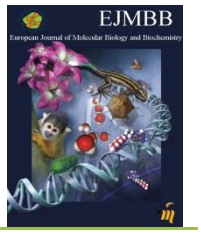
e - ISSN - 2348-2206
Print ISSN - 2348-2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



SERUM N-ACETYL-BETA-GLUCOSAMINIDASE LEVEL IN DIABETES MELLITUS

Davina Hijam*, Sonam Tashi Dolma, Wahengbam Diana Devi, Prabita Oinam

Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur.

Article Info

Received 23/01/2020
Revised 16/05/2020
Accepted 19/06/2020

Key words:- Diabetes, N-Acetyl β-Glucosaminidase, Blood sugar, Complication.

ABSTRACT

Lysosomal enzyme beta-n-acetyl-glucosaminidase (NAG) show an increase in diabetes mellitus and this is closely related to diabetic metabolic alterations. To estimate serum NAG levels in diabetic patients (with and without complications) and to compare the levels with that of controls. This was a cross- sectional study conducted on 50 diabetic subjects (Group A - 25 patients without complications and Group B - 25 patients with complications) in RIMS hospital during the period from September 2016 to August 2018. Twenty five age and sex matched healthy individual were taken as controls. Glycosylated haemoglobin (HbA1c) was estimated by Fast Ion Exchange Resin Method. Serum NAG was estimated by Sandwich ELISA. The difference in the level of fasting blood sugar (FBS), postprandial blood sugar (PPBS) and glycosylated haemoglobin (HbA1c) between control and diabetic groups (A and B) was statistically significant ($P<0.001$) indicating a poor control of diabetes in the study group. There was a significant difference in the level of NAG in control, group A and group B (11±2.9, 26.36±5.41 and74.87±24.55)U/L respectively. The comparison of serum NAG in male and female subjects in the study groups was insignificant. Statistically significant increase in serum NAG level was observed in group B compared to group A ($p <0.001$). There was a significant positive correlation of serum NAG with FBS, PPBS and HbA1c. Serum NAG can be used as an index for the development and prognosis of diabetic complications.

INTRODUCTION

Diabetes Mellitus (DM) is a disorder characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both andits effects include long-term damage, dysfunction and failure of various organs [1].WHO estimates that diabetes was the seventh leading cause of death in 2016 and an estimated 1.6 million deaths were directly caused by diabetes [2].

Lysosomal enzymes beta-n-acetyl-glucos aminidase (NAG) show change in diabetes mellitus. The increase in these enzymes is closely related to diabetic metabolic alterations. Knowledge of the pathogenesis of the vascular complications that accompany diabetes is important for the early detection, rational treatment and prevention of deterioration [3].

Diabetic nephropathy is perhaps the most serious complication, and is known to increase morbidity and mortality rates in diabetic patients. These rates could be lowered by the early detection and monitoring of the progression of diabetic complications using the techniques of enzymology, which are simple and inexpensive. For several years, many studies demonstrated that excreted urinary enzymes are useful biomarkers for evaluation and diagnosis of tubular dysfunction or injury, especially n-acetyl-beta-glucosaminidase (NAG) [4-6]. NAG is a lysosomalenzyme widely distributed in human tissue. This enzyme is mainly present in the proximal convulated tubule [7]. Urinary NAG assays have been used as a marker of early diabetic nephropathy. Many clinical studies have demonstrated positive correlation with the

Corresponding Author

Davina Hijam
Email: - davina_hijam@yahoo.co.in

development of diabetic complications like microangiopathy, retinopathy, nephropathy and increased urinary NAG level.

NAG is capable of degrading mucopolys accharides and glycoprotein compounds that accumulate in the walls of blood vessels in diabetics with vasculopathy[8].It has been postulated that the increased activity of NAG in diabetics could be the manifestation of an activation of lysosomes in tissues and its activity is correlated to the degree of clinical symptoms of diabetic vascular changes. Thus it may serve as an index differentiating the diabetic microangiopathy from other forms of microvessels.

On the basis of these considerations, the study was conducted to see the activities of serum NAG in diabetes with or without complicaionsin Manipur where incidence of diabetes is very high and as no such study has been carried out before. To estimate serum NAG levels in diabetic patients (with and without complications) and to compare the levels with that of controls.

MATERIALS AND METHODS

This was a Cross-sectional study carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, India during the period from September 2016 to August 2018.

The study population consists of 50 type 2 diabetes mellitus patients(Group A - 25 patients without complications and Group B - 25 patients with complications:microvascular and macrovascular) greater than 18 years of age, irrespective of sex, caste and creed, and willing to participate from different areas of Manipur and attending Diabetic Clinic or admitted in the Medical ward of RIMS, Imphal were included in the study group. Another 25 age and sex matched healthy individuals free from any systemic disease were taken as controls.

Exclusion Criteria

Individuals suffering from carcinoma, chronic diseases like, rheumatoid arthritis, COPD, coronary artery disease, active MI, sepsis, burn, viral hepatitis, hyperparathyroidism, diseases of blood cells, such as sickle cell disease or multiple myeloma renal disease like nephrotic syndrome, pyelonephritis, glomerulonephritis, and renal transplantation are excluded from participating in the study.

All the selected patients gave voluntary consent before start of the study. The study was done after obtaining the approval from Institutional Ethical Committee of RIMS.

A detailed history of the patients name, age, sex, duration of disease, age of onset of disease, presence of hypertension, associated symptoms like polyuria, chest pain, palpitation etc. was taken. Personal history of smoking, consumption of alcohol, presence or absence of obesity and family history of diabetes, hypertension or coronary heart disease were also recorded.

Weight, height, body mass index (BMI) was measured once at the beginning of the study. BMI was calculated as weight (kg) divided by the square of the height (m^2).

Nephropathy was defined as proteinuria >1+ on dipstick with no abnormal findings on urinary examination [9]. Neuropathy (peripheral) was defined as absent touch or vibratory sensations of the feet [10]. Retinopathy was defined by presence of varying degrees ofmicroaneurysms, haemorrhages, exudates, venous changes, new vessel formation, and retinal thickening [11]. Patients with a history of CAD and stroke were taken as having macrovascular complication.

Four ml of fasting blood was collected by venipuncture from each patient and control. The blood sample collected in the plain vial was centrifuged immediately. All the tests was carried out on the same day. The chemicals and reagents used in the study were of analytical grades.

Fasting Blood glucose was estimated by GOD PAP Method [12] using Liquicolor Kit manufactured by HUMAN, Germany.HbA1c was estimated by Fast Ion Exchange Resin Separation Method as described by Goldstein DE, Little RR & Widdmayer HM [13] by using Glycosylated Haemoglobin Test Kit manufactured by HUMAN WIESBADEN, Germany.

Serum N-Acetyl Beta Glucosaminidase was estimated by sandwich enzyme immunoassay using ELISA Kit manufactured by Uscn Life Science Inc. Wuhan [14].
Detection Range:- 1.56 -100 U/L. **Sensitivity:-** The minimum detectable dose of human NAGase is typically less than 1 U/L. **Specificity** This assay has high sensitivity and excellent specificity for detection of human NAGase.
Statistics: Statistical analysis was performed using SPSS version 17. Statistical tests like χ^2-test, independent t-test, ANOVA (F-test) and correlation coefficient 'r' were applied whenever found suitable and necessary. The P-value less than 0.05 is treated as significant.

RESULTS

It was observed that the number of females was more than that of males in both the control group (60%) and group A (52%) which is quite in contrast to group B where the number of males (76%) is more than that of females (24%). Majority of group A (32%) belong to age group of 50 to 59 years and 60 to 69 years whereas in group B, majority (36%) cases were in the age group of 60-69 years. Age expressed as mean ± SD in male and female controls were 65.2±8.44yrs, 56.27±5.93yrs and in group A and group B were 58 ± 9.4 yrs, 57.62±13.5 yrs and63.68±9.98 yrs, 59.17±9.02 respectively, but these difference are not statistically significant ($p>0.05$), indicating that both groups are of comparable age.

Table I shows that the difference in the level of FBS, PPBS and glycosylated haemoglobin between control and group A and control and group B were statistically

significant (P<0.001) indicating a poor control of diabetes in the study group.

The NAG level (expressed in mean±SD) in control and group A were 11±2.9 and 26.36±5.41 U/L. The highest level was found in group B(74.87±24.55 U/L). A statistically significant difference is detected in NAG levels between control and group A (P<0.001) as well as groupB (P<0.001).

Table II shows that the comparison of serum NAG level (expressed as mean±SD) in male and female subjects in both the study groups and control group was insignificant.

Table III shows that 46% diabetic cases have serum NAG level in the range 25 – 44.99 U/L, 30% cases have NAG level > 85U/L and 18% diabetic cases haveNAG level < 25U/L.

Table IV shows that there is significant increase of FBS, PPBS and HbA1c (P<0.001) and statistically significant increase in serum NAG level in group B compared to group A (P <0.001).

Table V shows the relationship between the parameters considered and NAG in all the three groups. In group A and group B, correlations of age, duration of disease are statistically insignificant but there is a significant positive correlation of NAG with fasting blood sugar, postprandial blood sugar and glycosylated haemoglobin.

Table 1. Summary of biochemical data in controls, group A and group B, (values expressed in terms of Mean ± SD)

Parameters	Control (n=25) Mean ± SD	Group A (n=25) Mean ± SD	P-Value	Group B (n=25) Mean ± SD	P-Value
Fasting BS (mg/dl)	89.12±6.24	126.68±15.70[a***]	<0.001	166.44±7.99[b***]	<0.001
Postprandial BS (mg/dl)	124.16±7.11	203.6±37.64[a***]	<0.001	271.12±26.16 [b***]	<0.001
HbA1c(%)	4.78±0.27	9.57±1.34 [a***]	<0.001	12.71±0.99 [b***]	<0.001
NAG (U/L)	11±2.9	26.36±5.41 [a***]	<0.001	74.87±24.55 [b***]	<0.001

a: comparison between control and group A
b: comparison between control and group B
*** P value <0.001

Table 2. Mean ± SD of Serum NAG level categorized on the basis of sex / gender

Study Subjects	**Sex**	Number(%)	**SerumNAGlevel (U/L)**	**P-Value**
Control (n=25)	Male	10(40)	10.68±2.87	0.661
	Female	15(60)	11.22±3.01	
Group A (n=25)	Male	12(48)	26.86±5.21	0.676
	Female	13(52)	25.92±5.78	
Group B (n=25)	Male	19(76)	75.09±23.71	0.937
	Female	06(24)	74.16±29.48	

Table 3. Distribution of diabetic cases by serum NAG levels

Serum NAG (U/L)	No. of diabetic cases	Percentage (%)	NAG Level(Mean ± SD)
<25	9	18%	20.4 ± 3
25-44.99	23	46%	32.5 ± 5.1
45-64.99	2	4%	64.3 ± 0
65-84.99	1	2%	77.8 ± 0
>85	15	30%	92.9 ± 4.5

Table 4. Comparison of serum FBS, PPBS, HbA1c and NAG level in group A and group B

Parameter mean ± SD	Group A (n=25)	Group B(n=50)	P value
FBS (mg/dl)	126.68±15.70	166.44±7.99	< 0.001
Postprandial BS (mg/dl)	203.6±37.64	271.12±26.16	<0.001
HbA1c(%)	9.57±1.34	12.71±0.99	< 0.001
Serum NAG (U/L)	26.36±5.41	74.87±24.55	< 0.001

Table 5. Correlation of serum NAG with age, diabetes duration, FBS, PPBS and HbA1c

Parameter	Control(n=25)		Group A(n=25)		Group B(n=25)	
	Correlation coefficient 'r'	P-value	Correlation coefficient 'r'	P-value	Correlation Coefficient 'r'	P-value
Age (yr)	-0.198	0.343	0.229	0.272	0.115	0.460
Diabetes duration(yr)	----	-----	-0.048	0.820	-0.065	0.759
Fasting BS(mg/dl)	-0.020	0.924	0.670**	<0.001	0.407*	0.043
Postprandial BS(mg/dl)	-0.338	0.098	0.536**	0.005	0.489*	0.013
HbA1c (%)	0.192	0.358	0.673**	<0.001	0.618**	0.001

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

DISCUSSION

This study was conducted to estimate serum NAG in diabetes without complications and with complications.

In the present study, 52% of diabetics were female and 48% were males. This finding is consistent with the statement that type 2 diabetes mellitus is more common in women [15]. Seventy six percent of diabetes with complication were males and 24% were females. This is consistent with the findings of **HoY**[16] who reported the occurrence of diabetes mellitus more in males (0.95%) than in females (0.20%).

In the present study, the mean FBS levels in diabetes without complication and diabetes with complication were 126.68±15.70mg/dland 166.44±7.99 mg/dl as shown in Table I. These differences were statistically significant (P<0.001) and consistent with the reports given by **Verma M *et al.,*** [17] and **Akinloye OA *et al.,*** [18].

In this study, there was a significant difference of blood glucose control as indicated by mean HbA1c among the control and both the diabetic groups (Table I). HbA1c was increased in diabetics without complication as compared to controls, and further increase in HbA1c was observed in diabetic patients with complication when compared to controls, which is a sign of poor glycemic status as described by **Selvin E *et al.,*** [19] and **Nakamura K *et al.,*.**[20] In diabetic patients, concentration of HbA1c is elevated as much as two fold and decreases with improvement of glycemic control.

In the present study, the NAG level in controls, diabetes without and with complication group were 11±2.9 and 26.36±5.41 and 74.87±24.55 U/L respectively (Table I). The highest level was found in diabetes with complication. There was a statistically significant increase in serum NAG level in diabetics without complication when compared to control group (P<0.001). This finding is consistent with the finding of **Poon PY *et al.,*** [21] and **Pitkanen P *et al.,*** [22] who found an increased NAG activity in NIDDM patients whereas the study was contradictory to the findings of **Perdichizzi G *et al.,*** [23] who found that there was no significant difference in serum NAG activity in NIDDM patients.

When the control group was compared to diabetes with complication group, a statistically significant increase in serum NAG level in diabetes with complication group was found (Table I), consistent with the findings of Deyuan Z *et al.,* in diabetic nephropathy [24] and **Teruo IA *et al.,*** [25] who found that the activity of serum NAG in NIDDM patients with various secondary complications was statistically significantly increased compared to the healthy individuals. Another study conducted by Edimar CP *et al.,* also found significantly higher NAG levels in diabetes mellitus patients with coronary artery disease (CAD) compared to those without CAD [26].

In the present study, the comparison of serum NAG level in male and female subjects in both the study groups and control group was insignificant (Table II).

When the diabetes without complication was compared to diabetes with complication group, a statistically significant increase in serum NAG level in diabetes with complication group was found (Table IV). This finding is in agreement to the findings of Clemenzia G *et al.,*[27] who reported that the total NAG activity was increased in the serum of diabetic patients with vascular complications, less so in diabetics without vascular complications. Additionally, Kuniko Y also reported that serum NAG activity was an independent and significant predictor of all-cause death [28]. The increased NAG activity in serum was due to the alteration in the rate of release or the rate of destruction of the enzyme. The finding was contradictory to the findings of Waters PJ *et al.,* [29] who found no difference in the activity of NAG between diabetes with or without microangiopathy. Increase levels of NAG results from exocytosis in hyperglycemia leading to the release of these enzymes in the serum from the cells. NAG is capable of degrading mucopolysaccharides and glycoprotiens that accumulate in the walls of blood vessels in diabetics with vasculopathy. Thus changes in NAG enzymes have been considered as an expression of a biochemical mechanism to protect blood vessels against excessive deposition of glycoproteins and mucopolysacchrides.

There was a significant positive correlation of NAG with fasting blood sugar and postprandial blood sugar in both the study groups (p<0.001)(table V). This finding is in agreement to the findings of **Brouhard BH *et al.,***[30] and **Ikenaga H *et al.,*** [31] who found excellent correlation between the activity of NAG and the content of

glucose in blood. There was also a significant positive correlation of NAG with glycosylated haemoglobin in both the study groups ($p<0.001$). This finding is in agreement to that of **Brouhard BH *et al.*,**[30] but contradictory to the findings of **Miralles JM *et al.*,**[32] who did not find any relationship between the activity of NAG and glycosylated haemoglobin. Therefore, NAG activity in diabetics appeared related to the degree of glycometabolic control, hyperglycaemia and to the presence of diabetes complications.

CONCLUSION

On the basis of all the results, it can be concluded that the increased activity of serum NAG was the consequence of poor metabolic control and increased exocytosis resulting from hyperglycaemia. Serum NAG may be used as an index for the development and prognosis of diabetic complications. Serum NAG levels along with HbA1c may give earlier and sensitive indication of the success achieved by the patient in controlling their diabetic condition.

DECLARATION OF INTEREST

There was no conflict of interest.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the patients, laboratory staffs and technicians for their participation, help and co-operation in this study.

REFERENCES

1. World Health Organization. *Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus.* Available at: http://www.who.int/iris/handle/10665/66040. Accessed on July 2, 2018.
2. World Health Organization. Key facts, *Diabetes. Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes.* Accessed on December 18, 2019.
3. Girach A, Manner D, Porta M. (2006). Diabetic microvascular complications: can patients at risk be identified? A review. Int J Clin Pract, 60, 1471-83.
4. Uslu S, Efe B, Alatas O. (2005). Serum cystatin C and urinary enzymes as screening markers of renal dysfunction in diabetic patients. J Nephrol, 18, 559-67.
5. Basturk T, Altuntas Y, Kurklu A, Ayidin L, Eren N, Unsal A. (2006). Urinary N-acetyl-beta glucosaminidase as an earlier marker of diabetic nephropathy and influence of low-dose perindopril / indapamide combination. Ren Fail, 28, 1125-8.
6. Jung K, Pergande M, Schimke E, Ratzmann KP, Ilius A. (1988). Urinary enzymes and low-molecular-mass proteins as indicators of diabetic nephropathy. Clin Chem, 34, 544-7.
7. Gatsing D, Garba IH, Godwin I, Adoga GI. (2006).The use of lysosomalenzymuria in the early detection and monitoring of the progression of diabetic nephropathy. *Indian J of Clin Biochem*, 21(2), 42-8.
8. Mahadevan S, Dillard CJ, Tappel AL. (1969). Degradation of polysaccharides and glycoproteins by lysosomalglycosidases. *Arch Biochem Biophys*, 129(2), 525-33.
9. Powers AC. Diabetes mellitus. In: Jameson JL, Longo DL, Braunwald E, Hauser SL, Kasper DL, Fauci AS editors. (2012). Harrison's principles of internal medicine 18th ed. USA: MC Graw Hill Companies, 2969-71.
10. Valk GD, Nanta JJ, Strijers RL, Bertelsmann FW. (1992). Clinical examination versus neurophysiological examination in the diagnosis of diabetic polyneuropathy. *Diabet Med*, 9, 716-21.
11. Basit A, Hydie ZI, Hakeem R, Ahmedani M, Waseem M. (2005). Glycemic control, hypertension and chronic complications in type 2 diabetic subjects attending a tertiary care centre. *J Ayub Med Coll Abbottabad*, 17(2), 63-8.
12. Barham D, Trinder P. (1972). GOD-PAP enzymatic colorimetric method of glucose estimation without deproteination. *Analyst*, 97, 312-22.
13. Goldstein DE, Little RR, Widdmayer HM. (1994). Glycated haemoglobin estimation and review of assay methods and clinical interpretation. *Diabetes Ann*, 193-212.
14. Porstmann T, Kiessig ST. (1992). Enzyme immunoassay techniques an overview. *J Immunol Methods*, 150(1-2), 5-21.
15. American Diabetes Association. (2004). Diagnosis and classification of diabetes mellitus. Diabetes Care, 27(Suppl 1), S5-S10.
16. Ho Y. (1959). The problem of diabetes in Singapore population and use of chlorpropamide in its management. *Proc Alum Assoc Mal*, 12, 84.
17. Verma M, Paneri S, Badi P, Raman PG. (2006). Effect of increasing duration of diabetes mellitus type 2 on glycated haemoglobin and insulin sensitivity. *Ind J Clin Biochem*, 21(1), 142-6.
18. Akinloye OA, Akinlade KS, Odetola AA, Raji AA. (2007). Relationship between fasting plasma glucose and glycated haemoglobin in adult diabetic Nigerians. *Afr J Biomed Res*, 10, 127-32.
19. Selvin E, Wattanakit K, Steffens MW, Coresh J, Sharret R. (2006). HbA1c and peripheral arterial disease in diabetes: the atherosclerosis risk in communities study. *Diabetes Care*, 29, 877-82.

20. Nakamura K, Yamagishi SI, Adachi H, Kurita Nakamura Y, Matsui T, Yoshida T *et al.*,. (2007). Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. *Diabetes Metab Res Rev*, 23(5), 368-71.
21. Poon PY, Dornan TL, Ellis RB, Turner RC. (1979). Increased plasma activities of n-acetyl-beta-d-glucosaminidases isoenzymes in human diabetes mellitus. *Clin Endocrinol*, 11, 625-30.
22. Pitkanen E, Kyllastinen M, Koivula T, Hormila P. (1980). Beta n-acetyl-glucosaminidase and beta-glucuronidase activities in insulin-dependent diabetic subjects with retinopathy. *Diabetologia*, 18(4), 275-278.
23. Perdichizzi G, Cucinotta D, Saitta A, Cavalieri A, Squadrito G. (1983). Serum and urinary activities of beta-n-acetyl glucosaminidase and beta-glucuronidase in diabetic patients. *Acta Dibetologica*, 20(3), 257-64.
24. Deyuan Z, Ye S, Pan T. (2019). The role of serum and urinary biomarkers in the diagnosis of early diabetic nephropathy in patients with type 2 diabetes. Peer J, 7, e7079.
25. Teruo IA, Riichiro MA, Shigenori MA, Yoshio UB. (2000). Serum n-acetyl-beta-d-glucosaminidase activity increases in association with insulin resistance in patients with coronary artery disease. *Atherosclerosis*, 149, 117-22.
26. Edimar CP, Marcelo CB, Andre AF, Osmar M, Hermes TX, Tiago VP, *et al.*,. (2015). Predictive potential of twenty-two biochemical biomarkers for coronary artery disease in type 2 diabetes mellitus. *Int J Endocrinol*, 146816.
27. Clemenzia G, Persichetti S, Sagliaschi G, Gallo G, Re M, Ronchi F, *et al.*,. (1988). Isoenzymes of serum n-acetyl-beta-d-glucosaminidase in insulin dependent diabetic subjects. *Minerva Med*, 79, 547-49.
28. Kuniko Y, Hisashi A, Yuji H, Mika E, Ako F, Kinuka O, *et al.*,. (2013). High serum n-acetyl-β-d-glucosaminidase activity is a predictor of 28-year mortality in a population of community-dwelling Japanese – the Tanushimaru study. *J Am Geriatr Soc*, 61(3).
29. Waters PJ, Flynn MD, Corall RJ, Pennock CA. (1992). Increase in plasma lysosomal enzymes in Insulin dependent diabetic mellitus: relationship to diabetic complications and glycemic control. *Diabetologica*, 35, 991-5.
30. Brouhard BH, LaGrone L, Travis LB, Pollard TG. (1984). Response of urinary n-acetyl-beta-d-glucosaminidase to rapid decreases in blood glucose. *Clin Chim Acta*, 140(2), 197-202.
31. Ikenaga H, Suzuki H, Ishii N, Itoh H, Saruta T. (1993). Enzymuria in non-insulin-dependent diabetic patients: signs of tubular cell dysfunction. *Clin Science*, 84(4), 469-75.
32. Miralles JM, Corrales JJ, Garcia-Diez LC, Cabezas JA, Reglero A. (1982). N-acetyl beta-D-glucosaminidase and alpha-L-fucosidase activities in relation to glycosylated haemoglobin levels and to retinopathy in diabetes. *Clin Chim Acta*, 121(3), 373-8.