International Journal of Medical Science in Clinical Research and Review
Online ISSN: 2581-8945
Available Online at http://www.ijmscrr.in Volume 05|Issue 06 (November-December)|2022 Page: 1170-1176

Original Research Paper

A PROSPECTIVE CLINICAL STUDY TO ESTIMATE GLYCOSYLATED HAEMOGLOBIN LEVELS IN NON DIABETIC SUBJECTS IN THIRD AND FOURTH DECADES OF LIFE

Authors:
[1]Dr. A. Gopal Rao. Associate professor, [2]Dr. Asha kiran Senior Medical Officer, [3]Dr. Bongu srinivas rao
[1,2,3]Department of General medicine. Andhra medical college King George hospital Visakhapatnam

Corresponding Author:
Dr. Asha Kiran, Senior Medical Officer, Department of General medicine. Andhra medical college King George hospital Visakhapatnam

Article Received: 05-08-2022 Revised: 25-08-2022 Accepted: 15-09-2022

ABSTRACT:
Hemoglobin A1c was initially identified as an unusual hemoglobin in patients with diabetes by Rahbar et al. in1960s. Around same time, there was a strong suspicion that hyperglycemia was related to the vascular complications observed in individuals with diabetes, but the association was difficult to prove due to lack of objective markers of glucose control[2]. As stated by Knowles in a paper reviewing the subject in 1964,"The most doubtful measurement of all is that of control… for it is impossible to determine with certainty the chemical state of patients during their day-to-day life and activity."[3]. From the discovery of HbA1c, multiple small studies were conducted correlating it to blood glucose measurements, with the hypothesis that it could be used as an objective measurement of glucose control.[2,4] It was introduced into wide spread clinical use in the 1980s and subsequently became a corner stone of clinical practice[5] .The Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) demonstrated that in Type1 and Type2 diabetes, respectively intensive glucose control, reflected in glucose and HbA1c measurements, decreased risk of complications[6,7]. To improve diabetes control worldwide by introducing a global standardization for HbA1c measurement, changes in the methods of reporting HbA1c have been proposed.

KEY WORDS : Glycosylated haemoglobin, Non diabetic subjects, complications,

INTRODUCTION:

GLYCOSYLATED HEMOGLOBIN : Hemoglobin (Hb) is made up of two globin dimers, each with an associated heme moiety. In most adults of total Hb, HbA (alpha$_2$, beta$_2$) comprises 97%, A2 (alpha$_2$, delta$_2$) comprises 1.5-3.5% and fetal hemoglobin (HbF, alpha $_2$, gamma $_2$) forms < 2%. The components of HbA identified by charge separation on cation exchange resin and named according to their order of elution as follows: A$_0$, A1a, A1b, A1c. A1c is the Hb compound that is composed chiefly of glyco-hemoglobin. In the setting of hyperglycemia the highly permeable erythrocyte cell membrane allows exposure of Hb to elevated intracellular glucose levels. Glyco-hemoglobin is formed by the non-enzymatic glycation of the N-terminal valine on the beta chain of Hb in a two step Maillard reaction. First , glucose forms a labile and readily reversible aldamine (Schiff base) with the N-terminal valine on the beta chain. The aldamine then undergoes an Amadori rearrangement to form a stable ketoamine, At greater levels of hyperglycemia glycation of N-terminal lysines and alpha chains may also occur to a variable extent. On average erythrocytes survive 117 days in men and 106 days in women. At any given time blood contains erythrocytes of varying ages, with different degrees of exposure to hyperglycemia. HbA1c is affected by a number of genetic, hematologic, and illness related factors. Genetic factors – studies demonstrate that individuals without diabetes and with comparable glucose tolerance have a range of HbA1c levels within normal parameters, even without factors known to alter HbA1c discussed below are excluded. Twin studies suggest that 69% of this interindividual HbA1c variance can be attributed to genetic factors, whereas the remainder is related to age and environment. Approximately one-third of this inherited variance is related to the "glycation gap", the difference between the HbA1c predicted by the glycation of serum proteins and the actual HbA1c.Variation in the glycation gap is reported to be related in part, to differences in the erythrocyte trans membrane

gradient, suggesting variations in the degree of glucose entry into the erythrocyte, as well as 2,3-diphosphoglycerate and pH levels within the erythrocyte, although other influences have yet to be determined.

Factors influencing HbA1c	Increased HbA1c	Decreased HbA1c	Variable change in HbA1c
Erythropoiesis	Iron deficiency, Vitamin B_{12} deficiency, decreased erythropoiesis	Administration of erythropoietin, iron or vitamin B_{12}, reticulocytosis, chronic liver disease	-
Altered hemoglobin	-	-	Fetal hemoglobin, hemoglobinopathies, methemoglobin
Glycation	Alcoholism, chronic renal failure, decreased erythrocyte Ph	Ingestion of aspirin, Vitamin C, Vitamin E, certain hemoglobinopathies, increased erythrocyte Ph	-
Erythrocyte destruction	increased erythrocyte life span, splenectomy	decreased erythrocyte life span, hemoglobinopathies, splenomegaly, rheumatoid arthritis, drugs such as antiretrovirals, ribavarin and dapsone	-
Assays	Hyperbilirubinemia, carbamylated hemoglobin, alcoholism, large doses of aspirin, chronic opiate use	Hypertriglyceridemia	Hemoglobinopaties

Advantages of HbA1c testing compared with FPG or 2hour plasma glucose (2HPG) for the diagnosis of diabetes:

- Standardized and aligned to the DCCT/UKPDS; measurement of glucose is less well Standardized
- Better index of overall glycemic exposure and risk for long term complications
- Substantially less biological variability
- Substantially less pre analytic instability
- No need for fasting or timed samples
- Relatively unaffected by acute (e.g. stress or illness related) perturbations in glucose levels
- Currently used to guide management and adjust therapy

AIM OF THE STUDY:

AIM OF STUDY: To see if individuals with pre diabetes diagnosed by FPG criteria adapted from ADA 2007 criterion would have excessive glycosylation
MATERIALS AND METHODS
STUDY PERIOD: July 2021– December 2021
SAMPLE SIZE: 60 PATIENTS

ELIGIBILITY CRITERIA;

INCLUSION CRITERIA:

- Subjects attending outpatient department of medicine
- Subjects between 20 and 40 years of age

EXCLUSION CRITERIA:

- Subjects already diagnosed to be diabetic
- Patients with illnesses like heart failure, renal failure and other severe illnesses
- Subjects with newly detected diabetes

MATERIAL AND METHODS:

A total of 60 subjects without known diabetes and between 20 and 40 years of age were randomly selected from outpatient department of general medicine. Patients already diagnosed to be diabetic; those with illnesses like heart failure, renal failure and other severe illnesses are excluded. Patients newly diagnosed with diabetes are also excluded from study. Name, age, sex, occupation, height, weight, family history of diabetes mellitus, h/o hypertension, habits of smoking, alcoholism, tobacco chewing are collected. Fasting blood sugar, post lunch blood sugar, lipid profile, HbA1c were measured in all subjects. Body mass index (BMI) is calculated using formula, weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position in the right arm with a mercury sphygmomanometer. Fasting blood sugar and post lunch blood sugar are

measured by glucose oxidase- peroxidase method. Serum cholesterol by cholesterol oxidase-peroxidase-amidopyrine method, serum triglycerides by glycerol phosphate oxidase-peroxidase-amidopyrine method, HDL cholesterol by direct method, LDL cholesterol calculated using the Friedewald equation. HbA1C is measured by Ion exchange high performance liquid chromatography

Definitions and diagnostic criteria of diabetes used:

Diabetes is diagnosed using criteria adapted from American Diabetes Association, 2007, i.e., FPG≥126mg/dl and/or 2 hour plasma glucose≥200 mg/dl during an OGTT.

IGT was defined as 2-h post load plasma glucose between 140 and 199 mg/dl adapted from ADA, 2007

Impaired fasting glucose defined using ADA criteria if FPG was≥100mg/dl and < 126 mg/dl by ADA 2007 criteria

NGT – Normal glucose tolerance if FPG < 100mg/dl by ADA 2007 criteria

Pre diabetes is individuals with IGT and /or IFG

In this study post lunch blood sugar is used instead of 2 – h post load plasma glucose. The subjects are classified into 2 groups, pre diabetes group and normal glucose tolerance group. The mean and standard deviation of age , BMI , systolic BP , diastolic BP , fasting blood sugar(FBS), post lunch blood sugar(PLBS), total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, HbA1c are calculated and compared. Relationship between smoking, alcoholism, family history of diabetes and disease (pre diabetes) is analyzed and the p value is calculated. The range of HbA1c in normal glucose tolerance group is calculated. The upper limit of normal (ULN) OF HbA1c was defined as mean plus 2 SDs. The FPG concentrations lower than 100 mg/dl, 100-125 mg/dl, are cross tabulated with HbA1c levels of less than ULN, ULN to ULN plus 1%, and greater than ULN plus 1%. We divided elevated HbA1c values into 2 intervals of ULN to ULN plus 1% and more than ULN plus 1% for the following reasons:

- Patients whose glycosylated hemoglobin levels are less than ULN plus 1% do not have or have little development or progression of retinopathy or nephropathy[6,41,42,43,44]
- Patients with glycosylated levels of 1% above the normal or more have 90-95% chance of meeting OGTT criteria for diabetes[45,46]
- Individuals with lesser degrees of hyperglycemia (i.e, those with IFG or impaired glucose tolerance) almost always have glycosylated hemoglobin levels of less than 1% above the ULN

OBSERVATIONS AND RESULTS:

60 normal subjects of third and fourth decades who attended outpatient department of RIMS GENERAL HOSPITAL SRIKAKULAM selected randomly have been included in the study. The subjects had no previous history of diabetes. Five subjects were newly detected to be diabetic and are excluded from the study. Hence 55 subjects who were non diabetic were taken up in the study. Off the 55subjects, 37 are male and 18 are females. Off the 37 male subjects, 16 had pre diabetes and 21 had normal glucose tolerance. Off the 18 females, 8 had pre diabetes and 10 had normal glucose tolerance. Off the 55 subjects, 31 (56.4%) had normal glucose tolerance (NGT), 24 (43.6%) subjects had pre diabetes. 23 subjects had impaired fasting glucose, 11 had impaired glucose tolerance and 10 subjects had both IFG and IGT. Subjects with glucose intolerance (i.e, pre diabetes) were older than subjects with NGT. BMI, fasting blood sugar, post lunch blood sugar, serum cholesterol. Serum triglyceride, LDL cholesterol, HbA1c were also higher among subjects with glucose intolerance (pre diabetes) than in those with NGT.

Mean ± SD values of HbA1c among subjects with NGT and pre diabetes, were 5.1 ± 0.2and 6.4 ± 0.8, respectively

Mean ± SD values of BMI among subjects with NGT and pre diabetes were 22.3 ± 1.8 and 26.3 ± 2.3 respectively.

Mean ± SD values of age among subjects with NGT and pre diabetes were 30.9 ± 5.8 and 36.5 ± 3.7 respectively.

Mean ± SD values of fasting blood sugar among subjects with NGT and pre diabetes were 93 ± 4.5 and 115.4 ± 4.9 respectively.

Mean ± SD of post lunch blood sugar among subjects with NGT and pre diabetes were 104.6 ± 17.0 and 136.7 ± 19.9 respectively.

Mean ± SD of serum cholesterol among subjects with NGT and pre diabetes were 168.3 ± 136.7 and 178.8 ±29.9 respectively.

Mean ± SD of serum triglycerides among subjects with NGT and pre diabetes were 147 ± 64.8 and 191.1 ± 122.7 respectively.

Mean ± SD of serum HDL cholesterol among subjects with NGT and pre diabetes were 38.2 ± 7.5 and 39.8 ± 7.7 respectively.

Mean ± SD of serum LDL cholesterol among subjects with NGT and pre diabetes were 101.2 ± 26.7 and 106.6 ± 25.4 respectively.

13 subjects of the total of 22 who smoked had pre diabetes and 9 subjects had normal glucose tolerance. 11 subjects of the total of 33 who do not smoke had pre diabetes while 22 subjects had normal glucose tolerance. P value of association is calculated to be 0.04 which is significant 15subjects of the total of 26 who consumed alcohol had pre diabetes while 11 had

normal glucose tolerance. 9 of the 29 who did not consume alcohol had pre diabetes while 20 had normal glucose tolerance. P value of the association calculated to be 0.03 which is significant 15 of the total of 26 who had family history of diabetes had pre diabetes while 11 had normal glucose tolerance. 9 of 29 without family history of diabetes had pre diabetes while 20 had normal glucose tolerance P value of the association is 0.03 which is significant. HbA1c of 5.5% has sensitivity of 87.5% and specificity of 100% when used to detect pre diabetes. HbA1c of 6% has sensitivity of 66.7% and specificity of 100% to detect pre diabetes. HbA1c of 6.5% has sensitivity of 41.7% and specificity of 100% to detect pre diabetes. Range of HbA1c in subjects with NGT was 4.7% to 5.5%. Excessive glycosylation was rare in those with normal fasting plasma glucose concentrations. 96.9% of subjects with NGT had HbA1c level <5.5%, i.e. less than ULN of range of HbA1c in subjects with NGT. 12(52.2%) subjects with IFG had mild elevation of HbA1c while 10 (43.5%) subjects had marked elevation of HbA1c. 1(4.3%) subject with IFG had <5.5% HbA1c. 95.7% of pre diabetes had HbA1c >5.5% (ULN of range of HbA1c in subjects with NGT). HbA1c > 5.7% detected 13 (54.2%) of 24 subjects as pre diabetes. Fasting blood sugar detected 23 (95.8%) of 24 subjects as pre diabetes. Post lunch blood sugar detected 11 (45.8%) of 24 subjects as pre diabete.

Statistical Method Statistical analysis was performed:

TABLES AND GRAPHS:

SEX DISTRIBUTION OF SUBJECTS

SEX	NO	PERCENTAGE
MALE	37	67.3
FEMALE	18	32.7
TOTAL	55	100%

	NO	PERCENTAGE
NGT	31	56.4%
PREDIABETES	24	43.6%
Total	55	100%

Mean ± SD of Clinical and bio chemical characteristics of study subjects

Clinical & Biochemical characteristics	NGT	Pre diabetes
N	31	24
Age	30.9±5.8	36.5±3.7
BMI	22.3±1.8	26.3±2.3
FBS	93±4.5	115.4±4.9
PLBS	104.6±17.0	136.7±19.9
HBA1c	5.1±0.2	6.4±0.8
Sr. Cholesterol	168.3±136.7	178.8±29.9
Sr. Triglycerides	147±64.8	191.1±122.7
HDL Cholesterol	38.2±7.5	39.8±7.7
LDL cholesterol	101.2±26.7	106.6±25.4

Association of risk factor smoking and disease i.e. pre diabetes

Risk factor	Pre diabetes	NGT	Total
H/O smoking	13	9	22
No H/O smoking	11	22	33
Total	24	31	55

Association of risk factor alcoholism and disease i.e. pre diabetes

Risk Factor	Pre diabetes	NGT	Total
H/O alcoholism	15	11	26
No H/O alcoholism	9	20	29
Total	24	31	55

Association of family history of diabetes with disease i.e. pre diabetes

Risk Factor	Pre diabetes	NGT	Total
Family H/O diabetes	15	11	26
No family H/O diabetes	9	20	29
Total	24	31	55

Hb A1c	Pre diabetes	NGT	Total
≥5.5%	21	0	21
<5.5%	3	31	34
Total	24	31	55
Sensitivity	87.5%		
Specificity	100%		

Hb A1c	Pre diabetes	NGT	Total
≥6%	16	0	16
<6%	8	31	39
Total	24	31	55
sensitivity	66.7%		
Specificity	100%		

Hb A1c	Pre diabetes	NGT	Total
≥6.5%	10	0	14
<6.5%	14	31	46
Total	24	31	60
sensitivity	41.7%		
Specificity	100%		

Distribution of Hemoglobin A1c levels according to Fasting Plasma Glucose Concentrations					
Diagnosis	Fasting Plasma Glucose mg/dl	No of Subjects	HbA1C (%)		
			<5.5	5.5 to 6.5	>6.5
NGT	<100 mg/dl	32	31 (96.9%)	1 (3.1%)	0
IFG	100-125 mg/dl	23	1 (4.3%)	12 (52.2%)	10 (43.5%)

DISCUSSION:

Our study showed mean ± SD values of HbA1c among subjects with NGT and pre diabetes as 5.1 ± 0.2 and 6.4 ± 0.8 respectively. A study done by Viswanathan Mohan, MD, PHD, et al[47]. in participants without known diabetes who were randomly selected from Chennai urban rural epidemiology study showed mean ± SD values of HbA1c among subjects with NGT and IGT were 5.5 ± 0.4 and 5.9 ± 0.6 respectively. The same study showed 78.2% participants with NGT among 2188 participants and 11.8% with IGT. Our study showed 56.4% subjects with NGT and 43.6% subjects with pre diabetes. The percentage of subjects with IGT are higher in our study when compared to study done by Viswanathan Mohan, MD,PHD, et al[47]. This difference may be because the study done by Viswanathan Mohan, MD, PHD, et al[47] is population based study done in 2188 subjects whereas the sample in our study is small in size and may not be representative of general population. Subjects with glucose intolerance were older than subjects with NGT. Fasting blood sugar, post lunch blood sugar,

HbA1c, serum cholesterol, serum triglycerides, and LDL cholesterol were also higher among subjects with glucose intolerance than in those with NGT. This result is similar in our study. Our study showed that 54.2% of subjects with pre diabetes were detected with HbA1c levels > 5.7% which is slightly less than that in study done by Viswanathan Mohan, MD, PHD, et al[47]. Study done by Viswanathan Mohan, MD, PHD, et al[47]. Showed HbA1c ≥ 5.7% detected two thirds of individuals with IFG and/or IGT. In a study titled Relationship between fasting plasma glucose and glycosylated hemoglobin done by Mayer B.Davidson, MD, et al [48] which compared data from NHANHES III and MRG HbA1c levels in subjects with normal fasting plasma glucose concentrations were normal in 97.3% (96.2%), slightly elevated in 2.7%(3.6%) and high in 0.1% (0.2%). HbA1c levels in subjects with IFG were normal in 86.7% (81.4%), slightly elevated in 13.1% (16.4%) and high in 0.2% (2.2%). Our study showed similar results in subjects with normal fasting blood sugar concentrations. But HbA1c levels in subjects with IFG were normal in 4.3%, slightly elevated in 52.2% and high in 43.5% in our study. This difference may be because of small sample size of our study that may not be representative of general population. NHANES III data set included 2836 persons whereas MRG data set included 8917 subjects. In The Insulin Resistance Atherosclerosis Study (IRAS) done by Carlos Lorenzo, MD, et al[49]. 23.6% of subjects were identified to be at increased risk of diabetes by HbA1c levels 5.7 – 6.4%, 69.1% of subjects identified by IFG, 59.5% subjects identified by IGT. Our study showed HbA1c > 5.7% detected 54.2% of subjects as pre diabetes, Fasting blood sugar detected 95.8% as pre diabetes and post lunch blood sugar detected 45.8% as pre diabetes. The IRAS study included 855 participants whereas in our study sample size is small which may not be representative of general population which may be the cause of difference.

CONCLUSIONS AND SUMMARY:

Because there is now strong evidence that lifestyle management of those with IGT can reduce the rate of progression to diabetes[50], it is important to correctly identify those with pre diabetes so that prevention efforts may be implemented, without missing those who would benefit from intervention. HbA1c > 5.7% identifies a much smaller proportion of individuals at increased risk of diabetes than do IFG. HbA1c levels of 5.5%, 6.0% and 6.5% have low sensitivity, but high specificity in detecting individuals at increased risk of diabetes. Thus HbA1c levels may be inadequate as the only criterion for detecting individuals at increased risk of diabetes. Recognition of individuals with a high risk of diabetes can be made on criteria other than glucose regulation only for example over weight, other elements of metabolic syndrome which can help to identify individuals who would need lifestyle modification advice. Smoking, alcoholism have significant association with pre diabetes. Hence lifestyle modification in relation to habits of smoking and alcoholism may help in prevention of progression to diabetes. Family history of diabetes has significant association with pre diabetes which cannot be modified.

REFERENCES:

1. Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Commun*. 1969; 36: 838-43.

2. Koenig RJ. Hemoglobin A1c and diabetes mellitus. *Annu Rev Med* . 1980; 31: 29-34.

3. Knowles HC. The problem of the relation of the control of diabetes to the development of vascular disease. *Trans Am Clin Climatol Assoc*. 1964; 76: 142-7.

4. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N Engl J Med*. 1976; 295: 417-20.

5. Gonen B, Rochman H, Rubenstein A, Tanega S, Horwitz D. Hemoglobin A1: an indicator of the metabolic control of diabetic patients. *The Lancet*. 1977; 310: 734-7.

6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *N E ngl J Med*. 1993; 329: 977-86.

7. UK Prospective Diabetes study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet*. 1998; 352: 837-53.

8. Review of hemoglobin A1c in the management of Diabetes. Journal of Diabetes. 1 (2009) 9-17.

9. RSSDI Text book of diabetes mellitus , 2nd ed , pp 841 , pp 674.

10. John WG. Hemoglobin A1c: analysis and standardization. *Clin Chem Lab Med*. 2003; 41: 1199.
11. Goldstein DE, Little RR, Lorenz RA et al. Tests of glycemia in diabetes. *Diabetes Care*. 2004; 27: 1761-73.

12. Little RR, Wiedmeyer HM, England JD et al. Inter-laboratory standardization of measurements of glycohemoglobins. *Clin Chem*. 1992; 38: 2472-8.

13. John WG,Mosca A,Weykamp C, Goodall I. HbA1c standardization: history, science and politics. *Clin Biochem Rev*.2007; 28: 163-8.

14. Mongia SK,Little RR, Rohlfing CL et al. Effects of hemoglobin C and S traits on the results of 14 commercial glycated hemoglobin assays. *Am J Clin Pathol*. 2008; 130:136-40.
15. Little RR, Rohlfing CL, Hanson S et al. Effects of hemoglobin E and Hb D traits on measurements of glycated Hb by 23 methods. *C LIN Chem*. 2008; 54: 1277-82.