

Glycosylated Haemoglobin Testing: A Review

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Abstract: The following article discusses the discovery, biochemistry, laboratory determination, clinical applications, and error for glycosylated haemoglobin. There is no one test method that is appropriate for all applications, and the development of widely recognized standards and reference ranges is unlikely in the near year. Nonetheless, the introduction of glycosylated haemoglobin tests represents a significant step forward. They provide the most accurate way of determining diabetic management. As a result of a growing awareness that chronic hyperglycemia is a substantial predictor of long-term issues, there has been a surge in interest in diabetes management monitoring. As a result, the discovery of glycosylated haemoglobin came at an ideal moment; measuring it should provide a more objective control evaluation than previously achievable. Therefore, before this promise can be fully fulfilled, technological difficulties must be solved.

Keywords: Haemoglobin, hyperglycemia, fasting haemoglobin

I. INTRODUCTION

Researchers started looking into the gel electrophoresis and chromatography variation of haemoglobin in non-diabetics in the 1950s. Several other types of haemoglobin were discovered in tiny amounts and were proven to be genuine. The most significant of these minor fractions is HbA, which had a hexose moiety attached to the N-terminus of the beta-globin chain, according to Bookchin and Gallo 1968. According to Rahbar, only two of the 1200 people tested in Tehran exhibited an abnormal rapid-moving haemoglobin percentage; both had diabetes. Huisman and Dozy earlier linked tolbutamide treatment to a rise in rapidly moving haemoglobin variation in excessively large amounts in diabetes, which was identical to the HbA found by Allen. Tattersall reported that the high proportion of fast haemoglobin observed in diabetes was an acquired expression of metabolic abnormalities, instead of a congenital sign of diabetes, in a study of identical twins concordant and discordant for diabetes. The finding by Koenig that HbA percentage was related to fasting blood glucose and glucose tolerance was the single most important result that led to the development of abnormal haemoglobin testing as a diabetes treatment monitoring platform. They also discovered that when diabetes regulation was improved through medication, Hb A concentration was decreased.

II. REVIEW OF LITERATURE

2.1 Biochemistry

2.1.1. Structure

There are several types of "glycosylated haemoglobin" as we know. The table below shows the structure and nomenclature.

Name	Globin subunit composition	Proportion found in	
		Non-diabetics	Diabetics
Non-glycosylated ("native") haemoglobins			
Hb A (Hb A ₀)	$\alpha_2\beta_2$	>80%	60-80%
Hb A ₁	$\alpha_1\beta_2$	2%	2%
Hb F	$\alpha_2\gamma_2$	<1%	<1%
Glycosylated derivatives not specifically named			
	$(\alpha\text{-Val-1-deoxyfructose})_2\beta_2$?8-10% (ref 14)	Unknown, but increased
	$(\alpha\text{-}(Lys\text{-glucose})_n)_2\beta_2$		
	$(\alpha_1\beta\text{-}(Lys\text{-glucose})_n)_2$		
Hb A _{1a1}	often named (and assayed) collectively as Hb A ₁	$\alpha_2(\beta\text{-Val-fructose diphosphate})_2$	<1%
Hb A _{1a2}		$\alpha_2(\beta\text{-Val-glucose-6-phosphate})_2$	<1%
Hb A _{1b}		? (ref 96)	<1%
Hb A _{1c}		$\alpha_1(\beta\text{-Val-1-deoxyfructose})_2$	4%

Carbohydrate moiety i.e., glucose or a derivative which is a part of glycosylated haemoglobin linked to the globin chain at one of its ends. N-terminal amino acids are linked by either alpha or beta globin chains with carbohydrates within each chain.

The binding to the N-terminus of the atoms has practical importance because it causes the physical features to alter, which are employed in the tests. For example, chromatographic mobility is enhanced, hence these chemicals are commonly referred to as FAST HAEMOGLOBIN. Adducts on other sections of haemoglobin molecules have less effect on their properties.[1]

2.1.2 Formation

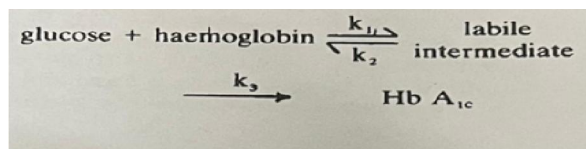
Simple chemical interactions between haemoglobin and sugars result in glycosylated haemoglobin, known as post-translational changes after complete haemoglobin production. Because of its practical relevance, the synthesis of Hb A has garnered a lot of attention. The procedure is divided into two parts and does not include the use of enzymes.

1. When glucose interacts with the amino group of the valine position at the N-terminus of globin chains, an aldimine molecule is formed because normal haemoglobin and glucose can be easily separated, this is a reversible process.
2. Through internal rearrangement of the aldimine intermediate, the Amadori reaction yields a stable ketamine derivative.

2.1.3 Kinetics of Reaction

According to preliminary research, glycation begins during erythropoiesis and continues slowly throughout the life of haemoglobin in the circulation; the levels achieved in diabetic red cells are compatible with their stated duration of 120 days.

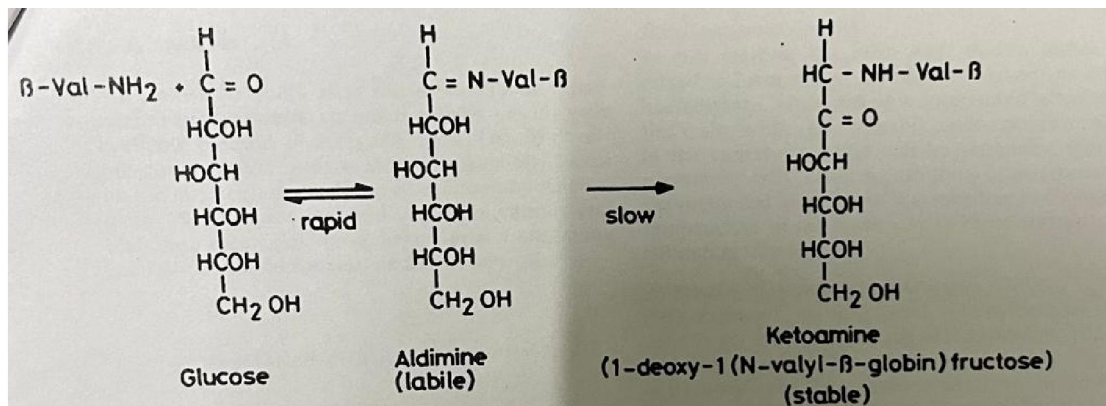
A simple equation describes the formation of Hb A:



2.2 Clinical studies

Various investigations aimed to strengthen the relationship between glycosylated haemoglobin concentrations and diabetes care after early demonstrations. [8] The endeavor has been made more difficult by two obstacles.

1. At best, previous diabetes control metrics were wrong. As a result, there hasn't been a straightforward, trustworthy, or universally acknowledged way to evaluate the new tests. No one has the gold standard of continuous and accurate blood glucose records over lengthy periods.
2. The role of unstable intermediates in skewing test findings is becoming more well recognized. The hypothesis that glycosylated haemoglobin measurements wouldn't represent short-term variations in blood glucose levels was partially right.



2.2.1 Time Course of Changes

Glycosylated haemoglobin levels represent mean blood sugar levels during the prior seasons when glucose levels were stable (whether normal or pathological), with the mean value in the red cell population being that maintained by cells with half their normal life span—45-60 days.

Recent research discovered a significant delay between advances in diabetic treatment and reductions in Hb A levels. According to Koenig, better management only resulted in a drop in Hb A after 3-4 weeks. Such a lag is expected based on theoretical arguments. [3] The stable Hb A intermediate, but not the unstable Schiff base intermediate, is retained in red blood cells until it is removed from circulation.

2.2.2 Hypoglycaemia

Previous glycosylation will not be reversed by a time of perfect management. As a result, a brief bout of hypoglycemia before the Hb A test has no impact on the results. Reduced glycosylation during prolonged hypoglycemia, on the other hand, may mask the effect of intermediate episodes of hyperglycemia, resulting in Hb A levels that are close to normal or even low. [2] These findings might be misinterpreted as confirmation of long-term normoglycemia. Regular nocturnal hypoglycemia, which is prevalent in insulin-treated individuals, is highly prone to giving false findings.

2.2.3 Fast Glycosylation

In a study of diabetic children who were closely monitored by regular blood and urine testing, Dunn discovered that although Hb A levels were associated with various measures of glycemia over several weeks, they were most closely related to blood glucose during the week immediately before blood was drawn for Hb A determination. According to Svendsen, HbA_{1c}, or a component of it, is produced at a higher pace than originally believed, as measured by chromatography. After so much discussion about fast glycosylation, Ditzel proposed and later shown in several studies that the reversible short-term intermediate Schiff base responds.

2.3 Assay techniques

Chromatography is a non-destructive method of resolving a multi-component combination of gases and liquids. While chromatography may be used for both quantitative and qualitative purposes, it is mostly used for separation. However, some additional analytical approach, such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), or mass spectrometry, is generally required for definitive identification (MS). The area of the chromatographic peak can be measured for quantitative analysis. As a result, chromatography may be utilized for both qualitative and quantitative research. Chromatography is a relatively recent technology that was created in 1906 in Warsaw by M. Tswett, a botanist. By percolating vegetable extracts down a column of calcium carbonate, he was able to separate chlorophyll, xanthophyll, and numerous other colored compounds in that year.[4]

The calcium carbonate column served as an adsorbent, allowing different compounds to be adsorbed to varying degrees, resulting in colored bands at various locations on the column. After the Greek words, chroma and graphics, which mean "color" and "writing," Tswett named this arrangement of colored bands the chromatogram and the procedure chromatography. Nonetheless, in the vast majority of chromatographic operations, no colored products are created, thus the word is misleading; however, much progress has been made since then, and the technology is now utilized to separate both colored and colorless compounds. The stationary phase refers to the calcium carbonate column employed in Tswett's technique, which stays motionless.[4] Chromatography may be defined as a separation procedure in which solutes are separated between a stationary phase and a mobile phase. As a separation method, chromatography in the form of thin-layer chromatography and ion-exchange chromatography was invented in the 1930s. Martin and Syngé invented partition and paper chromatography in 1941. Gas chromatography was first developed in 1952. During the next decade, chromatography as a separation technique became commonplace, and it was used in a variety of fields, including chemistry, biology, and medicine. Apart from its usage in analysis, it is emerging as a promising approach for the manufacturing of ultrapure substances, such as in the pharmaceutical business or the production of pure chemicals.[2]

The chromatographic methods of separation of biomolecules are responsible for the recent remarkable achievements in the field of biosciences. Chromatography is the most significant single analytical method utilized today, and it will most

likely remain so for the foreseeable future.[1] It is particularly important in molecular analytical chemistry. Its applicability to elemental analysis has recently been expanded thanks to its linkage with atomic absorption spectroscopy.

2.3.1 Cation Exchange Chromatography

Anionic groups are securely retained in the resin structure in cation exchange resins, but the cation is diffusible and hence participates in ion exchange. Sulphonic or carboxylic acids are found in most cation exchange resins, and they exchange their H⁺ ions with other cations.

Several factors influence the quantity of electrostatic interaction between an ion exchange resin and a solvent. The amount of exchange rises with increasing valency of the exchanging ion, i.e., Na⁺Ca²⁺Al³⁺, at low concentrations and room temperature.

For the same valency and under the same circumstances, i.e.Li⁺Na⁺K⁺, the number of interactions increases as the atomic number increases.

2.3.2 HPLC (high-resolution liquid chromatography)

HPLC is still in its early stages, but with the development of novel support materials and more sensitive detectors, it has the potential to grow in importance. It is already replacing several chromatographic techniques for gases. The advantages of HPLC are speed, resolution, and sensitivity. It is possible to reuse the column. It's particularly beneficial for isolating high molecular weight chemicals that have a low vapor pressure or pyrolyze when exposed to the higher temperatures necessary by gas chromatography.

The method has been used on a wide range of natural and artificial materials, including nucleic acids, urine, serum, carbohydrates, lipids, amino acids, and bile acids, as well as medicines, pesticides, herbicides, surfactants, and antioxidants. The separation of barbiturates by HPLC is illustrated in chromatography.

HPLC has become the most common method of chromatography due to its broad application, speed, and sensitivity, and it has been used to purify practically all sorts of biological substances. Reverse-phase partition HPLC facilitates the separation of polar substances such as medicines and their vitamins, metabolites, polyphenols, steroids, and peptides.

Before the invention of this type of chromatography, it was difficult to separate such polar chemicals and typically needed pre-derivatization to less polar molecules. Because biological fluids like serum and urine may be applied daily to the column, ideally via a guard column, technology is commonly employed in clinical and pharmaceutical work. One of two techniques can typically enhance the separation of some highly polar substances that are difficult to resolve properly by reverse phase chromatography, such as amino acids, organic acids, and catecholamines.

The first is ion-suppression, which involves chromatographing at a suitable high or low pH to prevent the chemical from ionizing. An acidified mobile phase, for example, can be used to chromatograph weak acids. The second method is ion-pairing, which involves adding a counter ion with the opposite charge to the one to be separated to the mobile phase such that the resulting ion-pair has enough lipophile character to be maintained with the reverse phase systems non-polar stationary phase. A quaternary alkylamine ion such as tetrabutylammonium would be employed as the counterion to facilitate the separation of acidic compounds present as conjugate anions, whilst an alkyl sulphonate such as sodium heptane sulfonate would be used to aid the separation of bases present as a cation.[1] Although the process by which ion-pairing improves separation is unknown, two possibilities have been offered. The first one suggests that the ion pair acts exactly like a single neutral atom, while the second suggests the forming of an active ion-exchange surface, with the counter ion, which has considerable lipid-soluble characteristics, as well as the ions to be differentiated adsorbed by the water-insoluble, non-polar stationary phase. The success of the ion-pairing strategy in practice is uneven and somewhat empirical. The size of the counterion, its concentration, and the pH of the solution are all variables that can have a significant impact on the separation's result.

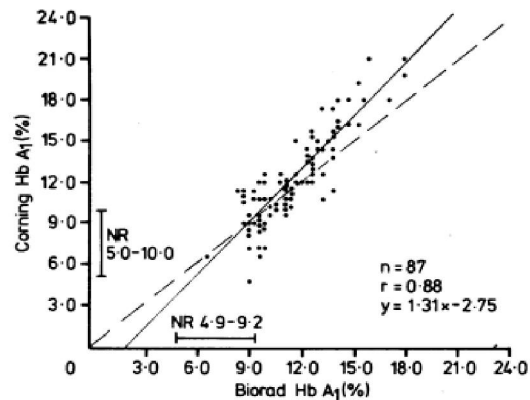
2.3.3 Isoelectric focusing

Because of the high resolution that may be achieved, isoelectric focusing is beneficial as a research method. Commercially available equipment can be utilized to identify haemoglobins eluted with Hb A_{1c} in microcolumn methods. Another advantage is how simple it is to separate stable Hb A from the intermediate Schiff base.

2.3.4 Electrophoresis/Electroendosmosis

The isoelectric point of glycosylated haemoglobin is only changed by one pH unit. It's hardly surprising, then, that traditional electrophoretic techniques for detecting Hb A have failed. Menard et al., on the other hand, developed an electroendosmosis technique for eliminating Hb A from agar gel plates. Although reading the plates necessitates careful calibration of the scanning densitometer to the right baseline, the technology, which is now commercially accessible, is highly simple, rapid, and temperature independent. Key breakthroughs were the use of plastic-supported cellulose acetate membranes and the concept of mobile affinity electrophoresis.[6] Haemoglobin's affinity for the sulfate groups of dextran sulfate in electrophoresis buffers is reduced by glycosylation, leaving Hb A immobile. When the plates are dyed with protein, scanning is more reliable.

Temperature, pH, and buffer strength had little influence on the procedure, and it appears to be cost-effective. Microcolumns or the endosmosis method are used in most laboratories that do routine glycosylated haemoglobin testing. Colorimetric, spectrophotometric, and batch chromatographic procedures are all viable options, but less common. Boucher et al. recently looked at the various approaches. They did not endorse any of the contenders and instead focused on their benefits and drawbacks. They emphasized the lack of established standards and the significance of doctors being aware of technique variations that may alter findings.



2.4 Source of error

All generally used approaches, except the colorimetric approach, measure the labile aldimine intermediate in addition to Hb A. A brief period of hyperglycemia before blood collection causes an acute increase in the formation of aldimine, which can increase glycosylated haemoglobin concentrations by 10-20% — for example, from 9% to 11% of total haemoglobin — lowering the test's reliability as a measure of long-term diabetic control. Blood samples should be treated to remove aldimine before the test.

2.5 Use in clinical practices

2.5.1 Blood Glucose Test

As the name indicates, the primary purpose of a blood sugar test is to assess the glucose concentration in the bloodstream. Glucose, often known as dextrose, is a simple sugar. Glucose is classified as a monosaccharide, which is the simplest unit of a carbohydrate. The name "glucose" comes from the Greek word "glucose," which means "sweet." "Blood sugar" refers to the amount of glucose in the blood. Glucose's chemical formula is C₆H₁₂O₆. A blood glucose test is frequently conducted on diabetics. It aids in the detection and monitoring of a person's diabetes type as well as the concentration of glucose/sugar in their blood. Insulin is a hormone that helps the body regulate the amount of glucose in the blood by turning ingested carbs into glucose for energy generation. Diabetes is a chronic disease characterized by elevated blood glucose levels.[8] Diabetes is a life-threatening disease. It's a sickness that, if left untreated, may cause lifelong problems. There are several forms of diabetes. The amount of insulin in our body is intricately tied to diabetes. Diabetes is caused by a lack of insulin in the body or an inability of the body to properly utilize the insulin it generates. Insulin is produced by the pancreas, which is an organ in the human body. Insulin is in

charge of transporting glucose into cells so that it may be converted into energy. The pancreas is the organ in the human body that produces insulin. Insulin is directly responsible for delivering glucose into cells via the circulation for energy production. If the body no longer produces or uses insulin, for example, the body's glucose level will ultimately increase, causing catastrophic injury. Hypoglycemia and diabetes patients both have their blood glucose levels checked. Hypoglycemia is the medical term for low blood glucose levels. Patients with this illness are usually advised to consume sugar or sugar-containing meals on a more frequent basis since failure to do so can lead to fatigue or even coma. A blood glucose test, in a word, detects the quantity of glucose in the blood. This test is frequently used by doctors to detect underlying illnesses, the most common of which being diabetes. Diabetics utilize blood glucose testing to keep track of their health.

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The quantity of glucose in the blood is determined via a blood sugar test. Doctors regularly use this test to uncover underlying disorders, the most prevalent of which is diabetes. Blood glucose tests are used by diabetics to monitor their health. The risk of developing diabetes varies from person to person. Some of these characteristics include: -

- Age (45+)
- obesity
- Sedentary lifestyle
- low HDL (high-density lipoproteins) or high triglycerides
- High blood pressure

Previous insulin resistance or gestational diabetes irrespective of if there is or has been a diabetic in one's family.

Some races are also more prone to diabetes than others. Asians, for example, are more likely than Irish people to get diabetes.

2.5.2 Blood Glucose Test Purpose

To assess the quantity of glucose in the blood, a blood glucose test is utilized. A blood glucose test is frequently ordered by a doctor to determine whether or not a person has diabetes or has a high risk of developing diabetes. Glucose is required for cell activity because it produces energy. To do so, the body breaks down carbohydrates, such as multigrain, fruits, and other foods, into energy-producing chemicals, called glucose.

Diabetic individuals can use basic gadgets like glucometers to monitor their blood glucose levels at home. Patients can change their food plan, exercise routine, and medicines based on the results.

Hypoglycemia is commonly diagnosed when someone has a low blood sugar level. Hypoglycemic individuals are more likely to experience mental disorientation, weariness, hunger, and perspiration.[2] They could also have headaches, convulsions, drowsiness, or even a coma. Patients with high blood sugar levels, also known as hyperglycemia, may exhibit signs and symptoms such as weakness, abdominal discomfort, headaches, hunger pains, and so on. Ketoacidosis, for example, can develop in severe situations. The body produces an excessive amount of ketones in ketoacidosis. The body begins to use fat as its only source of energy, resulting in rapid weight loss. Insulin levels fall. There is also a high danger of contracting additional ailments. Hyperglycemia can pose a significant threat to the eyes, kidneys, nerves, and other organs if left untreated.[2]

2.5.3 Blood Glucose Test Types

There are several types of blood glucose tests.

AT HOME TEST: When using a glucometer, all that is required is a tiny drop of blood obtained by finger pricking. A syringe is not used to draw blood. Instant results are available. However, this doesn't help determine whether or not someone has diabetes. In the case of an at-home test, the following tests are performed.[4]

Conventional Home Blood glucose level monitor: A single blood droplet collected by pricking one's finger with a needle, sometimes called a lancet, is placed on a test strip, which when inserted into a blood glucose leveling meter, indicates the level of blood glucose. The price, magnitude, clarity, motility, and speed of these blood glucose meters vary. These devices produce results and save data in around 15 -18 seconds. A few meters can also be used to calculate a typical glucose level over time. Some also highlight programming units that display diagrams and graphs based on data from the meter. These equipment and testing strips are usually available at any pharmacy.

Blood glucose level is monitored via different body parts: Blood glucose levels can be determined not only from the finger, but also from the thigh, forelimb, area between the wrist and elbow, and the bottom part of the thumb. However, as compared to the traditional finger pricking procedure, this one's results are less accurate. The reason for this is that body parts are unable to demonstrate a significant or simultaneous change in blood glucose levels when measured after eating or exertion or movement. If you have hypoglycemia or have symptoms that indicate hypoglycemia, you should avoid this test.

Continuous blood glucose monitoring approach: This method combines blood glucose monitoring devices and insulin pumps. This procedure is not as precise as finger pricking, nor is the blood glucose level monitored via various body locations. These, on the other hand, can help you keep track of your blood glucose levels.

Urine ketone testing: This test determines how much ketone is present in a urine sample. A urine sample is put into a testing strip. If there is a lot of ketone in the sample, it suggests the body has an insulin shortfall, and if there are too many ketones, it means the body has an insulin threshold. Both situations necessitate medical intervention.

GLUCOSE TEST IN THE LABORATORY

Blood is drawn using a syringe in this situation. Blood is taken and delivered to a testing center, where the glucose levels in the blood are measured. Blood glucose levels are also linked to underlying illnesses such as diabetes. It's worth mentioning that blood glucose level lab tests are commonly used to diagnose a patient's type of diabetes.

Blood glucose test after fasting: The patient must fast for 8–10 hours or overnight for this test. During this time, no food or water should be eaten.

Ranges:-

Nondiabetics – 70 – 100mg/dL

Prediabetes - 100 – 125mg/dL

Diabetes - >126 mg/dL

Postprandial examination: In this test, individuals will have their blood glucose levels checked two hours after eating.

A1c haemoglobintest: Haemoglobin A1c test determines how much blood glucose is linked to RBCs. There was no fasting done. A blood sample can be taken and measured at any moment. This test also keeps track of prior months' blood sugar levels.

Ranges:-

Nondiabetics - <5.8%

Prediabetes - 5.8 - 6.3%

Diabetes - >6.4%

Blood sugar testing at random: A blood sample was taken at any moment of the day and analyzed at the lab. Fasting is not required here either.

Ranges:-

Nondiabetics – below 125 mg/dL

Prediabetes - 140– 199 mg/dL

Diabetes - 200 mg/dL or higher

Glucose tolerance test in the mouth: In the oral glucose tolerance test, blood glucose, fast glucose, and after-meal glucose are utilized to identify diabetes. Pregnant women are regularly subjected to this procedure. Patients are advised to fast for at least 8 hours before receiving 75grams of anhydrous glucose solution for pregnant women and 100 grams for ordinary patients. The blood glucose level is checked after 2 hours.[4]

2.5.4 Diseases Associated with Blood Glucose Level

TYPE 1 DIABETES

When the pancreas produces no or a small quantity of insulin, type 1 diabetes develops. Viruses and genetics are the most prevalent causes of this disorder. Teenagers and children are the most vulnerable. This affects adults as well. There is currently no treatment for this chronic condition. Blood glucose levels can only be controlled via food and lifestyle modifications.[1]

Patients with type 1 diabetes will need to use an external medium to keep their insulin levels in check. They will need to be given frequent insulin doses by several procedures, including a syringe or a tablet.

Some of the symptoms include weight loss, mood swings, increased hunger, uncontrolled bladder, dehydration, hazy vision, weakness, sexual difficulties, and infections.

TYPE 2 DIABETES

Among all types of diabetes, type 2 diabetes is the most frequent. We've seen how in type 1 diabetes, the body, primarily the pancreas, is unable to produce insulin for glucose absorption and energy production. In the case of TYPE 2 diabetes, however, the situation is the opposite. In type 2 diabetes, the pancreas generates the hormone insulin rather efficiently, but it is unable to utilize it for its original function.[1]

This causes a person to become insulin resistant. As a result, Type 2 diabetes patients are insulin resistant. People in their mid-30s and above are the age group most prone to type 2 diabetes. Obese children have a higher risk of developing Type 2 diabetes later in life. Type 2 diabetes usually becomes worse as you get older. Men with low testosterone levels are also more likely to get this type of diabetes.

Mood fluctuations, increased appetite, thirst, weight loss, frequent urination, weakness, unhealed wounds, and increased infections are some of the symptoms.

Genetics, obesity, poor cell communication, excess glucose in the liver, sexual issues, and vaginal infections are all possible causes.

GESTATIONAL DIABETES

Diabetes that develops during pregnancy is known as gestational diabetes. The body can't utilize glucose effectively to produce energy when you have gestational diabetes. If a pregnant mother has gestational diabetes, she may also have hyperglycemia, which is defined as a high blood glucose level that affects both the mother and her offspring's bodies.[1]

The condition lasts until the delivery, after which it begins to deteriorate before finally disappearing. However, the woman is now in danger of acquiring Type 2 diabetes in the future because of this. Only by taking proper care of one's health can this be averted. Patients with gestational diabetes should spend their time doing mild workouts, eating nutritious foods, and monitoring their blood sugar levels.

Dehydration, mood swings, and weakness are some of the symptoms.

Causes are unknown, however, they are most likely linked to hormonal changes during pregnancy.

Prevention includes leading an active lifestyle, maintaining a healthy weight, and eating a nutritious diet.

PREDIABETES

Prediabetes is a condition in which blood glucose levels are high but not high enough to be classified as TYPE 2 diabetes. It's high, but not enough to be diagnosed with diabetes. Correcting one's diet and establishing a good eating plan can often prevent this, as can exercising regularly to lose weight.

For example, if normal blood glucose levels are below 99 and above 70 mg/dL, prediabetics' blood glucose levels would be around 126. It will cause diabetes if it rises any further, to a level of 140 or above. As a result, the term "borderline diabetes" was coined.

HYPOGLYCEMIA (LOW BLOOD GLUCOSE LEVEL)

Hypoglycemia is commonly diagnosed when someone has a low blood sugar level. Hypoglycemic patients are more likely to experience mental disorientation, weariness, hunger, and perspiration. [1] They might also have headaches,

convulsions, drowsiness, or even a coma. Patients with high blood sugar levels, also known as hyperglycemia, may exhibit signs and symptoms such as weakness, abdominal pain, headaches, hunger pangs, and so on. Ketoacidosis, for example, can develop in severe situations. The body produces an excessive amount of ketones in ketoacidosis. The body begins to use fat as its only source of energy, resulting in rapid weight loss. Insulin levels fall. There is also a high danger of contracting additional ailments. Hyperglycemia can cause serious harm to the brain, eyesight, kidneys, and other organs if left untreated. Hypoglycemia is defined as a blood glucose level of less than 70 mg/dL.

Tools Used to Calculate Blood Glucose level

Blood glucose meter: It monitors the blood glucose level present in the blood. It is available in different sizes, designs come with different functions which depend on the brands that manufacture them.

Test strips: These strips are responsible for converting blood glucose into signals that can be read by a glucometer. This is made feasible by the presence of particular compounds embedded on the strip, which facilitate the process.

Lancets: Lancets are the sharp needles used to prick fingers to draw out blood samples during blood glucose testing.

Lancet device: Lancet device holds the needle that is to be used for finger pricking to draw out blood samples for blood glucose testing.

2.6 Experiments Performed on Patients

EXPERIMENT 1

Aim: - Random blood glucose test

Materials Required: -Glucometer, lancet, test strip, alcohol, cotton

Procedure: -

Step 1: Wash your hands: hand washing is necessary to kill harmful bacteria and sanitize your hands. Wash your hands thoroughly with a glob of handwash. Clean between your fingers, under your nails, and rub your palms together. Continue for at least 1 minute before rinsing your hands with clean water. After that, pat dry.

Step 2: Activate the machine and insert a strip into the glucometer.

Step 3: Using alcohol, clean the fingertip and puncture it.

Step 4: Place the blood droplet on the strip.

Step 5: Pay attention to the reading.

Step 6: Wipe the place of the poked finger to sterilize it again and avoid infection.

Result– SUBJECT (A) RANDOM BLOOD GLUCOSE LEVEL = 101 mg/dl

Nondiabetics – below 125 mg/dL

Prediabetes - 140– 199 mg/dL

Diabetes - 200 mg/dL or higher

Hence, subject (A)'s blood glucose level is normal. i.e., non-diabetic

2.6.2 EXPERIMENT 2

Aim:-Fasting blood glucose level

Materials Required:-A glucometer, a lancet, a test strip, alcohol, and cotton are all needed.

Procedure:-

Step 1: Wash your hands: hand washing is necessary to kill harmful bacteria and sanitize your hands. Wash your hands thoroughly with a glob of handwash. Clean between your fingers, under your nails, and rub your palms together. Continue for at least 1 minute before rinsing your hands with clean water. After that, pat dry.

Step 2: Insert a strip into the glucometer and turn it on.

Step 3: sterilize the tip of the finger with alcohol and pierce it.

Step 4: Place the blood droplet on the strip

Step 5: Pay attention to the reading.

Step 6: Wipe the place of the poked finger to sterilize it again and avoid infection.

Result– Subject (B) FASTING BLOOD GLUCOSE LEVEL = 84 mg/dl

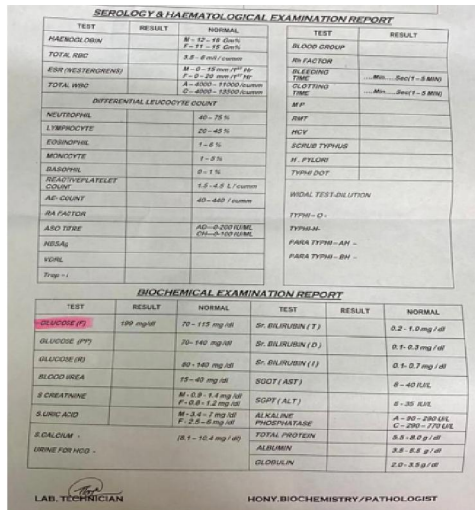
Nondiabetics – 70 – 100mg/dL

Prediabetes - 100 – 125mg/dL
 Diabetes - 126 mg/dL or higher
 Hence, subject (B)'s blood glucose level is normal i.e., non-diabetic

2.6.3 EXPERIMENT 3

Aim:-

FASTING BLOOD GLUCOSE TEST



The image shows two laboratory reports. The top report is a 'SEROLOGY & HAEMATOLOGICAL EXAMINATION REPORT' with columns for TEST, RESULT, and NORMAL. The bottom report is a 'BIOCHEMICAL EXAMINATION REPORT' with columns for TEST, RESULT, and NORMAL. In the biochemical report, the 'GLUCOSE (F)' test result is 199 mg/dl, which is highlighted in red.

Patient (C)'s blood glucose level result shows 199 mg/dL.
 Nondiabetics – 70 – 100mg/dL
 Prediabetes - 100 – 125mg/dL
 Diabetes - 126 mg/dL or higher
 Hence, subject (C)'s blood glucose level is normal i.e., Diabetic

2.7 Myths Surrounding Diabetes

1. Type 2 diabetes affects people who are overweight. This is untrue. When it comes to the occurrence of Type 2 diabetes, it is important to consider not only weight but also genetics, nutrition, and age.[10]
2. Excess sugar intake is the primary cause of diabetes. This is also inaccurate since, in addition to sugar, any meal that contains unhealthy fats, such as burgers, fried chicken, and other fast foods, can lead us to become obese, which is a big risk factor for diabetes.
3. Individuals with diabetes should develop healthy eating plans. This isn't always the case, since diabetics may consume a basic, healthy diet just like everyone else. Of course, factors such as the amount of time, the amount of food, and the meal's quality are taken into account.
4. Diabetic people should avoid sweets and chocolates for the rest of their lives. This is untrue. This is because diabetics may enjoy sweets on occasion when they are accompanied by a healthy diet, exercise, and lifestyle. They will not be harmed in any manner.
5. Diabetics should stay away from starchy foods. This statement is also untrue. Diabetics can eat starchy meals as long as they don't overdo and keep their calorie intake under control to avoid undesirable weight gain.

2.8 Treatment for Diabetes

1. The main cause of diabetes is excessive sugar consumption. This is also untrue because, in addition to sugar, any item containing harmful fats, such as burgers, fried chicken, and other fast foods, can make us obese, which is a major risk factor for diabetes.

2. Diabetic individuals should have a healthy eating plan. This isn't always the case, as diabetics, like everyone else, can eat a basic, healthful diet. Of course, certain concerns like the amount of time, the amount of food, and the quality of the meal are taken into account.
3. For the remainder of their life, diabetic individuals should avoid sweets and chocolates. This is completely untrue.[10] This is because diabetics can enjoy sweets on occasion if they are combined with a balanced diet, exercise, and lifestyle. It will not hurt them in any way.
4. Fruits should be ingested in large quantities by diabetics since they are healthful, include certain nutritional benefits, and contain fiber, vitamins, and other nutrients. This is also untrue because fruits, as healthy as they may appear, contain a lot of sugar, which can lead to diabetes in the long run.

2.9 Diabetes Management

Diabetes is a chronic and life-long condition in the majority of patients. As a result, sufficient care must be given to diabetes patients throughout their lives. Because there is no treatment for diabetes, diabetics must manage their blood glucose levels throughout their lives to live a healthy lifestyle.

Overall, diabetes can only be managed by controlling blood glucose levels by exercise, diet, correct insulin delivery, and other blood-glucose-lowering drugs such as metformin.

Patients with type 1 and type 2 diabetes use insulin in the form of a pill, inhaler, or injection to keep their blood glucose levels from becoming too high.[9] Excessive insulin use can produce a decline in blood glucose levels, which can lead to serious consequences. As a result, when using insulin, necessary measures should be taken.

III. CONCLUSION

Glycosylated haemoglobin measurement sheds new light on metabolic problems characterized by wide changes by giving a diabetes management indicator that successfully integrates blood glucose concentrations over several weeks. In practice, methodological limitations limit the potential of glycosylated haemoglobin, with each of the various test procedures having its own set of drawbacks. Simple methods are prone to faults that can lead to severe inaccuracy, and comparing data generated by multiple methods isn't always straightforward. Expertise, technological development and adjustment of testing, and a larger supply of dependable standards are all expected to help overcome these obstacles.

The most common use of blood glucose tests is to determine any underlying condition, such as diabetes. Different types of blood glucose tests can be used to detect diabetes. Hyperglycemia refers to a high blood glucose level, while hypoglycemia refers to a low blood glucose level. The outcomes of the experiments vary from person to person. The result of Experiment One, which was based on a random blood glucose test, was 101 mg/dL, showing that the individual (A) has a normal blood glucose level. Experiment B, a fasting blood glucose test, revealed a blood glucose level of 84 mg/dL, which is also within normal limits. However, the subject's blood sugar level was 199 mg/dL in experiment 3, indicating that he or she is diabetic. Numerous diabetes myths have been debunked. Hypoglycemia can be addressed by ingesting sweet beverages that offer immediate glucose. In severe situations, glucagon injections are frequently recommended. Because diabetes has no permanent cure, diabetics must manage their blood glucose levels with exercise, a good diet, and medication.

Methodology

This is prospective analytical correlation research. Diabetic patients who had been sent to the laboratory were among those investigated. Pregnancy, splenectomy, anemia, any sort of blood transfusion in the previous 3 months, and drug use were all exclusion factors. (salicylates). This article's material came from several sources, including Antibiotic Discovery & Development and others. Other online venues where the data was acquired include Google Scholar, E-libraries, and Mendeley. A review of various research publications served as the foundation for the study.

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