NGUYEN THI BAO NGOC

BLOOD GLUCOSE AND ENZYME ANALYSIS METHODS OF BLOOD GLUCOSE

Case in Vietnam

Thesis

CENTRIA UNIVERSITY OF APPLIED SCIENCES

Degree Program in Chemistry and Technology

November 2018

ABSTRACT

Unit	**Date**	**Author/s**
Kokkola - Pietarsaari	December 2018	Nguyen Thi Bao Ngoc
Degree programme		
Chemistry and Technology		
Name of thesis		
ANALYSIS METHODS OF BLOOD GLUCOSE: Case in Vietnam		
Instructor		**Pages**
Jana Holm + Nina Hynynen		**27+2**
Supervisor		
Jana Holm		

Glucose is known as a single sugar in the blood. The glucose index, or glycaemic index, is the concentration of glucose in the blood. Blood glucose levels are one of the most important indicators of whether a person has diabetes or not.

Blood sugar or diabetes is one of the chronic diseases that can be encountered at any age and is a concern of a significant number of developed countries today. Diabetes is the cause of diseases such as blindness, hemodialysis, cardiovascular events such as myocardial infarction, stroke, and death.

The main purpose of this study was to provide a basic understanding of glucose and glucose in the blood. Besides, the research introduces the most commonly used method of glucose measurement in blood, is glucose oxidase. This method is widely used with semi-automatic biochemical analysers, provide high results in fast time and at low cost.

Key words:

Glucose, blood glucose, diabetes, colorimeter, glucose oxidase.

TABLE OF CONTENTS

ABTRACT

1 INTRODUCTION

Diabetes is the most common chronic disease among metabolism and endocrine diseases. Diabetes is becoming a major medical problem, causing a number of socially detrimental effects such as increasing the burden of medical costs, reducing social labour, increasing the rate of death and shorten the life of the patient.

Nowadays, diabetes is increasing in developed countries, where urbanization gradually changes the lifestyle, eating habits and physical activity. Diabetes mellitus is associated with many chronic and acute complications of cardiovascular complications. These complications, together with psychological stress, not only diminish the quality of life of the patient but also endangers life expectancy, leaving many serious and permanent sequelae, resulting in increased mortality. For that reason, it can be seen that the high diabetes rate is a burden to the community and society.

It was found that from 40 to 49-year-old with a diagnosis of type 2 diabetes would lose an average of ten years of life. Patients with diabetes mellitus have 2-3 times the risk of coronary heart disease than those without diabetes. On the other hand, at the time of diagnosis, the majority of diabetic patients had complications, including retinopathy of 35%, peripheral neuropathy 12%. According to the World Health Organization (WHO), in 2008 the world had 135 million diabetics (4%) of the world population, only two years (2010) the number of people with diabetes to 221 million (5.4%). In Vietnam, in recent years, the rapid development of diabetes has become a major problem in the health sector. According to calculations by the Association of diabetes educators in Vietnam, the rate of diabetes in 2002 accounted for 2.7% of the population, by 2008 (after 6 years) has doubled 5.7% of the population. (Timon, Collantes, Galindo & Gomez, 2014.)

Blood sugar or blood glucose is a basic test used to assess glucose metabolism disorders such as diabetes mellitus or hypoglycaemia. Testing is done by two methods which are chemistry and enzyme. However, chemical methods are currently unavailable for measuring non-specific and high time-consuming

glucose levels. Instead, nowadays the most common method is to use enzymes. The enzyme method has higher specificity and time is also faster, giving the best results. Currently, the three most commonly method used enzyme to quantify blood glucose that are hexokinase, glucose oxidase and glucose dehydrogenase. This research focus primarily on the use of glucose oxidase enzymes to quantify glucose in the blood. This is a common method with chemistries for semi-automatic biochemistry and some biochemical machines. This method combines the use of glucose oxidase and peroxidase.

The paper is divided into four main sections. The first part is the basic introduction to glucose and the nature of glucose in the blood. The second part introduces the method used today to quantify glucose in the blood. Experimenting the method of using glucose oxidase to quantify glucose in blood will be introduced in the third part and the research concludes with a survey of the interest in the blood glucose index of young people nowadays.

2 GLUCOSE AND INFLUENCING FACTORS

In the body, glucose is known as an important carbohydrate source for energy. The glucose concentrations are maintained by gluconeogenesis and glycogenolysis in starving state while the glucose circulating is found in the fed state. The majority amount of glucose is found in complex carbohydrates which are separated to monosaccharides during the digestive period while a small amount of glucose is performed in fed state as glucose. (McMillin, 1975.) This section will show the glucose transporter and the way to control the blood glucose.

2.1 Glucose transporter

Glucose is used effectively by a significant number of different cell types under normal conditions, however, its content in the blood must be controlled accurately. Glucose plays a central role in the metabolism and homeostasis of the cell. Most of the cells in the body need continuous supply of glucose in the form of ATP to provide energy. The glucose balance disorders are known as a reason of the diabetes. Glucose is absorbed to the cells through the cell membrane. Molybdenum molecules cannot travel through the cell membrane by diffusing simply because the high molecular pathway cannot pass through the infinity matrix of the double lipid phosphorus layer. An efficient transport system is required to move molecules into and out of the cell for the glucose molecules used by cells. In certain absorbed cells, such as epithelial cells of the small intestine and tubules, glucose crosses the cell membrane (active transport) against the concentration gradient, injected by Na^+/K channel system. However, glucose is transported passively to almost all cells in the body by a mediated transport mechanism without energy. The transporter protein involved in this process is called glucose transport, abbreviated as GLUT. (Wood & Trayhurn, 2003.)

There are twelve different transport proteins have been identified along with their genetic codes. Genome project assisted in this identification because all transport proteins share a similar structure and sequences in their genomes. Approximately 28% of amino acids are common in the transporter protein group. Each GLUT is a protein that separates, penetrates and extends the lipid bilayer of the cell membrane and pass

through the membrane several times. (Goodsell, 2017.) The pattern of glucose transport in the molecular space is illustrated in Figure 1.

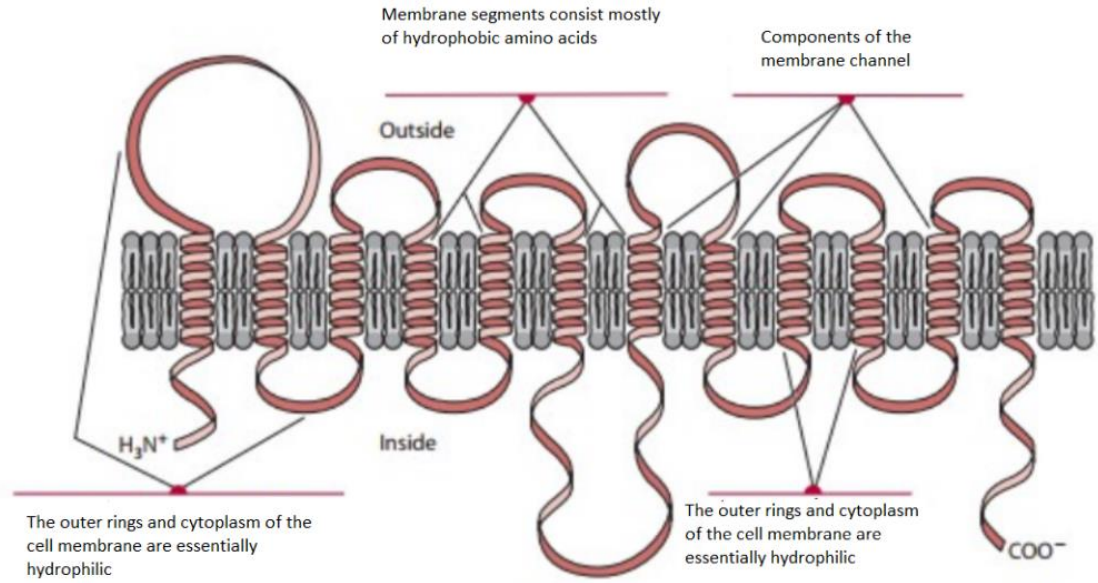

FIGURE 1: Glucose Transporter in Red Blood Cell. (Dam, 2018.)

In the simplest form, a transport protein has a specific location for the molecule being transported. Moreover, it also undergoes a configuration change when it binds to the molecule, allowing the molecule to pass through the other side of the membrane and be released. Protein transport is also capable of reversing process can be repeated. All cells express at least one GLUT isotope on their plasma membrane. Other isoforms have distinct tissue distribution and cell biochemistry, and they contribute to the precise processing of glucose under different physiological conditions. (Wood & Trayhurn, 2003.)

TABLE 1: Twelve isoforms of glucose transport. (Dam, 2018.)

Protein transporters	Insulin correction	Main expression location
GLUT 1	NO	Haemoglobin, blood brain barrier, placenta, fetal tissues.
GLUT 2	NO	Liver, β cell of the pancreas, intestines, kidneys, small intestines.
GLUT 3	NO	Brain, neurons.
GLUT 4 *	YES	Muscle, heart, brown fat and white fat.
GLUT 5	NO	Gut, testicle, kidney.
GLUT 6	NO	Spleen, white blood cells, brain.
GLUT 7	NO	Unknown
GLUT 8	NO	Testicles, embryonic, brain.
GLUT 9	NO	Liver, kidney
GLUT 10	NO	Liver, pancreas
GLUT 11	NO	Heart, muscle
GLUT 12	NO	Heart, prostate

GLUT 1 is expressed in the red blood cells and endothelial cells of the brain with the responsibility of providing basic glucose to the cells. GLUT 2 is involved in the transport of glucose from the intestinal mucosa into the bloodstream via the portal and, moreover, it can also transport fructose from the intestinal lining cells, the rate of transportation dependent on glucose concentration in the blood. GLUT 3 is considered a highly valued tissues dependent on glucose concentration such as the brain. In contrast, GLUT4 is quite sensitive with insulin and concentrate on the cell membrane that increases the hormones. The increase glucose transport across the membrane is accompanied by an increase in glucose uptake by insulin stimulating cells. The presence of GLUT 4 in skeletal muscle and in fat tissue, makes these tissues respond to insulin. The liver, brain, and erythrocytes lack GLUT 4, and, therefore, insensitive to insulin. Besides, fructose transport is characteristic of GLUT 5. (Navale & Paranjape, 2016.)

Molecular biology techniques have been applied to the study of the activity of some GLUTs. GLUT has also been found at glucose level in certain tissues such as stem cells of the pancreas. Moreover, following

ribosomal mRNA synthesis on the endoplasmic reticulum, the protein transported into the Golgi system, where it was synthesized in the tubular structure of the trans-Golgi network. In the absence of oocyte stimulation, GLUT4 is located in these structures as well as in cytoplasmic pouches. In skeletal muscle cells, there is also the distribution of GLUT 4. The balance between intestinal absorption of glucose and the absorption and metabolism of peripheral tissues have maintained the blood glucose level within a narrow range. (Navale & Paranjape, 2016.)

2.2 Insulin

Insulin plays an important role in the metabolism of lipids and amino acids. It is a powerful anabolic hormone and involved in the synthesis, and storage of glucose, lipids and amino acids or protein. Moreover, insulin promotes the expression or activity of enzymes that catalyse the synthesis of glycogen, lipids, and protein. The enzymes that catalyse the metabolism of glycogen, lipids, and amino acids, are inhibits the expression or activity. (Ogbru & Williams, 2016.) The anabolic and catabolic effects of insulin on glucose and glycogen, fatty acids and triacylglycerols, and amino acids and proteins is illustrated in Figure 2.

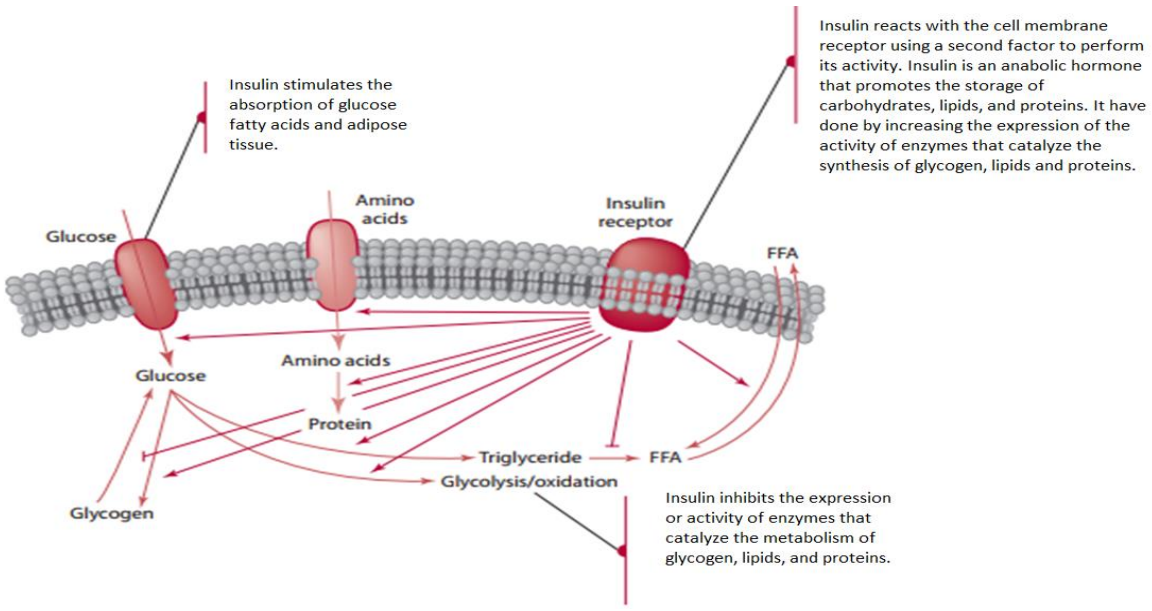

FIGURE 2: Insulin Regulate Metabolism. (Dam, 2018.)

Recently, the mechanism of insulin is used to control blood sugar and its complications. It also is known as an important role in regulating blood sugar. (Hellman, 1978.) The pancreatic β cells release the insulin when blood sugar rises. Insulin links to a specific receptor located on the muscle tissue membrane and

adipose tissue and stimulate the absorption of glucose by muscle and fat. Moreover, it also suppressed the synthesis of glucose (gluconeogenesis) in the liver and stimulates the GLUT 4 transport tubules to surround as a tubular cell that moves to carry the cells. The function of insulin through a second communication system and belongs to a subfamily of tyrosine kinase including receptor which relates with insulin (IRR) and growth factor as insulin (IGF). (Kahn, 1985.) The mechanism is illustrated in Figure 3.

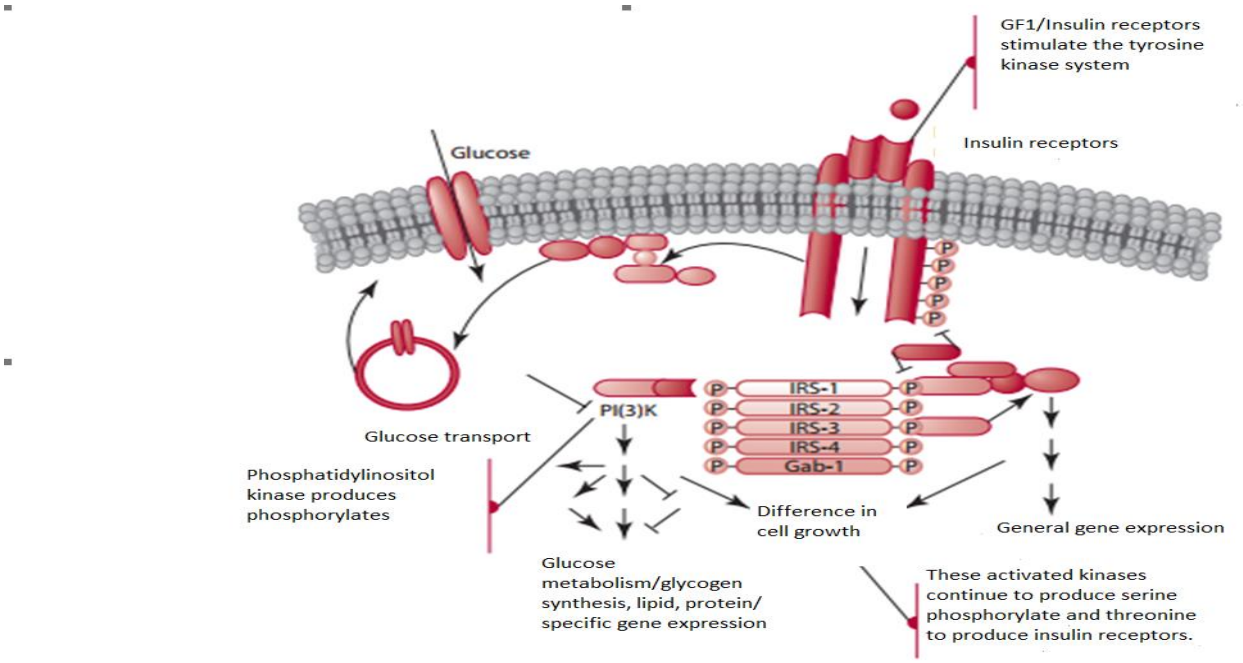

FIGURE 3: Insulin Mechanism (Dam, 2018.)

Insulin is an important hormone in signalling “Does the body have a lot of time?”. During this time, insulin stimulates the synthesis of glycogen in the liver and fatty acids synthesized in adipose tissue. Glucose is transported into the cells of the body by the isotopes of the glucose transporting proteins, and the cells of the various organs having specific glucose transport linked to them. The GLUT 4 is found mainly in skeletal muscle and adipose tissue, is dependent in insulin. (Huang & Czech, 2007.)

2.3 HbA1C Quantify

Hb is known as the haemoglobin in red blood cells. Hb includes HbA, HbA2 and HbF with HbA accounting for 95-97%. A small amount of HbA will combine with glycosylated carbohydrates to form HbA1. HbA1 is a normal in the blood about 5-7%. HbA1 includes HbA1a, HbA1b and HbA1c. HbA1c is caused by HbA-bound glucose, which accounts for 75-80% of HbA1, the highest glucose in the blood,

or similar 4-6%. HbA1 increases when the blood sugar increases during long period. The HbA1c rate increases, the blood glucose rate of patients has increased over time. On the other hand, the life of red blood cells is 120 days, because the amount of HbA1c will reflect the blood glucose of patients in the past 2 or 3 months. This test is very useful for monitoring and evaluating the therapeutic effect in patients with diabetes. The test should be done at least every 3 months. (Davis, 2018.)

The normal value of the test is 4-6%. Considered as an increase in HbA1c > 6.5%. Maintenance of HbA1c < 6% would be of great benefit in reducing the complications caused by the disease. It has been found that the correlation between HbA1c and glucose levels for every 1% HbA1c increase in equivalent to an increase in blood glucose levels of 1,7 mmol/l. For example, 4% HbA1c is equivalent to 3.3 mmol/l glucose, 5% HbA1c is equivalent to 5.0 mmol/l glucose, 6% HbA1c is equivalent to 6.7 mmol/l glucose. There is a significant method to quantify HbA1c such as turbidity (photometric), high-pressure liquid chromatography, ion exchange chromatography, electrolysis. However, there is two most common methods are turbidity measurement available on automatic biochemical machines and high-performance liquid chromatography (HPLC system) or high-pressure liquid chromatography. HPLC method is confirmed as the most accurate. (Davis, 2018.)

High performance liquid chromatography is a method pf separation in which the mobile phase is a liquid and the stationary phase contained in the column is a solid that has been partitioned in the form of a particulate or a liquid covering a solid carrier, or a substance are modified by chemical bonding to organic functional groups. Liquid chromatography is based on adsorption, distribution, ion exchange, or size classification, molecular sieve. In HPLC method, stationary phase is an important factor determining the nature of chromatography process and type of chromatography. On the other hand, if the stationary phase is an adsorption, the adsorption chromatography is positive, or phase reversed. If the stationary phase is ion exchange, ion exchange chromatography is collected. Besides, the mobile phase, organic solvent is passed through a chromatographic column containing stationary phase under high pressure. Depending on the affinity of the analyte with the stationary phase, the retention time varies. The results are determined by the retention time, the time from the injection of the sample to the time when the analyte is removed from the stationary column, and the area of the peak. Different substances will have different retention time and different concentration will results in different peak size. This technique will separate each type of HbA and quantify their concentration accurately. The turbidity method is performed on automatic biochemical machines. First use a chemical to break down

red blood cell and release HbA1c. After reaction, Hb and A1c will be measured separately by analyser. The Ac1 rate on Hb and HbA1c concentration will be calculated by machine. (Little & Robert, 2009.)

2.4 Fructosamin Quantify

Fructosamine is known as ketoamine, a common name of 1-amino-1deoxy-fructose, or isoglucosamine, a derivative of the enzyme-free sugar (mainly glucose) and protein (primarily albumin). Albumins and proteins are bound to glucose and product glycated which is abbreviated as GSA (glycated serum albumin) and GSP (glycated serum protein). The term glycation is used to refer to any reaction that connects glucose with proteins. Glycated proteins are also used instead of glycosylated or glucosylated proteins. Fructosamine has been used in biochemical literature since 1982 as a general term replacing the term glycated protein. (Nansseu, Domgue, Noubiap, Balti, Sobngwi, Kengne, 2018.)

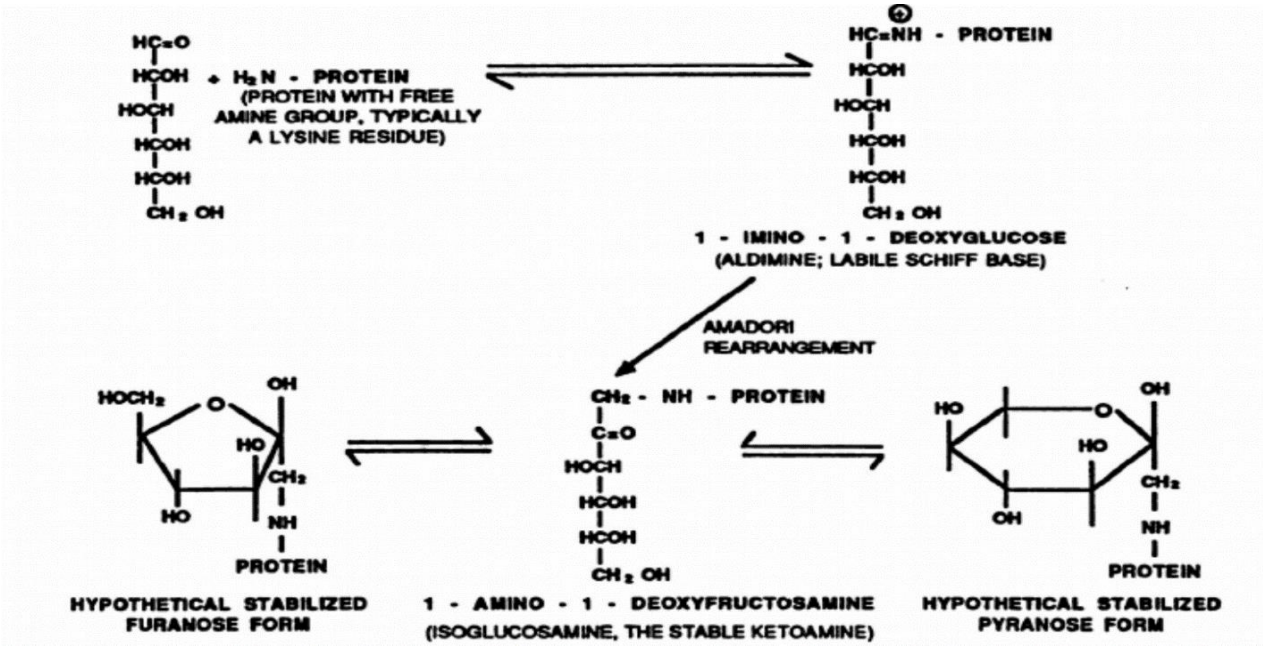

FIGURE 4: The reaction between glucose and protein to produce fructosamine. (Luong, 2013.)

The carbonyl group of glucose combines with the amino group of the protein to form an aldimine, which develops into a stable ketoamine or fructosamine. The unstable aldimine or schiff base (aldimine) is immediately rearranged in an amadorium rearrangement that translates the N-glycosides of aldoses into N-glycosides of the corresponding ketose to form a stable fructosamine. Plasma albumin and plasma protein increase in diabetes mellitus. Therefore, fructosamine is used to assess the patient's blood glucose rate over a period of one to three weeks. Fructosamine is a product formed by the glycosylation

reaction between glucose and serum proteins. Blood albumin level are so high that fructosamine is usually glucose bound. The amount of fructosamine used to assess indirect blood glucose levels in the past 2-3 weeks, because the half-life of albumin for 2-3 weeks. The normal fructosamine concentration in blood is determined about 2,0-2,8 mmol/l. Normal fructosamine levels may show that a person is either not diabetic or they have food diabetes control. A high to normal level of fructosamine also indicate that their treatment regimen is effective. (Nansseu, Domgue, Noubiap, Balti, Sobngwi, Kengne, 2018.)

Fructosamine is increased in patients with diabetes and based on that to assess the patients' blood glucose status in the previous 2-3 weeks. The test will be more stable and accurate than the amount of blood glucose, because the blood sugar is fluctuating and change in the short time, depend on patient's diet, the cycle of medicine. Fructosamine quantification is also fairly simple based on the principle of optical measurement by endpoint measurement. Research can be done on automated or semi-automatic biochemical systems. (Nansseu, Domgue, Noubiap, Balti, Sobngwi, Kengne, 2018.) In conclusion, both HbA1c and Fructosamine testing will help to evaluate patient's blood glucose rate in the past 2-3 months or the recent 2-3 weeks, respectively.

3 BLOOD GLUCOSE

Diabetes is known as a common disease nowadays. As the society development, the loss of control in use of food, chemicals, drugs are the reason of diabetes. Especially, nowadays, diabetes is rejuvenating. Anyone can get diabetes, and if it is not detected and controlled, the disease can cause of serious complication in the eyes, kidneys, blood vessels, or limbs. For that reason, blood glucose test should be done to find out early diabetes and the blood sugar can be controlled to avoid any unfortunate complication. (Cassobhoy, 2017.)

3.1 Diagnostic tests for blood glucose

Diabetes is a common disease in society nowadays. As developments of society, the use of food, chemicals, medicines or drugs is not controlled leading to increase the number of people with diabetes. Diabetes is especially rejuvenated. Anyone can have diabetes, and if it is not detected and controlled early, diabetes will cause serious complications in the eyes, kidneys, blood vessels, and limbs. This section shows the four basic blood glucose tests used nowadays.

3.1.1 Blood Glucose randomized testing

According to WHO, one of the criteria for the diagnosis of diabetes is a blood glucose test at any time of ≥ 200 mg/dl (≥11,1 mmol/l) with plasma or ≥180 mg/dl (≥10,0 mmol/l) with total blood. Therefore, blood glucose can be measured in anytime without regard to whether the patient has eaten or eaten for how long. In this case, if blood glucose is bigger than 11,1mmol/l, the patient is confirmed as diabetes. However, if the blood glucose result is measured as less than 7.8 mmol/l, blood glucose test should be confirmed again. (Barrell, 2018.)

There are two main ways to determine blood glucose. Firstly, blood is centrifuged to collect the plasma which is tested on semi-automatic or automatic biochemistry systems. Secondly, blood glucose meter is

known as a common method nowadays, which is used to measure the whole blood from the capillaries. (Barrell, 2018.)

3.1.2 Fasting Blood glucose

This is the most commonly used diagnostic test for diagnosing diabetes. If plasma glucose is between 4,4 mmol/l and 5,0 mmol/l, the result is confirmed as standard. However, if the test result shows that glucose blood is over 7,0 mmol/l, it is considered diabetes. Moreover, the blood plasma level is determined higher than whole blood approximately 10%-15%. (Norman, 2016.)

It means that, if the plasma glucose test is less than 5,6 mmol/l or less than 4,4 mmol/l in total blood, so no diabetes. If plasma glucose is between 5,6 mmol/l and 6,4 mmol/l or between 4,4 mmol/l and 5,5 mmol/l in total blood, then the risk of diabetes mellitus is not high and does not require hyperglycaemia. On the other hand, if the plasma glucose test is higher than 6,5 mmol/l – 7,0 mmol/l or 5,6 mmol/l – 6,6 mmol/l in total blood, then the risk of diabetes mellitus is high and need to be tested for hyperglycaemia. Finally, if the plasma glucose test is determined higher than 7,0 mmol/l or over 6,7 mmol/l in total blood, the diabetes mellitus is the last result. (Falck, 2017.)

3.1.3 Blood glucose tolerance test

This test is used common with pregnant women between weeks 24 and weeks 28 of pregnancy to determine the blood glucose level and create the suitable treatment and avoid the pregnancy complications. (Kinman, 2018)

The testing is performed by collect the patient's blood after 2 hours of patient's meal. The patient's meal will have about 100 grams of carbohydrates and other nutrients. After 2 hours, if the blood glucose test shows a plasma glucose result of $\geq$ 11.1 mmol/l, it will be considered as diabetes mellitus. If glucose blood level is less than 6,7 mmol/l are considered normal. However, this method is not popular because it is difficult to control the patient's meal composition or the time of the meal. It also is difficult to control food absorption. There are patients with fast absorption capacity, while others have slow absorption capacity. For that reason, the result of blood sugar may not accurate. (Norman, 2016.)

3.1.4 Blood glucose increased by oral administration

The method is valuable and used to confirm in the patient with plasma glucose > 6,4 mmol/l and < 7,0 mmol/l. Firstly, in this method, the blood is collected before drinking and still hungry. Secondly, the adult patient is required to use 75 grams of glucose solution while children are asked to use approximately 1,75 gr/kg of body weight. After that, the patient's blood is collected to quantify the glucose concentration at the times of 30, 60, 90, and 120 minutes after ingestion. (Brown & Yanovski, 2014.) If the result shows that the measurement is bigger than 11,1 mmol/l, the patient is considered as diabetes. On the other hand, in normal people, the blood glucose level rise to about 8,3 mmol/l after the blood glucose and then gradually lowered and returned to normal after 3 hours. However, in this method, the patient is not allowed to use glucocorticoid or diuretics. Besides, the patient is required to have meal and normal activities 3 days before the test. The procedure is performed after the patient had fasted for at least 8 or 10 hours. (Wiener, 1992.)

3.2 Effect factors to blood glucose test results

Firstly, patients are not eligible for blood glucose testing such as not fasting and not stopping medication before taking blood. On request, the patient must eat at least 8 hours before taking the blood. If the patient has eaten, the blood sugar rate will increase. Depending on the time from the meal to the blood and the amount of the food that will increase the blood glucose rate more or less. In addition, diabetics also need to stop the medication before taking the blood. Usually, the blood is taken in the morning and the patients will only take the medicine after the blood is taken. Secondly, during the blood collection or maintenance, technicians break the red blood cells that affect the results of the test. When the red blood cell ruptures, the enzymes in the red blood cells are released and it will use glucose very quickly, resulting of decrease of glucose concentration. Thirdly, when insufficient amount of sample leads to wrong anticoagulant, increased concentration of anticoagulant or blood is diluted, which will affect to the result. Particularly with sodium fluoride antifiction, when inadequate blood levels increase the level of anticoagulant effect of the test. (Ginsberg, 2009.)

Besides, blood samples should be centrifuged for testing. Because long-term use of glucose reduces glucose concentrations by about 3-5% every hour at room temperature. Currently, to avoid this decrease in glucose levels, sodium fluoride is used to inhibit enolase enzyme in the glycolytic cycle, preserving blood glucose levels stable for up to 48 hours at 4 degrees Celsius. In some research, the volume of red blood cells (hematocrit) affects the results of blood glucose measurement. Specifically, hematocrit is bigger than 55%, results is decreased, on the other hand, hematocrit is smaller than 35%, results are increased blood glucose levels. In addition, low oxygen levels will also affect the blood glucose test results. Emotional and physical disability, shocks, burns and infections can increase blood glucose levels. The reason is that the metabolism increases, the body needs more energy to increase the glucose rate in the blood. Moreover, some hormones increase, resulting the increase of glucose in blood. Medications that can increase fasting plasma glucose levels are psychiatric medications, azathioprine, basiliximab, diazoxide, adrenaline, estrogen, furosemide, gemfibrozil, isoniazid, leveothyroxine, lithium, niacin, protease inhibitors, thiazide. Some drugs that can reduce fasting plasma glucose levels are acetaminophen, basilizimab, carvedilol, desipramine, ethanol, gemfibrozil, hypoglycemic tablets, insulin, phenothiazine, risperidone, theophylline. (Ginsberg, 2009.)

4 DETERMINATION OF BLOOD GLUCOSE BY ENZYME METHODS

Glucose metabolism disorder is assessed by using a blood glucose test, which is done by chemical and enzyme methods. However, nowadays, enzymes are known as the most common method to determine the glucose in the blood. The enzyme methods have high specificity, fast time and effective. There are three main types of enzymes which commonly used to quantify blood glucose, are hexokinase, glucose oxidase and glucose dehydrogenase. (Yoo & Lee, 2010.)

4.1 Glucose oxidase method

Glucose oxidase is a common method with chemistries for semi-automatic biochemistry and some biochemical analyser. This method combines the use of enzyme glucose oxidase and peroxidase. (Mcmillin, 1990, 141.)

Glucose is oxidized to gluconic acid and hydrogen peroxide (H_2O_2) by glucose oxidase method. Oxygen will be released by formation of a peroxidase degrading enzyme (POD) from hydrogen peroxide. Oxygenate liberates 4-aminophenone (4-AAP) and the quinonimine phenol complex is reddish. Colour intensity is proportional to the amount of glucose. (Megazyme, 2018.) The principles of the method are as follows:

$$Glusoce + H_2O \xrightarrow{GOD} Gluconic\ acid + H_2O_2$$

$$2H_2O_2 + Phenol + 4\ amino - Antipyrin \xrightarrow{POD} Quinonemin + 4H_2O$$

The method includes two steps. The first step, glucose in the sample is oxidized by the enzyme glucose oxidase to produce peroxide H_2O_2. However, glucose oxidase is highly specific to β-glucose while serum also contains both α-glucose and β-glucose with ratio 1:2 respectively, for that reason, mutarotase also is added in some chemicals to convert α-glucose to β-glucose. H_2O_2 is formed directly proportional to glucose concentration. Then peroxide H_2O_2continues to react with phenols and 4 amino-Antipyrines to produce quinonimine. Quinonimine has a lotus pink colour, the colour intensity is proportional to the

glucose concentration. Quinonimine was measured at 540 nm. Nowadays, the amount of peroxide can be measured immediately without the second stage such as blood gas machine or hand blood glucose meter. (Bostick & Hercules, 1975.)

In this research, phenol and 4 amino-antipyrine are used to form oxidizing colouring agent.

$$Colour\ reducing\ agent + H_2O_2 \rightarrow Oxidizing\ coloring\ agent + H_2O$$

However, the serum also contains other reducing agents such as uric acid, vitamin C, bilirubin, which will inhibit this reaction by competing with the colouring agent such as phenol, an effect to the result. For that reason, 4 amino -antipyrine is added to remove the disturbance by uric acid, creatinine or haemoglobin. Besides, the reaction also can be affected by catalase which decomposes H_2O_2. (Yoo & Lee, 2010.)

This method is known as an effective method with fast time and low cost. However, the other side is that many of the influencing factors can affect the response and often decrease the glucose concentration in comparison to the fact. (Yoo & Lee, 2010.)

4.2 Hexokinase method

Hexokinase method is known as the most common method nowadays in automated analyser systems. Hexokinase enzyme gives the most accurate result because it is very glucose specific without interference from other carbohydrates. (Dohnal, Kalousova, Zima, 2010.) There are two phases in this method:

$$Glucose + ATP \xrightarrow{Hexokinase} Gluconic - 6 - Phosphate + ADP$$

$$Glucose - 6 - Phosphate + NADP^+ \xrightarrow{G6PD} 6 - Phosphogluconate + NADPH + H^+$$

In the first step, Glucose-6-Phosphate was produced by glucose phosphorylation with hexokinase reagent. In the second step, G6PD catalysed glucose-6-phosphate oxidation to produce NADPH. The glucose concentration is in proportional to the increase of the optical density of NADPH and measured at 340 nm. (Dohnal, Kalousova, Zima, 2010.)

Hexokinase catalyses the phosphorylation of both fructose and mannose, however, the concentration of these sugars in the blood is too small and insufficient to interfere with the reaction. However, the serum rupture of red blood cells may affect this reaction because G6PD and 6-phosphogluconate dehydrogenase in red blood cells also use $NAPD^+$ as the substrate. Nowadays, G6PD of bacteria (instead of fungi) is used to reduce the effect to the reaction. The reason is known as that the G6PD of bacteria will use NAD^+ instead of $NADP^+$. Moreover, nowadays, instead of measuring the change in optical density of NADPH, some chemicals use an indicator such as phenazine methosulphate (PMS) or idonitrotetrazolium (INT) to react with NADPH to produce colorimetric products is at 520 nm. (Dohnal, Kalousova, Zima, 2010.)

In conclusion, this method is known as the most common method nowadays because of its high specificity and the results are less affected by other factors. However, the disadvantage of this method is the high chemical cost. (Dohnal, Kalousova, Zima, 2010.)

4.3 Glucose dehydrogenase method (GDH)

Glucose dehydrogenase method (GDH) is used on hand glucose meter. There is only one phase in this method:

$$\beta - D - Glucose + NAD^+ \xrightarrow{GDH} D - Glucono - \Delta - lacton + NADH + H^+$$

NADH is created in the step 340 nm in the kinetics or endpoints. Besides, mutarotase also is added to convert from α-Glucose to β-Glucose to increase the accuracy. However, GDH also reacts with other sugar such as maltose, galactose or xylose. Therefore, if patients use some of those sugar in the treatment, the result will not accurate. For that reason, nowadays, GDH from the strain of Bacillus cereus is used to avoid the error. (Staiano & D'Auria, 2017.)

5 THE RESEARCH RESULTS

Vietnam is known as the developing countries in Asia area and diabetes is also rapidly increasing. Since the 1990s, the incidence of diabetes has increased in the large cities of Vietnam. In 1990, Hanoi had confirmed with a high rate of diabetes, 1,2% with 1,44% in the inner city and 0,63% in the rural. In 1993, in Hochiminh city, the rate of diabetes was 2,52%. In Hue, in 1996, there was 0,96% with 1,05% and 0,6% in inner city and suburbs respectively, the proportion of women was higher than man. In the early year of the 21st century, Vietnam has promoted extensive diabetes research to provide effective prevention and treatment. The results of the study show that the incidence of diabetes was higher than in the 1990s. In 2001, an international epidemiological survey of diabetes was conducted at four major cities in Vietnam which were Hanoi, Haiphong, Danang, and Hochiminh city, resulting in 4,0%. In 2002, in Hanoi, the proportion of diabetes patients was 2,16%. The result was nearly double compared to the 1990s although the research was conducted in the same location, the same age group and the same research method. The national diabetes rate was 2,7% with 4,4%,2,1%, and 2,7% in the city, mountainous and mid-urban, and suburb respectively, in 2002. However, there was approximately 64,9% of patients were undetected and were not properly treated. (Luu, Trinh, Tran, Nguyen, Le, Pham, & Nguyen, 2018.) This section will show the result of research about blood glucose situation in Binh Giang hospital from September 2017 to September 2018.

5.1 Research subjects

The researching subjects included 89 patients who had been diagnosed with diabetes for more than three months in hospital treatment. Patients are divided into two main groups. The first group consisted of 18 patients whose diabetes was diagnosed at the time of research was less than 1 year. The second group consisted of 71 patients who had diabetes for at least one year.

Criteria for selecting patients based on the criteria of the study. Inpatient hospital treatment in Binh Giang General Hospital, from September 2017 to September 2018. Firstly, patients who have been

diagnosed with diabetes according to WHO criteria for more than 3 months prior to initiation of research. Secondly, patients with normal mental health and ability to communicate and interact directly with the researcher. Lastly, patients voluntarily agreed to participate in the study. Patients included in the study who were diagnosed with diabetes mellitus according to WHO criteria included fasting glucose level above 7 mmol / l, resulting in 2 consecutive times; or any blood sugar above 11 mmol / l, combined with clinical symptoms such as thirst, multiple urinary, weight loss. Besides, Blood glucose levels were elevated to 11.2 mmol / l

** HbAlC is greater than 6.7% according to new ADA standards.*

Some exclusion criteria are given according to the criteria of the study. Firstly, the patient did not agree to participate in the interview. The doctors or researchers should not force or discriminate against these patients. Secondly, patients diagnosed and treated for diabetes for less than 3 months. Thirdly, patients with coma or cognitive dysfunction, dementia, lack of ability to communicate affect the ability to hear or provide information to the researcher; or patients were interviewed once during the study. Criteria are defined and implemented to produce the most accurate results.

The experiment was conducted with the consent of the hospital's leaders and the help of the technicians at Binh Giang hospital's blood analysis department. The research process was started from September 2017 to September 2018. The whole research process was conducted and researched at the Binh Giang General Hospital, Hai Duong province, Viet Nam. The research will be conducted in a cross-sectional manner.

In this research, a sample of 89 patients was selected according to the criteria of the research. The sample was selected based on a total sampling method, in turn selected patients who had been diagnosed with diabetes for more than 3 months for inpatient treatment at Binh Giang General Hospital from September 2017 to September 2018. Data was collected based on the information collected from 89 patients enrolled in this research. The information will consist of four main parts. Part A, the information is collected, related to the demographic characteristics of the participant. Part B, the collected information relevant to the patient's perceptions of their illness. Part C, patient is asked about information on treatment adherence. Lastly, part D, some basic reasons make the patient unable to follow the doctor's instructions.

Organizing data collection is done through the following two steps. Firstly. in step 1, the research tools were developed, tested and refined. The questionnaire was designed and developed based on the practice of treatment adherence, including compliance with nutrition, exercise and medication. Next, the questionnaires will be revised, supplemented and finalized to achieve the best results for the study before being included in the study for the study. Secondly, step 2, after the questionnaire is ready, the survey will be conducted with the selected subjects for the research. The research was based on the general information variables of the selected research subjects. These include age, sex, occupation, education, regular information, HbA1C at the hospital. The team must follow the doctor's treatment regimen which includes following diet and exercise; adherence to oral medications and injections.

Results are collected and evaluated according to some criteria. Firstly, patients routinely follow the nutritional guidelines of their physician. During the treatment, the patients were not allowed to use drugs or food without the doctor’s permission. Secondly, patients who practice more than 30 minutes per day under the guidance of a physician. Moreover, patients taking oral medications as directed by health care workers. Patients taking the injection as prescribed by the doctor. It is the right kind of injection, right time, involves eating the right foods, preserving the medicine properly, taking the right and sterile drug when injected properly. During the research period, the patient was entitled to refuse to participate in the research without any difference in care and treatment during treatment in Binh Giang General Hospital. Information obtained from participants will only be used for research purposes. Lat but nor least, research only for the purpose of protecting and improving the health of the people, not for personal or other purposes.

Based on the time from the onset of the disease to the time of the research, the patients were divided into two main groups. Group 1 included patients with diabetes who had a disease detection time to the research time less than one year. Group 2 included patients with diabetes who had a disease detection time to the research time more than one year.

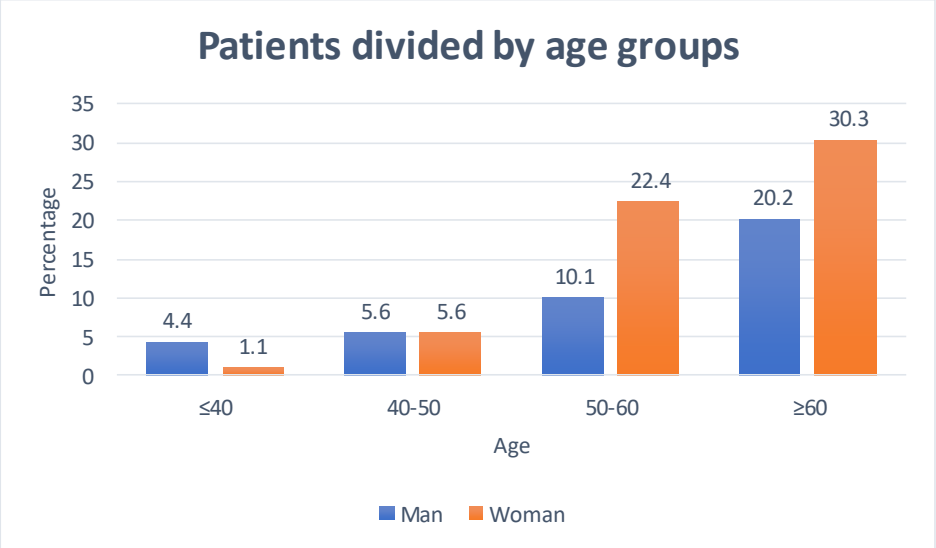

FIGURE 5: The patients were divided by age. (adopted from blood testing room, Binh Giang hospital, 2018.)

The figure 5 shows the division patients following the age group from September 2017 to September 2018. Under 40 years old, it is noted that the proportion of male patients is four times higher than women group. On the other hand, over the age of 50, the diabetes patients in women group tend higher than man group. In the research, the patients were mainly in the age group over 60 years old, in which the female patients accounted for 30.3% and man patients accounted for 20.2%.

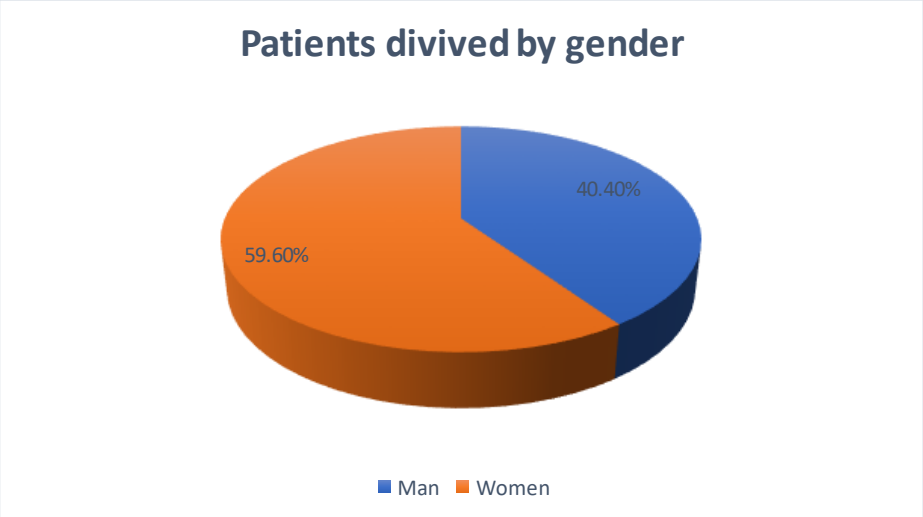

FIGURE 6: Patients were divided by gender. (adopted from blood testing room, Binh Giang Hospital, 2018.)

Figure 6 illustrates the division of patients by gender. Based on the gender charts, the number of male patients was only 40.4%, while the number of female patients in the study was 59.6%. There are two main reasons why women have higher risk of diabetes than men. Firstly, during the pregnancy, the increasing insulin caused of the diabetes. Secondly, menopause is one of the causes of hormonal changes, which makes women more susceptible to increased blood glucose.

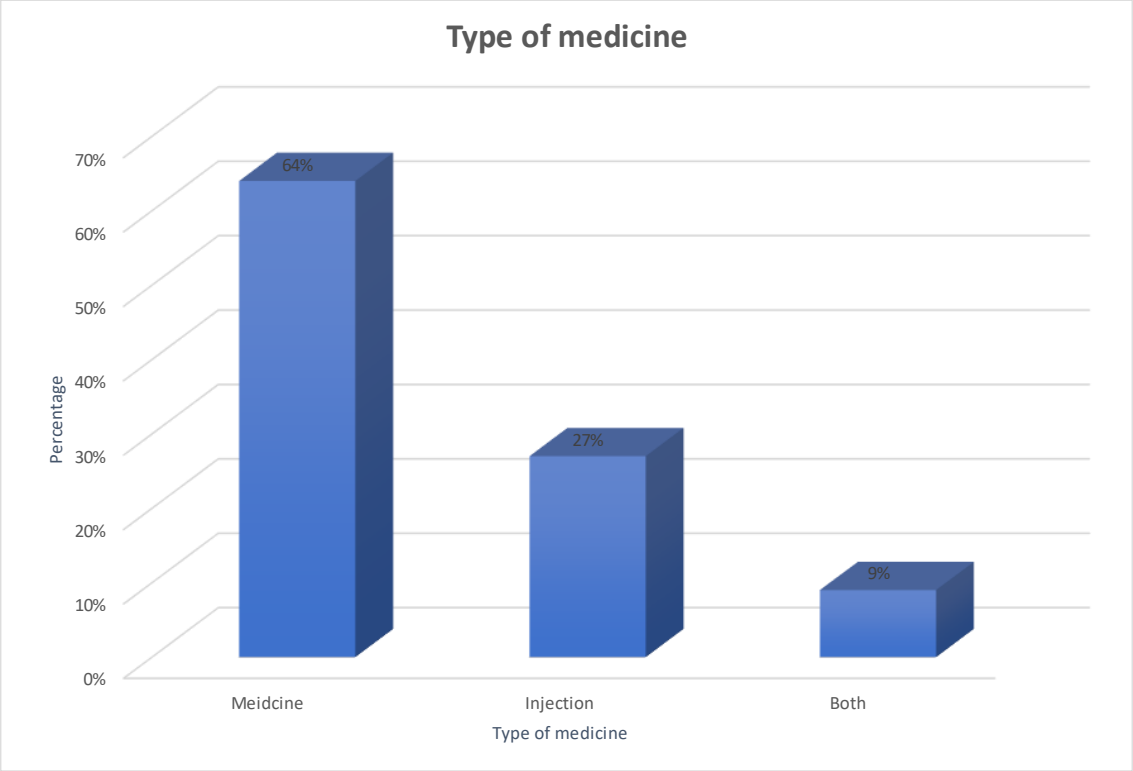

FIGURE 7: Patients were divided by using the type of medicine. (adopted from blood testing room, Binh Giang hospital, 2018.)

The type of medicine used in diabetes treatment is described in figure 7. Patients in the research who mainly controlled blood glucose daily with medicine, accounted for 64% while only 9% of patients controlled by both medicine and injection. It can be seen that the patients take tablets in order to control blood glucose levels because of their ease of use and ease of control. Patients do not need high expertise.

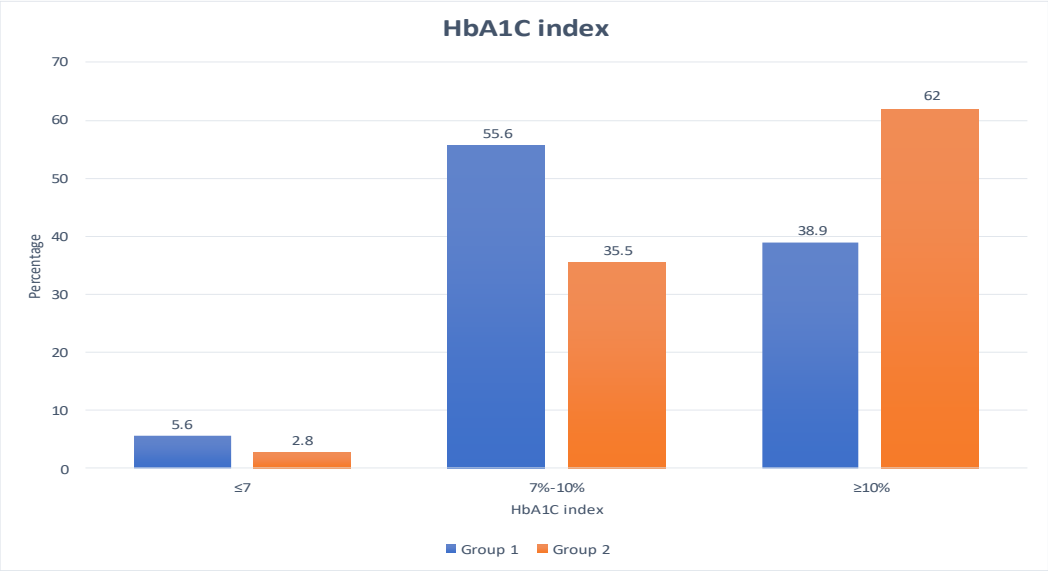

FIGURE 8: The patients were divided by the HbA1C index. (adopted from blood testing room, Binh Giang Hospital, 2018.)

Figure 8 shows the HbA1c index following the group. In the research, Group 1 included patients with diabetes who had a disease detection time to the research time less than one year. Group 2 included patients with diabetes who had a disease detection time to the research time more than one year. In the figure, the HbA1C index was higher than 10%, found in both patients group 1 and patient group 2, approximately 38.9% and 62% respectively.

TABLE 2: How to detect the diabetes. (adopted from blood testing room, Binh Giang hospital, 2018.)

Situation	**Patient number (N=89)**	
	N	**%**
Accidental medical examination	38	42.7
Fatigue	17	19.1
Thirsty more often	7	7.9
Lose weight fast	4	4.5
Urinary abnormalities	10	11.2
Other	13	14.6

The table 2 show how the patients found diabetes. The research was done in five main ways. Based on the research, most patients are diagnosed by accidental medical examination, approximately 42.7%. A small number of patients recognize the disease through rapid weight loss symptoms.

5.2 Survey results

The survey was designed to find out the concerns of young Vietnamese people with blood glucose, one of the current diseases in developing countries nowadays. Questions were designed as multiple choice with a completion time of less than 2 minutes. There was 8 question in the author's online survey and concentrate on the Vietnamese under 30 years old. The survey question was created by Google Form in Google Driver, and formed in English from 16th November 2018 to 20th November 2018. The questionnaire focuses on personal information and their attention to the blood glucose nowadays. The survey question is showed in the Appendices.

In 22 respondents, there are 40,9% of male and 59,1% of female. The people aged 18-24 accounted for the majority with 54,5%, followed by the range age of 25-30 and over 30 with 31,8% and 13,6% respectively. On the online survey, 68,2% of respondents answered that their job is less related to physical activity and 31,8% of audiences said that their work involve a lot to the physical activity. In this survey, only 31,8% of participated had a blood glucose test and 68,2% said no. Unsurprisingly, in the question regarding the frequency of blood glucose testing, 72,7% said that they had never tested and only 18,2% checked at least once per month. The results are illustrated in figure 9.

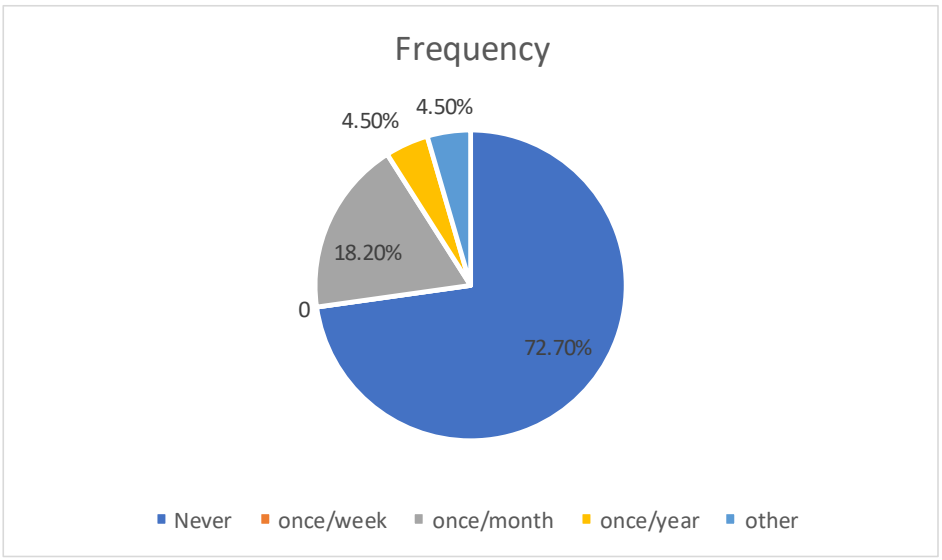

FIGURE 9: Frequency of blood glucose test.

From the frequency of the blood glucose testing in figure 9, it is not surprising that 72,7% of the participants did not know their blood glucose levels, approximately 22,7% had a blood glucose index of

less than 6,0mmol/l, which meant they do not have diabetes, only 4,5% said that they had blood glucose levels of 6,1-6,9mmol/l, and were in Type 1 of diabetes. Only 13,6% of audiences check their blood glucose at home by hand blood glucose meter, about 22,7% come to hospital to check the blood glucose and 63,6% said that they never check their blood sugar. On the subject of the diabetes history of family, 72,2% of participated answered that their family do not have high blood sugar index, approximately 27,3% said that their family had a history of high blood glucose level.

In conclusion, the majority of respondents are under 30 years of age and have less work related to physical activity. Most of them have never tested their blood sugar and do not know their blood glucose index. From the survey results, it can be seen that the young people still have careless to regular health checks, only few people join in the regular healthcare, which results in their uncontrolled blood sugar index or late detection.

6 CONCLUSIONS

Glucose is the source of energy for all cells in the human body. Blood sugar changes naturally, depending on the type of food, the amount of human consumption, as well as the timing of the meal. However, the cells cannot absorb glucose in the blood when the body has problems, resulting in hyperglycaemia. Hyperglycaemia can damage nerves, blood vessels, and organs in the body, leading to many complications such as reduced vision, infection, poor digestion, kidney damage, poor memory, high risk of strokes. The purpose of this thesis is to give the reader a basic knowledge of glucose, blood glucose, and current blood glucose testing techniques, and help the reader understand the importance in controlling blood glucose for human health.

The paper introduces readers to the basic concept of transport proteins and their types. They are responsible for transporting the glucose molecules into and out of the cells as needed. In addition, insulin is also described for the function of synthesizing and storing glucose, lipids, and amino acids, which play an important role in the lipid and amino acid transport. There are currently many methods used to quantify blood glucose, however, this thesis focused on three common methods of enzymes used to measure blood glucose nowadays. It is hexokinase, glucose oxidase and dehydrogenase. The hexokinase method is considered to be the fastest and most accurate method available nowadays and is commonly used on automated systems, while the glucose oxidase method result is in fast and low cost. However, this method is still affected by some factors that reduce glucose concentration compared to reality. The dehydrogenase method also yields the exact result as same as the hexokinase method because of using of GDH isolated from the Bacillus cereus.

In conclusion, according to statistics from the Ministry of Health in Vietnam, the number of people with hyperglycaemia is increasing rapidly, leading to reduced life expectancy. The main reason for this is lack of knowledge about how to control blood glucose as well as inaccessible to the technique of diagnosis and treatment in time. Through this thesis, the reader should make the routine health checks to be able to detect the disease and appropriate treatment.

REFERENCES

Amrita, 2012. Estimation of blood glucose by glucose oxidase method.

Ayyanar.K., Pichandi.S., Janakiraman.P., 2018. Evaluation of Glucose Oxidase and Hexokinase methods. International Journal of Biotechnology and Biochemistry. 51-58.

Barrell, A. 2018. What is random glucose test? Medical News Today.

Bostick, D.T. & Hercules, D.M. 1975. In quantitative determination of blood glucose using enzyme induced chemiluminescence of luminol. 447-452.

Brown.R.J. & Yanovski.J.A. 2014. Estimation of insulin sensitivity in children methods, measures and controversies. US National Library of Medicine National Institutes of Health. USA.

Cassoobhoy, A. 2017. High blood sugar, diabetes, and your body. WebMD Medical.

Cell Metobolism. Huang. S & Czech.M.P. 2007. The GLUT4 glucose transporter. Cell Metabolism.

Dam, H. 2018. Glucose Transporter Thesis. Glucose Transporter in Red Blood Cell. Insulin Mechanism and Insulin Regulate Metabolism.

Davis.C., 2018. Hemoglobin A1c Test (HbA1c, A1c, Hb1c) for diabetes. In P.Cunha. Hemoglobin A1c definition and facts. MedicineNet.

Dohnal.L., Kalousova.M., Zima.T., 2010. Comparison of Three Methods for Determination of Glucose. Charles University in Prague. Czech Republic. 42-54.

Falck, S. 2017. What to know about fasting blood sugar? Medical News Today.

Ginsberg.B. 2009. Factors affecting blood glucose monitoring: sources of errors in measurement. US National Library of Medicine National Institutes of Health. 903-913.

Goodsell.D. 2017. Glucose transporters. PDB-101.

Hellman.B.1977. Regulatory Mechanisms of Carbohydrate Metabolism. Mechanism of Insulin secretion. 11th Meeting Copenhagen. 41-50.

Kahn.C.R. 1985. The Molecular Mechanism of Insulin Action. Research division, Joslin Diabetes Center, and Department of Medicine, Brigham and Women's Hospital, Harvard Medical school, Boston, Massachusetts 02215. 429.

Kinman, T. 2018. Glucose tolerance test: Purpose, Procedure, and Risks. Healthline. Healthline. Glucose tolerance test for diabetes.

Little.R., & Robert.W. 2009. A review of variant haemoglobins interfering with haemoglobin A1c measurement. 446-451.

Luong, T. 2013. Fructosamine. The reaction between glucose and protein to produce fructosamine.

Luu.H., & Trinh.A., & Tran.A., & Nguyen.H., & Le.H., & Pham.H., & Nguyen.H., 2018. Knowledge, Attitude, Practice of treatment of diabetes patients. Clinical radiology of Radioactive Medicine and Military Artery.

McMillin, J. 1990. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Blood Glucose. Chapter 141.

Megazyme. 2018. Glucose Oxidase. Assay Procedure. In Bray Business Park, Bray. Ireland. 1-8.

Nansseu.J., & Domgue.J., & Noubiap.J., & Balti.E., & Sobngwi.E., & Kengne.A., 2018. Fructosamine measurement for diabetes mellitus diagnosis and monitoring: a systematic review and meta-analysis protocol.

Navale.A., & Paranjape.A. 2016. Glucose transporters: physiological and pathological role. In US National Library of Medicine National Institutes of Health. 5-9.

Norman,J. 2016. Diagnosing Diabetes. Endocrine. The two primary tests and their results, which combine to make the diagnosis of diabetes.

Ogbry.O. & Williams.E. 2016. Insulin. In W.Mark (eds.) Insulin. MedicineNet.

Staiano.M., & D'Auria.S. 2017. Methods in Enzymology. Glucose Dehydrogenase.

Strecker.H.J. 1955. Methods in Enzymology 1. Enzymatic Assay of Glucose dehydrogenase.

Thien Luc, 2018. Glucose Transporter. In Glucose and Transport protein. Chapter 2.

Timon, I & Collantes, C & Galindo, A & Gomez, J. 2014. In two type of diabetes and cardiovascular disease. Have all risk factors the same strength?

Varadaraju.C & Tamilselvan.G & Enoch.I & Selvakumar.M. 2018. Phenol sensing studies by 4 amino-antipyrine method. Department of chemistry, School of Science and Humanities, Karunya University, India.

Wiener.K. 1992. The diagnosis of diabetes mellitus, including gestational diabetes. In Department of Clinical Biochemistry, North Manchester General Hospital, Manchester M8 6RB, UK. 481-493.

Wood.S & Trayhurn.P. 2003. Horizons in Nutritional Science. Glucose transporters (GLUT AND SGLT): expanded families of sugar transport protein. 3.

Yoo.E.H & Lee.S.Y. 2010. Glucose Biosensors: An overview of use in clinical practice. US National Library of Medicine National Institutes of Health. 4558-4576.

BLOOD GLUCOSE

1. **What is your gender?**
   - Male
   - Female
2. **What is your age?**
   - 12-17
   - 18-24
   - 25-30
   - Over 30
3. **What is the nature of your current job?**
   - Less related to physical activity
   - Much related to physical activity
4. **Have you ever tested blood glucose?**
   - Yes
   - No
5. **Do you regularly have blood glucose test?**
   - Never
   - Once/week
   - Once/month
   - Once/year
   - Other
6. **What was your last blood glucose index?**

- < 6.0 mmol/l
- 6.1-6.9 mmol/l
- 7.0-11.1 mmol/l
- >11.1 mmol/l
- Other

7. Where do you usually check for your blood sugar?

- At home, by hand glucose blood meter
- At hospital
- Never test before

8. Does your family have a history of diabetes?

- Yes
- No