is a chronic disease in which the pancreas secretes little or no insulin. Type 1 DM presents with various symptoms like extreme hunger, increased thirst, frequent urination, increased weight, fatigue, and weakness. Type 1 DM can be caused by several factors, including genetics, and age. Type 1 DM is more frequent in children. Still, there is a lack of treatment for type 1 DM. To avoid complications, treatment gives emphasis on controlling Blood Glucose (BG) levels with insulin, food, and lifestyle changes (Ojiako and Chikezie, 2015, Roep et al., 2021).

1.2.2 Type II Diabetes

Type II DM is the most common type of DM. It occurs when the BG level is too high in the bloodstream. Glucose is the main source of energy and comes mainly from food. In Type II DM, insulin is not produced by the pancreas so excess glucose stays in the bloodstream and is not reached cells. Type II DM presents with increased thirst and urination, increased hunger, blurred vision, sores that do not heal, and unexplained weight loss (Saisho, 2014, Artasensi et al., 2020). Approximately, 90% of diabetes cases are of Type II. The established risk factors for Type II diabetes include a combination of metabolic, environmental, and genetic factors that relate to each other and contribute to its increased prevalence (Berbudi et al., 2020; Galicia-Garcia et al., 2020). It commonly occurs in adults but recently it has been reported to increase in children as well because of unhealthy lifestyles such as obesity, lack of physical activity, and diet (Ismail et al., 2021).

1.2.3 Gestational Diabetes

Gestational diabetes is a type of DM that develops during pregnancy. Gestational DM means that the BG level is high only during pregnancy (Palmer and Clegg, 2019). Gestational DM is usually diagnosed in the 24^{th} to 28^{th} week of pregnancy. The management of gestational DM is very necessary for the health of the baby (Long and Endocrinology, 2021). Increased levels of BG during pregnancy can cause problems for the baby such as preterm birth of baby and breathing problems. The baby can also develop Type II DM and obesity if the mother has gestational DM. If pregnant women have gestational DM, then there is a strong chance of developing hypoglycemia right after the delivery. Gestational DM also increases the chance of miscarriages or a stillborn baby. (Uzoma et al., 2021).

According to an estimate, in pregnant women, there is a great chance of developing preeclampsia with gestational DM. Preeclampsia is defined as high blood pressure and the presence of protein in urine during the second trimester of pregnancy (Sudarmaji et al., 2020). Preeclampsia can cause serious problems for the mother and baby. The only cure for preeclampsia is to give birth early. If the mother has preeclampsia till 37 weeks of pregnancy, then early delivery of the fetus is recommended (Morais et al., 2020).

1.3 Risk factors

DM is a multifactorial disease. It is caused by many factors. Major factors are including genetic predisposition and environmental factors. Many other risk factors are also associated with DM such as age, obesity, weak physical fitness, high triglycerides levels and hypertension, smoking, and polycystic ovarian syndrome.

1.3.1. Genetic influences

In the progression of type II DM, genetic factors play a significant role. The most commonly occurring type II DM is polygenetic which means it is caused by mutations in many genes. Some other forms of type II diabetes mellitus are monogenetic. This means they are caused by a mutation in a single gene. Type 1 DM also has genetic influences, but it is important to understand and not to be confused with each other (Ali, 2013).

1.3.2. Environmental factors

In the development of Type II DM, many environmental factors are involved. Type II DM is associated with a person's lifestyle and their gut microbiota. The microbiota composition can reshape the intestinal barrier. They induce different signaling pathways associated with resistance to insulin (Sharma & Tripathi, 2019).

1.3.3. Age

Age is now considered a major risk factor in developing Type II DM. Recently, DM was found to be present in old age and adults. This was associated with less muscle with more adipose tissue, less physical activity, and the capacity for less sugar burning. Nowadays, DM s even present in children because of less physical activity and obesity.

1.3.4. Obesity

Obesity is defined as "A person with a body mass index (BMI) equal to or greater than 30 kg/m2 is generally considered obese". The deposition of adipose tissues especially in the abdominal site is directly related to obesity which in turn is the cause of developing DM.

The fatter cells will result in insulin resistance in the human body. According to an estimate, around 80% of patients with Type II DM have obesity (Apovian, 2016).

1.3.5. Poor physical fitness

Physical activities are very important for the human body. They control the body weight, and reduced glucose, and triglyceride levels in the blood. An inactive lifestyle will lead to developing Type II DM (Wu et al., 2014, McGovern et al., 2018).

1.3.6. High triglycerides levels and hypertension

Hypertension and high triglyceride levels are commonly related to insulin resistance. The chance of developing Type II DM with these conditions is quite common.

1.3.7. Smoking

Smoking is also associated with Type II DM. Smoking badly affects health and is the cause of many diseases such as DM, cancers, and heart failure.

1.3.8. Gestational diabetes

During pregnancy, gestational DM is common, increasing the chance of developing Type II DM in these women.

1.4 Diagnosis of Diabetes

An imbalance in BG levels is a sign of diabetes. Blood sugar levels are monitored and controlled by insulin. If DM is left untreated it can lead to severe complications, so it is important to make a timely diagnosis of DM to prevent the serious consequences of the disease. The main symptoms of diabetes are:

i. High levels of BG
ii. Recurring urination
iii. Elevated thirst, and
iv. Increased hunger.

A typical fasting BG level is characterized as less than 100 mg/dL and ordinary 2-hour postprandial plasma glucose under 140 mg/dL. BG levels are over the typical level

however the basic layout for DM demonstrates weakened glucose homeostasis. As per the American Diabetes Association (ADA), an individual is considered to have DM at the point when one of the two conditions is met.

1.4.1 Fasting Plasma Glucose

By calculating glucose readings that are independent of hematocrit and that reflect the glucose concentration to which the body's tissues are exposed, the serum or Plasma from venous blood tests has an advantage over the total blood. It is necessary to collect plasma in the fluoride-containing tube (Fluoride inhibits glucose metabolism). As comparison to a full blood sample, the concentration of glucose is 10–15% higher in plasma or serum. The value of glucose in the fasting state that is less than 126 mg/dL more than once (after no calorie intake for at least eight hours and up to 14 hours) is regarded as diagnostic.

1.4.2 Random Plasma Glucose

A random blood glucose reading of 2:200 mg/dL obtained during the day at any time after the previous feast and showing the classic symptoms of excessive urine, thirst, and unexplained weight loss is regarded as diagnostic.

1.4.3 Oral Glucose Tolerance Test

OGTT should be feasible in that situation if the plasma glucose is not crucial. Based on the use of 75 g of glucose weight, an OGTT result of 2:200 mg/dL was obtained in the two-hour test.

There is some necessary prevention that patients should follow before the OGTT.

i. The patient ought to consume around 150-200 g of carbohydrates for three back-to-back days before the test procedure.
ii. Patients ought to notice short-term fasting for no less than 8 hours.
iii. Espresso, tea, exercise, and cigarette smoking are permitted during the test.
iv. In 300 mL of water, 75g of glucose is required around five minutes before the test.

v. BG ought to be looked at during fasting and nearly two hours after taking the glucose.

1.4.3.1 Interpretation of OGTT

Table 1 shows the concentration of BG in fasting and two hours after taking glucose.

Table 1: Concentration of Glucose

	Concentration mg/dL
Fasting plasma glucose	126
2-hour postprandial glucose	200

There is a chance of false positive results in those who use contraceptive pills, corticosteroids, excess thyroxin, and diuretics.

1.4.4 Hemoglobin A1c (HbA1c)

It has a 4-6% overall HbA1c content. When diabetes is uncontrolled, the HbA1c percentage is unusually elevated. As glycohemoglobins circulate inside Red Blood Cells (RBC), which have a lifespan of up to 120 days, they largely reflect the glycemia during the previous 8–12 weeks, providing a better method for assessing diabetes management. Estimates should be taken at 3- to multi-month intervals in individuals with any type of DM so that treatment can be changed if HbA1c is not at a healthy level.

The ADA has used HbA1c as a lagging indicator of diabetes. Because there is a significant increase in the risk of retinopathy above this level, a cutoff value of 6.5% was chosen. The advantage of using the HbA1c to assess diabetes is that there is a strong case for doing so because it has less intra-individual variability than the oral glucose tolerance test and fasting blood sugar. A high prevalence of hemoglobinopathies or diseases with increased red cell turnover make HbA1c measurement inappropriate. Likewise, the testing ought to be conducted utilising National Glycohemoglobin Standardization Program assured technique and normalised to the Diabetes Control and Difficulties Preliminary measure.

RBC endurance has an impact on HbA1c values. Hence, when RBC count throughput is low, resulting in an unequal amount of older red cells, high values of mean BG values can

be obtained. Those who are lacking in folate, iron, or vitamin B12 experience this issue. Nevertheless, rapid red cell turnover misrepresents low HbA1c values and suggests a greater proportion of younger RBCs. Patients with hemolysis, those on erythropoietin treatment, and those with iron, vitamin B12, or folate deficiencies are all included in the models. Vitamins C and E have also been implicated in faking negative test results, possibly by preventing haemoglobin glycation.

1.4.5 Serum Fructosamine

When serum proteins are non-enzymatically glycosylated, serum fructosamine is created. Because serum albumin has a substantially shorter half-life than haemoglobin, serum fructosamine only accurately represents glycemic management for one to two days. The serum fructosamine value decreases when serum albumin levels drop (for example, due to nephrotic condition or hepatic disease). Serum fructosamine tests can be useful when unusual haemoglobins or hemolytic conditions affect our comprehension of glycohemoglobin or when a shorter time period is needed, such as glycemic control during the time of conception in a diabetic woman who has recently gotten pregnant.

When the serum albumin level is 5 g/dL, typical attributes alter in accordance with the serum albumin focus and are 200-285 mcmol/L. Values of serum fructosamine and HbA1c have a strong correlation. In light of linear regression analysis, the associated relationship between serum fructosamine levels and HbA1c has been taken into account: HbAlc = 0.017 × the amount of serum fructosamine (mcmol/L) plus 1.61.

1.4.6 Glycosuria (Urine Test**)**

The paper strip impregnated with glucose oxidase and a chromogenic foundation, which is sensitive to just 0.1% glucose in urine, is a unique and useful method of diagnosing glycosuria. Understanding requires a typical renal glucose threshold as well as trustworthy bladder drainage. Because there is a precise renal limit for glycosuria, glucose in urine may not appear in urine despite mild hyperglycemia in many other benign or obsessive

conditions besides diabetes. In this way, the indicative purpose largely does not require urine glucose.

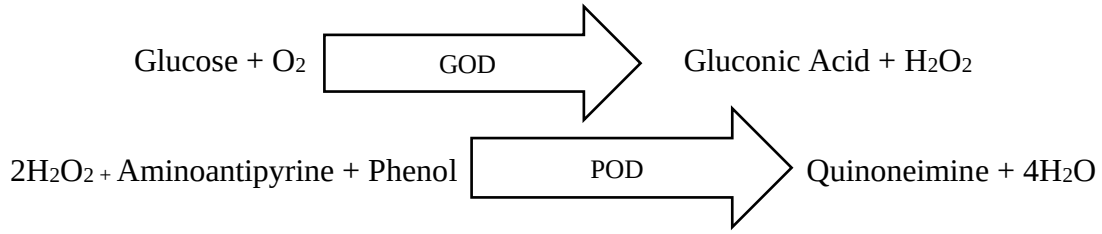
1.5 BG Estimation Methods

Diabetes is a serious worldwide problem that has no cure. Therefore, diabetic patients must maintain near-normal BG levels by regularly monitoring their BG concentration levels (Rachim and Chung, 2019; Rodr´ıguez-Rodr´ıguez et al., 2019). BG concentration can be estimated using different methods: Automated Chemistry Analyzer which includes Oxidase and Hexokinase method and Portable Glucometer. The estimation of BG concentration using the hexokinase and oxidase method is performed in the laboratory using an automated chemistry analyzer. They are very specific and precise methods but as the test is conducted in the laboratory, the results are not available timely (Harish et al., 2015). Recent technology has made it possible to test BG levels at home using a portable Glucometer. It has become a common practice due to its rapid results (Liyanage et al., 2019; Thilakarathna, 2021). Generally, laboratory methods for the measurement of BG levels are considered more accurate and reliable but recently the use of Glucometers has increased as they are commercially available and easy to use (Vanavanan et al., 2010; Lascaris et al., 2022; Proulx et al., 2022).

1.5.1 Automated Chemistry Analyzer

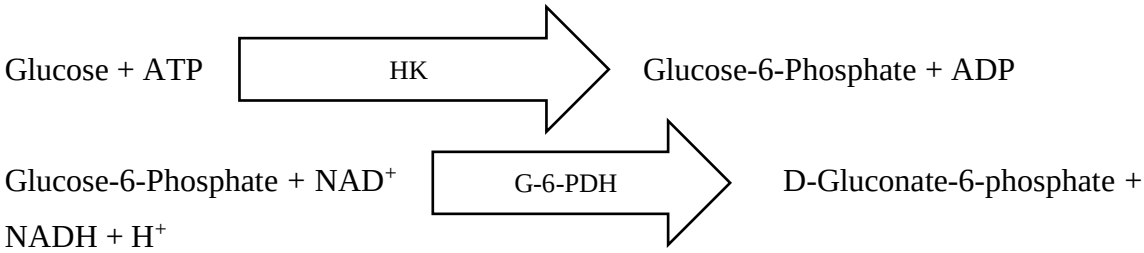
1.5.1.1 Oxidase Method

The estimate of BG following enzymatic oxidation by the enzyme glucose oxidase is the basic idea behind the glucose oxidase method. The colorimetric indicator, quinone imine, is produced by hydrogen peroxide and the catalytic reaction of peroxidase from phenol and 4-amino antipyrine.



1.5.1.2 Hexokinase Method

The following reaction is the basis for the hexokinase method's estimation of glucose:



GOD = Glucose oxidase

HK = Hexokinase

POD = Peroxidase

G-6-PDH = Glucose-6-phosphate dehydrogenase

1.5.2 Portable Glucometer

The Procedure for using a glucometer is:

1. First, check the expiry date on the test strip container.
2. Take the test strip out of the container it was stored in.
3. Next, put the test strip's metallic end inside the meter. There is power to the meter.
4. When the flashing drop symbol appears, wait for it to occur before using the lancing tool on a finger stick.
5. To help the blood flow, gently squeeze the finger.
6. Apply the blood drop on the test strip's yellow edge. As the hourglass sign starts to flash, take your finger off the test strip.
7. An arrow points to the test's outcome. The direction of this arrow indicates whether the outcomes are above, within, or below the target.
8. Remove the used test strip and threw it away.

1.6 Aim of the Study

The aim of the present study was to evaluate the agreement between BG concentration measured using Oxidase Method and a Portable Glucometer with Automated Chemistry

Analyzer Hexokinase Method, used as a reference in Type II Diabetes patients. The reason for using Hexokinase as a reference method is because it correlates closely to definitive mass spectrometry.

Chapter 2 Literature Review

Tewabe et al.2019 developed a cross-sectional investigation in which they tracked BG levels by contrasting readings from glucometers and the reference hexokinase technique. 52 patients who are suspected of having diabetes mellitus have been taken for this reason. SPSS software was used to examine the data that was obtained. Based on ISO 15197:2003 & ISO 15197:2013 standards, the glucometer's final and highest level of precision was evaluated. They found that the typical serum glucose readings obtained using the reference hexokinase and the glucometer were 130.94 mg/dl and 132.52 mg/dl, respectively. The statistical difference observed was (p-value < 0.001). They discovered that glucometer values were higher than those obtained using the hexokinase method, indicating that the glucometer did not meet the likely requirements established by ISO. As a result, glucometers need to be further verified.

Link et al. 2015 The accuracy of self-monitoring of blood glucose levels (SMBG) was assessed and tested using three test strip lots from the Accu-Chek® Aviva, Contour®XT, GlucoCheck XL, and GlucoMen® LX PLUS systems. Comparative analysis was carried out using the hexokinase (cobas® c111) and glucose oxidase (YSI 2300 STAT PlusTMglucose analyzer) methods. The lot-to-lot variability was present in all systems to some extent; however, two systems (Accu-Chek Aviva and ContourXT) showed very modest differences in relative preference across the three evaluated lots.

Wolde et al. 2018 they developed a paradigm in which they chose four randomly chosen PoCG devices (CareSens N, DIAVUE Prudential, On Call Extra, and i-QARE DS-W) for BG monitoring of 200 randomly chosen study participants (100 members with diabetes and 100 healthy controls), which were assessed against the hexokinase method and ISO 15197:2003 and ISO 15197:2013 protocols. CareSens N (21 mg/dl) and the hexokinase technique (498.8 mg/dl) were used to record the lowest and maximum blood sugar levels, respectively. As compared to the reference hexokinase technique, all portable devices' mean glucose levels had showed substantial variances, except for ON Extra call. On the

other hand, neither the ISO 15197:2003 nor the ISO 15197:2013 standards' minimum accuracy level have been met by any portable device.

Corstjens et al. 2006 designed an observational paradigm in which they evaluated 3 different glucometers in a seriously ill 12-bed medical ICU. Patients who have a stay of at least 48 hours and more were included in this study. Starting with the validation of ABG analyzer ABL715. In the second phase, arterial blood was taken from the artery line and investigated on Precison PCx and ABL715. Paired measurements were analyzed and plotted by the Bland & Altman method and expressed and correlation coefficient. The correlation coefficient was 0.95. ABL715 is a swift and precise substitute for lab monitoring and served as a standard blood glucometer.

Chapter 3 Methodology

This section describes the methodology of the present work.

3.1Study Design

We performed a cross-sectional study, comprising 150 participants using a 95% confidence interval to compare the BG measurement of a portable Glucometer and an Automated Chemistry Analyzer (Oxidase and Hexokinase Plasma method) in patients with Type II diabetes. The Hexokinase method was used as a reference.

3.2 Study Area

This study was conducted at Mayo Hospital and samples were collected from June 2022 to September 2022.

3.3 Participants Recruitment

Patients with Type II diabetes that attend the hospital were randomly selected for this study. To each patient, the study's purpose and significance were explained in detail. we followed inclusion and exclusion criteria for the recruitment of subjects, Figure 1 shows the inclusion-exclusion criteria. The patients who volunteered to be part of this study were reviewed and those patients who had a history of other known metabolic disorders were excluded. Permission from the respective institution was taken. The data was kept confidential and used only for study purposes.

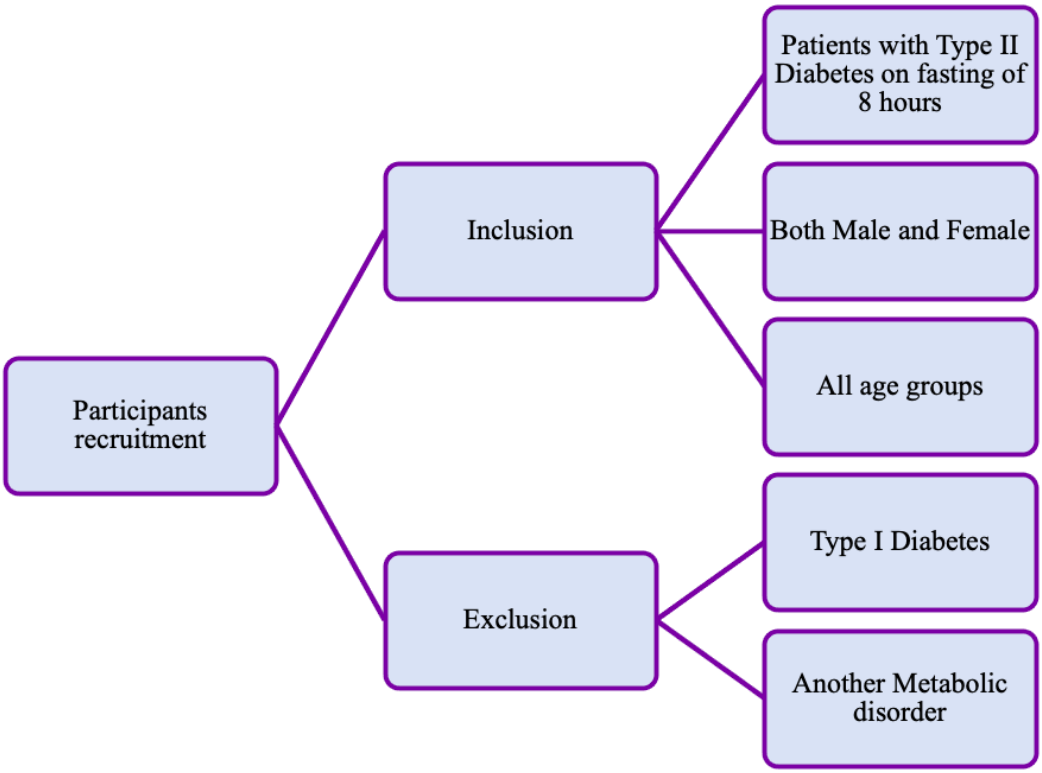


Figure 1: Inclusion and Exclusion Criteria

3.4 Sample Collection

After written consent was obtained from subjects, they were requested to fill out a questionnaire to collect socio-demographic information from the individuals. The questionnaire was comprised of demographic data of individuals like name, age, sex, clinical features, and study variables. Blood sample collection was done by a skilled medical laboratory scientist from the capillary of fingers for a portable Glucometer and from veins for Hexokinase and Oxidase Plasma methods after overnight fasting of 8 hours.

3.5 Measurement of The BG Concentration Using a Portable Glucometer

BG levels in capillary blood were assessed using the Glucometer ACCU-CHEK Instant S and the manufacturer-recommended protocols. Using biosensor technology, the blood glucose levels were measured using a finger stick on a glucometer. A drop of blood was

applied to the strip which was provided by the manufacturer having lot number and expiry date and the reading was noted on the digital window of the glucometer.

3.6 Measurement of BG Concentration Using Automated Chemistry Analyzer

BG levels using the Selectra fully automated chemistry analyzer in venous blood were measured following standard procedures described by the manufacturer. At the same time, about 3ml of venous blood was taken and added to the Sodium fluoride (NaF) vacutainer and sent to the chemical pathology laboratory. Plasma was separated by centrifugation, at a speed of three thousand rounds per minute for a period of ten minutes and was analyzed on an automated chemistry analyzer by Oxidase and hexokinase methods. Figure 2 shows the experimental setup for laboratory-based Hexokinase and Oxidase Methods and Figure 3 shows the overview of our methodology.

Figure 2: Experimental setup in Laboratory

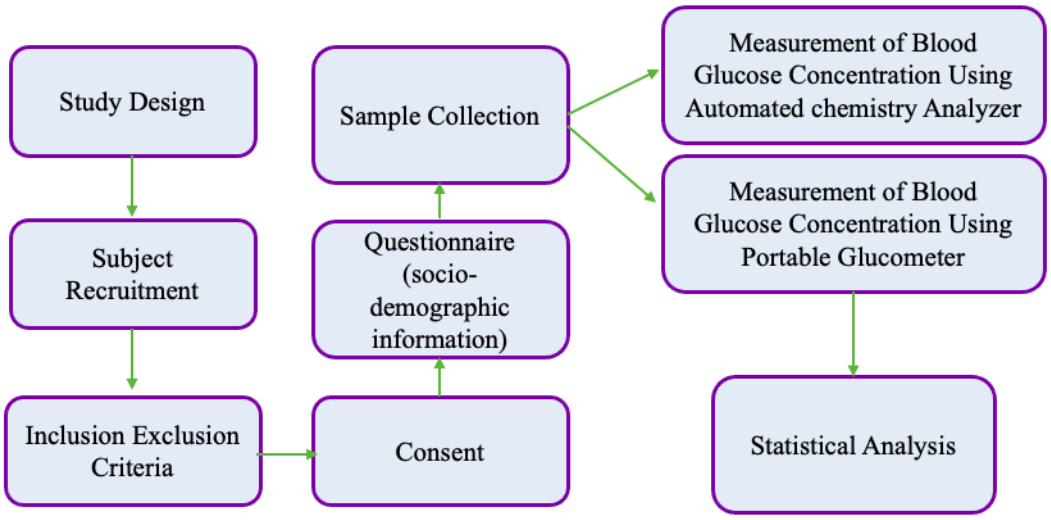


Figure 3: Summary of present work

3.7 Data Analysis

Data collected from the study were analyzed using Statistical Package for the Social Sciences 25.0 (SPSS 25.0). The quantitative variables like age were summarized as mean and standard deviation. The categorical value was expressed in the form of frequency and percentages. Appropriate statistical tools such as the Independent t-test and Pearson correlation were applied to analyze the data.

Chapter 4 Results

This section provides the statistical analysis of this study. A total of 150 subjects were enrolled in this study. Table 2 shows the mean of BG levels measured using three methods in 150 participants and their standard deviation, illustrated in Figure 4.

Table 2: Mean BG levels measured using three methods and their standard deviation in 150 participants.

Method	N	Mean BG levels (mg/dL)	Standard Deviation	Standard Error Mean
Hexokinase	150	128.78	61.164	4.994
Oxidase	150	128.31	61.365	5.010
Glucometer	150	122.53	59.568	4.864

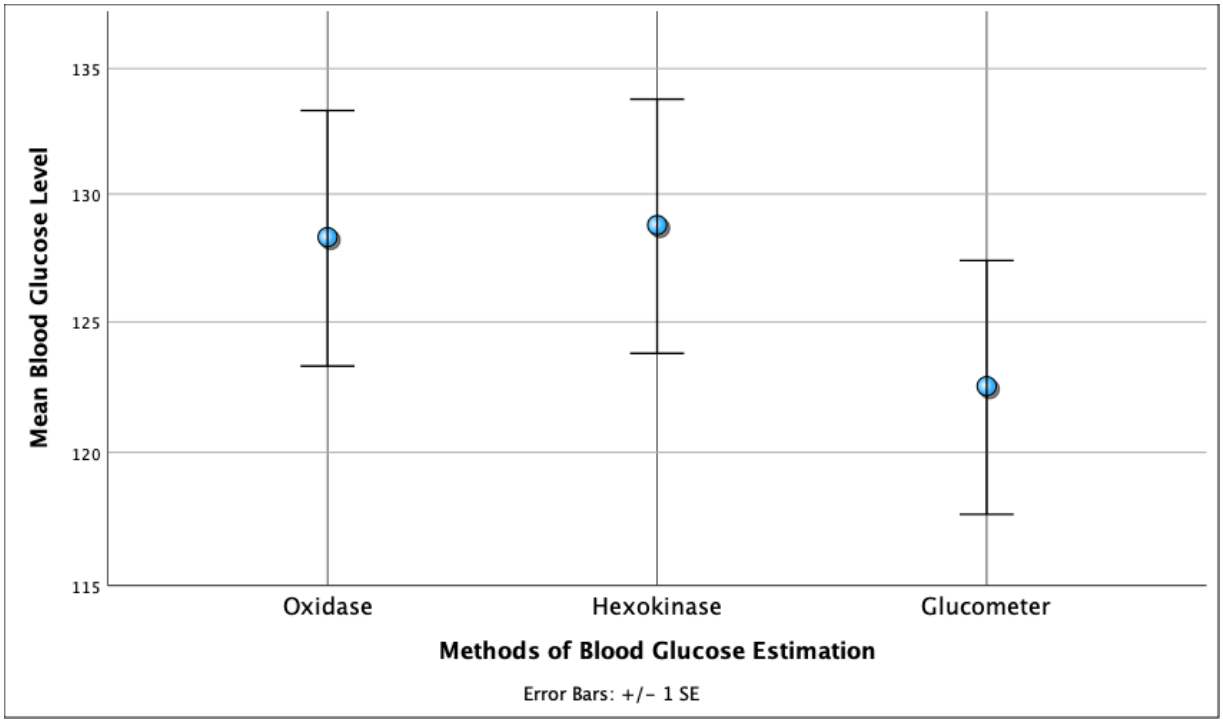


Figure 4: This Figure shows the Mean of BG levels of 150 participants measured with 3 methods (Hexokinase as reference) and their standard deviation from mean.

More than half (67.3%) were females. The mean age of the participants was 47.78 (ranging from 17 to 80). We divided the participants in 4 age groups 11 to 30, 31 to 50, 51 to 70, and 71 to 90 respectively. 49.3% of patients belonged to age group of 31-50 and 35.3% belonged to age group 51-70. Table 3 shows the demographic data and frequency of participants.

Table 3: Demographic data and frequency of participants

Parameters		**Number of participants(N=150)**	**Frequency (%)**
Gender	Males	49	67.3
	Females	101	32.7
Age	11 - 30	15	10.0
	31 - 50	74	49.3
	51 - 70	53	35.3
	71 - 90	8	5.3

4.1 Independent Sample t-test

We performed an independent t-test to compare the mean values of Oxidase and Portable Glucometer readings with the reference method (Hexokinase) using a confidence interval of 95% and alpha = 0.05 and differences were regarded statistically significant if the p value was less than 0.05.

4.1.1 Comparison of BG levels measured with Hexokinase and Oxidase Method

Based on our study, we formulated 2 hypotheses; Null Hypothesis (Ho): There is no difference in means of BG levels measured with Hexokinase and Oxidase Methods. Alternative Hypothesis (H1): There is a significant difference in means of BG levels measured with Hexokinase and Oxidase Methods. Table 4 shows the mean BG levels

measured using Hexokinase (reference) and Oxidase method and their standard deviation, between males and females, different age groups, and p-values obtained from the t-test. The results are illustrated in Figure 5.

Table 4: Mean BG levels measured using Hexokinase and Oxidase methods in Type II Diabetes patients.

Parameters		Mean BG (mg/dL) ± SD		t-value	Sig. (2-tailed)
		Hexokinase	Oxidase		
Mean (N=150)		128.78 ± 61.2	128.31 ± 61.4	0.067	0.947
Gender	Females	128.53 ± 64.8	128.47 ± 64.9	0.008	0.994
	Males	129.29 ± 53.4	127.98 ± 53.8	0.121	0.904
Age	11 - 30	87.53 ± 19.5	87.13 ± 23.8	0.050	0.960
	31 - 50	122.23 ± 47.2	121.96 ± 47.9	0.035	0.972
	51 - 70	146.92 ± 78.2	146.28 ± 77.6	0.040	0.966
	71 - 90	146.50 ± 55.8	145.13 ± 59.8	0.048	0.963

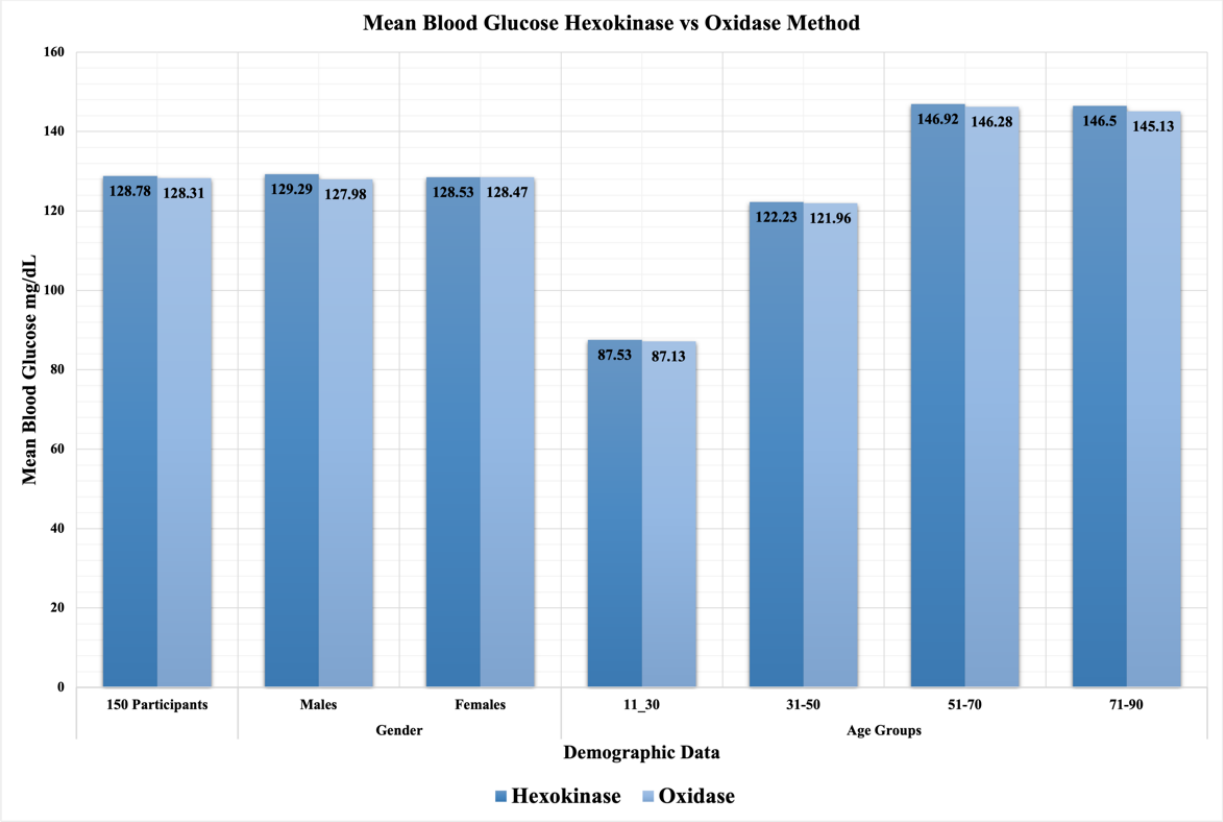


Figure 5: Histogram of Mean BG levels measured using Hexokinase and Oxidase methods in Type II Diabetes patients

The results of the independent t-test indicated no statistically significant difference (p = 0.947) between means of BG levels obtained with the Hexokinase Method (128.78 + 61.2 mg/dL) and Oxidase Method (128.31 + 61.4 mg/dL). We further accessed the BG Levels measured with these two methods according to sex and age. The results showed no statistically significant difference in BG levels obtained Hexokinase and oxidase methods within patients aged 11-30 (p = 0.960), 31-50 (p = 0.972), 51-70 (p = 0.966) and 71-90 (p = 0.963). Also, the difference in BG levels measured using the two methods was highly similar in the case of gender (p = 0.994 for females and p = 0.904 for males). Based on our results, we accept our Null Hypothesis and state that there is no difference in means of BG levels measured with the Hexokinase and Oxidase Method.

4.1.2 Comparison of BG levels measured with Hexokinase and Portable Glucometer Method

BG levels were also obtained using a portable Glucometer. To check if there is any difference in readings obtained using Hexokinase and a portable Glucometer, an Independent t-test was applied. Two hypotheses were formulated; Null Hypothesis (Ho): There is no difference in the means of BG levels measured with Hexokinase and Portable Glucometer Method. Alternative Hypothesis (H1): There is a significant difference in means of BG levels measured with Hexokinase and Portable Glucometer Method. Table 5 shows the mean BG levels measured using Hexokinase (reference) and Portable Glucometer method, and their standard deviation between males and females, different age groups, and p-values obtained from the t-test. Figure 6 shows the results.

Table 5: Mean BG levels measured using Hexokinase and Glucometer methods in Type II Diabetes patients

		Mean BG (mg/dL) ± SD		t-value	Sig. (2-tailed)
Parameters		**Hexokinase**	**Glucometer**		
Mean (N=150)		128.78 ± 61.2	122.53 ± 59.6	0.897	0.370
Gender	Females	128.53 ± 64.8	122.48 ± 63.2	0.673	0.502
	Males	129.29 ± 53.4	122.64 ± 51.9	0.625	0.533
Age	11 - 30	87.53 ± 19.5	83.933 ± 21.8	0.477	0.637
	31 - 50	122.23 ± 47.2	115.88 ± 46.3	0.826	0.410
	51 - 70	146.92 ± 78.2	140.40 ± 75.5	0.437	0.663
	71 - 90	146.50 ± 55.8	138.00 ± 59.3	0.295	0.772

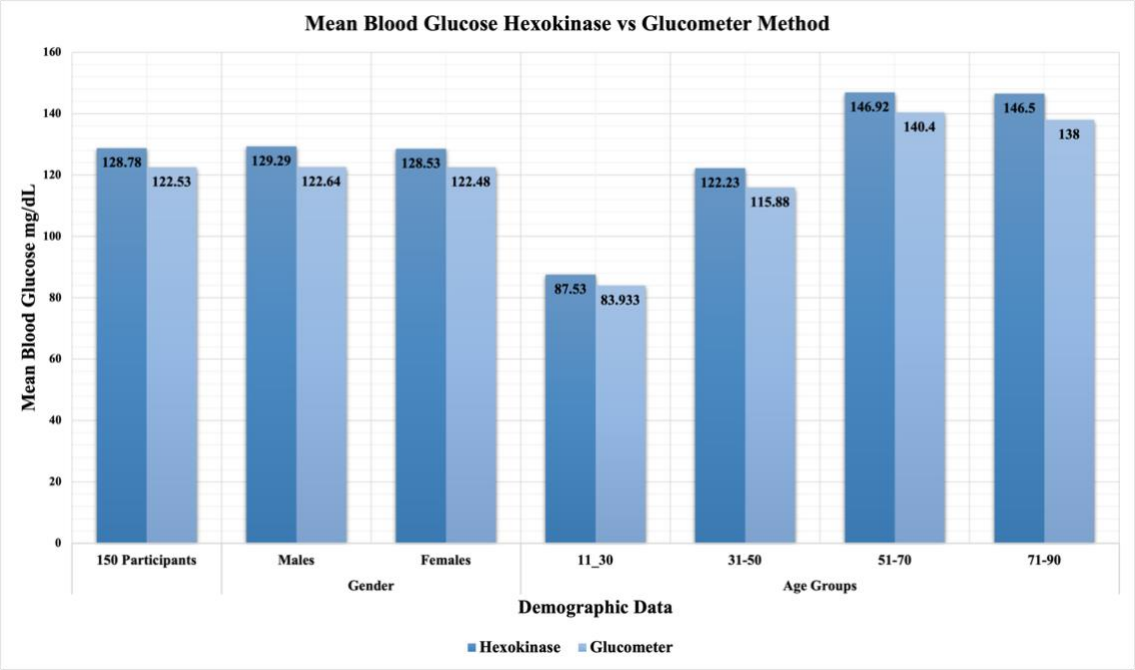


Figure 6: Histogram of Mean BG levels measured using Hexokinase and Glucometer methods in Type II Diabetes patients

In the case of Hexokinase and Portable Glucometer readings, the mean of BG levels obtained with Hexokinase was 128.78 + 61.2 mg/dL, and with Glucometer was 122.53 + 59.6 mg/dL. The means of both methods were compared using a t-test and it yielded a p-value of 0.370 which shows there is no statistically significant difference in both methods even though measurements of the Portable Glucometer were slightly less than the reference Hexokinase method. In the case of gender, there was no statistically significant difference in BG levels obtained with both methods ($p = 0.502$ for females and $p = 0.533$ for males). Also, the readings of BG levels in patients aged 11-30 ($p = 0.637$), 31-50 ($p = 0.410$), 51-70 ($p = 0.663$), and 71-90 ($p = 0.772$) were highly comparable. Based on our results, we accept our Null Hypothesis and state that there is no difference in means of BG levels measured with Hexokinase and portable Glucometer Methods.

4.2 Bivariate Correlation of three Glucose Estimation Methods

To check the association of the Oxidase Method and a portable Glucometer Method with our reference Hexokinase Method, we applied Pearson Correlation. The results are described in Table 6. Results showed a positive and significant correlation between the

reference Hexokinase method and Oxidase method (r = 0.997, p = < 0.001) as illustrated in Figure 7. Also, the BG readings of the Portable Glucometer showed a significant correlation with the Hexokinase Method (r = 0.996, p = < 0.001) shown in Figure 8.

Table 6: Pearson Correlation

Correlations				
		Hexokinase	**Glucometer**	**Oxidase**
Hexokinase	Pearson Correlation	1	.996**	.997**
	Sig. (2-tailed)		<.001	<.001
	N	150	150	150
Glucometer	Pearson Correlation	.996**	1	.999**
	Sig. (2-tailed)	<.001		<.001
	N	150	150	150
Oxidase	Pearson Correlation	.997**	.999**	1
	Sig. (2-tailed)	<.001	<.001	
	N	150	150	150

**. Correlation is significant at the 0.01 level (2-tailed)

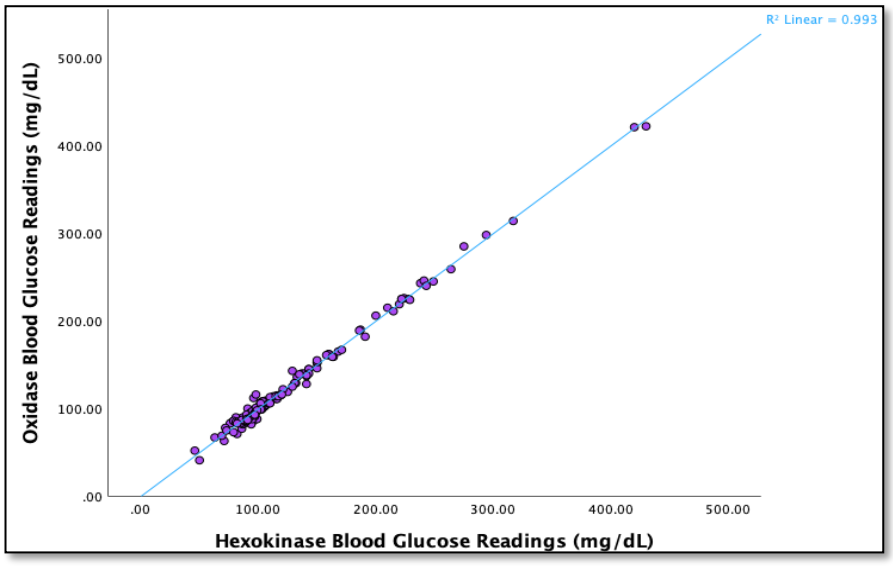


Figure 7: Regression curve between Oxidase BG readings and Reference Hexokinase BG Readings

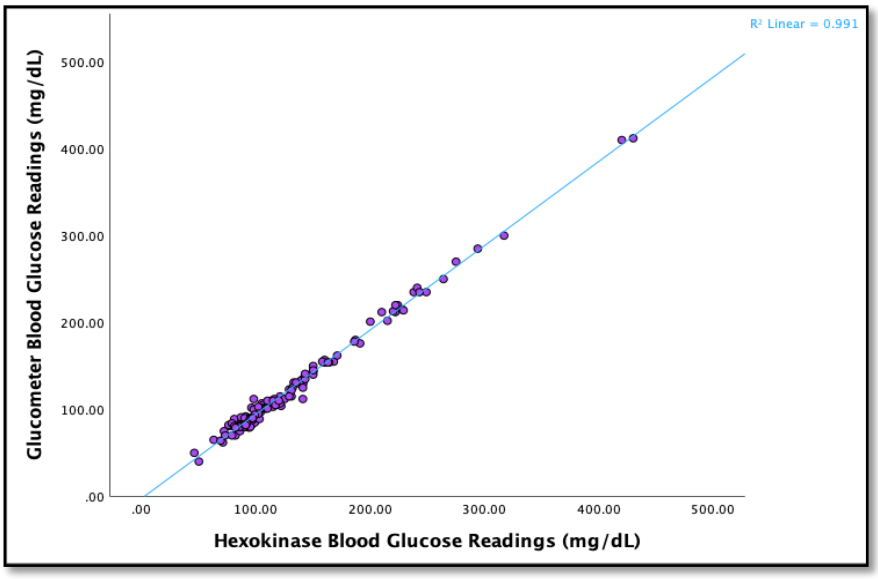


Figure 8: Regression curve between Portable Glucometer BG readings and Reference Hexokinase BG Readings

Chapter 5 Discussion

Monitoring of BG is very essential in the management of diabetes, especially in Type II diabetes patients. Although laboratory Chemistry analyzer methods are considered a gold standard, but their use has limitations in several situations, such as the amount of blood required delay in results in case of emergency, and the cost of repeated measurements (Proulx et al., 2022). Whereas Potable Glucometers provide quick results with less amount of blood samples. This is the first study in which we provide a comparison of the world's available glucose estimation methods in the Pakistani population with type II diabetes. The outcomes of this study will help pathologists and the diagnostic community with a better treatment of diabetic patients. Our results indicate that there is no significant difference in readings of both laboratory BG methods, Hexokinase and Oxidase. The readings obtained with both methods are highly comparable ($p = 0.947$). The mean difference between these two was 0.47. The accuracy of a portable Glucometer is a major concern. This study was designed to evaluate the accuracy of a portable Glucometer with the reference Hexokinase method. Many studies have been conducted to check the accuracy of Glucometers as their use as a home-based self-monitoring Glucose device has become prominent. According to our results, there was no significant difference ($p = 0.370$) in means of BG readings of the portable Glucometer (122.53 + 59.6) and Reference Hexokinase method (128.78 + 61.2). Glucometers slightly underestimated the BG levels in almost all Type II diabetes patients in this study, but a good correlation was observed between the 2 glucose estimation modalities. Bimenya et al., 2003 and Sudan, 2014 also reported that the glucometer gave lower glucose readings but there was a good correlation between the Chemistry analyzer and the glucometer. This difference can be because of various reasons as the readings of glucometers are usually affected by temperature and humidity. Also, the BG levels in veins vary from that in capillary (Kanwugu et al., 2017). Baig et al., 2007 stated that a glucometer does not accurately measure high and low level BG concentration. (Kanwugu et al., 2017) results concluded that although there was no significant difference in readings of glucometer and Hexokinase methods, Glucometer overestimated the BG levels. This disagreement with our results can be due to different brands of glucometers and the amount of blood used. This study showed no significant

difference in glucose levels recorded by Glucometer and reference Hexokinase methods in case of age and gender. The results show a strong positive correlation (r = 0.996) between Glucometer and Hexokinase methods, which shows that the glucometer used in our study is accurate in measuring BG. Hyperglycemic or hypoglycemic patients can rapidly be tested and managed in an emergency using a glucometer. Our results indicate a good correlation between glucometer readings and laboratory-based methods readings in the Pakistan population which will help our pathologist in the selection of BG estimation methods depending upon the condition and situation of diabetic patients.

Chapter 6 Conclusion

The findings of this study show that Portable Glucometer (ACCU-CHEK Instant S) efficiently measures BG levels when compared with the automated Laboratory based chemistry analyzer (Oxidase and Hexokinase Methods). Hexokinase Method remains the gold standard for glucose measurement and should be used whenever possible but for rapid results of BG, glucometers can be used as a home-based easy-to-use glucose measuring device for continuous monitoring of diabetes patients. We recommend further studies should be conducted on different brands of glucometer as this study has been using ACCU-CHEK Instant S glucometer only so the results cannot be generalized for all brands.

6.1 Limitations

This study was conducted only on Type II diabetes patients. For future studies, testing should be conducted on both Type I and Type II diabetes patients and for a fair comparison Non- diabetic participants (control group) should also be included.

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