Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/20205
DOI URL: http://dx.doi.org/10.21474/IJAR01/20205

RESEARCH ARTICLE

HBA1C STATUS IN TYPE II DIABETES MELLITUS WITH AND WITHOUT IRON DEFICIENCY ANAEMIA

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Manuscript Info

Manuscript History
Received: 08 November 2024
Final Accepted: 14 December 2024
Published: January 2025

Key words:-
Iron Deficiency Anemia, Hemoglobin A1c (HbA1c), Diabetes, IDA

Abstract

Diabetes is an endemic global illness that is on the rise in both developed and developing countries. The American Diabetes Association has suggested glycated haemoglobin (HbA1c) as a possible substitute for fasting blood glucose in diagnosing diabetes. HbA1c is an important measure of long-term glycaemic control since it can represent the total glycaemic history of the two to three months before. In addition to providing a reliable measure of chronic hyperglycemia, HbA1c is highly correlated with the risk of long-term complications from diabetes. Elevated HbA1c has also been identified as an independent risk factor for coronary heart disease and stroke in people with or without diabetes. However, recent research has demonstrated that inadequate iron levels and glycemia contribute to high HbA1c.Therefore, decreasing circulating iron levels might be a worrying risk factor for heart attacks and strokes. The HbA1c test is an impartial biomarker for the diagnosis and outlook of diabetes since it offers valuable information with just one test. This study's results have demonstrated the significance of HbA1c in the diagnosis and prognosis of diabetic patients.

Introduction:-

Diabetes mellitus and other lifestyle-related illnesses have become an important health issue for the public in the modern period. Diabetes mellitus is a prevalent metabolic disease that causes both micro and macrovascular problems and has a high rate of fatality[1]. Even though this is still the case, the infection has received little attention as a consequence of diabetes, especially in low- and middle-income nations where infections frequently occur as symptoms of untreated diabetes. Infections are more common and have a more complex course in people with diabetes than in the general population[2]. Diabetes mellitus (DM), sometimes referred to as just diabetes, is a collection of metabolic disorders characterised by persistently elevated blood sugar levels. The symptoms of increased appetite, thirst, and more frequent urinating are brought on by this elevated blood sugar. Diabetes can lead to several consequences if left untreated.Diabetic ketoacidosis and nonketotic hyperosmolar coma are examples of acute complications. Heart disease, stroke, renal failure, foot ulcers, and eye damage are serious long-term

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consequences. Diabetes results from inadequate insulin production by the pancreas or inadequate insulin cellular response. Diabetes mellitus arises in three primary forms[1,2].

Type-I

The loss of the beta cells in the pancreatic islets of Langerhans that produce insulin is a hallmark of type I diabetes mellitus, which results in insulin insufficiency. This kind can also be classified as idiopathic or immune-mediated.The essential function of the human immune system in the development of diseases is highlighted by the recent regulatory approval of the first immunotherapy that targets T cells to prevent the autoimmune destruction of pancreatic β-cells. This also tends to open the door for other immune-targeted therapies for type 1 diabetes[3].About 10% of instances of diabetes mellitus in North America and Europe are caused by it. Whenever commencement occurs, the majority of the individuals affected are otherwise healthy and at a healthy weight. Particularly in the early phases, insulin sensitivity and response aretypically normal. Although type I diabetes can strike adults or children, it was formerly known as "juvenile diabetes" since most incidences of the disease occurred in youngsters.

Originally, the term "brittle diabetes" referred to a life "disrupted by episodes of hypoglycemia or hyperglycemia." Small case reports of primarily young women with psycho-social instability, recurrent diabetic ketoacidosis, poor patient compliance, or maladaptation were the main emphasis of early descriptions. We reinterpret "brittle diabetes" as affecting four separate life periods, each with unique traits and related diseases that lead to extremely unpredictable glycaemic control and unfavourable consequences. However, these influences may frequently be reversed or much reduced after they have been recognised[4].Several genes, including certain HLA genotypes, are known to affect the risk of diabetes type I, which is partially hereditary. Diabetes can develop in genetically predisposed individuals as a result of one or more environmental variables, such as food or a viral infection. There is some evidence that the Coxsackie B4 virus and type-I diabetes are related. The emergence of type I diabetes is independent of lifestyle, in contrast to type II diabetes.

Type II

One component of metabolic syndrome (MS), which we have dubbed metabolic dysfunction syndrome (MDS), is type II diabetes (T2D), which is defined by a heterogeneously increasing loss of islet β cell insulin production. This loss often occurs after the presence of insulin resistance (IR). It is unclear how T2D develops, however IR and β cell dysfunction are key factors in its pathophysiology. Inflammation, endoplasmic reticulum stress (ERS), oxidative stress, and ectopic lipid deposition are some of the common routes by which dyslipidaemia, hyperglycemia, and other metabolic diseases cause IR and/or islet β cell dysfunction. Although there isn't a cure for type II diabetes at this time, lifestyle changes and/or medicines can prevent it or put it in remission[5].It is assumed that the insulin receptor is involved in the impaired sensitivity of bodily tissues to insulin. Nevertheless, the precise flaws remain unknown. Cases of diabetes mellitus brought on by a known defect are categorised differently. The most prevalentkind is type II diabetes.

In the first stages of type II, decreased insulin sensitivity is the most common defect.

There are several subtypes of diabetes. Type II diabetes accounts for almost 80% of all instances of diabetes (T2D). T2D is a polygenic illness that has a 30–70% inheritance rate. The risk of type II diabetes is increased by genetic and environmental/lifestyle variables, including obesity and a sedentary lifestyle. We examine the history of the genetic studies of diabetes, how they grew with the availability of exome and whole-genome sequencing and genome-wide association studies, and the current issues in these investigations[6].Insulin or medication alone can be used to treat type II diabetes. Low blood sugar can be brought on by insulin and certain oral drugs. People with type II diabetes who are obese might benefit from weight loss surgery[7].

Dietary and lifestyle choices that involve consuming large amounts of sweetened drinks are linked to weight gain and the prevalence of type II diabetes. Excessive sugar consumption affects the risk factors for T2DM's macrovascular consequences[8].

Gestationaldiabetes:

The third major kind, gestational diabetes, which is brought on by elevated blood glucose levels in pregnant women who have never had diabetes before [9].According to studies, GDM is one of the best indicators of type II diabetes since it increases a woman's risk of developing diabetes by more than seven times, and around half of moms with GDM will have diabetes within ten years[10,11].

Although it is entirely treatable, gestational diabetes needs close medical monitoring during the whole pregnancy. Blood glucose monitoring, dietary modifications, and in certain situations, the use of insulin are all possible forms of management[11,12].Untreated gestational diabetes can harm the mother's or fetus's health, even if it may only be temporary.The baby is at risk for congenital heart and central nervous system defects, muscular skeletal deformities, and macrosomia (large birth weight).Foetal surfactant synthesis may be inhibited by elevated foetal insulin, leading to respiratory distress syndrome. It is possible for red blood cell breakdown to cause hyperbilirubinemia.

Diagnosis:
Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following[33]:
Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

Table1:-Blood test levels for diagnosis of diabetes and prediabetes.

	Normal	Prediabetes	Diabetes
A1C(Percent)	5≥	5.5-6.5	6.5 ≤
Fasting Plasma Glucose(mg/dl)	99≥	100-125	126
Oral Glucose Tolerance Test(mg/dl)	139≥	140-199	200≤

*mg = Milligram, dl = deciliter

If there is no obvious hyperglycemia, a positive result should be verified by repeating any of the aforementioned procedures on a separate day.The simplicity of measurement and the significant time commitment of formal glucose tolerance testingwhich takes two hours to complete and provides no predictive benefit over the fasting testmake it preferable to assess a fasting glucose level[6,11]

The current definition of diabetes mellitus states that two fasting glucose readings greater than 126 mg/dl (7.0 mmol/l) are diagnostic.This significant change in the definition of metabolic syndrome positions insulin hypersecretion as the common factor. If shown to be accurate, this new conceptual framework has significant ramifications for the prevention and treatment of metabolic syndrome in the future, as well as for the illnesses that are linked to it, such as PCOS, ASCVD, obesity-related T2D, and NAFLD[10,12].The most common kind of anaemia in India is iron deficient anaemia. Based on their blood glucose levels over the previous three months, patients with diabetes use haemoglobin A1c (HbA1c) as a gauge of their glycaemicmanagement[10,11,12].

Material and Methods:-

A. Study design:
A total number of 50 subjects between the ages of 30 to 55 years enrolled for the present study. Detailed medical history relevant clinical examination data and written consent were obtained from all subjects by explaining the study procedure.

The estimated sample size is 50 which includes 25 cases (clinically diagnosed patients of diabetes mellitus with iron deficiency anemia that is IDA) & 25 age and sex-matched controls (clinically diagnosed patients of diabetes mellitus without IDA). The cases and controls included in the present study were selected from patients attending outpatient departments (OPD) and indoor patient departments (IPD) of internal medicine. Samples were assessed at the Central Clinical Laboratory (CCL), Department of Biochemistry.

B. Selection of study subjects – inclusion and exclusion criteria

a. Inclusion criteria:

- Non-diabetic patients
- Patients with complications of diabetes mellitus
- Hemolytic anemia
- Chronic alcoholism
- Pregnancy

- Blood transfusion within 6 months.

Collection Of Blood Sample:
About 2 ml of fasting blood sample was collected from the Median cubital vein in a fluoride bulb for fasting plasma glucose estimation (BSL-F).

A 2 hr postprandial venous sample of the patients was also collected in a fluoride bulb for post-prandial plasma glucose estimation (BSL-PP).

A 2 ml venous sample was collected in an EDTA bulb for estimation of complete blood count (CBC) and Glycated haemoglobin (HbA1c)

A 3 ml venous sample of the case group was collected in a plain bulb for estimation of serum ferritin.

The serum/ plasma was separated by centrifugation at 3000 rpm for 10 minutes. Venous blood samples were processed within 2 hours of collection.

Methods:-

The following parameters were evaluated:

- ❖ Estimation of Blood Glucose Level By GOD- POD or Hexokinase Method
- ❖ Estimation of Glycated hemoglobin by HPLC Method
- ❖ Hematological Parameters

Estimation Of Blood Glucose Level By God-Pod
Quantitative estimation is done using the glucose oxidase peroxidase endpoint (GODPOD) method.

Principle: Glucose is oxidised by glucose oxidase (GOD) to produce gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4amino-antipyrine (4-AAP) and 4 hydroxybenzoic acid [4-HBA] in the presence of peroxidase (POD) to yield a pink quinoneimine dye that is measured at 540 nm.

Glucose + O2 + H20 ⟶ Gluconic acid + H2O2

H2O2 + 4- AAP + 4 HBA ⟶ 2 H2O +Quinoneimine dye

AAP: 4 – Aminoantipyrine. 4 HBA: 4-Hydroxy benzoic acid. The intensity of the pink colour formed is proportional to the glucose concentration when measured colourimetrically at 540 nm.

Table 2: Reagent composition(Glucose reagent)

Glucose oxidase	20000 lU/L
Peroxidase	3250 lU/L
4-Aminoantipyrine	0.52 mmol/L
4-Hydroxybenzoic acid	10 mmol/L
Phosphate buffer	110 mmol/L

Also contain nonreactive fillers and stabilizers pH 7.0 ± 0.2 at 25°c.

Table 3: Glucose standard

Glucose Standard	100mg/dl

Reagent reconstitution:
The vial is allowed to attain room temperature (15 – 30ºc). The contents of each vial are dissolved using glucose diluents with a special lipid-clearing agent.

The final volume made is 500 ml and it is transferred to a clean coloured bottle. The reconstituted reagent is stable for 90 days at 2-8ºc or 14 days at 25 ºc.

SAMPLE: Fasting and 2 hr postprandial venous blood samples are collected in a fluoride bulb. Glucose in the plasma is stable for 24 hrs at 2 – 8 ºc and for 4 hrs at 30 ºc.

Table 4: Assay procedure:

Tubes	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10 µl

mix well and incubate at 37 ºc for 15 minutes.

Calculation:

$$\text{Glucose} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard (mg/dl)}} \times \text{concentration of standard (mg/dl)}$$

Normal Range: Fasting Plasma Glucose: 70 – 110 mg/dl

Estimation Of Glycated Haemoglobin:

HPLC Technology:

High-Performance Liquid Chromatography, or HPLC, is the gold standard method for haemoglobin A1c testing. Using Tosoh's proprietary, non-porous column, our ion-exchange methodology provides chromatographic results in high resolution chromatograms without loss of precision.

Figure1:-Biorad-10 (GHB ANALYZER)

High-performance liquid chromatography, or HPLC, is an analytical chemistry technique to separate, identify, and quantify each component in a mixture. In ion-exchange chromatography, the glycated haemoglobin components are separated according to their different electrical charge. As fractions elute, the time it takes to separate that fraction is called the retention time. The retention times for each fraction determine the identity of the component.

Tosoh's ion-exchange methodology utilizes a proprietary, in-house developed, non-porous polymer resin column that provides high resolution chromatograms and high-efficiency separation without loss of precision. The HbA1c measurement yields a direct determination of stable HbA1c through clear separation between labile HbA1c and stable HbA1c by generating a chromatogram that contains key valuable information about the patient including the presence of a haemoglobin variant or hereditary persistence of fetal Hb- a feature unique to the HPLC ion-exchange methodology.

Features and benefits

- Gold Standard Technology
- Ion-exchange HPLC is the gold standard for HbA1c measurement
- Ion-exchange HPLC was used in the Diabetes Control and Complications Trial, or DCCT, study undertaken in the United States

Clinical Interference

- Clear separation between L- HbA1c and s- HbA1c
- HbAD, HbAS, and HbAC separated from Ao peak
- No clinical interference with HbAD, HbAS, HbAC, and HbAE
- No clinical interference with labile A1c, acetylated Hb, aldehyde Hb, and carbamylate Hb.

HAEMATOLOGICAL PARAMETERS:-
PCV, MCV, MCH, MCHC, RDW (Coulter Principle + VCS technology).

Cell count:
Red blood cells, white blood cells and platelets are counted electronically in the system based on aperture impedance or light scattering technology.

Mean corpuscular volume (MCV) and pack cell volume (PCV/Hct): when a cell passes through the aperture or a beam of light, the high electrical pulse generated is proportional to the cell volume.

Cell indices:
Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations are derived parameters. MCH is derived from hemoglobin and RBC count and MCHC is derived from hemoglobin and hematocrit.
MCH = [Hb (g/dl) / RBC count (million/cmm)] × 10 (Unit- pictogram)
MCHC = [Hb(g/dl) / Hematocrit(%)]× 100 (Unit- g/dl)
RDW: is derived from pulse high analysis. It indicates a quantitative measurement of variation in cell volume, an equivalent of the microscopic assessment of the degree of anisocytosis.

Result and Discussion:-

Scholars that investigate the HbA1c status in Type II Diabetes Mellitus (T2DM) in conjunction with and without Iron Deficiency Anaemia (IDA) typically concentrate on the effects of IDA on HbA1c levels, which are a crucial indicator for sustained glucose control. Summary of Results: HbA1c Comparison Levels: Using IDA: In T2DM patients with IDA, artificially increased HbA1c readings are frequently observed. This is because alterations in the lifespan and turnover of red blood cells impact the glycation process. Lacking IDA: Patients with type 2 diabetes who do not have IDA usually have HbA1c values that more closely correspond to their glycemic control. Glycemic Management Evaluation: Deceptive Markers: HbA1c may not be a good measure of glycemic control in T2DM patients with IDA, which could cause average blood glucose levels to be overestimated.HbA1c is still a useful indicator for evaluating long-term glycemic management in those without IDA. Effects of Iron Supplementation: Normalisation: When iron supplements are used to treat IDA, patients' HbA1c values typically drop and more accurately reflect their true glycemic state.

Every year, thousands of individuals worldwide suffer from diabetic complications that result in permanent organ damage or death. As such, accurate diagnosis and patient monitoring are critical and need great care. One test used in the diagnosis and follow-up of diabetes patients is the HbA1c, which gives information on blood glucose levels during the previous three months. In diabetic patients, it serves as the fundamental marker for assessing long-term glucose levels91,92, and 93. If the test is performed in particular centres that offer standard and reference procedures for the analysis, the American Diabetes Association has recommended using HbA1c for the follow-up and diagnosis of DM. Nonetheless, issues with this method's standardisation continue to exist.

HbA1c ≥6.5% should be used for the diagnosis of DM5, according to a standardisation study published by the ADA in 2010. According to the American Diabetes Association (ADA), the HbA1c test can only be used if it is approved by the Diabetes Control and Complications Centre (DCCT) and is performed by a lab that is a member of the National Glycohemoglobin StandardisationProgramme (NGSP) 92,100,101. The National Glycohemoglobin Standardisation Program102 recommended that the crucial value for HbA1c be adjusted to ≥ 6.3% in early 2010, notwithstanding the ADA's previous report that this number should be ≥ 6.5%.

Conclusion:-

The research was done in the Department of Biochemistry from October 2023 to January 2024 on the HbA1c status in type II DM with and without IDA. A total of 50 subjects were randomly enrolled; 25 of the subjects were type II

DM cases with IDA and the remaining 25 patients were age and sex-matched type II DM controls without IDA. The subjects' ages ranged from 30 to 55 years, with the case and control groups having respective means of 42.80 and 43.72 years. In each group, there were 11 females and 15 men. The glycemic status was in control for both groups. Comparing the case group to the control group, there was a substantial increase in RDW and a significant drop in Hb, MCV, MCH, and MCHC levels. Significant negative correlations were found between HbA1c and MCV, MCH, MCHC, and Hb. The study revealed that patients with diabetes mellitus and iron-deficient anaemia had higher HbA1C values than controls.

The HbA1c level is impacted by iron deficiency anaemia in addition to blood glucose. Given the prevalence of both diabetes mellitus and IDA in India, IDA should be taken into account when interpreting HbA1c results for the diagnosis and ongoing care of diabetes mellitus. Before making changes to DM medication, rule out IDA in individuals with increased HbA1c levels despite glycemic management. HbA1c values would drop with IDA treatment.

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