GLYCOSYLATED HAEMOGLOBIN LEVEL IS A DEFINITIVE PREDICTOR OF DIABETIC NEPHROPATHY- A STUDY

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ABSTRACT

BACKGROUND

Diabetes Mellitus is a part of a metabolic syndrome, which leads to severe morbidity and mortality. It has emerged as an illness which is beset with numerous complications and incapacitation. Out of all complications Diabetic Nephropathy takes a heavy toll on our health system. Therefore, it is imperative to find not only a cure, but also a feature which predicts the onset of the disease. Fortunately, microalbuminaria (Excretion of minute quantities of albumin in urine) is one such factor. Moreover, it is also linked to glycemic control which in turn is reflected by glycosylated haemoglobin levels which can be measured with reasonable accuracy. Also the stage of MA is reversible. Our study was an attempt at correlating the two factors.

MATERIALS AND METHODS

The study was conducted on 100 patients (50 T2DM patients without DN and 50 T2DM patients with DN) who attended the Medical and Nephrology In-Patient and Out-Patient Departments of Gandhi Hospital.

Clinical DN was diagnosed by the presence of albumin excretion and creatinine clearance. Estimation of glycosylated hemoglobin was done by Cation-Exchange chromatography. Micral-Test strip was used to screen for Microalbuminuria Percentage of hemoglobin was estimated by modified Sahli-Adam's method.

RESULTS

Significant correlation was found between levels of HbA1c and occurrence of MA as all MA cases in patients of T2DM without DN had >8% HbA1c levels. Mean MA levels were compared between in T2DM and Diabetic Nephropathy which showed statistical significance.

CONCLUSION

Our study reiterates the correlation between glycosylated haemoglobin levels and microalbuminuria. As MA occurs prior to overt Diabetic Nephropathy, it predicts the onset of the latter. Also MA is a disturbance in renal function in early DN, which is reversible and hence can be targeted for preventive measures.

KEYWORDS

HBA_1C, Diabetic Nephropathy, Micro-Albuminuria.

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INTRODUCTION

Diabetes mellitus is a global health problem. According to World Health Organization (WHO), at least 171 million people worldwide have diabetes. Assuming that age-specific prevalence remains constant, the number of people with Diabetes in the world is expected to approximately double between 2000 and 2030, based solely upon demographic changes.[1] Currently in India, 40.9 million people are victims of this disease. Type 2 Diabetes Mellitus (T2DM) is more seen in younger, slimmer persons in India than in the Western Countries.

Diabetes mellitus, especially T2DM is associated with several alarming complications. One such which should receive a greater attention is End-Stage Renal Disease (ESRD) or Kimmelstiel Wilson syndrome or Diabetic Nephropathy (DN).

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It is seen in about 30 to 40% of all diabetic patients and poses a severe burden on already fragile resourced health care system in India.

Several studies were done to identify an accurate predictor of DN. One such entity which predicts the onset of overt Diabetic Nephropathy before its actual onset is microalbuminuria.

Microalbuminuria (MA) is persistent elevation of albumin in the urine of 30-300mg/day (20-200microg/min). These values are less than the values detected by routine urine dipstick testing, which does not become positive until protein excretion exceeds 300-500mg/day. The presence of MA is a marker of endothelial dysfunction and a harbinger of markedly enhanced cardiovascular risk. As uncontrolled elevation of blood sugars is the main cause for MA, Glycosylated Hemoglobin (HbA1c) levels (indicators of the degree of control of blood sugars) can be used as a marker for occurrence of DN.

Our study was an attempt to emphasize the need for tight glycaemic control to prevent the onset of Diabetic Nephropathy. It assesses the percentage of occurrence of Microalbuminuria when Glycosylated Hemoglobin (HbA1c) levels are high in T2DM. Glycosylated haemoglobin level (HbA1c) of less than 7% has been set as a target by the American Diabetes Association to minimize the chances of complications.[2]

MATERIALS AND METHODS

The study was conducted on 100 patients (50 T2DM patients without DN and 50 T2DM patients with DN) who attended the Medical and Nephrology In-Patient and Out-Patient Departments of Gandhi Hospital. The former were grouped under group A and the latter were grouped under group B. The duration of study period was one year from January 2013 to January 2014. Patient age group was 45 years to 70 years and gender distribution was random. Duration of Diabetes Mellitus was from 6 years to 15 years.

Clinical DN was diagnosed by the presence of albumin excretion and creatinine clearance based on standardized 4 hours collection and by periodic iothalamate clearance assessment.

Estimation of glycosylated hemoglobin was done by Cation-Exchange.

Chromatography. Normal range is between 4.27% and 6.07%.

Micral-Test strip was used to screen for Microalbuminuria defined as 30-300mg.

Albumin in two different 24-hour urine samples.[3]

Percentage of hemoglobin was estimated by modified Sahli-Adam's method.

RESULTS

The data obtained was subjected to suitable statistical analysis. The mean duration of Diabetes was found to be 9.6 years and mean age 56.1 years for onset of DN. (Table I); 74% of Diabetic Nephropathy cases had more than 8% of HbA1c as compared to 24% in T2DM. (Table II, Chart I, II, III). The mean levels in T2DM were 7.01 and in Diabetic Nephropathy 9.27 with standard deviation of 1.141 in T2DM and 1.69 in Diabetic Nephropathy with P value <0.01 showing extreme significance. Significant correlation was found between levels of HbA1c and occurrence of MA as all MA cases in Group A had >8% HbA1c levels (Table III). Mean MA levels were compared between T2DM and Diabetic Nephropathy with mean value of 20 and 92 with significant P value of <0.01 considered extremely significant (Chart IV).

DISCUSSION

Glycosylated Hemoglobin is formed by the slow non-enzymatic attachment of sugars to the N terminal valine residue of each beta chain of hemoglobin (Hb A). This binding remains for the rest of the life of the red blood cell. The level of the glycosylated haemoglobin depends on the long-term level of glucose present in the blood and its duration.

Glycosylated hemoglobin is a measure of integrated glycemic control over the preceding 8 to 12 weeks (Lifespan of red blood cell).

Proteinuria was first recognized in diabetes mellitus in the late 18th century and 40 years later Bright.[4] postulated that this form of renal disease was specific to angiopathy of capillaries leading to intercapillary glomerulonephritis. Finally, nodular glomerulosclerosis and nephritic syndrome associated with proteinuria and Hypertension result (Kimmelsteil and Wilson).

Clinically, overt DN is defined by the presence of persistent Albuminuria >300mg/24 hours or 200 micrograms/min in a diabetic patient.

The prevalence of diabetic nephropathy in Type 2 DM is 5%-9%.[5] Among type 2 DM, ethnicity and gender also appear to be important factors in the development of nephropathy. Asian diabetic subjects have significantly higher prevalence (52.6%) of ESRD when compared with the Caucasians (36.2%) (Young et al.).[6] Incidence of ESRD, 10 years after onset of proteinuria is seen in 65% of Pima Indian T2DM patients.[7] Familial clustering of diabetic nephropathy in type 2 DM and affected sib pair linkage identified in type 2 DM may also be involved.[8]

Diabetic nephropathy has no symptoms in the early course of the disease. They develop in late stages and may be a result of excretion of high amounts of protein in the urine or due to renal failure. Earliest known manifestation of diabetic kidney disease is the presence of small amounts of albumin in urine called microalbuminuria. It is also called as Incipient Nephropathy. More importantly it is reversible and hence treatable.

Urinary albumin excretion within the microalbuminuric range (30-300mg/24 hours) in at least two out of three consecutive non-ketotic sterile urine samples is the generally accepted definition of persistent microalbuminuria. When the albumin level exceeds 300mg/24hrs. condition, it is known as Overt Nephropathy.

Uncontrolled hyperglycemia as indicated by HbA1C levels is associated with microalbuminuria prior to the development of overt DN. It is a well-known fact that tight metabolic control can abolish microalbuminuria and prevent the diabetes related renal damage as the rate of progression of nephropathy is correlated with metabolic control and hence delay its progression. Nephropathy is uncommon in patients with HbA1c constant less than 7.5% to 8%.[9] which may retard the complications of microvascular complications, as there is a positive relationship between the microvascular complications and hyperglycemic milieu of diabetics.

Sustained hyperglycemia leads to non-enzymatic glycosylation. Glycation results from condensation of reducing sugars with e-amino group's lysine residue of proteins. The resulting Schiff base undergoes rearrangement to form relatively stable ketoamines, the Amadori products. These glycated proteins undergo progressive dehydration, cyclization, oxidation and rearrangement to form Advanced Glycation End Products (AGE's). Once these AGE's are formed, the reaction is not reversible and they gradually accumulate over the lifetime of the protein accumulation of AGE's parallels, the degree of renal insufficiency. Glucose is reduced to sorbitol by enzyme aldose reductase.[10] Sorbitol functions as a tissue toxin and is a probable cause of the pathogenesis of nephropathy.

With sorbitol formation, there is relative depletion of myoinositol and Na+/K+ - ATPase activity. Increased sorbitol interferes with inositol signaling and depletes intracellular NADPH store leading to oxidative injury.[11]

Rate of synthesis of matrix and glomerular basement membrane is increased in diabetic nephropathy. The activity of the enzyme, protein kinase C, increases in various diabetic tissues including the glomerulus. LY333531, an orally active synthetic inhibitor of the II isoform of protein kinase C prevents the development of hyperfiltration and albuminuria.[12]

Heparin sulphate is the principal Glycosoaminoglycans (GAG) in glomerular basement membrane. Heparin sulphate together with sialic acid contributes to the negative charges of glomerular capillary wall and charge selective properties of the barrier. The sialo proteins are negatively charged and coat the glomerular epithelial cells, their foot process, epithelial slit and diaphragm. Glomerular basement membrane glycosylation leads to increase in degree of disulfide bridges between type 1V collagen components via increased oxidation of sulfhydryl groups. This affects the assembly and architecture of glomerular basement membrane by the presence of reactive carbonyl group of glucose attached to these structures. This accounts for linear deposition of albumin and IgG observed along glomerular and tubular membranes.

Glycosylation of the structural or circulating proteins trapped in glomerular structure reduce their degradation, which

results in accumulation and expansion of mesangial matrix and glomerular basement membrane. These monokinins initiate a cascade of stimuli, which results in increased protein synthesis cell proliferation and vascular permeability.

Diabetic Nephropathy is a consequence of extracellular matrix accumulation including type IV and type VI collagen, laminin and fibronectin.[13] The number of restrictive pores leads to reduced ultra-filtration capacity (Meyers and Coworkers).[14] A defect in the glomerular barrier size selectivity also compounds the problem.

The mesangium plays a key role in the pathogenesis of basement membrane thickening, which arises from either augmented synthesis by the epithelium and/or diminished removal by the mesangium. Increased concentrations of alpha-2 macroglobulin inhibit mesangial proteases, thus retarding the removal of basement membrane.

Thickening of the glomerular basement membrane is the first change that can be quantified afferent and efferent glomerular arteriolar hyalinosis can also be detected with three to five years after the onset of diabetes glomerular epithelium podocyte cell structure and number are also related to glomerular permeability alterations in diabetes.

CONCLUSION

DN is a complication of Diabetes, which leads to severe morbidity and increased mortality rate. Fortunately, it manifests with microalbuminuria in the early and reversible stages. As HbA1c levels govern the occurrence of microvascular complications and hence microalbuminuria, tight glycemic control is imperative for prevention of overt DN. Therefore, early control of hyperglycemia and screening for microalbuminuria at the time of diagnoses of diabetes can prevent this serious disorder.

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PATIENTS	Group A	Group B
No. of Patients	50	50
Sex (Male/Female)	31/19	35/15
Mean Age	58. 68 years	56 years
Duration of DM	7.64 years	9.7 years
Standard Deviation	1.8	3.3

Table 1: Parameters Studied among Group A and Group B subjects

HbA1c%	Group A	Group B
6 to 7%	27 (54%)	3 (6%)
7 to 8%	11 (22%)	10 (20%)
>8%	12 (24%)	37 (68%)

Table 2: Distribution of HbA1c Levels among Group A and Group B subjects

	Group A		Group B	
HbA1c % ages	Number of Subjects	Subjects with Microalbuminuria	Number of Subjects	Subjects with Microalbuminuria
6-7	27	0	3	0
7-8	11	0	10	9
8-9	12	10	37	37
Total	50	10	50	46

Table 3: Distribution of MA among Group A and Group B subjects

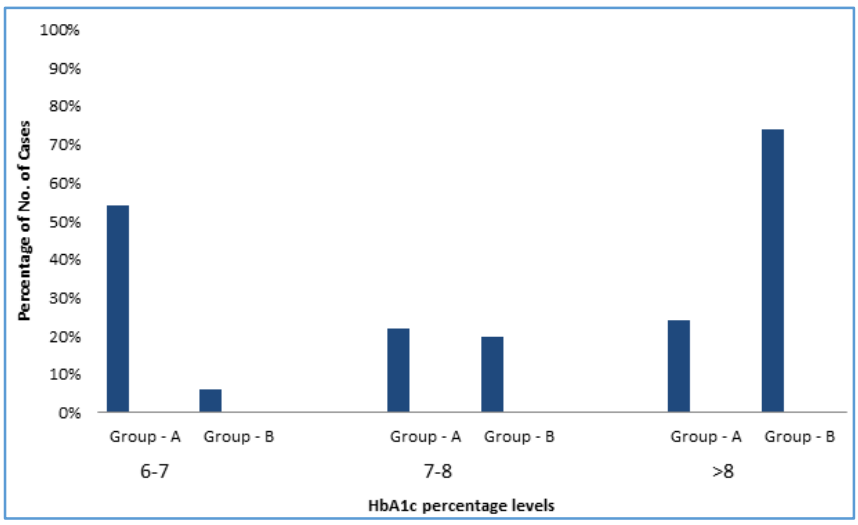

Chart I: Distribution of HbA1c Levels among Group A and Group B subjects

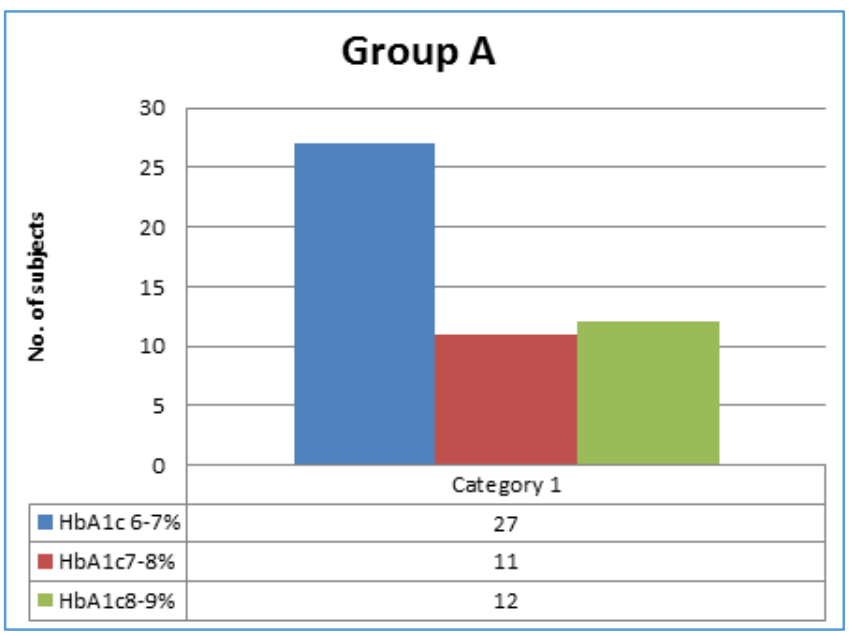

Chart II: Individual Distribution of HbA1c levels among Group A subjects

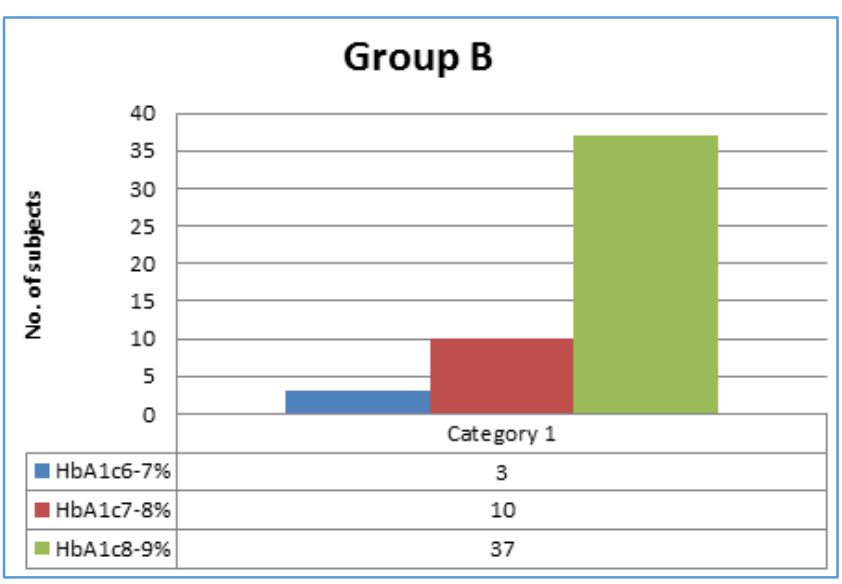

Chart III: Individual Distribution of HbA1c among Group B subjects

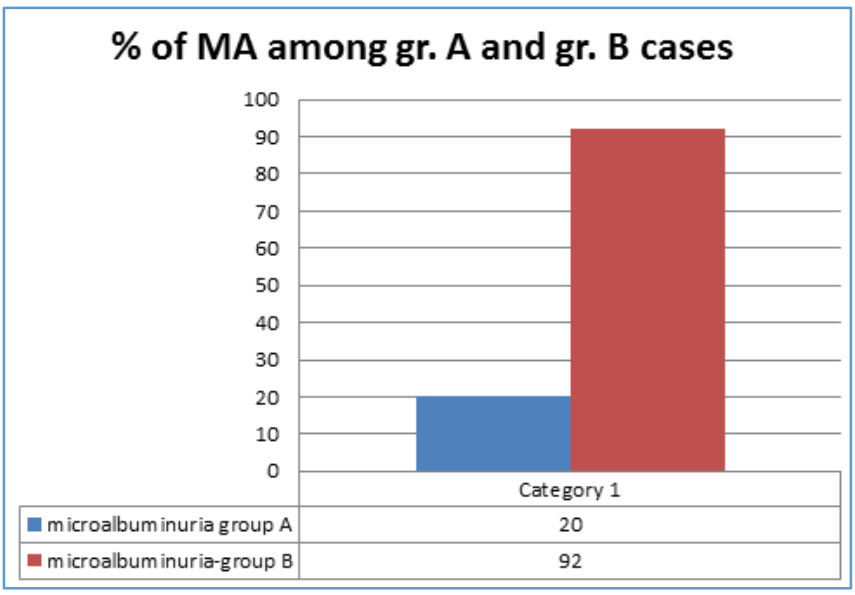

Chart IV: Distribution of MA among Group A and Group B subjects