Available online through

www.jbsoweb.com

Research Article

GLYCATED ALBUMIN AND MICROALBUMINURIA AS RISK FACTORS IN DIABETIC RETINOPATHY OF TYPE 2 DIABETES MELLITUS

Kondaveeti Suresh Babu[1]*, R. Aravind Kumar [2], I. Anand Shaker[3]

[1]Dept of Biochemistry, Anna Medical College & Research Centre, University Of Technology, Mauritius

[2]Dept of Physiology, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur, Tamil Nadu, India

[3]Dept of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur, Tamil Nadu, India

***Correspondence**

Suresh Babu Kondaveeti

Department of Biochemistry, Anna Medical College & Research Centre, University Of Technology, Mauritius

DOI: 10.7897/2321-6328.01120

Article Received on: 01/05/13

Revised & Accepted on: 12/06/13

Abstract

Diabetes mellitus (DM) is a major health problem with long-term micro and macrovascular complications. Diabetic retinopathy (DR) is a sight-threatening chronic complication of diabetes mellitus in adults. In this study, we determined the frequency of DR and the relationship between Glycated Albumin (GA) levels, duration of diabetes and Microalbuminuria with DR in type 2 diabetic patients. Two hundred and fourty seven type 2 diabetic patients participated in this study. In the first examination, retinopathy was evaluated by ophthalmoscopy through dilated pupil by experienced ophthalmologist. Based on their optic fundi findings they were classified into three groups; without retinopathy, had non-proliferative DR (NPDR) and had proliferative diabetic retinopathy (PDR). In addition, the patients were classified in three groups according to their GA levels; below 17 %, between 17 and 25% and above 25%. According to the duration of diabetes the patients were divided into three groups. First group consisted of patients who were diabetic for less than five years, the second group consists of patients who had diabetes for a period 6-10 years and the third group, who were diabetic for more than 10 years. According to our results, there was a significant relationship between duration of diabetes and DR (both nonproliferative and proliferative) ($p<0.001$). A similar relationship between PDR and Microalbuminuria($p<.001$), between NPDR and Microalbuminuria($p<.01$) was found. These results imply that duration of diabetes, GA level and Microalbuminuria are important risk factors for onset or progression of DR in type 2 DM.

Keywords: Diabetes mellitus (DM), diabetic retinopathy (DR), glycosylatedAlbumin, GA, Microalbuminuria

INTRODUCTION

Diabetes mellitus is a major health problem in Western countries as well as in India. India, with its population of 180 million, is estimated to have about 15 million people with diabetes mellitus. Currently it is 5th in the world according to WHO estimation for prevalence of diabetes mellitus and by the year 2030 is expected to rise to the 2nd position[1]. Many people with type 2 diabetes have macrovascular and microvascular complications such as diabetic retinopathy (DR) at the time of first diagnosis of diabetes [2-5]. DR is the most frequent cause of blindness among adults aged 20-75 years and it remains a significant health problem worldwide as reported by the ADA [6]. Improvements in diabetic care and earlier detection of the disease can reduce the incidence of visual impairment and blindness [7,8]. By the time of clinical diagnosis of type diabetes, some individuals already show evidence of DR, indicating that diabetes may have been present for several years [9]. Duration of diabetes, glycemic control, hypertension, dyslipidemia, obesity, proteinuria, pregnancy and socioeconomic status play important role for development of Dr. Duration of diabetes and inadequate glycaemic control are most important [10]. Currently, monitoring HbA1c levels is the gold standard for assessing average blood glucose concentration over three months [11-13]. The target level of HbA1c which is needed for adequate glycemic control in type 2 DM is unknown. galbumin (GA) and glycated hemoglobin (HbA1c) are closely correlated, and that values for glycated albumin accurately represent the equivalent values for HbA1c in diabetic patients not subject to physiological conditions that disturb hemoglobin metabolism. In these cases, glycated albumin has been found to be a better indicator of glycation than HbA1c. As expected, levels of glycated albumin change more rapidly over time in response to changes in treatment (as reflected by changes in fasting plasma glucose) than do levels of HbA1c.[14,15]

There are no reports available on levels of glycated albumin at different stages of diabetic retinopathy in Indian population. therefore this study was planned to assess frequency of DR and the relationship between GA levels, duration of diabetes, Microalbuminuria and DR with type 2 diabetic patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted from December 2010 to January 2012 in two tertiary health centers located at Kancheepuram and Melmaruvathur, Tamil Nadu respectively. A total of 300 (M: F; 185:115) type 2 diabetic

subjects were selected for this cross sectional study from the outpatient department of diabetes care in MAPIMS&R and were compared with 80 (M: F; 54:26) non diabetic control subjects. After the full explanation of the study, written informed consent was obtained from each study subject. The study was approved by the Ethics committee of the institution prior (EC No-MAPIMS/RC/SEP/2010/42) the informed consent was obtained from all the subjects. Demographic and anthropometric details like age, weight, height, duration of diabetes, duration of diabetic nephropathy were recorded for all the study subjects. Family history of diabetes and hypertension, smoking and alcohol consumption habits were obtained from the medical records of the study subjects. Blood pressure was measured in all the study subjects using a standard mercury sphygmomanometer. Blood samples were collected for the biochemical estimations. Fasting and post prandial samples were collected from the known cases of diabetes and other subjects underwent a standard oral glucose tolerance test. The diagnosis of diabetes was based on previous history of diabetes or on the criteria of World Health Organization for the classification of glucose tolerance.[16]

Retinopathy was evaluated by ophthalmoscopy through dilated pupil by experienced ophthalmologist. Based on their optic fundi findings they were classified into three groups; Group 1 without retinopathy, normoalbuminuric subjects having random urinary albumin by creatinine ratio (ACR) of <30ug/mg creatinine, Group 2 non-proliferative DR (NPDR) microalbuminuric subjects having urinary albumin by creatinine ratio of 30 - 300ug/mg creatinine. And Group 3 proliferative diabetic retinopathy (PDR) macroalbuminuric subjects having massive proteinuria of expected protein excretion rate of >500mg/day with the presence of diabetic nephropathy. In addition, the patients were classified in three groups according to their GA levels; below 17 %, between 17 and 25%, and above 25%. According to the duration of diabetes the patients were divided into three groups. First group consisted of patients who were diabetic for less than five years, the second group consists of patients who had diabetes for a period 6-10 years and the third group, who were diabetic for more than 10 years.

GA and HbA1c estimation

Plasma glycated albumin (GA) levels were measured by an enzymatic method using albumin specific protease, ketoamine oxidase and albumin assay reagent on the Hitachi autoanalyser 912 (Lucica GA-L, Asahi Kasei Pharma Corp, Tokyo, Japan)[17,18] GA was hydrolyzed to amino acids by albumin specific protease and then oxidized by ketoamine oxidase to produce hydrogen peroxide, which was measured quantitatively. The GA value was calculated as the percentage of GA relative to total albumin, which was measured with bromocresol purple method. The measured values of GA was not influenced by the substances such as bilirubin F up to 14.6 mg/dl, bilirubin C up to 15.2 mg/dl, glucose up to 1000 mg/dl, ascorbic acid up to 100 mg/dl. Glycosylated haemoglobin (HbA1c) was estimated by the turbidimetric inhibition immunoassay using haemolyzed whole blood on the Hitachi autoanalyser 912.

All the biochemical estimations were done by using standard enzymatic procedures. Plasma glucose was estimated by glucose oxidase peroxidase method. Renal parameter like urea was estimated by kinetic enzymatic UV assay, serum creatinine was estimated by Jaffe's kinetic method and serum albumin was estimated by bromocresol. The statistical analysis was done by using SPSS version 16.0. One Way ANOVA method was applied to observe association of microalbuminuria with GA and duration of diabetes. P value < 0.0001 was considered as statistically significant.

Table 1: Comparison of GA levels and HbA1c between Group A, Group B, and Group C

Parameters	Group A(NDR) (n=100)	Group B(NPDR) (n=100)	Group C(PDR) (n=100)
GA(%)	16.5±0.05	20.2±4.80	25.4±1.24
HbA1c(%)	5.93±0.339	7.94±0.37	9.01±0.88

(NDR=Non Diabetic retinopathy, NPDR=Non progressive Diabetic retinopathy PDR=Progressive Diabetic retinopathy)
GA:F ratio-490.41,df-2, pvalue<0.0001(significant); HbA1c:F ratio-392.34, df-2, pvalue<0.0001(significant)

Table 2: Comparison of albuminuria between Group A, Group B, and Group C

Parameters	Group A(NDR) **(n=100)** Normoalbuminuriic	Group B(NPDR) **(n=100)** microalbuminuric	Group C(PDR) **(n=100)** Macroalbuminuric
Albuminuria	23.12±0.24	47.14±39.15	131±49.89
P value	0.012	0.096	0.157

(NDR=Non Diabetic retinopathy, NPDR=Non progressive Diabetic retinopathy PDR=Progressive Diabetic retinopathy)
Albuminuria:F ratio-77.82, df-1; value<0.0001(significant)

Table 3: Duration of Type 2DM with Retinopathy and GA

Duration of Diabetes	GA(%)	Diabetic Retinopathy (%of prevalence)
<5 years (n=150)	17.45±0.26	< 20
6 to 10 years (n=80)	20.00±5.25	> 45
>10 years (n=70)	25.02±2.45	> 85

Duration of Diabetes:F ratio-408.5, df-2; P value < 0.0001 significant.

RESULTS

The normal cut off value for GA was derived using control group (mean+1SD). The cut off value for GA (rounded off) in this population was 15% (Range 7-17%).There was no significant difference between the study groups with respect to age and duration of diabetes. No significant difference was noted in the blood pressure values between the study groups. Of the 100 patient in the sample, the mean age of patient with diabetic retinopathy group was 52.5 ± 7 years while it was 52 ± 8 years for non diabetic retinopathy group. Duration of diabetes was it is 9 ± 5 years in diabetic retinopathy group and 8 ± 5 years in non diabetic retinopathy group.

Among the 300 Type 2DM patients studied [controlled and uncontrolled groups], 45% had a family history of diabetes and Male:Female ratio was 1.17:1. In Table.1, the glycemic control of Group A, Group B and Group C were compared

between diabetic patients and healthy controls with serum GA levels. Microalbuminuria was compared between non-proliferative DR (NPDR) and proliferative diabetic retinopathy (PDR) in Table 2. Based on Table.1 and Table.2, the Microalbuminuria increased significantly with poor glycemic control and correlated with elevated serum GA levels indicating retinal damage ($p<0.0001$).

The parameter of the studied groups according to duration of diabetes was summarized in Table.3. In Type 2DM patients, Microalbuminuria and GA have shown a significant linear correlation with duration of diabetes ($p<0.0001$).

DISCUSSION

We analyzed the association between diabetic retinopathy, Glycated albumin, microalbumionuria and found that patients with proliferative diabetic retinopathy (PDR) had significantly higher GA levels and microalbuminuria(>500mg/day) than non-proliferative DR (NPDR).progression of DR influenced by hyperglycemia, characteristic of diabetes which causes glycation of compounds such as HbA1c and albumin. All proteins in the body can be modified by non- enzy-matic glycation. In diabetes mellitus the extent of the non- enzymatic glycation of proteins increases, compared with non-diabetic subjects, which may comprise at lea st a part of diabetic complications[19]. Among these modified proteins, measurement of HbA1c has been applied for clinical use in order to monitor chronic glycemic control in diabetic patients. But now we try to provide the better indicator of glycemic control in DR patients which is Glycated albumin, is a kind of early-stage amadori-modified reaction products formed from Schiff's base adducts and has been implicated in the pathogenesis of diabetic complications and better indicator[20]. Stable fraction of glycated hemoglobin (HbA1c) is rou-tinely measured in the majority of patients with diabetes around the world; Since HbA1c reflects glycemic control over the preceding 2-3 months[21]. However HbA1c may not be suitable for evaluation of short term variation in gly-cemic control because of long life span of erythrocytes (120 days). Because the turnover of human serum albumin is much more rapid (half life of 15-20 days) than that of hem-globin, the measurment of glycated albumin (GA) provides an index of glycemic control over a short period of time than the measurment of HbA1c[22,23].

A recent article on proliferative diabetic retinopathy (PDR) discusses the involvement of glycated albumin in stimulating angiogenesis in the retina. Involvement of AP-1 (Activator Protein-1) has been implicated in both in vitro and in vivo studies of angiogenesis. The study shows that glycated albumin stimulates the phosphorylation of c-Jun, a component of the transcriptional factor AP-1 in retinal glial cells. AP-1 up regulates the mRNA level of cytokine vascular endothelial growth factor (VEGF), stimulating increased levels of VEGF and proliferation of unregulated capillary growth. When the newly formed capillaries invade the retina, leakage of blood plasma damages the retinal area, inducing macular degeneration. The result is a loss of vision in the central retinal area.[24]

The present study was conducted on 300 Diabetic patients[100 cases without DR, 100 Non Prolifearative and 100 Prolifearative DR groups]. The serum glucose is a continuous variable, rising and falling about two-fold throughout the day in people without diabetes, and up to some 10-folds in people with diabetes. Hence, the higher mean value of Glycated Albumin in Prolifearative DR group was expected in view of increase in microalbuminuria levels. Also, the level of mean blood glucose, effectiveness of treatment and risk of development of possible long term chronic complications are typically associated with suboptimal or poor glycemic control in uncontrolled Diabatic. This finding was supported by the present study, which showed that elevated levels of microalbuminuria and serum creatinine levels were seen in patients with poor glycemic control. We also observed that there is relative risk reduction, when the mean GA levels were with in the range (7-17%) [Table.2]. This was substantiated by the reduction in the levels of microalbuminuria and serum creatinine levels in controlled diabetics. From the foregoing, it is obvious that good glycemic control is the key to preventing and/or forestalling microalbuminuria and subsequently, diabetic retinopathy amongst other chronic complications of DM. Also in our study, we observed increase in the levels of GA and DR with increase in the duration of the disease. The possible reason could be that during long course of disease, the patient might have default treatment. Therefore regular screening for DR for every 4-5 months in addition to continuous GA estimation are important tools in the management of DM.

One of the limitations of our study is the relatively small number of cases. Therefore only the strongest associations with retinopathy are expected to be detected.

CONCLUSION

The study results suggest that there is strong association between diabetic retinopathy and GA levels. Being a developing country; there is a dire need that GA testing should be done in both, newly diagnosed as well as already diagnosed Type 2DM patients as an early marker of retinal risk factor. Hence, patients and health care givers should give very high priority to improving glycemic control sufficiently to prevent Diabetic Retinopathy.

ACKNOWLEDGEMENT

The authors are thankful to the Management, MAPIMS&R, M.Sachithanandam, Vishnu Diagnostics Center, No. 1 Ennakara street, Kancheepuram 631501 Tamilnadu for providing the necessary facilities and permitting to carry out this research work. Also the authors are also very much thankful to all the Physicians who had referred the cases to this Diagnostic Center.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H: Global prevalence of diabetes:estimates for the year 2000 and projections for 2030. Diabetes Care 2004, 27:1047-1053. http://dx.doi.org/10.2337/diacare.27.10.2569-a PMid:15111519
2. Basile F. The increasing prevalence of diabetes and its economic burden. J Manag Care 2000; 6: 1077-81.
3. Bloomgarden ZT, American Diabetes Association 60th Scientific Sessions. Diabetes Care 2000; 24: 779-84. http://dx.doi.org/10.2337/diacare.24.4.779
4. Brinchmann-Hansen O, Dahl-Jorgensen K,Hanssen KF,Sandvik L: The response of diabetic retinopathy to 41 months of multiple insulin injections, insulin pumps, and conventional insulin theraphy. Arch Opthalmol 1988; 106: 1232-46. http://dx.doi.org/10.1001/archopht.1988.01060140402041
5. Buse JB. Management of glycemia in type 2 diabetes. Clin Cornerstone 1998; 1: 39-57. http://dx.doi.org/10.1016/S1098-3597(98)90017-5
6. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris III FL, Klein R, Diabetic Retinopathy . Diabetes Care 2003; 26 (Suppl 1): 99-102. http://dx.doi.org/10.2337/diacare.26.1.226

7. 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. NEJ Med 1993; 329: 977-86. http://dx.doi.org/10.1056/NEJM 199309303291401 PMid:8366922
8. Mohammad Javadi et.al The Eye Disease Prevalance Research Group in tehran. Arch Opthalmol 2004; m 122: 552-63.
9. Tapp RJ, Shaw JE, Harper CA, Balkau B, McCarty DJ, Taylor HR, Welborn TA, Zimmet PZ, The prevalence of and Factors Associated with diabetic retinopathy in the Australian Population, Diabetes Care 2003;26:1731-7.http://dx.doi.org/10.2337/diacare.26.6.1731 PMid:12766102
10. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, Klein R, Retinopathy in diabetes. Diabetes Care 2004; 27: 84-87. http://dx.doi.org/10.2337/diacare.27.10.2540
11. Evans J, Newton R, Ruta D, Macdonald T, Stevenson R, Morris A. Frequency of blood glucose monitoring in relation to glycemic control. BMJ 1999; 319: 83-86. http://dx.doi.org/10.1136/bmj.319.7202.83 PMid:10398627 PMCid:28155
12. Little RR. Recent progress in HbA1c testing. Diabetes Care 2000; 23: 265-6. [PMID: 10868848] http://dx.doi.org/10.2337/diacare.23.3.265 PMid:10868848
13. Matz R. The target for good glycemic control should be an HbA›C concentration of less than 0.07. West J Med 2000; 173: 179-80 http://dx.doi.org/10.1136/ewjm.173.3.179PMid:10986180 PMCid:1071058
14. Tahara, Y. and Shima, K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care. 1995;18(4): 440-447. http:// dx.doi.org/10.2337/diacare.18.4.440 PMid:7497851
15. Takahashi, S., et al. Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. Endocrinology Journal. 2007;1507: 6-103.
16. World Health Organization 1999 Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. Geneva, World Health Organization.
17. Kouzuma T, Usami T, Yamakoshi M, et al. An enzymatic method for the measurement of glycated albumin in biological samples. Clin Chim Acta 2004; 324: 61-71. http://dx.doi.org/10.1016/S0009-8981(02)00207-3
18. Kouzuma T. Study of glycated amino acid elimination for an improved enzymatic glycated albumin measurement method. Clin Chim Acta 2004; 346: 135-143 http://dx.doi.org/10.1016/j.cccn.2004.02.019 PMid:15256314
19. Cohen, M.P., Nonenzymatic glycation: a central me-chanism in diabetic microvasculopathy., J Diabetes Compli-cations. 1998; 2:214–217. http://dx.doi.org/10.1016/S0891-6632(88)80012-6
20. Higai, K., Shimamura, A., and Matsumoto, K., Ama-dori-modified glycated albumin predominantly induces E-selectin expression on human umbilical vein endothelial cells through NADPH oxidase activation., Clin. Chim. Acta., 2006; 367: 137–143. http:/ /dx.doi.org/10.1016/j.cca.2005.12.008 PMid:16455066
21. Rohlfing, C.L, Wiedmeyer, H.M., England, J.D., Tennill, A., and Goldstien, D.E., Defining the relationship between plasma glucose and HbA1c: analysis of glucose profile and HbA1c in diabetes control and complication trial., Diabetes. Care. 2002; 25: 275-278. http://dx.doi.org/ 10.2337/diacare.25.2.275 PMid:11815495
22. Dolhofer, R., and Wieland, O.H., Increased glycosyla-tion of serum albumin in vivo. J. Biol. Chem., 1980; 261, 13542-13545.
23. Tahara, Y., and Shirma, K., Kinetics of HbA1c, gly-cated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level., Diabetes. Care., 1995;18: 440-447. http:// dx.doi.org/10.2337/diacare.18.4.440 PMid:7497851
24. Okumura, A., et al. Glycated albumin induces activation of activator protein-1 in retinal glial cells. Journal of Ophthalmology. 2007; 51: 231–243.

Source of support: Nil, Conflict of interest: None Declared