Original Research Article

Assessment of glycated haemoglobin, total protein and albumin levels in patients with type 2 diabetes mellitus visiting NAUTH, Nnewi

R A Analike[1,*], A C Ihim[2], S C Iweanya[2], E C Ogbodo[2], C E Onah[3], A L Asomugha[4], A K Amah[5], P C Obi[6], E I Nwobodo[7], C O Timothy[8]

[1]Dept. of Chemical Pathology, Nnamdi Azikiwe University, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi, Anambra, Nigeria
[2]Dept. of Medical Laboratory Science, Faculty of Health Sciences Technology, Nnamdi Azikiwe University, Nnewi, Anambra, Nigeria
[3]Dept. of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra, Nigeria
[4]Dept. of Human Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi, Anambra, Nigeria
[5]Dept. of Physiology, College of Medicine, Imo State University, Owerri, Imo, Nigeria
[6]Dept. of Internal Medicine, Federal Medical Centre, Owerri, Anambra, Nigeria
[7]Dept. of Biochemistry, Chukwuemeka Odimegwu Ojukwu University, Uli, Anambra, Nigeria
[8]Dept. of Human Physiology, College of Medicine, Abia State University, Uturu, Abia, Nigeria

A R T I C L E   I N F O

A B S T R A C T

This is a case-control study designed to assess the glycated haemoglobin, total protein and albumin levels in patients with type 2 diabetes mellitus visiting NAUTH, Nnewi. A total of 114 subjects comprising of 57 diabetic subjects and 57 controls aged between 40 and 73 years were recruited for the study. The patients and controls were aged and sex matched. Subsequently, structured questionnaire were used to obtain patients' biodata and thereafter, 5mls of blood sample was collected from each patients and 1ml was dispensed into EDTA for the estimation of glycated haemoglobin, and 4ml was dispensed into plain containers for the determination of serum albumin and total protein levels using standard laboratory methods. HbA1c, serum albumin and total protein levels were assayed by using immunoturbidimetric method, Bromo Cresol green Method and Biuret Method respectively. The result revealed that the mean level of HbA1c was significantly higher in the diabetic subjects when compared with the non diabetic subjects (9.62 ± 1.27 Vs 5.58 ± 0.62; $p=<0.01$). However, the mean serum of levels of Albumin and total protein did not differ significantly when compared between the diabetic patients and control subjects ($p>0.05$). This finding implies that there was a poor glycemic control in the diabetic subjects studied. Therefore, there is need for better management of diabetic patients through medication and use of diet and exercise.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease which results from diminished or absent secretion of insulin or even by reduced tissue sensitivity to insulin (Report of WHO Consultation, 1999;[1] International Diabetes Federation, 2015; American Diabetes Association, 2016).[2] Diabetes is a global endemic with rapidly increasing prevalence in developing countries such as Nigeria. Type 2 diabetes mellitus is one of the leading causes of preventable death in the world, with stroke, myocardial infarction and other cardiovascular diseases being the most common causes of death for adults with diabetes (Newman *et al.*, 2017).[3] A number of factors including less glycemic control, smoking, high blood pressure, elevated cholesterol levels, obesity, and lack of regular exercise are considered to be risk factors that accelerate the deleterious effects of

* Corresponding author.
E-mail address: rose_analyke@yahoo.com (R. A. Analike).

diabetes (Elfaki *et al.*, 2014).[4] According to International Diabetes Federation (IDF), in 2017, approximately 425 million adults were living with diabetes and it is estimated to affect up to 629 million people by the year 2045 (IDF, 2017).[5] Diabetes Mellitus has become a major public health problem in Nigeria accounting for a prevalence of 2.4% with total number of mortality amounting to 3028 deaths in 2017 (IDF, 2017).[5] In the world, WHO estimates that, globally, 422 million adults aged over 18 years were living with diabetes in 2014 accounting for a prevalence of 8.5% among the adult population (WHO, 2016).[6] Therefore, this continued increase in the prevalence of diabetes globally has become a matter of great concern, and hence, the management of diabetic complications is particularly important. (Yang *et al.*, 2010; Danaei *et al.*, 2011; Soriguer *et al.*, 2012).[7–9]

In individuals diagnosed of diabetes mellitus, glucose monitoring is essential for glycemic control (Yoon *et al.*, 2015). Also, in diabetes mellitus, compared with non-diabetes, glycation of various proteins is known to be increased, and some of these glycated proteins are thought to be involved in the onset and progression of chronic diabetic complications (Cohen, 1998).[10] Currently, the laboratory tests used to diagnose Diabetes Mellitus are glycated hemoglobin (A1C), fasting plasma glucose (FG) and two-hour plasma glucose (2hG) after a 75g oral glucose tolerance test (OGTT) (Sacks *et al.*, 2011; ADA, 2016).[11] Of the glycemic indices, the American Diabetes Association recommends glycated hemoglobin (HbA1 c) testing in all diabetic patients as an initial assessment and then as a part of continuing care (ADA, 2014).[12]

This recommendation is derived from clinical data that shows that HbA1 c reflects average glycemicstatus over 2-3 months and predicts diabetic complications (Lee *et al.*, 2013).[13]

Albumin is one of the most abundant plasma proteins (Tiwari *et al.*, 2015).[14] The glycation of albumin to form glycated albumin (GA) is ten times more than the glycation of hemoglobin in type 2 DM (Tahara *et al.*, 1995).[15] GA is a marker reflects a short-term glycemic control (Yoon *et al.*, 2015).[16] One advantage of utilizing serum albumin as a measure of glycemic control is its shorter half-life of 21 days, which renders its serum concentration more sensitive to recent change in average blood glucose level than HbA1C (Guerin-Dubourg *et al.*, 2012).[17] Studies have shown decreased albumin levels associated with increased HbA1c and total protein (Shalbha *et al.*, 2015; Malawadi and Adiga, 2016; Nazki *et al.*, 2017).[18,19] Therefore, the present study evaluated the glycated haemoglobin, total protein and albumin levels in patients with type 2 diabetes mellitus visiting NAUTH, Nnewi.

2. Materials and Methods

2.1. Study Site

This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi.

2.2. Study design

The present study is a case control study aimed at evaluating the glycated haemoglobin, total protein and albumin levels in patients with type 2 diabetes mellitus visiting NAUTH, Nnewi. The protocol was explained to the subjects and those who gave their informed consent were recruited for the study. A total of 114 subjects comprising of 57 diabetic subjects and 57 controls aged between 40 and 73 years were recruited for the study. The patients and controls were aged and sex matched. Subsequently, structured questionnaire were used to obtain patients' biodata and thereafter, 5mls of blood sample was collected from each patients and 1ml was dispensed into EDTA for the estimation of glycated haemoglobin, and 4ml was dispensed into plain containers for estimation of serum albumin and total protein levels.

2.3. Inclusion and exclusion criteria

Known diabetic subjects aged between 40 and 73 years were recruited for the study, whereas those younger than 40 or older than 73years and non-diabetic subjects were excluded from the study.

2.4. Ethical consideration

The ethical clearance for this study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi.

2.5. Determination of glycated haemoglobin level

Glycated Haemoglobin level was determined using immunoturbidimetric method as described by Wolf *et al.*, (1984).[20]

2.6. Estimation of serum albumin level

Serum albumin level was estimated Bromo Cresol green Method as described by Doumas *et al.*, (1971).[21]

2.7. Estimation of total protein

Estimation of serum total protein level was done using Biuret Method according to Weichselbaum, (1946).[22]

2.8. Statistical analysis

The data were presented as mean±SD and the mean values of the control and test group were compared by Students t-test and pearson correlation using Statistical package for

social sciences (SPSS) (Version 20) software. Statistical significance was tested at P<0.05.

3. Results

The mean level of HbA1c was significantly higher in the diabetic subjects when compared with control group (9.71±1.30Vs 5.58±0.65; p=0.000). There was no significant differences observed between the age, the serum levels of Albumin and Total protein in the test and control subjects (p>0.05).Table 1

Table 1: Levels of HbA1c, total protein and albumin in diabetic and control subjects

Parameters	Control	Diabetic subject	t-test	p-value
Age(years)	57.69±8.89	57.51±8.85	-0.082	0.935
HbA1c(%)	5.58±0.65	9.71±1.30	1.712	0.000*
Protein(g/L)	74.25±3.80	72.86±3.36	1.649	0.104
Albumin(g/L)	38.23±3.35	38.38±3.24	0.191	0.849

*Statistically significant at P<0.05.

Table 2 shows that there is no significant correlation between age, HbA1c, total protein and albumin in diabetic subjects.

Table 2: Correlation of HbA1c with age, total protein and albumin in diabetic subjects

Parameters	R	p-value
HbA1c Vs age	0.078	0.647
HbA1c Vs Total protein	0.096	0.571
HbA1c Vs Albumin	-0.162	0.338
Age Vs Total protein	-0.044	0.797
Age Vs Albumin	0.085	0.615
Total protein Vs Albumin	-0.007	0.966

*Statistically significant at P<0.05.

4. Discussion

In this study, the mean level of HbA1c was significantly higher in the diabetic subjects than in control. This is in consonance with the report of some previous similar studies (Shalbha *et al.*, 2015; Malawadi and Adiga, 2016; Nazki *et al.*, 2017). This increase can be attributed to hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism that results from abnormalities in insulin secretion, insulin action or even both (IDF, 2015; ADA, 2016).[5,23] This finding implies that there is a poor glycemic control in the diabetic subjects under study. Furthermore, our finding shows a higher mean value of HbA1c (9.71±1.30) than the recommended cut-

point (<7%) in diabetic patients (ADA, 2010). It follows therefore, that these patients may be at greater risk of long-term complications due to diabetes if the glycemic level is not properly controlled and this call for concern in the management of diabetic patients.

However, there was no significant difference between the mean serum level of Albumin in the test subjects when compared with control subjects (p>0.05). This is in line with the report of previous studies (Malawadi and Adiga, 2016). This may be as a result of Insulin resistance which is a principal cause of type 2 diabetes (Kahn, 1994)[24] and previously, serum albumin has been associated with insulin resistance (Hostmark *et al.*, 2005; Ishizaka *et al.*, 2007).[25,26] In diabetic patients, plasma albumin concentration has been reported to be inversely related with HbA1c levels, revealing a large proportion of poorly controlled diabetes in patients with lower plasma albumin concentrations (Rodriguez-Segade *et al.*, 2005; Hemangi *et al.*, 2012).[27,28] This inverse relationship may also be explained by the fact that poorly controlled type 2 diabetes has been associated with a further decrease in insulin production and secretion by the pancreatic β-cell (Marshak *et al.*, 1999; Kahn, 2003).[29,30]

Furthermore, our finding shows no significant difference between the serum levels of total protein in the diabetic patients and control subjects (p>0.05). This is in contrast with the findings of (Malawadi and Adiga, 2016; Nazki *et al.*, 2017).[18,19]

There is no significant correlation between age, HbA1c, total protein and albumin in diabetic subjects. This finding is not in agreement with the finding of Hemangi *et al.*, (2012)[27] in which plasma albumin levels were negatively correlated with HbA1c and low albumin levels was associated with increased plasma protein glycation and that albumin competes for glycation with other plasma proteins in diabetes.

5. Conclusion

In conclusion, the present study showed significantly higher mean levels of HbA1c in the diabetic patients compared with the control subjects. However, the mean serum of levels of Albumin and total protein did not differ significantly when compared between the diabetic patients and controls. This finding implies that there was a poor glycemic control in the diabetic subjects studied. Therefore, there is need for better management of diabetic patients through medication and use of diet and exercise.

6. Source of funding

None.

7. Conflict of interest

None.

703

References

1. World Health Organization, Definition, Diagnosis and Classification of Diabetes Mellitus and its complications. Geneva: WHO ; 1999,. Report of a WHO Consultation.
2. American Diabetes Association. Standards of Medical Care in Diabetes - 2016. *Diabetes Care*. 2016;39(Supplement1):1–112.
3. Newman JD, Schwartzbard AZ, Weintraub H. Primary prevention of cardiovascular disease in diabetes mellitus. *J Am College Cardiol*. 2017;70:883–893.
4. Raheem ME, Ahmed AM, E. Evaluation of Lipid Metabolism among Sudanese Patients with Type 2 Diabetes Mellitus. *Int J Pure Appl Sci Technol*. 2014;23(1):28–33.
5. International Diabetes Federation: IDF Diabetes Atlas ; 2017,. : International Diabetes Federation.
6. Global burden of diabetes: part 1; Geneva: World Health Organization ; 2016,. Available from: https://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf;jsessionid=BA98FB532201E9B110409C3652AA18EC?sequence=1,.
7. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2?7 million participants. *Lancet*. 2011;378:31–40.
8. Soriguer F, Goday A, Bosch-Comas A, Bordiu E, Calle-Pascual A. Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Diabetes Study. *Diabetologia*. 2012;55:88–93.
9. Yang SH, Dou KF, Song WJ. Prevalence of diabetes among men and women in China. *New Engl J Med*. 2010;362:2425–2426.
10. Nonenzymatic glycation: a central mechanism in diabetic microvasculopathy. *J Diabetes Complications*. 1998;2:214–217.
11. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, Mcdonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem*. 2011;57(6):1–47.
12. Standards of medical care in diabetes-2014. *Diabetes Care*. 2014;37:14–80. supplement 1.
13. Lee EJ, Kim YJ, Kim T. A1c variability can predict coronary artery disease in patients with type 2 diabetes with mean A1c levels greater than 7. *Endocrinol Metabol*. 2013;28(2):125–132.
14. Tiwari S, Bothale M, Hasan I, Kulkarni M, Sayyad M, Basu A. Association between serum albumin and glycated hemoglobin in Asian Indian subjects. *Indian J Endocrinol Metabol*. 2015;19(1):52–55.
15. Tahara Y, Shima K. Kinetics of HbA1c, Glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care*. 1995;18:440–447.
16. Yoon H, Lee Y, Kim K, Kim S, Kang E, Cha B. Glycated Albumin Levels in Patients with Type 2 Diabetes Increase Relative to HbA1C with Time. *BioMed Res Int*. 2015;p. 576306–576306.
17. Guerin-Dubourg A, Catan A, Bourdon E, Rondeau P. Structural modifications of human albumin in diabetes. *Diabetes Metabol*. 2012;38(2):171–178.
18. Malawadi BN, Adiga U. Plasma proteins in Type 2DM. *IOSR J Biotechnol Biochem*. 2016;2(5):1–03.
19. Nazki FA, Syyeda A, Mohammed S. Total proteins, albumin and HBA1c in type 2 diabetes mellitus. *Medpulse Int J Biochem*. 2017;3(3):40–42.
20. Wolf HU, Lang W, Zander R. Alkaline haematin D-575, a new tool for the determination of haemoglobin as an alternative to the cyanhaemiglobin method. II. Standardization of the method using pure chlorohaemin. *Clinica Chimica Acta*. 1984;136:95–104.
21. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*. 1971;31:87–96.
22. Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am J Clin Pathol*. 1946;10:40–49.
23. International Diabetes Federation: IDF Diabetes Atlas. 7th ed. Belgium: International Diabetes Federation ; 2015,.
24. Kahn CR, Lecture B. Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes*. 1994;43:1066–1084.
25. Hostmark AT, Tomten SE, Berg JE. Serum albumin and blood pressure: a population-based, cross-sectional study. *J Hypertens*. 2005;23:725–730.
26. Ishizaka N, Ishizaka Y, Nagai R, Toda E, Hashimoto H, Yamakado M. Association between serum albumin, carotid atherosclerosis, and metabolic syndrome in Japanese individuals. *Atheroscler*. 2007;193:373–379.
27. Hemangi SB, Arvind MK, Sachin SK, Sandeep BG, Ashok DC, et al. Low Plasma Albumin Levels Are Associated with Increased Plasma Protein Glycation and HbA1c in Diabetes. *J Proteome Res*. 2012;11(2):1391–1396.
28. Rodriguez-Segade S, Rodriguez J, Mayan D, Camina F. Plasma albumin concentration is a predictor of HbA1c among type 2 diabetic patients, independently of fasting plasma glucose and fructosamine. *Diabetes Care*. 2005;28:437–439.
29. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetol*. 2003;46:3–19.
30. Marshak S, Leibowitz G, Bertuzzi F, Socci C, Kaiser N, et al. Impaired beta-cell functions induced by chronic exposure of cultured human pancreatic islets to high glucose. *Diabetes*. 1999;48:1230–1236.