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HEMOGLOBIN A1C: BIOMARKER FOR DIABETES PREDICTION

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ABSTRACT

General information:Diabetes is a metabolic disorder in which glucose level in the body is elevated may be due to impaired insulin secretion or insulin resistance or increased glucose production. Various tests can be used glucose estimation in the body during diabetes, one of which also includes estimation Hb A1C% which gives precise level of glucose over a period of 2-3 months.What is HB A1C and test for it:The hemoglobin A1C test -- also called HbA1c, glycated hemoglobin test, or glycohemoglobin -- is an important blood test used to determine how well diabetes is being controlled. Hemoglobin A1C provides an average of blood sugar control over a six to 12 week period. Hemoglobin is a substance within red blood cells that carries oxygen throughout body. When your diabetes is not controlled (meaning that blood sugar is too high), sugar builds up in blood and combines with hemoglobin, becoming "glycated." Therefore, the average amount of sugar in blood can be determined by measuring a hemoglobin A1C level. If glucose levels have been high over recent weeks, hemoglobin A1C test will be higher. The amount of hemoglobin A1C will reflect the last several weeks of blood sugar levels.Studies suggest that the lower the hemoglobin A1C level, the lower the incidence of diabetic complications (eye, kidney, heart, blood vessel, and nerve disease). The American Diabetes Association (ADA) recommends keeping the hemoglobin A1C less than 7%. The result of hemoglobin A1C test can also be used to estimate average blood sugar level.How HB A1C value helps in prediction: The table below shows hemoglobin A1C with estimated average glucose.

Hemoglobin A1C (%)	*Estimated average glucose (mg/dL)*
6%	126
7%	154
8%	183
9%	212
10%	240
11%	269
12%	298

Conclusion: Since Hb A1C level remains normal for long duration of about 2-3 months, its level increase with glucose elevation in body, can be used as a bio-marker for prediction of diabetes.

Objective:

Glycosylated hemoglobin estimation is a useful index for assessment of diabetic control. This review shows that Glycosylated hemoglobin is useful as an objective marker for monitoring blood glucose level.

Introduction:

Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. It is a group of syndromes characterized by:

(a) Hyperglycemia altered metabolism of lipids carbohydrates and protein.

(b) An increased risk of complication from vascular disease. [1]

Diabetes causes about 5% of all deaths globally each year. Around 80% of people with diabetes live in low and middle income countries. Most people with diabetes in low and middle income countries are middle-aged group between 45 to 64yrs, but not elderly (above 65yrs). It is estimated that death due to diabetes are likely to increase by more than 50% in the next 10 years if any immediate action is not taken for its prevention.

Diabetes is of two types:

- Type I Diabetes Mellitus (insulin- dependent diabetes mellitus, IDDM): It usually develops before the age of 30 and may also occur suddenly in a normal weight person. This type occurs due to destruction of pancreatic B cells. Autoimmunity is believed to be the major mechanism involved in this form of diabetes. [3]

- Type II Diabetes Mellitus (non-insulin- dependent diabetes mellitus, NIDDM): This type accounts for 90-95% of diabetes cases and usually starts later in life in overweight people. [2] The etiology of type II is less clearly understood. It occurs due to two factors i.e. impaired insulin release and insulin resistance.

Risk of diabetes increases if a person has any of the following: [2]

- Excess body weight (especially around the waist)
- Any family history of diabetes.
- HDL cholesterol level less than 35 mg/dL
- A high level of triglycerides (a type of fat molecule), in the blood i.e. 250 mg/dL or more.
- High blood pressure (>= 140/90 mmHg)
- Any metabolic syndrome and impaired glucose tolerance.

Epidemiology of diabetes: [4]

- Prevalence of diabetes As per WHO during year 2000 was found to be 17.1 million amongst whole world and if proper precaution is not taken then diabetic population is predicted to become double in future.
- According to a study conducted by ADA in 2007 it was found that 23.6 million in the United States which accounts for 7.8% of the population were diagnosed with diabetes.
- Total Prevalence of diabetes [4]: Population that is known to have diabetes

a) Based on age group:

Age Group	Statistics
< 20 years	Around 186,300 i.e. 0.22% of total population. One in every 400 to 600 children and adolescents has type I diabetes. 2 million adolescents (or 1 in 6 overweight adolescents) aged 12-19 have pre-diabetes.
>= 20 years	23.5 million Or 10.7% of all people.
>=60years	12.2 million Or 23.1% of the total population.

b) Based on Gender

Gender	Statistics
Men	12.0 million or 11.2% of all men aged 20 years or older have diabetes.
Women	11.5 million or 10.2% of all women aged 20 years or older have diabetes.

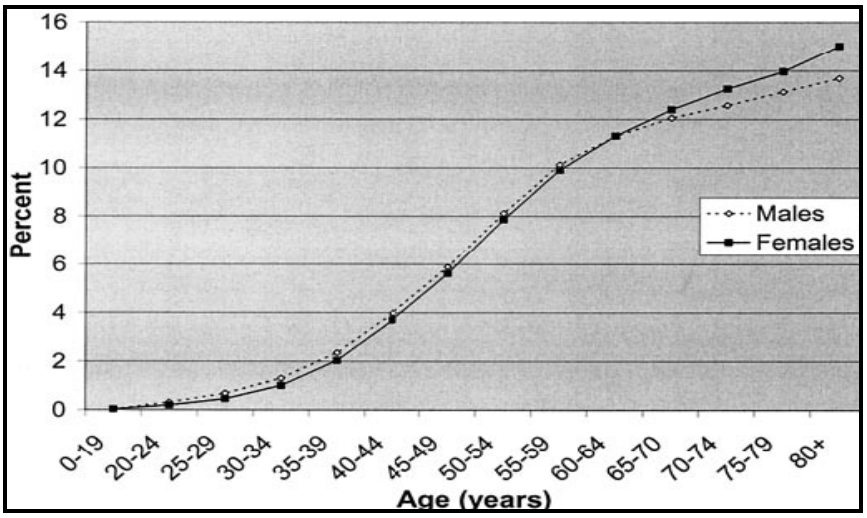


Fig 1: Total prevalence of diabetes based on gender [2]

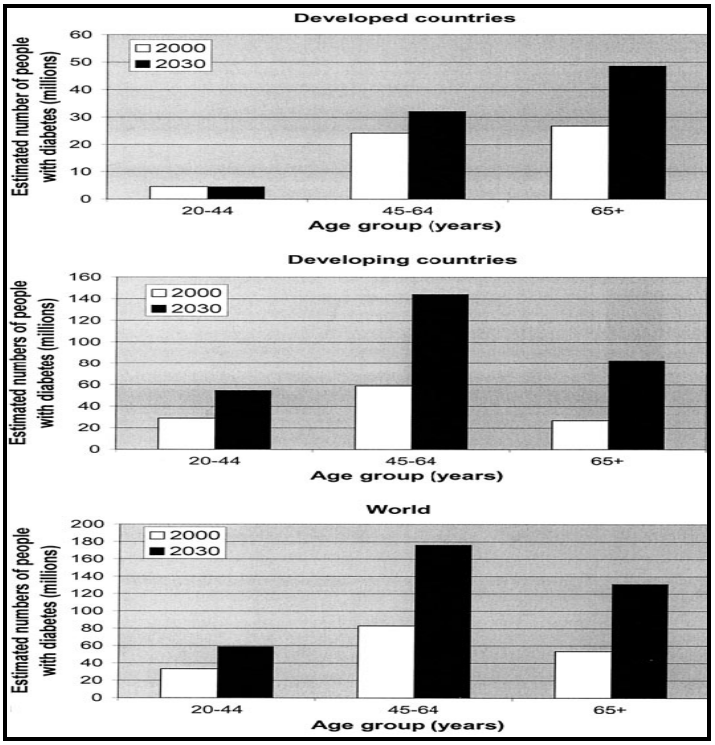


Fig 2: Total prevalence of diabetes based on age group [2]

Some major complications associated with diabetes are as follows: [3]

- MACROVASCULAR COMPLICATION

a) Coronary Artery Disease:

Coronary heart disease (CHD) is highly prevalent and is the major cause of morbidity and mortality in diabetic patients. Coronary artery disease is one of the leading causes of death among both men and women in the Western world.

Nonetheless, women are at a much lower risk for heart disease mortality than men are. Data from the World Health Organization show that the ratio of coronary disease mortality in men to women is consistently close to 2, even though the prevalence of coronary disease varies widely between countries.

Coronary artery disease (CAD), also called coronary heart disease, is a condition in which plaque builds up inside the coronary arteries. These arteries supply your heart muscle with oxygen-rich blood. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. When plaque builds up in the arteries, the condition is called atherosclerosis.

b) Strokes:

If a person has diabetes, there is atleast twice as likely as someone who does not have diabetes to have heart disease or a stroke. People with diabetes also tend to develop heart disease or have strokes at an earlier age than other people. Some studies suggest that Middle-aged with type 2 diabetes, chances of having a heart attack is as high as someone without diabetes who has already had one heart attack.

Women who have not gone through menopause usually have less risk of heart disease than men of the same age. But women of all ages with diabetes have an increased risk of heart disease because diabetes cancels out the protective effects of being a woman in her child-bearing years.

People with diabetes who have already had one heart attack run an even greater risk of having a second one. In addition, heart attacks in people with diabetes are more serious and more likely to result in death.

- MICROVASCULAR COMPLICATION

a) Kidney damage (Nephropathy):

About 20% of those with type 2 diabetes eventually develop some kidney damage and later kidney failure. The damage occurs in tiny vessel (called capillaries) throughout the kidney, which acts as filters to remove waste products from bloodstream.

The first detectable sign of kidney damage is appearance of small amount of protein called albumin in the urine (microalbuminuria), usually develops after you have diabetes for 5-10 years. After a period of 8-10 years, worsening of kidney damage causing release of larger protein into the urine (proteinuria). It leads to accumulation of waste product in the blood (azotemia), and finally kidney failure, which can be treated with dialysis or a kidney transplant.

b) Retinopathy:

Almost everyone with type 1 and more than 70% of people with type II diabetes eventually develop some evidence of retinopathy. In most cases there is no vision loss.

'Retinopathy' refers to damage in the retina, a light sensitive nerve tissue at the back of the eye that transmits visual images to the brain. The damage is caused in the tiny blood vessel that carries blood to retina.

In the early stage – called nonproliferative retinopathy blood vessel weakens and develops bulges (microaneurysms) releasing tiny amount of blood or fluid (microhemorrhages or exudates) into surrounding tissue. When damage worsens, fragile new blood vessel begins to grow on retina and into vitreous humor, condition called as proliferative retinopathy.

c) Nerve damage (Neuropathy)

Neuropathy is serious consequence of diabetes. About 60-70% of people with diabetes develop neuropathy. Neuropathy due to diabetes can be of three types

Peripheral neuropathy: It refers to damage of peripheral nerves that attach spinal cord to different parts of body.

Mononeuropathy: Disruption of blood supply to a single nerve or nerve group leads to pain or weakness.

Autonomic neuropathy: It develops when diabetes damages the autonomic nervous system which regulates body function that is not under voluntary control like digestion or heart rate.

Diagnosis of Diabetes: [3]

Four types of blood test available in laboratory for diagnosing pre-diabetes and diabetes are:

A) Casual plasma (blood) glucose: This test measures blood glucose level at any time of the day. The criteria for diagnosis include blood glucose level of 200mg/dl or higher.

B) Fasting plasma glucose test: It is a preferred method for diagnosing diabetes in children, men and non pregnant women.

C) Oral glucose tolerance test: this test is done when diabetes is suspected, but has normal result on fasting plasma glucose test.

D) Hemoglobin A1c test: It measures amount of glucose attached to hemoglobin. As the blood glucose level rises, so does the amount of glucose attached to the hemoglobin. ADA recommends keeping A1C level at less than 7%.

GLYCOSYLATED HEMOGLOBIN

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates, and it is also available in the tissues of some invertebrates. [5]

Various types of hemoglobin include:

A) Hemoglobin A (α2β2) - The most common with a normal amount over 97%.

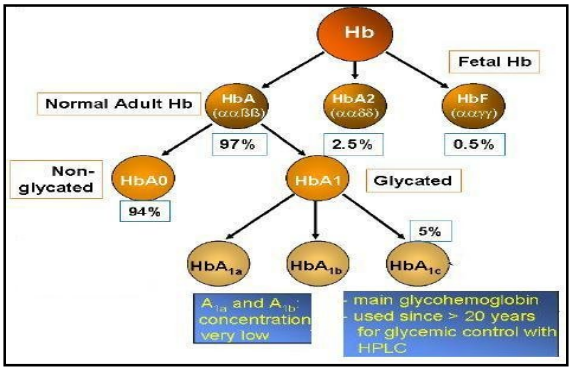
B) Hemoglobin A2 (α2δ2) - It has a normal amount about 2.5%.

Fig 3Various types of Haemoglobin

C) Hemoglobin F (α2γ2) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells.

A small percentage of hemoglobin (the pigment in red blood cells) binds to glucose resulting in Glycosylated Hemoglobin in the blood. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose .This binding remains for the rest of the life of the red blood cell, which is nearly 3-4 months.

Hence more glucose in the blood, the more hemoglobin A1c or HbA1c will be present in the blood. [12] Glycation of



hemoglobin has been associated with cardiovascular disease, nephropathy and retinopathy in diabetes mellitus. Monitoring the HbA1c in type-1 diabetic patients may improve treatment. [6]

History:

Hemoglobin A1C was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column. [7] It was first characterized as a glycoprotein by Bookchin and Gallop in 1968. [8] Its increase in diabetes was first described in 1969 by Samuel Rahbar and co-worker. [9]

The reactions leading to its formation were characterized by Bunn and his co-workers in 1975. [10]The use of hemoglobin A1C for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and co-workers. [11]

Methods and Analysis:

Before the measurement of glycosylated hemoglobin, or HbA1c, was available, physician could infer concentration of glucose in blood only by extrapolation of information from a series of fasting or random blood glucose measurement. The introduction of the HbA1c test has made it possible to make an accurate assessment of glycemic control over a definite time period.

HbA1c is most commonly measured because it comprises the majority of glycosylated hemoglobin and is least affected by recent fluctuation in blood glucose. HbA1c measures the percentage of HbA that has been irreversibly glycosylated at the N-terminal amino group of the β-chain. The value is determined by the level of plasma glucose and the life span of red blood cells). Thus HbA1c is indicator of glycemic control over the preceding 2 to 3 months. [13]

HbA1c is measured by taking Blood from a vein (venupuncture) and sent to the laboratory where it is tested by various methods such as:

1. Hemoglobin electrophoresis (separation of different types of hemoglobin over a gel). [6]

2. Column chromatography (separation of different types of hemoglobin over a column).

The principle behind these methods is reaction of glucose molecules with hemoglobin, to form glycated hemoglobin. Once a hemoglobin molecule is glycated, it remains the same. A buildup of glycated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle.

Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA1c level is proportional to average blood glucose

concentration over the previous four weeks to three months. In individuals with poorly controlled diabetes, the quantity of this glycated hemoglobin is much higher than in healthy people.

Each laboratory establishes its own normal values for HbA1c because different components of Hb A are measured by different assay methods. Because a 1% change in the glycosylated hemoglobin represents a 35-mg/dL change in the mean plasma glucose concentration, it is important to follow relative changes in HbA1c values measured by a single laboratory.

The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the HbA1c ≥ 7% as another criterion for the diagnosis of diabetes.

Following table shows the relationship between HbA1c % and estimated glucose level changes in blood, as per American Diabetes Association.

HbA1c (%)	Estimated glucose level (mg/dL)
6	126 (100–152)
7	154 (123–185)
8	183 (147–217)
9	212 (170–249)
10	240 (193–282)
11	269 (217–314)
12	298 (240–347)

Table 1: Relationship between HbA1c and Glucose level

Above data shows direct relationship between HbA1c and glucose level. As the level of glucose increases proportional increase in HbA1c is also observed.

Clinical use:

HbA1c can be measured without any special patient preparation (e.g., fasting) and generally is not subject to acute changes in insulin dosing, exercise, or diet.

Currently, the HbA1c value is used as an adjunct to assessing overall glycemic control in patient with diabetes. Often, it is used to verify clinical impression related to glucose control and patient adherence.

Some also have suggested the use of HbA1c values for diabetes screening and

diagnosis; however until the test is standardized and more studies are completed, it cannot be recommended for these purposes. HbA1c should be measured quarterly in patient who do not meet treatment goals, and at least semiannually in stable patient who are meeting treatment goals. [13]

Limitations:

Apart from its diagnostic use, it has certain limitations. HbA1c does not replace the day-to-day monitoring of blood glucose concentration, which is essential for evaluating acute changes in blood glucose concentration. These values are needed to adjust the meal plan or medication doses.

Alteration in RBC survival such as haemoglobinopathies, anemia, acute or chronic blood loss, and uremia may affect HbA1c values, resulting in inaccurate indication of glycemic control. Antioxidants such as vitamin C and E also may interfere with the glycosylation process.

Till date there is no gold standard assay method available [14], and in some countries standardize operating procedures are beyond reach, hence in those condition HbA1c test cannot be carried out and OGTT and FGT are preferred.

A) Kyoko Sunaga et al. study helps to predict complication like stroke by measuring Glycosylated hemoglobin level in the body. The relationship between HbA1c and stroke is not fully understood but few trials have been performed to for each stroke subtype. This study is a prospective cohort study which includes 32,726 subjects with an approximately 6 year follow-up. Categories of strokes were calculated using Cox-proportional hazard method. Hazard ratio of ischemic and hemorrhagic stroke showed tendencies to increase with HbA1c levels form mild, when HbA1c level is more than 6% to severe when HbA1c level is more than 7%. There is no significant increase in HbA1c level in case of hemorrhagic stroke. This study shows that when HbA1c level is above 7% chances for severe ischemic stroke increase and acts a marker in a diabetic patient having value of Hb A1c level more than 7%.

B) R.N.Roberts et al. study shows that diabetic women patient are at risk of developing hypertension during pregnancy, since pregnancy and hypertension both are associated with insulin resistance. Study was carried out to investigate the relationship between glucose metabolism in the first and second trimester and hypertensive complication of pregnancy. Study included 1334

diabetic women subjects with a age range of 15 to 45 years, Study shows that when HbA1c level goes as high as 6.56% patient develops pre-eclemsia (i.e. about 3.28%) and when it is about 6.52% gestational hypertension develops(i.e. about 16.86%). These indicate that during gestational diabetes, value of HbA1c helps in prediction of hypertensive complication.

Discussion:

Diabetes is a metabolic disorder in which glucose level in the body is elevated may be due to impaired insulin secretion or insulin resistance or increased glucose production. Various tests can be used glucose estimation in the body during diabetes, one of which also includes estimation HbA1c% which gives precise level of glucose over a period of 2-3 months.

Normal range for HbA1c is 4-6% and during diabetes the value goes up because Glycosylated Hemoglobin is formed by attachment of glucose molecule to hemoglobin and hemoglobin is circulating in the body and thus glucose which is bind to hemoglobin also circulates and cause the increase in level of glucose and hence HbA1c. As per ADA value more than 7 % indicates the diabetes.

There are several complications are also associated with the diabetes and most commonly associated includes retinopathy, nephropathy, CVS complication, neuropathy, etc. When Glycosylated Hemoglobin level increases above certain level it indicates the presence of complication. Above studies shows that higher level of HbA1c is associated with various complication like decreased GFR, Proteinuria, etc.

Thus value of HbAlc can be use a bio-marker for the prediction of diabetes as well as complication of diabetes.

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