Biochemical Investigation of Glycosylated Haemoglobin in Diabetes Associated Nephropathy in Chhattisgarh Population

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ABSTRACT

Diabetes mellitus is a worldwide major health problem mostly associated with End Stage Renal Disease (ESRD). In most countries diabetic nephropathy has become the single most frequent cause of ESRD which requires Renal Replacement Therapy. The incidence of reported ESRD is 4.3% with type-1 diabetes mellitus and 4.5% with type-2 diabetes mellitus. The Glycosylated haemoglobin (HbA1c) is widely accepted and used as the most reliable test for assessing chronic glycemia. The HbA1c reflects overall blood glucose levels over a period of 2-3 months and further, used to monitor diabetic treatment. Therefore, in the present investigation a biochemical approach of the HbA1c in diabetes associated nephropathy is proposed to explore in Chhattisgarh population. The study was undertaken including both male and female subjects and the fasting blood sugar, post prandial blood sugar, HbA1c, urea and creatinine were analyzed in the blood. The result indicated that the levels of fasting, post-prandial blood sugar and glycosylated haemoglobin were elevated significantly (P<0.05) in diabetes associated nephropathy compared to control and nephropathy, however, there was no change compared to diabetes. Further, urea level increased significantly (P<0.05) in both nephropathy and diabetes associated nephropathy compared to both control and diabetes. Similarly, creatinine level increased significantly (P<0.05) in both nephropathy and diabetes associated nephropathy compared to both control and diabetes. Moreover, the creatinine level decreased significantly (P<0.05) in diabetes associated nephropathy compared to nephropathy only. In conclusion,
**Keywords:** Diabetes, nephropathy, glycosylated haemoglobin and creatinine.

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**INTRODUCTION**

Diabetes mellitus is a major health problem worldwide. It is a serious debilitating and deadly disease that has now reached epidemic proportion and the prevalence rates are expected to go even higher in the future. Diabetic patients may reach End Stage Renal Disease (ESRD) if diabetes mellitus is not treated early and adequately. In most countries diabetic nephropathy has become the single most frequent cause of ESRD and should undergo hemodialysis. The ESRD requires Renal Replacement Therapy (RRT), is one of the most serious complication of diabetes mellitus [1]. Recent data suggests that a number of patients suffering from diabetes nephropathy and ESRD who are admitted to dialysis units are increasing dramatically. The incidence of reported ESRD was 4.3% with type-1 diabetes mellitus and 4.5% with type-2 diabetes mellitus.

The principal pathological feature of diabetic nephropathy occurs in the glomerulus. Glomerular enlargement occurs due to basement membrane accumulation and mesangial expansion [2]. It has been suggested that during diabetes, the volume of the whole kidney and of individual glomerulus is increased at the time of diagnosis [3]. Basement membrane thickening occurs within 2 years of diagnosis and it has been recognized as a pathological hallmark of diabetes [4]. Further, it has been accepted that the high level of micro-albuminuria is associated with fractional mesangial expansion, leads to diabetic nephropathy [5]. Early microalbuminuria is due to an increase in transglomerular pressure and decreased in glomerular filtration rate (GFR; [6]).

The Glycosylated haemoglobin (HbA1c) is widely accepted and used as the most reliable test for assessing chronic glycemia [7]. The HbA1c reflects overall blood glucose levels over a period of 2-3 months and further, used to monitor diabetic treatment. It has been recognized that the HbA1c as an essential adjunct to regular self-blood glucose measurement assisting in the achievement of the best possible glycemic control. The major use of the HbA1c assay is to assess changes in metabolic control that follow an alteration in treatment. Moreover, diabetes treatment is adjusted based on the HbA1c result, expressed as the percentage of haemoglobin that is glycated. The HbA1c does not require fasting blood sample and it is not affected by recent meals [8].

Therefore, in the present investigation the effect of the HbA1c in diabetes associated nephropathy is proposed to explore in Chhattisgarh population.

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**MATERIALS AND METHODS**

**1.1. Subjects:**
Subjects with both sex were belonging to different age groups (30 to 70 years), different economic groups (upper, middle and lower), different dwellings (urban, semi-urban, rural),
different occupations (professionals, farmers, businessmen and students), from the patients of Chhattisgarh population those who are suffering from the Type-2 Diabetes mellitus. Sample collection was normally carried out during the working hours i.e. in between 8.00 am to 5.30 pm. every day. A consent letter has been taken from all the subjects and the experiment is approved by Institution Ethics Review Board, Chhattisgarh Institute of Medical Science, Bilaspur, India.

1.2. Chemicals and Reagents:
All chemicals and reagents of Excellar quality of Roche diagnosis Ltd., (Germany and USA), Randox (UK), Bayer & Accurex (India) have been used for various chemical analyses and estimations.

1.3. Experimental Design:
The study was undertaken in 160 diabetic patients during 2009-10. The experiment was carried out in four different groups and was divided into control (CON), diabetes (DM), nephropathy (NP) and diabetes associated nephropathy (DM+NP) groups. The overall mean values have been determined for 20 diabetic patients in the period of two years including 20 control patients values were subjected for statistical analysis.

1.4. Sample Collection:
The blood samples were collected in the morning on fasting (8-12 hrs fasting after their dinner the previous night) and post-prandial (1.5 hrs. to 2 hrs after lunch). The same procedure was followed for each patient on his/her every visit. The Hb1Ac was estimated in EDTA anti-coagulated specimen, as it has to be done in the whole blood with preparation of haemolysate sample, while other parameters were estimated in serum or plasma samples. Special care was taken during sample collection from different patients to maintain and keep up time.

1.5. Biochemical estimation:
The fasting and post prandial blood sugar, urea and creatinine were estimated by following end point colorimetric assay method [9] and the Hb1Ac was estimated by following Turbidometric inhibition Immuno assay method [10]. All the readings were taken by using Hitachi-912 fully automatic chemical analyzer.

1.6. Statistical analysis:
The results are expressed as Mean ± S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by Post-hoc Student Newman Keuls test. P < 0.05 was considered to be statistically significant.

RESULT

3.1. Effect on fasting and post-prandial blood sugar levels:
The effect of fasting blood sugar level is illustrated in Fig-1 (A). Statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (3, 76) = 2.68, P<0.05]. Post hoc analysis by Student Newmann keuls test revealed that diabetes (DM) and diabetes associated nephropathy (DM+NP) groups were significantly increased fasting blood sugar levels.

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and no significant change in fasting blood sugar levels was observed in nephropathy (NP) group compared to control. Further, there was significant decrease in fasting blood sugar in NP and was found to be no significant change in fasting blood sugar levels in DM+NP compared to DM, indicating that nephropathy has no role in the levels of fasting blood sugar level. Furthermore, DM+NP showed significant increase in fasting blood sugar levels compared to NP.

Fig-1 (B) depicts the effect on post-prandial blood sugar levels. Statistical analysis by One way ANOVA revealed that there was significant difference among groups \( F(3, 76) = 2.31, P<0.05 \). Post hoc analysis by Student Newmann keuls test revealed that DM and DM+NP groups were significantly increased in post-prandial blood sugar levels and no significant change in post-prandial blood sugar levels was observed in nephropathy (NP) group compared to control. Further, there was significant decrease in post-prandial blood sugar levels in NP and was found to be no significant change in post-prandial blood sugar levels in DM+NP compared to DM, indicating that nephropathy has no role in the levels of post prandial blood sugar levels which is similar to the effect of fasting blood sugar levels. Furthermore, DM+NP showed significant increase in post-prandial blood sugar levels compared to NP.

![Graph](image)

Fig. 1. The effect on fasting (A) and post prandial (B) blood sugar level is illustrated in control, DM, NP and DM+NP. All values are Mean±SEM. *P<0.05 compared to control, @P<0.05 compared to DM and #P<0.05 compared to NP [One-way ANOVA followed by Student Newmann keuls test].
3.2. Effect on glycosylated hemoglobin (Hb1Ac) level in blood:
The effect of Hb1Ac levels is illustrated in Fig-2. Statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (3, 76) = 2.58, P<0.05]. Post hoc analysis by Student Newmann keuls test revealed that DM and DM+NP groups were significantly increased in Hb1Ac levels and no significant change in Hb1Ac levels was observed in NP group compared to control. Further, there was significant decrease in Hb1Ac levels in NP and was found to be no significant change in Hb1Ac levels in DM+NP compared to DM, indicating that nephropathy has no role in the levels of Hb1Ac. Furthermore, DM+NP showed significant increase in Hb1Ac levels compared to NP.

Fig. 2. The effect on glycosylated haemoglobin (Hb1Ac) level is illustrated in control, DM, NP and DM+NP. All values are Mean±SEM. *P<0.05 compared to control, @P<0.05 compared to DM and #P<0.05 compared to NP [One-way ANOVA followed by Student Newmann keuls test].

3.3. Effect on urea and creatinine level in blood:
Fig-3 (A) depicts the effect on urea levels. Statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (3, 76) = 3.88, P<0.05]. Post hoc analysis by Student Newmann keuls test revealed that NP and DM+NP showed significant increase in urea level in blood however, DM group did not show any change in blood urea level compared to control. Further, there was significant increase in blood urea level in NP and DM+NP group compared to DM. Moreover, there was no significant change in blood urea level in DM+NP compared to NP.
Fig. 3. The effect on urea (A) and creatinine (B) level is illustrated in control, DM, NP and DM+NP. All values are Mean±SEM. *P<0.05 compared to control, ‡P<0.05 compared to DM and §P<0.05 compared to NP [One-way ANOVA followed by Student Newmann Keuls test].

The blood creatinine level is depicted in Fig-3 (B). Statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (3, 76) = 3.08, P<0.05]. Post hoc analysis by Student Newmann Keuls test revealed that NP and DM+NP showed significant increase in creatinine level in blood however, DM group did not show any change in blood creatinine level compared to control. Further, there was significant increase in blood creatinine level in NP and DM+NP group compared to DM. Moreover, there was significant decrease in blood creatinine level in DM+NP compared to NP.

DISCUSSION

In the present investigation, diabetes associated nephropathy (DM+NP) showed significant elevated levels of HbA1c in blood. The present study gains critical importance as HbA1c is an important tool in clinical investigation and would guide in the pathogenesis of DM+NP.

It has been observed from the present investigation that there was a strong relationship between fasting blood sugar level, post-prandial blood sugar level and HbA1c level in diabetic patients.
Similarly, it has been reported that the blood glucose and HbA1c levels considerably increase in diabetic patients [11]. The findings of the current study are in agreement with who has reported elevated levels of HbA1c in diabetes [12]. The diabetes is one of the leading causes of renal failure. The patients suffering from diabetes mellitus are prone to develop diabetic nephropathy which implicates the microvascular complications in diabetes involving Kidney, Nerves, and Blood vessels. The nephropathy is common in diabetic patients and usually associated with vascular complications. High systemic blood pressure and increased cholesterol, both are associated with a rapid rate of renal disease. Diabetic patients with nephropathy are at high risk of death. The risk of diabetes associated nephropathy is similar in both type-1 and type-2 diabetes. The long-term complications of diabetes have major consequences for individual and health care providers. The good glycemic control is more potent factor and is being assessed by the measurement of glycosylated haemoglobin. This assay plays central roles in diabetic management, patients clinical guidance etc. The blood glucose was considered as a prime test for optimizing treatment of diabetes mellitus. But the HbA1c determination is the new better method to monitor the long term glucose control irrespective of glucose measurement for patient management. It would prevent or delay the further diabetic complications. Diabetic patients with oral hypoglycemic therapy should go for HbA1c test as recommended by American Diabetes Association.

Present findings suggested that there was significant elevation in urea and creatinine levels in DM+NP and the findings were similar to that of previous reports [13, 14]. It could be due to family history of cardiovascular disease and pre-disposition and this factor would increase the risk of nephropathy in diabetics. Hyperglycemia causes renal damage by several mechanisms including non-enzymatic glycation of protein, activation of hexosamine and increased intracellular reactive oxygen species. Also other factors such as increased glomerular capillary pressure and proteinuria itself play an important role in the pathogenesis of kidney disease in diabetes.

CONCLUSIONS

The present findings suggested that glycosylated haemoglobin along with creatinine level would be an important tool in the pathobiogenesis and management of diabetes associated nephropathy. An effective self-management can reduce the microvascular complications in type-2 diabetes. Life style measures can also make an important contribution to the control of diabetes with nephropathy.

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