Study on Association of Glycation Gap in Diabetes Mellitus with Renal Complication

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ABSTRACT

Aim of the study: Type 2 Diabetes Mellitus is a chronic disease characterized by insulin resistance, impaired insulin secretion and increased glucose production. Due to increased blood glucose level, the blood proteins are non-enzymatically conjugated with glucose. HbA1c & fructosamine are formed, they reflect the mean blood sugar. Glycation Gap is calculated as the difference between HbA1c and HbA1c value predicted from fructosamine value. Glycation Gap is related to onset of renal complication. The objective of the study is to find out the association of Glycation Gap with renal complication.

Material & Method: The study included 59 diabetic patients without renal complication, 60 diabetic patients with renal complication and 64 healthy controls. The different biochemical parameters, urinary albumin excretion rate (UAER) were estimated and compared.

Result: HbA1c, fructosamine, Glycation Gap are increased significantly in diabetes without and with renal complication. There is significant increase in urinary albumin excretion in diabetes mellitus with complication in compare to diabetes without complication. A positive correlation is found between Glycation Gap & urinary albumin excretion rate. There is significant relation between Glycation Gap & occurrence of renal complication in diabetes.

Conclusion: Glycation Gap can reflect presence of disease and diabetic complication.

Keywords: fructosamine, Glycation Gap, HbA1c, UAER

INTRODUCTION

Type 2 Diabetes Mellitus (DM) is a heterogenous group of disorder characterised by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. Distinct genetic & metabolic defect in insulin action and/or secretion give rise to the phenotype of hyperglycemia.

The global prevalence of Type 2 DM was 8.5% in 2014. The prevalence rate has risen faster in underdeveloped & developing countries over past decades. India ranks third position with prevalence rate is 9% in males & 8.3% in females respectively in year 2016.

The American Diabetes Association (ADA) has proposed following criteria for diagnosis of DM:

HbA1c ≥ 6.5%
FPG ≥ 126 mg/dl
2 hour ≥ 200 mg/dl

Presence of clinical symptoms of hyperglycemia or crisis, random blood sugar ≥ 200 mg/dl

It is established fact that diabetic complications are due to prolong exposure to excess glucose. So long control of blood glucose is required. The process of protein glycation is understood to be both a marker for the progress of diabetes complication & underlying cause of most serious complication.

Proteins react spontaneously with glucose to form glycated derivatives. The concentration of glucose in blood influence
the extent of glycation of proteins. A variety of proteins are subjected to non-enzymatic glycation. The amount of glycated protein is increased in diabetes mellitus and contributes to complications. [6-9]

HbA1c reflects the circulating plasma level of glucose with a normal red blood cell life span [10] and average glycemia of previous 12-16 weeks as this is the half-life of red blood cell. [11]

HbA1c is a modified hemoglobin where glucose is linked to N-terminal valine of β-chain. [12] There occurs non-enzymatic attachment of glucose to hemoglobin. [13] This occurs first by formation of a labile adduct aldimine (Schiff base) than changed to form more stable ketimine form. [11] Recently the American Diabetes Association & WHO have recommended HbA1c ≥ 6.5% to diagnose diabetes mellitus. [14]

DCCT (Diabetes control and Complication Trial) demonstrated that a 1% rise in HbA1c level reflects a rise in blood glucose level by 36 mg/dl and is associated with microvascular complication. [15]

Fructosan was introduced into clinical chemistry literature in 1982 as a general form for glycated protein. [16] Similar to the other proteins, albumin undergoes glycation. [17] The main Amadori adduct formed is fructolysine, a reaction between glucose and lysine (59th position) in albumin. Among the fructosamines, Glycated albumin is the main constituent that represents about 80% of the total glycation in plasma. [18]

The Glycation Gap (GG) is the difference between measured glycated hemoglobin (HbA1c) and HbA1c predicted from fructosamine. Cohen et al defined glycation gap as the difference between the measured HbA1c and the HbA1c predicted from the measured fructosamine based on the HbA1c-fructosamine regression equation. [19] The study conducted by Ananth U. Nayak showed a positive GG directly associated with micro & macrovascular complications in diabetes. [20]

Thus, the study was undertaken to evaluate the correlation of GG with renal complication.

MATERIAL AND METHOD
This case control study was conducted in the Department of Biochemistry, SCB Medical College, Cuttack in collaboration with Department of Endocrinology, SCB Medical College, Cuttack. The study was approved by IEC. Written consents were obtained from study population.

A total 183 subjects were inducted for the study. They were grouped in to three groups. Group-1 (64 numbers) were chosen as control having euglycemia. Group-2 (59 number) were diabetic without renal complication and group-3 (60 number) were diabetic with renal complication. Following inclusion & exclusion criteria were chosen.

Inclusion criteria
- Patients between 30-80 years of age, clinically diagnosed as case of Diabetes Mellitus without & with renal complication.

Exclusion criteria
- Any other endocrinal disorder
- Hemoglobinopathy
- Chronic inflammatory disease
- Hypertensive patients

Collection of samples
5 ml fasting venous blood was collected. Of which 2 ml was kept in plain vacutainer for biochemical analysis, 2 ml was transferred to EDTA vacutainer for HbA1c estimation and 1 ml was kept for fasting blood sugar estimation. After 2 hour, 2 ml blood was collected for post prandial glucose estimation. 5 ml of urine sample was collected for urinary albumin estimation.

A). Estimation of all biochemical parameters
FBS, PPBS and serum creatinine were estimated using commercial kit adopted to autoanalyzer.

B). Estimation of urinary albumin (Pyrogallored method)
Protein in acidic medium combines with pyrogallolred and molybdate to form
blue purple colour complex. Intensity of which is directly proportional to amount of protein present in the sample compared with standard at 600 nm.

C). Estimation of HbA1c (HPLC)

The analyser uses pressure cation exchange high performance liquid chromatography (HPLC) in conjunction with gradient elution to separate human hemoglobin subtypes and variants from hemolysed whole blood. The separated hemoglobin fraction is monitored by absorption of light at 415 nm.

D). Estimation of Serum fructosamine (ELISA KIT)

The purified human antibody to coat microtiter plate well, make solid phase antibody. Then samples are added to wells combine with human fructosamin antibody which with HRP labelled become antibody-antigen-enzyme -antibody complex. After washing completely, TMB substrate solution is added. TMB substrate becomes blue colour which is terminated by addition of sulphuric acid and colour changed to yellow measured spectrophotometrically at 450 nm. The concentration of human fructosamine in the sample is determined by comparing the OD of samples to standard curve.

E). Calculation of Glycation Gap (GG)

Glycation Gap (GG) = HbA1c measured – HbA1c predicted from fructosamine

HbA1c can be predicted from value of fructosamine by the using the formula. \[ \text{HbA1c level} = 3.02 \times \text{fructosamine} + 2.29 \]

Statistical Analysis

All the data were expressed in mean ± SD. The statistical significance was found by one-way ANOVA (post hoc test) version 20. The ‘p’ value of < 0.05 was taken as significance. Pearson correlation was done to find out the correlation. Chi square test was used to find out the association.

RESULT

Demographic character & BMI

The demographic & BMI of all groups were shown in table -1. There was no observed significant difference in age, male to female ratio and BMI between all groups.

Biochemical Parameters

In table -2, fasting blood sugar was significantly raised (p<0.001) in group -2 & Group-3 study population when compared with group-1 study population. Similar finding of significant rise (p<0.001) in fasting blood sugar was seen in group-3 population compare to group-2 population. The PPBS also showed similar trend with ‘p’ value of <0.05.

There was a significant rise (p<0.001) in UAER value in group-3 study population with respect to group-2 population. Serum creatinine value was significantly raised (p<0.001) in group-2 and group-3 study population compare to group-1 study population, while there was no significant difference between group-2 and group-3 study population.

Special biochemical parameters

In table-3, HbA1c level in group-2 and group-3 study population was raised significantly (p<0.001) compared to group-1 study population and there also significant rise in group-3 with the group-2 study population.

The values of fructosamine and estimated HbA1c showed a significant rise (p<0.001) in group-2 and group-3 study population in compare to group-1 population. There was a significant rise in group-3 population when compare with group-2 study population. Glycation Gap was significantly high in group-3 study population when compare with group-2 population as well as with group-1 population, the negativity Glycation Gap decreases with complication i.e. it becomes positive.

Correlation of Glycation Gap with UAER

In table-4 we observed a positive correlation (r = +0.622) between Glycation Gap and UAER in DM patient with renal complication and it was significant statistically (p<0.001).
Association of Glycation Gap with occurrence of complication.

The table-5 we found a significant association between Glycation Gap with occurrence of renal complication (p<0.05).

Chi square test has shown the significant association of Glycation Gap of HbA1c to occurrence of renal complication. (p<0.05).

**DISCUSSION**

Insulin resistance, impaired insulin secretion and increased glucose production are the characteristics of Type 2 Diabetes Mellitus. [1]

Retinopathy, nephropathy, neuropathy, myocardial infarction and stroke are the complications of Type 2 DM. It has been documented that lowering of HbA1c concentration significantly reduces the onset and rate of progress of microvascular complications. [15,22]

Glycated hemoglobin reflects the mean glucose concentration over preceding 8-12 weeks [23,24] correlates with risk of developing micro & macrovascular complication. [25,26]

The half-life of albumin in the blood is 14-20 days and hence, the fructosamine concentration reflects the mean glucose concentration over 10-14 days, a much shorter period than that reflected by HbA1c. [27]

We observed, there is no significant difference between the study groups with
respect to BMI, it is in contrast to the study by Garber AJ et al. [28]

We observed a significant increase in HbA1c in both diabetes mellitus without complication and diabetes mellitus with renal complication, which corroborated with the finding of Rahber et al. [29] Poor glycemic control is well defined contributor to development and progression of microalbuminuria in Type 2 Diabetes Mellitus. Middleton RJ et al demonstrated the importance of increased HbA1c. [30]

We observed an increase urinary albumin excretion (UAER) in diabetes with renal complication. Pedersen EB et al demonstrated similar finding. [31]

We found in our study that a significant increase in fructosamine level in diabetes mellitus with renal complication compares to diabetes mellitus without renal complication. Our finding is in accordance with study conducted by Chen HS et al. [32]

In our study we found a positive GG in 21 patients with renal complication 3 positive GG in patients without renal complication. GG predicts the progression of nephropathy in Type 2 DM This is supported by the study conducted by Rodri’ guez-Segade. [32]

CONCLUSION

Serum fructosamine level and Glycation Gap reflect the presence of diabetic complication. So, a large group study is required further.

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