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EVALUATION OF GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS WITH AND WITHOUT MICROVASCULAR COMPLICATIONS

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ABSTRACT

Diabetes mellitus (DM) includes a group of metabolic disorders that share common phenotype of hyperglycemia. Assessing glycemia in diabetes has always been a challenge. Aim of this study is to evaluate glycemic control in patients with type 2 DM patients with and without microvascular complications. HbA1c, FBS and PP_2BS were estimated as measure of glycemic control. A cross sectional study consists of 150 subjects: 50 patients having type 2 DM without any microvascular complications (Group II), 50 patients with type 2 DM with one or more microvascular complications (Group III) and 50 normal healthy control (Group I) were selected. Study shows increased value of HbA1c, FBS and PP_2BS level in group II and group III patients compared to group 1 (higher than upper reference value in group III). These finding indicate that poor glycemic control plays important role in the pathophysiology and management of type 2 DM and its complications.

KEYWORDS: **Type 2 Diabetes Mellitus, Glycemic Control and Hyperglycemia**

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INTRODUCTION

Diabetes is undoubtedly one of the most challenging health problems in 21st century. With India having the highest number of diabetic patients in the world, it is posing an enormous health problem in the country. Calling India the diabetes capital of the world, the International Journal of Diabetes in developing countries says that there is alarming rise in prevalence. It is the fourth or fifth leading cause of death in most high-income countries.It is a chronic, incurable, costly, and increasing but largely preventable non communicable disease which is responsible for millions of deaths annually, debilitating complications, and incalculable human misery[1]. Thus, understanding the pathogenesis and preventing and/or ameliorating these long-term complications have been major goals of research in diabetes mellitus. Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycemia in type 2 DM[2]. Hyperglycemia not only defines the disease but is the cause of its most characteristic symptoms and long-term complications.

Complications from diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society[1]. The long-term complications of diabetes have major consequences for individual subjects and growing healthcare delivery and cost implications for society[3]. Because the development of complications is linked to the accumulation of glycation adducts in tissue proteins, any analytical method that serves as an index of the extent of glycation should clearly be used to guide therapy in diabetes and its complications[4]. Assessing glycemia in diabetes has always been a challenge. The monitoring of glycemia is an essential component of diabetic care. The core of the issue is glycemic control[5]. Optimal monitoring of glycemic control involves plasma glucose measurements and measurement of glycated hemoglobin. These measurements are complementary: the patient's glucose measurements provide a picture of short-term glycemic control; whereas the HbA1c reflects average glycemic control over the previous 3 months[2].Amongst the various markers of glycemic control, glycated hemoglobin has now been established as the most reliable[4].

Glycated hemoglobin is formed by a posttranslational, non-enzymatic, substrate-concentration dependent irreversible process of combination of aldehyde group of glucose and other hexose with the amino-terminal valine of the β-chain of hemoglobin[6]. HbA1c is the predominant fraction and gives an estimate of the blood sugar levels of an individual over the last three months. The American Diabetes Association has also recommended that the lowering of HbA1c reduces the risk of microvascular and neuropathic complications and possibly, macrovascular complications. HbA1c should thus be kept to less than 7% for patients in general and to less than 6% for individual patients. HbA1c is the primary target for glycemic control[7]. Aim of this study is to evaluate glycemic control by measuring HbA1c, FBS, and PP_2BS in patients with type 2 diabetes mellitus patients with and without microvascular complications.

MATERIALS AND METHODS

Study design and Subjects

This study was a hospital based cross sectional study conducted at shree sayajirao general hospital and medical college, vadodara (India) during period of January 2010 to October 2010. A cross sectional study consists of 150 subjects out of them 50 patients having type 2 DM without microvascular complications (Group II), 50 patients with type 2 DM with microvascular complications (Group III) and 50 normal healthy control (Group-I) were selected. Subjects were recruited according to simple

random sampling method meeting the selection criteria.

Selection Criteria

Inclusion Criteria: The subjects selected for study were grouped as follows:

Group I – Control group (n=50)

This group consisted of age and sex matched healthy subjects. They were free from any ailment which could affect the parameters under study. They were not on any medication. They were taken from general population.

Group II – Type 2 DM without microvascular complications (n=50)

This group consisted of patients with type 2 DM and frees from clinical evidence of any microvascular complications.

Group III – Type 2 DM with one or more microvascular complications (n=50)

This group consisted of patients with type 2 DM and associated with one or more microvascular complications (e.g. diabetic nephropathy, diabetic retinopathy, heart disease, diabetic neuropathy).

Exclusion Criteria: The patients with type 1 DM, hemolytic anaemia, hemoglobin variants, pregnancy, hepatic disease and infectious diseases like tuberculosis, sarcoidosis etc were excluded from this study.

Ethical Considerations

The objectives of study were explained to all eligible subjects for this study. Informed written consent of all subjects included in the study was obtained for involvement in study groups and for venipuncture. Emphasis was given that participation in this study was voluntary.

Questionnaire and Bio data Collection

A questionnaire was specifically designed to obtain information which helps to select individuals according to the selection criteria of the study. The questions mainly focused on age of patients, type and duration, mode of treatment and complications of diabetes.

Blood Sample Collection

A 5 ml of venous blood was drawn from each volunteer using a disposable vacutainer system in fasting condition (plain, EDTA and fluoride). Post prandial (2 hour) sample collected in fluoride vacutainer for PP_2BS estimation. Serum or plasma separated within half an hour and stored at 2-8° C temperature till analysis was done.

Analysis of Sample

Fasting and Post prandial (2 hour) blood sugar (FBS & PP_2BS) estimated by Glucose Oxidase-Peroxidase (GOD-POD) enzymatic end point method. (Kit: Quantitative determination by glucose oxidase peroxidase method (Trinder GOD-POD) Mfg by Spinreact)[8]. Glycated hemoglobin (HbA_1C) concentration was measured by Immuno turbidimetric method (Kit: Quantitative determination of glycated hemoglobin (HbA1c) in human blood by latex turbidimetry Mfg by Spinreact)[9]. Other biochemical parameters like total cholesterol, triglycerides, serum creatinine and ALT were also estimated. All biochemical investigation performed on fully automatic analyzer I.S.E. srl MIURA. Hemogram and Urine examination were done in pathology laboratory. Fundoscopy and Electrocardiogram were done in respective department.

Statistical Analysis

The data collected during the current study were recorded and analyzed statistically to determine the significance of different parameters by using GraphPad InStat statistics software. Statistical analysis was done by using t-test to find out significance of difference between two groups and correlation coefficient to find out statistical correlation between two variables and its significance. Interpretation was done according to p-value as follows:

$p < 0.05$ was considered significant

$p \geq 0.05$ was considered not significant

RESULTS

As shown in table 1 and 2, mean FBS, PP_2BS and HbA1c concentration in Group III were 194.26 mg/dl, 274.08 mg/dl and 8.26 % respectively which is significantly higher compared to values in Group II and Group I. (with p value <0.0001). Such values were also higher that upper reference range for this parameters. Also mean FBS, PP_2BS and HbA1c concentration in Group II were 108.22 mg/dl, 141.40 mg/dl and 6.15 % respectively which is higher compared to normal healthy

control (Group I), but within upper reference range. Further HbA1c concentration was significantly and positively correlated with FBS and PP2BS concentration in group II and group III (Table 3, Figure 2 and 3). Table 4 shows comparison of other parameters between study groups.

Table 1
Comparison of FBS, PP_2BS and HbA1c level between study groups

Parameter	Group I	Group II	Group III
Fasting blood sugar (FBS) (mg/dl)	97.02±16.18	108.22±19.01	194.26±56.59
Post prandial blood sugar (PP_2BS) (mg/dl)	127.88±21.17	141.40±20.31	274.08±63.00
Glycated Hemoglobin (HbA1c) (%)	5.71±0.57	6.15±0.44	8.26±1.10

Figure 1
showing concentration of HbA1c in study groups

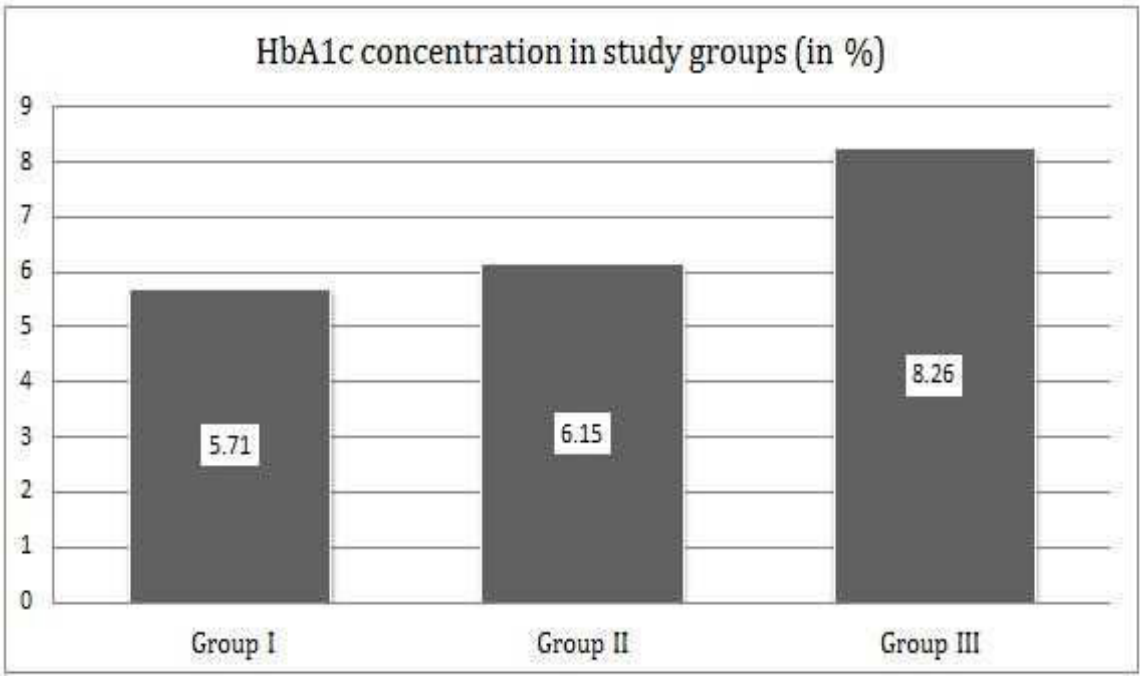

Table 2
Independent samples t-test study: HbA1c, FBS and PP_2BS between study groups

Study groups	HbA1c		FBS		PP_2BS	
	t value	p value	t value	p value	t value	p value
I and II	3.823	0.0018	3.171	0.002	3.258	0.002
II and III	12.519	<0.0001	10.190	<0.0001	14.172	<0.0001
I and III	14.432	<0.0001	11.681	<0.0001	15.553	<0.0001

p value is two tailed probability value and t value is test statistic t

Table 4
Correlation of HbA1c with FBS and PP_2BS

Correlation between		Group I	Group II	Group III
HbA1c (%) and FBS (mg/dl)	Correlation coefficient r	- 0.1745	0.7529	0.8549
	Significance (p value)	0.2255	<0.0001	<0.0001
HbA1c (%) and PP_2BS (mg/dl)	Correlation coefficient r	- 0.1930	0.7535	0.7024
	Significance (p value)	0.1590	<0.0001	<0.0001

Figure 2
showing correlation between HbA1c and FBS concentration in patients (N=100)

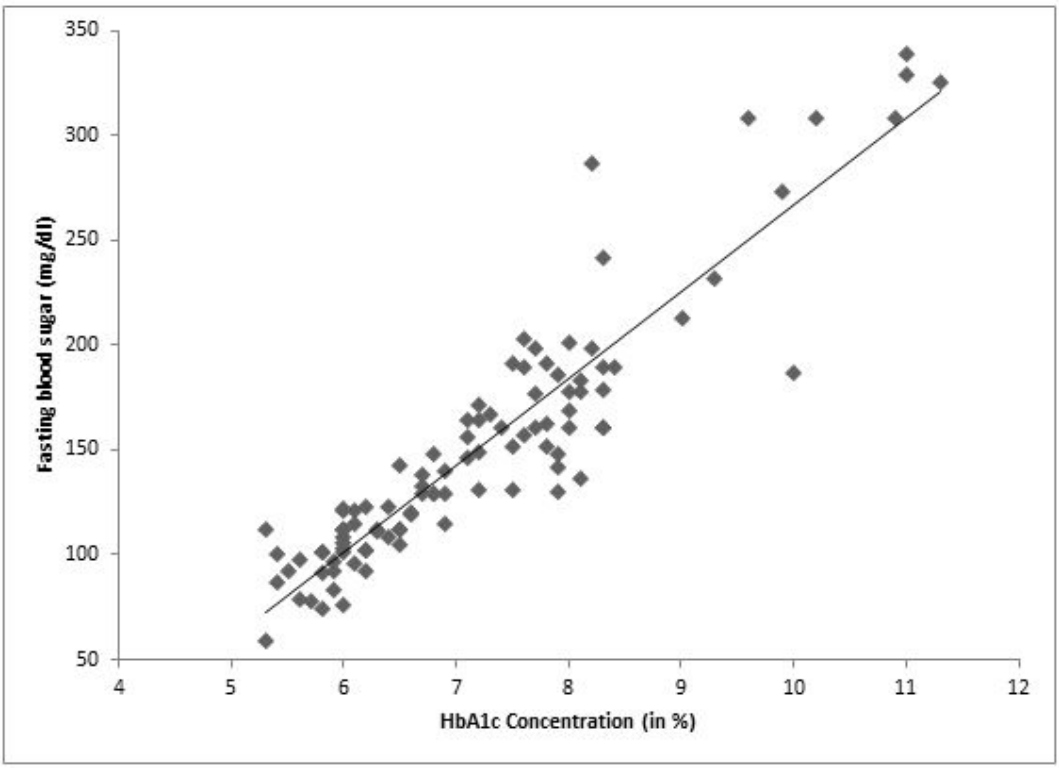

Figure 3
showing correlation between HbA1c and PP2BS concentration in patients (N=100)

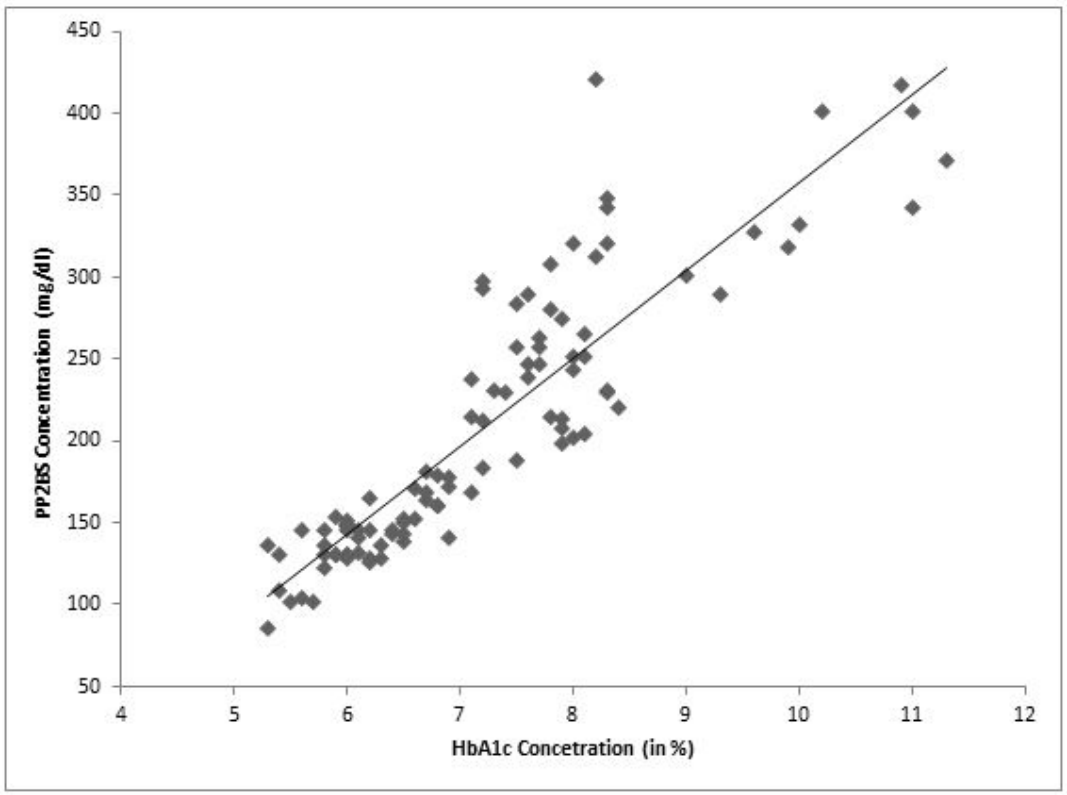

Table 4
Comparison of Other Variables between study groups

	Group I	Group II	Group III
Number of patients	50	50	50
Sex (M/F) %	60/40	44/56	56/44
Average age (years)	56±7.12	55±10.7	59±8.6
Average duration of DM (in year)	-	8.11±1.7	9.28±3.4
Prevalence of Hypertension (in %)	-	62	82
Prevalence of smoking (in %)	-	26	50
Average Height (cm)	155.9±8.03	154.3±4.22	157.4±4.41
Average weight (kg)	57.96±4.535	63.6±5.67	66.6±5.78
Average BMI (kg/m^2)	24.16±5.136	26.77±2.877	26.86±2.476
Mean Serum total cholesterol (mg/dl)	148±25.9	193.32±51.5	201.76±58.2
Mean Serum triglycerides (mg/dl)	103.36±23.2	130.98±32.1	140.56±65.2
Mean Serum Creatinine (mg/dl)	0.73±0.12	0.87±0.1	1.92±1.5
Mean Serum ALT (U/L)	19±6	21.46±7.95	43.11±74.8

DISCUSSION

Present study was undertaken to assess glycemic control in patients with type 2 diabetes mellitus with and without microvascular complications. Fasting blood sugar (FBS), postprandial blood sugar (PP_2BS) and glycated hemoglobin (HbA1c) was measured as a marker of short and long term glycemic control. Study shows patient with one or more microvascular complication had poor glycemic control which evidenced by significantly increased value of HbA1c, FBS and PP_2BS level in group III patients (higher than upper reference limit). FBS, PP2BS and HbA1c level is also higher in diabetic patients compared to normal healthy control, but they were within normal reference range of these parameters. Also HbA1c was correlated well with FBS and PP_2BS concentration in diabetic patients (group II and group III). This poor glycemic control lead to chronic complications in patients with type 2 diabetes mellitus. The mechanism(s) by which it leads to such diverse organ dysfunction is explained by following four theories.

1. Increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intra- and extracellular proteins. Nonenzymatic glycosylation results from the interaction of glucose with amino groups on proteins. AGEs have been shown to cross-link proteins (e.g. collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction, and alter extracellular matrix composition and structure. The serum level of AGEs correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines.
2. Via the Sorbitol pathway: Intracellular glucose is predominantly metabolized by phosphorylation and subsequent glycolysis, but when increased, some glucose is converted to sorbitol by the enzyme aldose reductase. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species, and likely leads to other types of cellular dysfunction.
3. Hyperglycemia increases the formation of diacylglycerol leading to activation of protein kinase C (PKC). Among other actions, PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons.
4. Hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter function by glycosylation of proteins such as endothelial nitric oxide synthase or by changes in gene expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1 (PAI-1).

A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the

mitochondria; these compounds may activate all four of the pathways described above. Although hyperglycemia serves as the initial trigger for complications of diabetes, it is still unknown whether the same pathophysiologic processes are operative in all complications or whether some pathways predominate in certain organs.[2]

Diabetes control and complications

The Diabetes Control and Complications Trial (DCCT) demonstrated that improvement of glycemic control reduced nonproliferative and proliferative retinopathy (47% reduction), microalbuminuria (39% reduction), clinical nephropathy (54% reduction), and neuropathy (60% reduction). Improved glycemic control also slowed the progression of early diabetic complications. There was a nonsignificant trend in reduction of macrovascular events.[2,10,11] The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that each percentage point reduction in A_{1c} was associated with a 35% reduction in microvascular complications. In patients with type 2 diabetes the risk of diabetic complications was strongly associated with previous hyperglycemia and there was a continuous relationship between glycemic control and development of complications. Any reduction in HbA_{1c} is likely to reduce the risk of complications, with the lowest risk being in those with HbA_{1c} values in the normal range (<6.0%)[2,12]. Intensive glycemic control can delay the onset and progression of the early stages of diabetic microvascular complications in Japanese patients with type 2 diabetes (Kumamoto study). [13]The findings of the DCCT, UKPDS, and Kumamoto study support the idea that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic microvascular complications. These landmark studies prove the value of metabolic control and emphasize the importance of intensive glycemic control in DM[2]. Of the several pathogenic mechanisms by which hyperglycemia may lead to altered tissue structure and function, non-enzymatic glycation changes the structure and function of several soluble and insoluble proteins in vivo and in vitro. Because cells and their extracellular matrix share a dynamic and reciprocal relationship, modulations of matrix components by glycation leads to altered cell behavior, including changes in cell spreading, phosphorylation of key intracellular signaling molecules, and expression of extracellular matrix proteins and their modulators. Extracellular matrix from diabetic patients is more extensively glycated than extracellular matrix from nondiabetic people. In addition, the accumulation of glycation products and the accompanying structural extracellular matrix modifications correlate with the development of functional complications of diabetes. These changes in tissue structure and function are slow and cumulative, producing a long time lag between the start of diabetes and the onset and progression of the complications. These mechanisms were promptly confirmed by determination of Glycated Hemoglobin[4].

The role of glycated hemoglobin, 25 years on…

The long-term complications of diabetes have major consequences for individual subjects and growing healthcare delivery and cost implications for society. Evidence for the benefits of good glycemic control, as monitored by glycated hemoglobin measurements, has been developed in the 25 years since they were introduced to the point where HbA_{1c} assays play central roles in patient management, clinical guidance and audit, and clinical trial design[3]. Glycated hemoglobin (GHb) is formed by the glycation of hemoglobin. Its value represents the glycemic status of a person over the last two to three months. It is measured in diabetics as well as in those with impaired glucose tolerance to assess the glycemic status over the last two to three months[7]. Formation of GHb is essentially irreversible, and the concentration in blood depends on both the lifespan of the red blood cell (average 120 days) and the blood glucose concentration. Because the rate of formation of GHb is directly proportional to the concentration of glucose in the blood, the GHb concentration represents the integrated values for glucose over preceding 6 to 8 weeks. This provides an additional criterion for assessing glucose control because GHb values are free of day to day glucose fluctuation and are unaffected by recent exercise and food

ingestion. The contribution of plasma glucose to GHb depends on the time interval, with more recent values providing a larger contribution than earlier values. The plasma glucose in preceding 1 month determines 50% of the HbA1c, whereas days 60 to 120 determine only 25%. After a sudden alteration in blood glucose concentrations, the rate of change of HbA1cis rapid during initial 2 months, followed by a more gradual change approaching steady state 3 months later[14]. GHb may also serve as a check on the accuracy of the patients meter and adequacy of the SMBG testing[4,5].

The HbA_{1c} test has been used in diabetics and reflects the glycemic status of a patient over the past two to three months. In the ADA guidelines 2007, HbA_{1c} has been referred to as A_{1c}. These guidelines recommend that A_{1c} should be performed at least twice a year in patients who are meeting their treatment goals (and who have stable glycemic control), and quarterly in patients whose therapy has changed or who are not meeting their glycemic goals. There is a correlation between the A_{1c} levels and mean plasma glucose levels on multiple testing over two to three months. For example, an A_{1c} value of 6% corresponds to a mean plasma glucose level of 135 g/dl. The American Diabetes Association has also recommended that the lowering of A_{1c} reduces the risk of microvascular and neuropathic complications and possibly, macrovascular complications. A_{1c} should thus be kept to less than 7% for patients in general and to less than 6% for individual patients. A_{1c} is the primary target for glycemic control[7]. Carlos Abraira and William Duckworth has emphasizes the need for glycemic trials in type 2 diabetes. They correlate glycemia with blood pressure and microangiopathies and has shown that Glycemic control is a well-established treatment objective in diabetes care. However, the effectiveness and specific goals of glycemic control are not yet known for older type 2 diabetic patients with advanced complications and suboptimal response to current treatments. Therefore, current glycemic guidelines for such patients are variable[15].

Nasir Ahmed et al shown overall glycemic control in type 2 diabetic patients by estimating HbA_{1c} and simultaneously measuring the blood glucose[16]. S. Patiakas et al has shown a correlation between the Glycated hemoglobin (HbA_{1c}) level, the arterial blood pressure (AP), and the body mass index (BMI) in diabetic patients and evaluate its utility as regulation criteria of these patients[6].ZeinabGhazanfari et al showed HbA_{1c} study as determinant of glycemic control in female diabetic patients to identify the factors affecting glycemic control[17]. Thus assessing glycemia and understating pathophysiological mechanism by which chronic hyperglycemia lead to development of complication in diabetes mellitus is still being a major challenge in patients with type 2 DM.

CONCLUSION

Patients with type 2 diabetes mellitus having one or more microvascular complications had poor glycemic control compared to normal healthy persons and patient with type 2 DM without any complications. These finding indicate role of poor glycemic control in development of microvascular complication in type 2 DM and control of hyperglycemia is very important in management of diabetes and prevention of diabetic complications. Further studies at the molecular level are required to know the how hyperglycemia modulate various pathway in producing diabetic complications.

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CONFLICT OF INTEREST

The author stated that there are no conflicts of interest.

REFERENCES

1. A call to action on diabetes: International Diabetes federation, November 2010.
2. Alvin C. Powers. Diabetes mellitus. In: Kasper, Braunwald, Fauci, Hauser, longo, Jameson (editors), Harrison's Principle of Internal Medicine, 16th Edition, McGraw-Hill, New York, 2005, pp. 2152-2179.
3. Jeffcoate SL, Diabetes control and complications, the role of glycated haemoglobin, 25 years on.Diabetes Medicine, 21(7):657-65, (2004)
4. H. B. Chandalia and P. R. Krisnaswamy, Glycated Hemoglobin. Current Science 83(12): 1522-1532, (2002)
5. S Thakur, V Chauhan, RC Negi, Role of HbA1c in Diabetes Mellitus. Journal of Indian Academy of Clinical Medicine 10(1&2):52-54, (2009)
6. S. Patiakas, C. Charalampous, Correlation between the Glycated Hemoglobin (HbA_{1c}) level, the arterial blood pressure (AP) and the body mass index (BMI) in diabetic patients and evaluation of its utility. Journal of Hypertension 28:e300 A, (2010)
7. Nitin S, HbA1c and factors other than diabetes mellitus affecting it. Review Article Singapore Med Journal 51(8): 616-622, (2010)
8. Quantitative determination of glucose by glucose oxidase peroxidase method (Trinder GOD-POD); Spinreact.
9. Quantitative determination of glycated hemoglobin (HbA1c) in human blood by latex turbidimetry; Spinreact
10. Results of the DCCT are reported in the New England Journal of Medicine, 329(14):234-243, (1993)
11. Diabetes Control and Complications Trial (DCCT): Results of Feasibility Study. Diabetes care, 10(1), (1987)
12. Irene M Stratton, Amanda I Adler, H Andrew W Neil, David R Matthews, Susan E Manley, Carole A Cull, David Hadden, Robert C Turner, Rury R Holman, Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. British medical journal 321, (2000)
13. Shichiri M, Kishikawa H, Ohkubo Y, Wake N, Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. Diabetes care, 23(2):B21-9, (2000)
14. David B. Sacks: Carbohydrates. In: Carl A Burtis, Edward R Ashwood, David E. Bruns (editors), Tietz Textbook of Clinical chemistry and Molecular Diagnostics, 4th Edition, Elsevier Saunders, 2006, pp. 879-883.
15. Carlos Abraira and William Duckworth, The Need for Glycemic Trials in Type 2 Diabetes. Clinical Diabetes 21(3):107-111, (2003)
16. Nasir Ahmed, Type 2 Diabetes Mellitus: How well controlled in our patients? J Ayub Med Coll Abbottabad 20(4):70-72, (2008)
17. ZeinabGhazanfari, Determinants of glycemic control in female diabetic patients, a study from Iran. Lipids Health Disorders9: 83, (2010)