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BIOCHEMICAL MARKER ANALYSIS IN DIABETIC PATIENTS

Samer Salim Abed[1], Fadhil Hamad Zaidan[1], Balasubramanian Sathyamurthy[2*]

[1]Department of Biochemistry, Indian Academy Degree College, Bangalore.

[2]Associate Professor, Department of Biochemistry, REVA University, Bangalore.

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***Correspondence for Author**
Dr. Balasubramanian Sathyamurthy
Associate Professor, Department of Biochemistry, REVA University, Bangalore.

ABSTRACT

Diabetes mellitus is a common disease of the world. Diabetes is a metabolic disorder where insulin is not produced or used in the human body. Insulin hormone is required to convert sugar, starches, and other food into energy. Diabetes mellitus is a disorder which is characterized by high levels of blood glucose (sugar). In human system, the blood glucose levels are regulated in a very narrow range, which is done with insulin and glucagon. The function of glucagon regulates the release of glucose in liver from its cells into the blood, for the production of energy. Biochemical markers are important in determining the disease status. However the data related to the correlation between different markers like fasting and post prandial glucose levels, HbA1c level and Vitamin D3 level with different age group is inadequate. In our study about 10 patients of different age groups were selected as experimental materials. The study was intended to mainly study the blood markers which can help in detecting the diabetic status of the people with informed consent. The markers of interest were Glucose levels both in fasting and post prandial, HbA1c levels in blood samples, and Vitamin D3 levels. All these are found to be positively correlated with different age groups. The levels of Glucose both in Fasting and post prandial state are estimated by spectrophotometry. The levels of vitamin D3 and their correlation among different age groups in serum samples were also studied. The HbA1C values in the serum were quantitatively estimated by Turbidimetric Inhibition Immunoassay (TINA, Tina-quant HbA1c II). The results obtained were found to be significant especially in the age group 56-60 and 61-65 age groups. All the results were confirmed and significance was given by the student's t test. ($p<0.05$). The vitamin D3 levels are inversely correlated to the age groups. This may be a finding that, the Vitamin D3 deficiency may be one of the causes of diabetes.

KEYWORDS: The function of glucagon regulates the release of glucose in liver from its cells into the blood, for the production of energy.

INTRODUCTION

Diabetes mellitus is a metabolic disorder due to the defects in either in insulin secretion or insulin action or both. It is characterized by hyperglycemia in which the patients will have a constant high glucose concentration in their blood. This disorder causes nearly 2 – 3 % of all deaths globally each year. The chronic hyperglycemia of diabetes is associated with long term complications such as failure of various organs, especially the eyes, kidneys and nerves.[1] The prevalence of diabetes for all age-groups worldwide was estimated 4.4% in 2030. By the recent studies it is projected that the total number of people with diabetes will be doubled in 2030 especially in urban population of developing countries.

In the recent World Health Organization (WHO) report, India has 31.7 million diabetic subjects at present which is expected to raise 79.4 million by 2030.[2] Glycemic control is an important aspect in managing diabetes and its related complications. Many randomized, prospective clinical trials studies in both the types of diabetes shown clearly that achieving glycemic control decreases the microvascular complications in diabetes. Each 1% reduction in haemoglobin A1c decreases 37% risk for microvascular complications and a 21% decrease of death related to diabetes.

HbA1c test is used commonly to measure chronic hyperglycemia. Due to its high cost, it is not available in resource for poor. There are some clinical disorders where red cell life span is decreased in which using the A1C test may provide unreliable information. But many studies shows the acceptable correlation between hemoglobin A1c level and fasting blood glucose (FBS) level[3], and very few studies showed a better correlation with Post prandial blood glucose (PPBS) levels.[4] This raises a question whether FBS or PPBS is a better predictor of glycemic control. Hence the objective of our present study was to find the correlation between HbA1c with FBS, PPBS & RBS in different age groups so as to assess their usefulness in monitoring the glycemic control in Diabetic patients.

The HbA1c and Diabetes

In blood, the red blood cells are made of a molecule, haemoglobin. Glucose interacts with the haemoglobin to form a 'glycosylated haemoglobin' molecule, known as haemoglobin A1C or

HbA1C. The increase levels of glucose in the blood directly proportionate the levels of haemoglobin A1C or HbA1C in the blood.[5]

Life span of red blood cells has 8 – 12 weeks which are then replenished by our physiological and anatomical system. By measuring the HbA1C we can predict the range of how much high blood glucose has been on average over a period of one to two months. A normal non-diabetic HbA1C level ranges from 3.5 – 5.5%. In diabetes it is about 6.5%.

Glucose levels fluctuate drastically but the HbA1C level changes slowly, over 10 weeks, and hence this can be used as a marker analysis. The HbA1C test is one of the best ways to check diabetes is under control at present which can be performed in routine biochemical analysis. Levels of HbA1C are not the same as the glucose level. Glucose levels averaging 6.5 mmols/l before meals is equivalent to 7% HbA1C (glucose levels are higher after meals).

For healthy adults the normal range for the A1c test ranges between 4 – 6 %. The ideal range for diabetes is generally less than 7 percent. For diagnostic purposes, two separate A1c tests at 6.5 percent are positive for diabetes. Patients found diabetic should have this test for every 3 months irrespective of their medication.[6]

Vitamin D_3 and Diabetes

Vitamin D_3 is synthesised in the skin. Vitamin D_3 production occurs between 295-297 nm. A UV index of more than 3 is more important for Vitamin D syntheses which are present in the tropics, every day during spring, summer, and parts of autumn. In recent studies it is proved that there is a link between the levels of vitamin D with insulin resistance which may lead to diabetes. This mechanism is related to the function of macrophages and also with the levels of cytokines. Macrophages are specialized immune cells that attack invaders. When fat cells get too large, they die, and macrophages move in to eliminate the dead tissue. Active Macrophages releases cytokines, which serve as signals to other parts of the body and can impair insulin action in the liver and muscle. Higher cytokines means more insulin resistance," a key factor in type 2 diabetes. It is also found that macrophages have special receptors for vitamin D which in the case of its deficiency making macrophages more active results in insulin resistance followed by inflammation. It is believed that increase Vitamin D reduces inflammation.[7]

MATERIALS AND METHODS

Estimation of blood glucose levels

After an overnight fast (8 – 12 hours), a specimen for fasting serum glucose were collected from the patients. Blood sample were collected with the anticoagulants EDTA. Serum was used to prevent glycolysis and for accuracy. The patient is then given a breakfast containing 100 grams of carbohydrate. Two hours later, blood is drawn into a red top tube for glucose determination. Fastings and post pradinal blood glucose levels are determined by glucose oxidase method.[8]

Estimation of HbA1c levels

Venous blood samples were collected in EDTA containing tubes. Capillary blood samples were obtained by finger prick with a sterile lancet. 30 µl of capillary blood samples of each were blotted on to the filter paper (Whatman number1) and allowed to dry at room temperature. HbA1c measurements were performed on ethylenediamine tetra-acetic acid (EDTA) blood samples using cation-exchange HPLC Adams A1c HA-8160, Diabetes Mode [Arkray, Inc., Kyoto, Japan (also known as Menarini)].[9]

Parameters calculated using the final results

Mean Blood Glucose (mg/dl) = HbA1c (%) × 26.7 – 46.7

Reference value

Non Diabetic: 4.5 – 6.3% HbA1c

Diabetics: Good Control: 6.1 – 6.8

Fair Control: 6.9 – 7.7

Poor Control : >7.7%

Detection limit: 3 - 18 % HbA1c

Estimation of Vitamin D3 levels

Most circulating 25(OH)D and 1,25$(OH)_2$D is transported in the circulation are bound to DBP (≈ 90%) and to albumin (≈ 10%). A very small fraction 25(OH)D and total 1,25$(OH)_2$D remains free or unbound. The vitamin D–DBP complex can be taken up by target cells via an endocytic process involving megalin and cubilin. Once in the cell, the DBP is proteolytically degraded, leaving the intracellular vitamin D metabolite available for further action or metabolism as indicators of vitamin D status.[10]

To 0.5 mL of serum, added 350 µL of methanol–2-propanol (80:20 by volume) and mix in a Multitube vortex mixer for 30s. 25(OH)D was extracted by mixing three times (60 s each time) with 2 mL of hexane. The phases were separated by centrifugation, and the upper organic phase was transferred to a conical tube and dried under nitrogen. The residue was dissolved in 100 µL of mobile phase. Calibration curves were constructed using four concentrations of 25(OH)D_3 (15–120 nmol/L; cat. no. H-4014; Sigma Chemical Co.) and human serum albumin (50 g/L; The Finnish Red Cross).[11, 12]

RESULTS

Fasting Glucose levels

Table: 1 Glucose (Fasting) levels based on their age group. Concentration noted as mg/dL. The values are the means of the experimental samples.

Age group	Average (mg/dL)
31-35	106.5
36-40	77
41-45	103.53
46-50	121
51-55	107
56-60	140
61-65	142

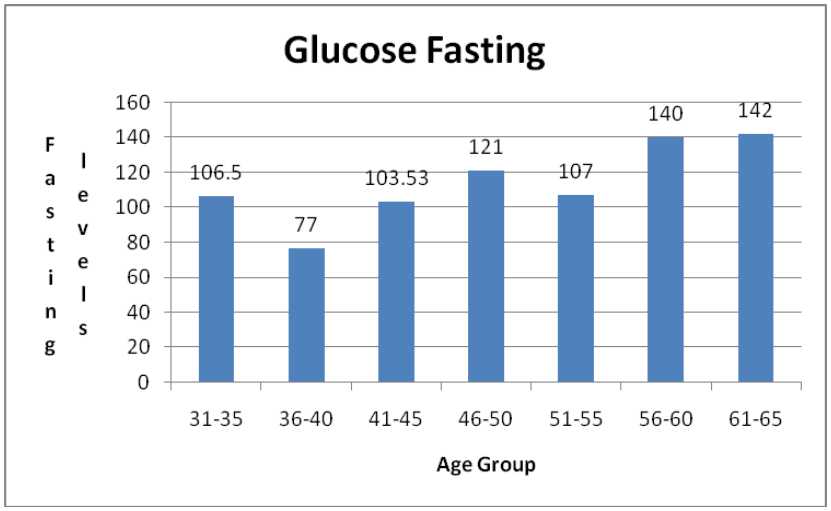

Fig 1: Graph showing the Glucose fasting values of different age groups

Table: 2 Statistical reports of the glucose fasting levels, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	4	113.8619
Variance	4.6666667	515.4446
Observations	7	7
Pearson Correlation	0.8018719	
Hypothesized Mean Difference	0	
df	6	
t Stat	-13.83415	
P(T<=t) one-tail	4.44E-06	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	8.879E-06	
t Critical two-tail	2.4469118	

Post Prandial Glucose levels

Table 3: Post Prandial Glucose levels based on their age group. The concentration noted as mg/dL. The values are the means of the experimental samples.

Age group	Average (mg/dL)
31-35	161.5
36-40	104
41-45	127.33
46-50	164
51-55	107
56-60	210
61-65	212.4

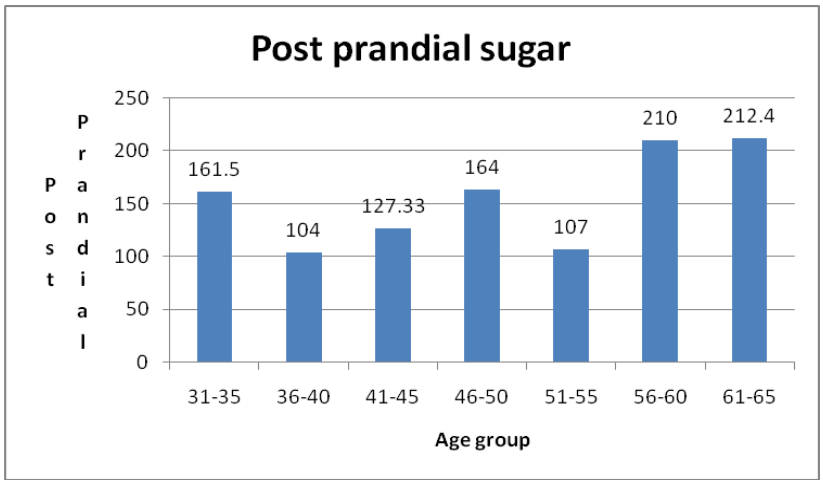

Fig 2: Graph showing the Post Prandial Glucose values of different age groups.

Table 4: Statistical report of the Post Prandial Glucose, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	4	155.17619
Variance	4.6666667	2018.8729
Observations	7	7
Pearson Correlation	0.5913056	
Hypothesized Mean Difference	0	
Df	6	
t Stat	-9.154989	
P(T<=t) one-tail	4.78E-05	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	9.56E-05	
t Critical two-tail	2.4469118	

HbA1C (Glycosylated haemoglobin) levels

Table: 5 HbA1C (Glycosylated haemoglobin) levels based on their age group. The concentration noted as mg/dL. The values are the means of the experimental samples.

Age group	Average
31-35	6.5
36-40	4.8
41-45	5.70
46-50	7.2
51-55	5
56-60	8.2
61-65	8.1

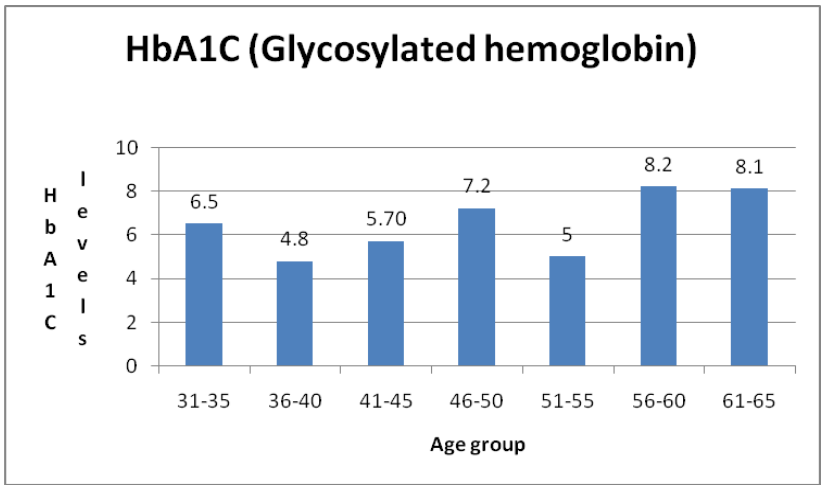

Fig 3: Graph showing the HbA1C (Glycosylated haemoglobin) values of different age groups.

Table: 5 Statistical report of the HbA1C (Glycosylated haemoglobin)levels, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	4	6.5
Variance	4.6666667	1.9533333
Observations	7	7
Pearson Correlation	0.6017051	
Hypothesized Mean Difference	0	
Df	6	
t Stat	-3.827328	
P(T<=t) one-tail	0.0043431	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	0.0086862	
t Critical two-tail	2.4469118	

Vitamin D3 Levels

Table 6: Vitamin D3 levels based on their age group. The concentration given as U/L. the values are the means of the experimental samples.

Age group	Average (U/L)
31-35	24.5
36-40	48
41-45	43.33
46-50	18
51-55	41
56-60	14
61-65	16.5

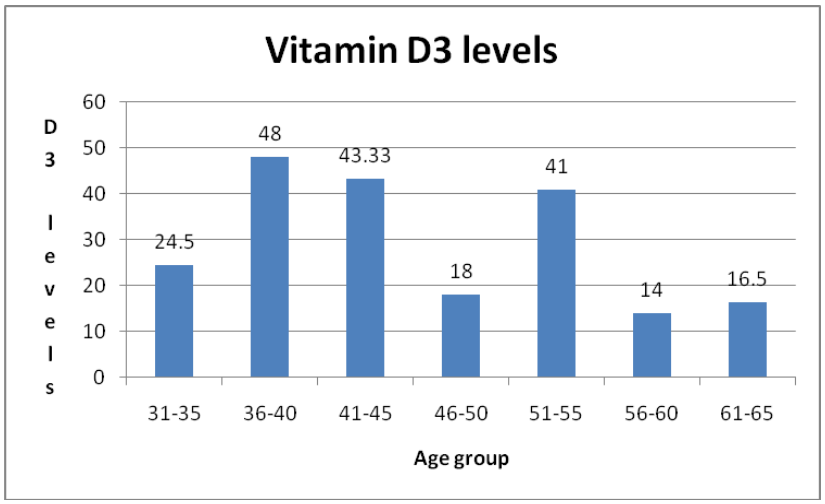

Fig 4: Graph showing the Vitamin D3 values of different age groups. Age group of 56-60 and 61-65 are found to be significant. All the values are means of the triplicates.

Table 7: Statistical report of the Vitamin D3 levels, done on Students t test, paired mean samples.

t-Test: Paired Two Sample for Means		
	Variable 1	Variable 2
Mean	4	29.333333
Variance	4.6666667	205.36111
Observations	7	7
Pearson Correlation	-0.507869	
Hypothesized Mean Difference	0	
Df	6	
t Stat	-4.313281	
P(T<=t) one-tail	0.0025097	
t Critical one-tail	1.9431803	
P(T<=t) two-tail	0.0050194	
t Critical two-tail	2.4469118	

DISCUSSION

Fasting Glucose levels: The prevalence of impaired fasting glucose in population reporting to tertiary care centre was found to be 18%. 18.36% pre-diabetics were found among males and 17.64% among females. Among males, the highest percentage of pre-diabetics was found in the above 65 age group and lowest among 36 - 45 age groups, whereas in females the highest percentage was found in the 56 - 65 age groups and lowest among the 46 - 55 and above 65 age group (Table I and Fig.1). No significant difference was found in the presence of pre-diabetics among men and women (p value > 0.05). Similarly, difference in prevalence of impaired fasting glucose (IFG) in the age group below 46 yrs compared to 46 yrs and above age group was non significant. (p value > 0.05) (Table 2).

Post Prandial Glucose levels: From the Table 3 and Fig. 2 the post prandial glucose concentration in the age group of 56 – 60 and 61 – 65 found to be higher in the serums. Though 46 – 50 age groups shows considerable increase, but the effect was very clear in the higher age group in the post prandial glucose concentration. This shows the intolerance of Insulin or deficiency of insulin is much exhibited at the old age (Table – 4). Fasting and random plasma glucose level found increased by 0.15 mmol/L and in post prandial plasma glucose level increased by 0.26 mmol/L per decade-increase in age.

HbA1C (Glycosylated haemoglobin) levels: To evaluate the relationship between glycohemoglobin and age in both sexes, we divided the subjects into five age groups: 31 – 35, 36 – 40, 41 – 45, 46 – 50, 51 – 55, 56 – 60, and 61 – 65 years of age. As shown in Table 4, glycohemoglobin increased gradually with age in men and also in women, while the largest

elevation of glycohemoglobin occurred in the 56 – 60 year old age group for both sexes. However, men exhibited a significant increment of glycohemoglobin with age only in the 55 – 60 year-old age groups, while women had a significant increase in the 41 – 45 year old and 46 – 50 year-old age groups. Men had higher average glycohemoglobin levels than women below the 45 – 54 year old age group (Table. 5).

In our study, glycohemoglobin was significantly higher in men than women under 55 years of age. Hormonal changes during the menstrual cycle may account for the differences in glycohemoglobin levels in men and women and women has shorter red cell survival, thus lowering glycohemoglobin levels compared with men. But as the ages increases these levels are almost identical. Evidence shows that estrogen is implicated in suppressing erythropoiesis in vitro and in vivo.[13, 14]

Vitamin D3 Levels: Vitamin D also known as the antiricketic factor and considered unique because of its ability to get derived from cholesterol and acts as a second messenger for maintain the calcium homeostatis and other biological functions such as cell growth and differentiation, inhibition, as well as in immune response. Vitamin D deficiency does indeed constitute an epidemic in many populations across the world, especially in India more than 90% of healthy individuals irrespective of their genders have subnormal 25(OH)D levels. Common causes of vitamin D deficiency in the general population are due to low dietary vitamin D intake and poor exposure to sunlight [12]. Sunlight is the major source of vitamin D and its synthesis are directly proportional on exposure to sunshine and also depends vitamin D intake through the diet or its supplements. Almost 90% vitamin D in the body is produced by the skin which is influenced ultraviolet radiation in the 300nm. Serum 25(OH)D, the major circulating form of vitamin D used for assess the status of vitamin D[10]. Decreased levels of Serum 25(OH)D are found in the individuals living at higher latitudes and having darker skin.

Alteration in the levels of vitamin D, especially when it is decreased it is found to be associated with developing Type 2 diabetes mellitus (DM) and other diseases related to circulatory system including heart and blood vessels[1]. About 82% of subjects of study population were found to be having vitamin D deficiency. In Indian population about 70% of adults in both rural and urban areas were found vitamin D deficiency which according to International Diabetes Federation, the diabetes prevalence in India is likely to be doubled in 2025.[2, 3]

Inverse relationship between vitamin D status and diabetes was not found in the present study from Table 6, 7 and Fig. 4. This can be attributed to ethnic variations and highly prevalent vitamin D deficiency in this area. Possibly other factors like small sample size, cross-sectional study design, and dietary habits can also be attributed for this phenomenon.

REFERENCES

1. Smaldone A, Glycemic control and hemoglobinopathy: When A1c may not be reliable. Diabetes Spectrum. 2008; 21: 46–9.
2. Malekiani CL, Ganesan A, Decker CF. Effect of Hemoglobinopathies on Hemoglobin A1c Measurements. Am J Med. 2008; 121.
3. Chu CH, Wang MC, Sun TH, Lee JK, and Lam HC. Very low HbA1c values in a patient with clinical silent hemoglobin variant (Hemoglobin J): A case report. J Intern Med Taiwan. 2007; 18: 45–50.
4. Nigam PK, Sharma S, Sareen R, Paul V, andLal A. Lack of precision in HbA1c values on Variant II in cases of Hemoglobin Q. India. Indian J ClinBiochem. 2006; 21: 72–5.
5. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem. 2001; 47: 153–63
6. Sabath DE. Case study: Artifactually low HbA1c in a patient with high Hemoglobin F. Clin Diabetes.2000; 18: 179–83.
7. Wolf G. "The discovery of vitamin D: the contribution of Adolf Windaus". J. Nutr. June 2004; 134 (6): 1299–302.
8. Bergmeyer, H.U. and Bernt , E., Methods of Enzymatic Analysis, H.U. Bergmeyer, Ed., New York, Academic Press, 2nd Edition, 1974; 1205-1212.
9. Higgins T, Stewart D, andBoehr E. Challenges in HbA1c analysis and reporting: An interesting case illustrating many pitfalls. Clin Biochem. 2008; 41: 1104–6.
10. Hollis BW. "Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it." Cacif Tissue Int. 1996; 58(1): 4.
11. Holick MF, Schnoes HK, Deluca HF, Suda T, Cousins RJ. "Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine", Biochemistry, 1971; 10(14): 2799–804.
12. Holick MF, Deluca H F andAvioli LV. "Isolation and identification of 25-hydroxycholecalciferol from human plasma". Archives of Internal Medicine, 1972; 129(1): 56–61.

13. Roberts WL, Safar-Pour S, De BK, Rohlfing CL andWeykamp CW, Little RR. Effects of Hemoglobin C and Hemoglobin S traits on glycohemoglobin measurements by eleven methods. Clin Chem., 2005; 51: 776–8.
14. Tsai LY, Tsai SM, Lin MN, and Liu SF. Effect of hemoglobin variants (Hb J, Hb G and Hb E) on HbA1c values as measured by cation-exchange HPLC (Diamat) Clin Chem. 2001; 47: 756 – 8.