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Original Research Article

Correlation of Hba1c With Microalbuminuria and Fundoscopic Finding in Type-2 Diabetes Mellitus Patients

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Abstract

Background: Diabetes is a very common disease now days. It has adverse effect on many human organs as its duration increases. Many studies exist to show its bad effects on body organs in different parts of world. We have studied is there any relation between microalbuminuria and fundoscopic finding with HbA1c levels.

Methods: Our study includes all the known case of type 2 diabetes mellitus patients of age group of 45 years and above. Five milliliters of venous blood is collected from all patients. For glycosylated hemoglobin estimation, EDTA blood samples were used. Blood sugar was investigated by the glucose oxidase method, glycosylated hemoglobin (HbA1c) by the cation exchange resin method and micro albumin levels in the urine sample by using the turbilatex method. Retinopathy was tested by using the Direct Fundoscopic Method.

Results:

HbA1c with microalbuminuria: Total 31 patients had Urinary albumin level more than 30 mg/dl out of which only 3% had Hb1Ac value less than 6.5 % whereas 96% had Hb1Ac value more than 6.5%. This association was found to be clinically significant (Pearson Chi-Square- 4.888, df is 1 and p value is 0.027, Fishers Exact test is 0.033)

Fundoscopic changes: It was seen that, 81 patients had grade 1 fundoscopic changes from which 14 (17.2%) shows Hb1Ac was less than 6.5% whereas 67(82.7%) had Hb1Ac level more than 6.5%. 15 patients had grade 2 fundoscopic changes from which 1 (6%) had Hb1Ac level less than 6.5% and 14 (94%) had Hb1Ac level more than 6.5%. 4 patients had grade 3 fundoscopic changes and all of them were having Hb1Ac level more than 6.5%.This Fundoscopic finding was not statistically associated when compared with Hb1Ac. (Pearson Chi-Square- 1.854 with df 2 and p value is 0.396).

Conclusion: Patients having microalbuminuria were associated with high level of glycosylated haemoglobin. Fundoscopic changes in patients was not found associated with glycosylated haemoglobin as p <0.3.

Keywords: Diabetes Mellitus, Microalbuminuria, Diabetic Nephropathy Glomerulopathy, Fundoscopic, Glycosylated Hemoglobin. Retinopathy.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders that share the phenotype of hyperglycaemia. Several distinct types of diabetes mellitus are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of diabetes mellitus, factors' contributing to hyperglycaemia includes reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic disregulation associated with DM causes secondary pathophysiologic changes in multiple organ system.

Diabetic Nephropathy: Diabetic nephropathy is a devastating chronic microvascular diabetic complication. The mechanisms leading to the development and progression of this most feared diabetic complication are mainly poor metabolic control. We should direct all our efforts into achieving good glycaemic control. The global number of diabetic patients is believed to be around 180 million and is set to increase to 350-400 million in the next two decades. Patients with type 2 diabetes will account for 90% of all cases, and we expect an increase of new cases of diabetic nephropathy (DN) within the current decade, given the predicted 30-40% prevalence of renal disease in the diabetic population. This is particularly so for populations of Afro-Caribbean and Asian origin, who are known to have a significantly higher risk for renal disease.

Diabetic Glomerulopathy: The endothelium plays a central role in the pathophysiology of diabetic glomerulopathy. Endothelial dysfunction precedes altered vascular permeability and albuminuria. Markers of endothelial dysfunction such as soluble intercellular and vascular adhesion molecules, Von Willebrand Factor, and altered microvascular reactivity can be observed in patients with diabetes before any clinical manifestation of DN [1]. Indeed many studies in experimental models of diabetes and hypertension and in the clinical setting have described an important synergistic effect of metabolic (hyperglycaemia) and hemodynamic (glomerular hypertension) perturbations in the development and progression of DN [2]. Typically a progressive accumulation of extracellular matrix is observd in the glomerular and tubular compartment resulting respectively in diffuse glomerulosclerosis and fibrosis of the tubular interstitium and tubular damage [3] Changes in Other Components of the Glomerular Filtration Barrier Parallel changes occur in the glomerular filtration barrier with glomerular endothelial cell injury, with loss of glycocalyx and cell apoptosis. The glycocalyx is an aqueous extracellular layer that covers the glomerular capillary lumen side, and plays an important role in glomerular vascular function and permeability [4]. Further thickening of the glomerular basement membrane and podocyte foot process effacement and loss of podocytes in the urine are other mechanisms that characterize the alteration of the permeability properties of the glomerular filtration barrier and disease progression. Podocyte detachment has been positively correlated with urinary albumin excretion and increasing podocyte detachment is associated with decreased permeability of the glomerulus and progressive albuminuria. Of note recently the Kidney Disease Improving Global Outcomes Initiative (KDIGO) has proposed that a better estimate of patients' expected outcomes in terms of renal and cardiovascular risk is obtained by combining albuminuria and GFR. Further the

classification of albuminuria has been revisited and 3 categories have been proposed: normal to mildly increased (normoalbuminuria or high-normal albuminuria), moderately increased (microalbuminuria) and severely increased (macroalbuminuria). It is widely accepted that the rise in albuminuria reflects an increased transglomerular flux of albumin as a consequence of an increased transglomerular pressure gradient and damage of the glomerular filtration barrier.

Diabetic Retinopathy: Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for more than 10,000 new cases of blindness every year in the United States alone. [5]

The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia. Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hypertension in the U.K. Prospective Diabetes Study (UKPDS), and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis. [6,7] Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy.

Aldose reductase may participate in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes. Treatment studies with aldose reductase inhibitors, however, have been disappointing [8]. Cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the nonenzymatic formation of advanced glycosylated end products (AGEs). In animal models, these substances have also been associated with formation of microaneurysms and pericytes loss. Evaluations of AGE inhibitors are underway.

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Animal studies have suggested that treatment with antioxidants, such as vitamin E, may attenuate some vascular dysfunction associated with diabetes, but treatment with antioxidants has not yet been shown to alter the development or progression of retinopathy or other microvascular complications of diabetes. [9]

Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy [10] Diabetic retinopathy is generally classified as either background or proliferative. It is important to have a general understanding of the features of each to interpret eye examination reports and advise patients of disease progression and prognosis.

Background retinopathy includes such features as small hemorrhages in the middle layers of the retina. They clinically appear as “dots” and therefore are frequently referred to

as “dot hemorrhages.” Hard exudates are caused by lipid deposition that typically occurs at the margins of hemorrhages. Microaneurysms are small vascular dilatations that occur in the retina, often as the first sign of retinopathy. They clinically appear as red dots during retinal examination. Retinal edema may result from microvascular leakage and is indicative of compromise of the blood-retinal barrier. The appearance is one of greyish retinal areas. Retinal edema may require intervention because it is sometimes associated with visual deterioration. [11] Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous hemorrhage. White areas on the retina (“cotton wool spots”) can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous hemorrhage and traction retinal detachment. With no intervention, visual loss may occur. Laser photocoagulation can often prevent proliferative retinopathy from progressing to blindness.

Glycated hemoglobin (HbA1c): It is a form of hemoglobin that is measured primarily to identify the three month average plasma glucose concentration, because the lifespan of a red blood cell is three months. It is formed in a nonenzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin.9, 10 Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous three months before the measurement as this is the lifespan of red blood cells. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuro pathy, and retinopathy.

Measuring HbA1c: A number of techniques are used to measure hemoglobin A1c. Laboratories use: High-Performance Liquid Chromatography (HPLC): The HbA1c result is calculated as a ratio to total hemoglobin by using a chromatogram. Immunoassay, Enzymatic, Capillary electrophoresis, Boronate affinity chromatography. The United States, HbA1c testing laboratories are certified by the National Glycohemoglobin Standardization Program (NGSP) to standardize them against the results of the 1993 Diabetes Control and Complications In Trial (DCCT)(12) An additional percentage scale, Mono S is in use by Sweden and KO500 is in Japan.13,14.

Table 1: Observations of Measuring HbA1c

HbA1C (%)	(mmol/mol)(15)	Estimated average glucose (mmol/L)	(mg/dL)
5	31	5.4 (4.2–6.7)	97 (76–120)
6	42	7.0 (5.5–8.5)	126 (100–152)
7	53	8.6 (6.8–10.3)	154 (123–185)
8	64	10.2 (8.1–12.1)	183 (147–217)
9	75	11.8 (9.4–13.9)	212 (170–249)
10	86	13.4 (10.7–15.7)	240 (193–282)
11	97	14.9 (12.0–17.5)	269 (217–314)
12	108	16.5 (13.3–19.3)	298 (240–347)
13	119	18.1 (15–21)	326 (260–380)
14	130	19.7 (16–23)	355 (290–410)

15	140	21.3 (17–25)	384 (310–440)
16	151	22.9 (19–26)	413 (330–480)
17	162	24.5 (20–28)	441 (460–510)
18	173	26.1 (21–30)	470 (380–540)
19	184	27.7 (23–32)	499 (410–570)

Inclusion Criteria:

1. All patients of type 2 diabetes mellitus 45 years and above

2. Patients with or without micro vascular complication.

Exclusion Criteria:

1. Patients with secondary hyperglycemic states like hypothyroidism,

2. Proteinuric conditions like congestive cardiac failure, renal failure and proven renal diseases,

3. Pregnancy.

Material and Method:

We have planned to select approximately 100 diabetic patients from OPD as well as IPD of medicine department. All the patients will go through the investigations for micro-albuminuria and HbA1C. The samples were centrifuged, separated and stored at 4°C until analysis. For glycosylated hemoglobin estimation, EDTA blood samples were used. Glycosylated hemoglobin (HbA1c) by the cation exchange resin method and micro albumin levels in the urine sample by using the turbilatex method. The present cross sectional study is conducted from January 2016 to March 2017 in a tertiary health care hospital located in Jhalawar, Rajasthan. Our study includes all the known case of type 2 diabetes mellitus patients of age group of 45 years and above (according to American diabetes association). All the patients were fully informed about the purpose, the procedures and the hazards of the study.

Result

HbA1c with microalbuminuria

Table 2: Comparison of HbA1c with microalbuminuria

HbA1c	U.alb		Total
	<30	>30	
Less than 6.5%	14 (20.2%)	1 (3%)	15
More than 6.5%	55 (79.7%)	30 (96%)	85
Total	69	31	100

Pearson Chi-Square- 4.888, df is 1 and p value is 0.027

Fishers Exact test is 0.033

Total 69 patients had Urinary albumin level less than 30 mg/dl out of which Hb1Ac was less than 6.5% present in 20.2% of patients and 79.7% of patients had more than 6.5%.

Total 31 patients had Urinary albumin level more than 30 mg/dl out of which only 3% had Hb1Ac value less than 6.5 % whereas 96% had Hb1Ac value more than 6.5%. This association was found to be clinically significant (Pearson Chi-Square- 4.888, df is 1 and p value is 0.027, Fishers Exact test is 0.033) (Table no-2, Graph no- 1)

These results can be compared with the study conducted by Mishra et al in which patients of DM having HbA1c ≤ 7% had mean urinary microalbumin level of 40.27 mg/24hrs and the patients of DM having HbA1c >7% had mean urinary microalbumin level of 67.95 mg/24 hrs($p \leq 0.03$). This is statistically significant. The patients of DM having HbA1c ≤ 7% had

mean serum microalbumin level of 1.7 mg/dl and the patients of DM having HbA1c >7% had mean serum microalbumin level of 1.64 mg/dl (p ≤0.53). [16].

One more study conducted by Alamdari et al on risk factors for microalbuminuria in T2DM patients and reported 30.5% prevalence of microalbuminuria. Alamdari et al also reported significantly high HbA1c levels in patients with microalbuminuria. [17]

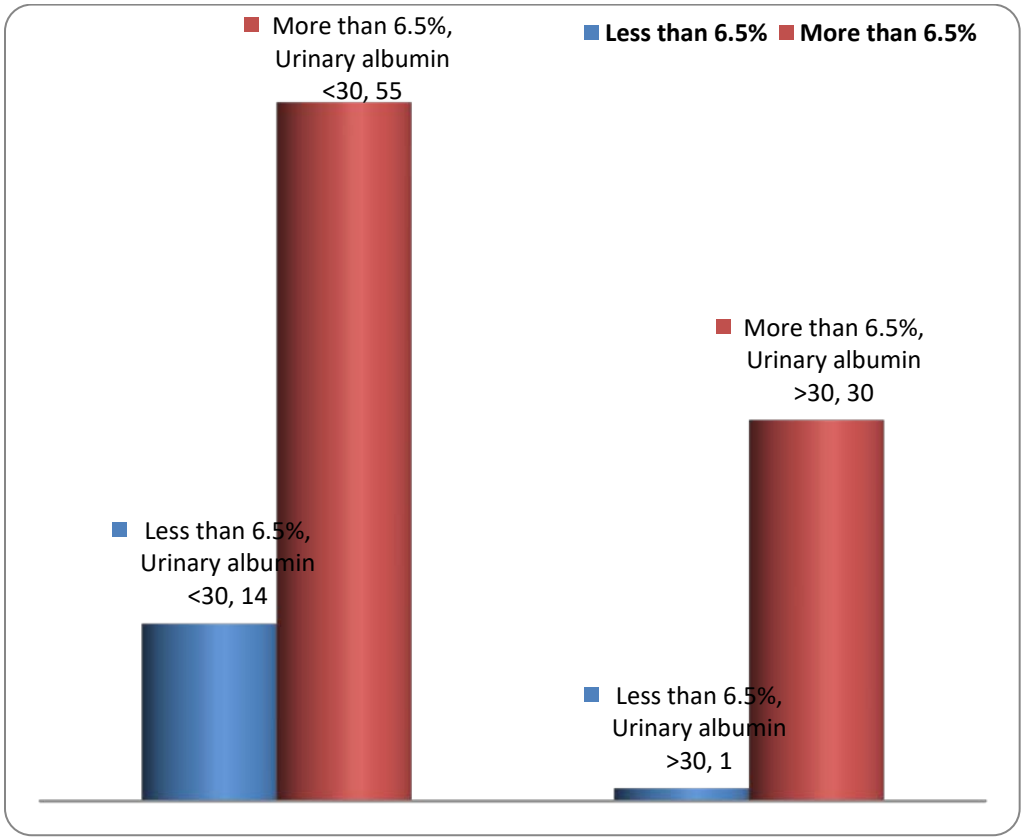

Figure 1: Comparison of HbA1c with microalbuminuria

Fundoscopic changes and Hb1Ac

14 and 67 patients had grade 1 fundoscopic changes and showing Hb1Ac level less than 6.5% and more than 6.5% respectively. 1 patient had grade 2 fundoscopic changes and showing Hb1Ac level less than 6.5% whereas 14 patients having grade 2 changes had Hb1Ac level more than 6.5%. 4 patients had grade 3 fundoscopic changes and showing Hb1Ac level more than 6.5%.

Table 3: Comparison of HbA1c with Fundoscopic change

Hb1Ac	Fundus			Total
	1	2	3	
Less than 6.5%	14 (17.2%)	1 (6%)	0	15
more than 6.5%	67 (82.7%)	14 (94%)	4 (100%)	85
Total	81	15	4	100

Pearson Chi-Square- 1.854 with df 2 and p value is 0.396

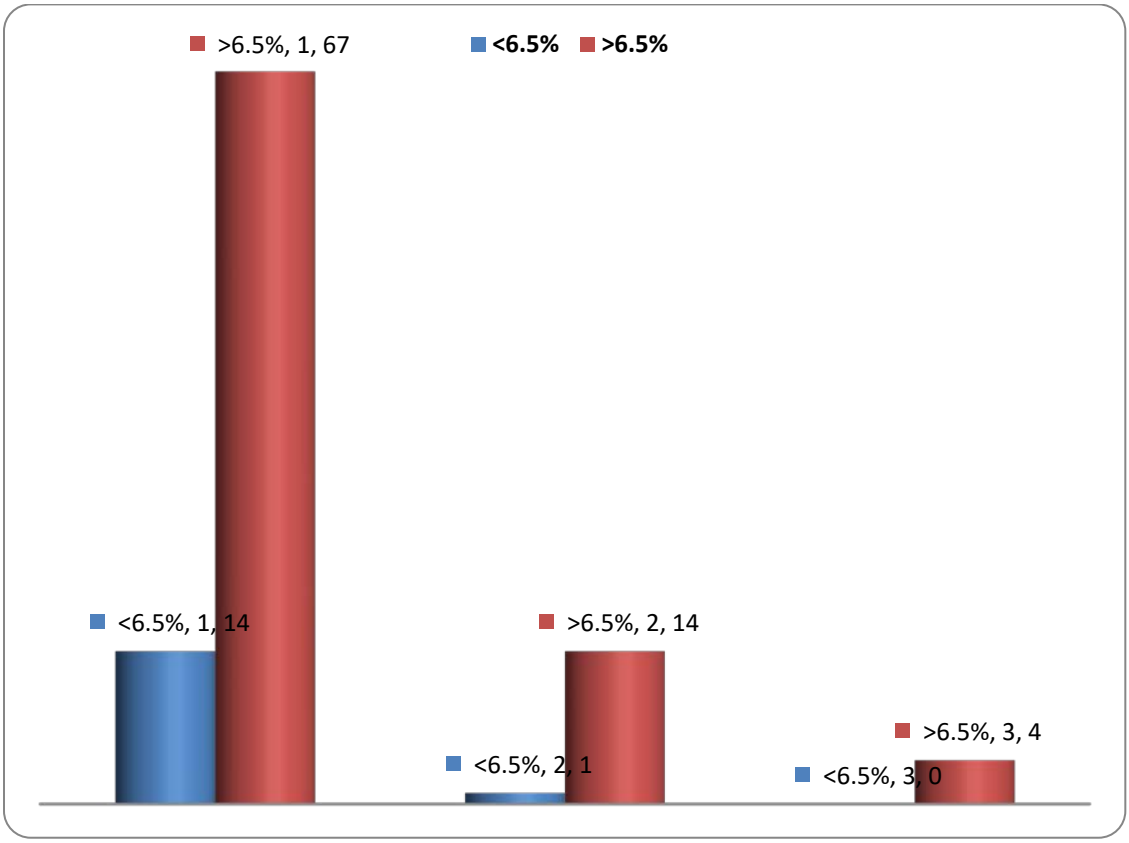

Figure 2: Comparison of HbA1c with Fundoscopic changes

Fundoscopic changes

It was seen that, 81 patients had grade 1 fundoscopic changes from which 14 (17.2%) shows Hb1Ac was less than 6.5% whereas 67(82.7%) had Hb1Ac level more than 6.5%. 15 patients had grade 2 fundoscopic changes from which 1 (6%) had Hb1Ac level less than 6.5% and 14 (94%) had Hb1Ac level more than 6.5%. 4 patients had grade 3 fundoscopic changes and all of them were having Hb1Ac level more than 6.5%.This Fundoscopic finding was not statistically associated when compared with Hb1Ac. (Pearson Chi-Square- 1.854 with df 2 and p value is 0.396). (Table no -3, graph no-2)

One study conducted in Turkey, shows The frequency of diabetic (both NPDR and PDR) retinopathy was lowest in the group of diabetes with the lowest HbA1c concentration <6% (4.8% or 5/104), 8.7% or 12/137 in the group with HbA1c values between 6.1% and 6.9%, 62.8% or 121/195 in the group with HbA1c values between 7% and 9.9% and highest (82.2% or 150/182) in the group with HbA1c concentrations over 10%. As seen by the logistic regression analysis, in patients who had HbA1c value of 7%-9.9% there were significant relationship between DR and HbA1c levels (p=0.001). [18]

Study conducted by Priyadharshini N., shows that HbA1c was found to be significantly associated with maculopathy (clinically significant macular oedema with p = 0.003). Distribution based on duration was found to be 45% in those with disease greater than 15 years, 40 % with disease greater than 10 years and 15%with disease less than 10 years. When the subgroups with maculopathy were

analysed, a statistical significant difference was noted with p=0.03 in those with high Hba1c levels but no difference was noted in terms of duration of the diabetes mellitus and the age groups affected [19,20]

Conclusion

Patients having microalbuminuria were associated with high level of glycosylated haemoglobin. Fundoscopic changes in patients was not found associated with glycosylated haemoglobin as p <0.3.

Limitations:

One of the biggest challenges for health care providers today is addressing the continued needs and demands of individuals with chronic illnesses like diabetes. During study, only 100 patients were agreed and included in the study. More sample size will require generalizing its effect on population. Many patients didn't agree to participate in study because of many problems like no time to participate in the study, have ignorance towards the diseases etc. We did not exclude obese patients. Ours is cross sectional study so we cannot compare efficacy of HbA1c levels in controlling and prevention of microvascular complication in diabetic patients.

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