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Original Article

A comparative analysis of fructosamine with other risk factors for kidney dysfunction in diabetic patients with or without chronic kidney disease

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ABSTRACT

Background: Hyperglycemia is the driving force for the development of diabetic nephropathy leading to the end stage renal disease. It is well known that in hyperglycaemic condition, serum proteins become glycated through non-enzymatic glycation. With the other risk factors, serum fructosamine may be an important risk factor for kidney impairment. To assess coexistence of frequently documented risk factors of kidney dysfunction with serum fructosamine in diabetic patients with chronic kidney disease (CKD).
Methods: In this study, total 150 individuals, as control, type2 diabetic patients without complication and with CKD were included. Blood samples were collected from all the samples to estimate blood glucose, HbA1c, serum creatinine, fructosamine levels and lipid profile. Statistical analysis i.e. regression and correlation between serum fructosamine and other documented risk factors for diabetic CKD has been done. $P < 0.001$ was considered significant.
Results: Serum fructosamine, HbA1c, creatinine levels, cholesterol and LDL were increased significantly ($P < 0.001$) in diabetic patients with CKD compared to without complications. Systolic and diastolic blood pressure and BMI were also significantly higher in diabetic patients compared to control. Serum creatinine, total cholesterol and LDL showed a significant positive correlation but HDL showed a negative correlation with fructosamine in CKD diabetic patients. No significant correlation was found with any risk factors in diabetic patients without complications expect HbA1c.
Conclusion: It is concluded that elevated serum fructosamine level is strongly associated with kidney dysfunction in diabetic patients. As there is a significant link between serum fructosamine and other risk factors for CKD diabetic patients.

1. Introduction

Diabetes has now become the most common single cause of diabetic nephropathy and about 40% of diabetic patients develop nephropathy [1]. In generic term diabetes nephropathy is referred to any deleterious effect on kidney structure and/or function caused by diabetes mellitus. Hyperglycaemia is the driving force in the development of diabetic complications, although the probable mechanism of how it causes is not clear. Nevertheless, non-enzymatic glycation has implicated excess protein glycation as a mechanistic link between hyperglycemia and the pathogenesis of diabetic nephropathy [2–6]. Chronic hyperglycemia leads to accelerate the non-enzymatic glycation in which glucose combines with many circulating and tissue proteins and formed an early stage stable Amadori product (Fructosamine) [7]. These products undergo further irreversible complex reactions to form advanced glycation end products (AGEs). Accumulation of Amadori as well as AGEs in tissues of diabetic patients, particularly with secondary complications like nephropathy, retinopathy and atherosclerosis are accompanied by increased free radical activity that contributes to the biomolecular damage [8]. Diabetic nephropathy is a metabolic disorder and several risk factors probably have been associated with this metabolic syndrome, including hypertension, poor glycemic control, central obesity, dyslipidemia and other metabolic glycated by products. Serum fructosamine is also a potential risk

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factor for the development of macro and micro vascular complications of diabetes.

As in general term fructosamine originally introduced for all glycated serum proteins that have become stable ketoamines. It has the ability to act as reducing agents in alkaline solution which can be measured by NBT (nitro blue tetrazolium) assay [9]. Fructosamine is not affected by any haemoglobin metabolism disorder but in protein turnover. Fructosamine concentration mainly depends on the concentration of each serum protein because it is generated from non-enzymatic glycation of mainly albumin (~90%) and various proteins [10].

This correlation based study was designed to investigate the significance of serum fructosamine in the development of chronic kidney disease in diabetic patients and to associate clinical relationship of serum fructosamine with other risk factors of diabetic nephropathy.

2. Ethical approval

The present study was approved by institutional ethical committee, Aligarh Muslim University, Aligarh. All patients were informed before the procedures and signed consents has been received.

3. Material and methods

3.1. Material

Human serum albumin (HSA) was purchased from the Sigma Chemical Company (St. Louis, USA). Nitroblue tetrazolium (NBT) and D-glucose were obtained from SRL, India. Polystyrene microtitre flat bottom ELISA modules were purchased from NUNC, Denmark. Creatinine kit was obtained from Avantor performance material India Limited, Dehradun. HbA_1C kit was purchased from BioRad, USA. Vacutainers were obtained from BD bioscience. All other reagents and chemicals used were of the highest analytical grade available.

3.2. Study design

A total of 150 previously diagnosed type 2 diabetic patients (50 with and 50 without CKD and 50 healthy subjects) attending/admitted in Rajiv Gandhi Center for Diabetes and Endocrinology, JN Medical College, Aligarh Muslim University, Aligarh were enrolled in this study. The selection criteria was based on American Diabetic Association 2010 [11]. In the present study, written informed consent was provided to all patients before participation.

3.2.1. Inclusion criteria

- Only T2DM patients aged 25–75 years.
- Hypertensive.
- Diabetes patients with CKD (stage 2–4).

3.2.2. Exclusion criteria

- T1DM & Gestational diabetes
- Mental illness
- Pregnant females
- Inflammatory or infectious diseases.
- Autoimmune and rheumatic diseases, cancer, haematological diseases.

50 individuals with no known history of hyperglycemia and renal disease were included as healthy subjects. A previously structured questionnaire was used to record the demographic features of all subjects. Blood pressure was measured with a standard mercury sphygmomanometer. Body weight and body height was measured and calculated BMI using the formula:

$$Body\ Mass\ Index(BMI) = Weight\ in\ kg/height\left(m^2\right)$$

3.3. Biochemical analysis

Serum fructosamine was estimated by nitro blue tetrazolium assay (NBT). Samples for A1C analysis were placed in EDTA tubes and estimated by HPLC Method. Fasting and postprandial blood glucose level were measured by glucose oxidase peroxidase enzymatic method. Serum creatinine was measured by the Jaffe's manners method. Serum triglycerides, HDL cholesterol, total cholesterol were measured by enzokits, Ranbaxy diagnostics and cholesterol reagent set supplied by Pointe Scientific Inc., MI, USA. Serum LDL cholesterol concentration was calculated indirectly by using Friedwalds equation:

$$\mathbf{LDL} = \frac{\mathbf{total\ cholesterol} - \mathbf{HDL\ conc} - \mathbf{triglycerides}}{5}$$

3.4. Statistical analysis

All results are presented as mean ± SD deviation. Statistical significance and difference from control and test values were evaluated by Student's *t*-test. P values < 0.001 was considered significant. Correlation coefficient was calculated by simple regression analysis. A scattergram was plotted by taking independent variable (Fructosamine) on the x-axis and y axis for the dependent variables.

4. Results

Fig. 1 represents all the risk factors showing a relation with serum fructosamine. There are no significance difference between the diabetic patients without CKD and healthy subjects. The prevalence of diabetic retinopathy with nephropathy patients was more frequent (82%) and in diabetic patients without complications it was (26%). Although diabetic patients without complications have

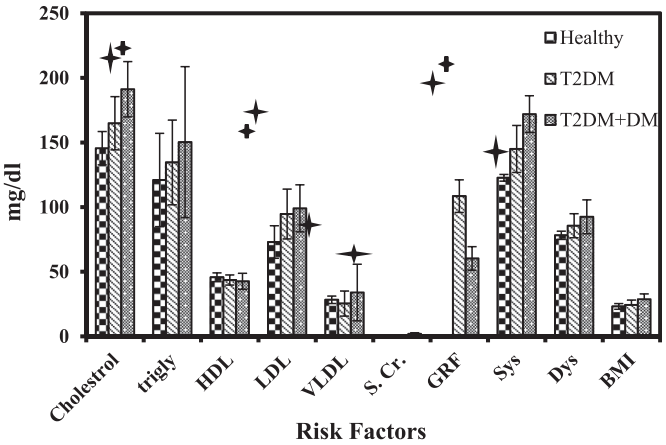

Fig. 1. Risk factors in patients with diabetes mellitus with or without nephropathy and controls individuals. All values are in mean ± SD deviation. p < 0.001 are significant compared to healthy subjects.

also high blood pressure (45.7%) but it was much higher in diabetic nephropathy patients, about 97.1% nephropathy patients had hypertension in our study. Systolic and diastolic blood pressure were raised significantly in ($p < 0.001$) in diabetic patients with or without nephropathy. Table 1 summarizes the glucose monitor parameters of three groups. As expected, the mean fasting, postprandial plasma glucose and A1C levels were higher in diabetic groups with or without nephropathy than in control group. These higher glucose levels corresponded to higher plasma fructosamine in both diabetic groups. Patients with diabetic nephropathy had higher serum fructosamine than diabetes without complication and controls (382.4 ± 60.3, 315.4 ± 35.3, 248.6 ± 24.5, $p < 0.001$) respectively. The high concentration of fructosamine in serum has a major role to play in secondary humoral response, which may associate with inducing the diabetic complication such as nephropathy. Blood plasma glucose (FPG, PP) and A1C were increased significantly ($P < 0.001$) in both diabetic and diabetic nephropathy patients as compared to control subjects. This elevation was higher in diabetic nephropathy patients compared to those without nephropathy. In diabetic nephropathy patients, serum creatinine levels and BMI were significantly increased ($P < 0.001$) compared to control subjects but no significant elevation was observed for both (serum creatinine and BMI) in diabetic patients without nephropathy compared to control shown in Fig. 1.

A regression and correlation analysis for all three groups was also performed, values are shown in Table 2. Regression and Pearson correlation analysis showed a positive correlation between serum fructosamine and serum creatinine ($P = 0.00000508$, $R = 0.595$) in diabetic nephropathy patients. A1C ($R = 0.90$, $P = 6.49$), Systolic ($P = 0.608$ $R = 0.0744$) and diastolic ($P = 0.518$, $R = 0.0936$) blood pressures and GFR ($P = 0.503$, $R = 0.097$) were also positively correlated with serum fructosamine in diabetic nephropathy. Our study showed a negative correlation between fructosamine and BMI ($P = 0.292$, $R = -0.152$), which is insignificant statistically (Table 2). A correlation was also observed between serum fructosamine and lipid profile: Cholesterol ($P = 0.035$, $R = 0.298$), triglycerides ($P = 0.06$ $R = 0.265$) and LDL ($P = 0.0003$ $R = 0.488$) have positive correlation. HDL ($P = 0.055$ $R = -0.273$) has negative correlation with fructosamine within nephropathy group. No significant correlation between serum fructosamine with serum creatinine, systolic and diastolic blood pressure, BMI and lipid profile was observed in diabetic patients without nephropathy (Table 2). But a significant correlation was observed fructosamine with A1C in without complications patients. The linear regression analysis showed positive linear correlation of serum fructosamine with glycosylated haemoglobin and serum creatinine in type2 DM with nephropathy as shown in Fig. 2 and Fig. 3. T2DM with and without nephropathy showed linear correlation of glycosylated A1C with fructosamine ($R^2 = 0.809, 0.305$) respectively, whereas the control group showed no linear correlation between them. The higher R^2 values are revealing that higher level of fructosamine is more closely related to A1C and serum creatinine. Total cholesterol (Fig. 4), triglyceride (Fig. 5) and LDL (Fig. 6) were not found to be linearly correlated with fructosamine. The serum fructosamine was also found to be inversely correlated with HDL in diabetic patients without complication and diabetic patients with nephropathy ($R^2 = 0.093$, 0.074) that is shown in Fig. 7.

Table 1
Different Sugar monitors parameters in diabetic patients with or without CKD and healthy subjects.

Sugar parameters	Control	T2DM	T2DM + CKD
FB	86.98 ± 11.36	$128.52 \pm 44.01^*$	$131.88 \pm 44.58^*$
PG	122.94 ± 11.4	$183.64 \pm 52.56^*$	$212.76 \pm 73.66^*$
HbA1C	4.89 ± 0.66	$7.04 \pm 0.92^*$	$9.8 \pm 2.5^*$
Fructosamine	255.86 ± 27.47	$315.42 \pm 35.3^*$	$395.28 \pm 74.82^*$

All results are in mean ± SD. $P < 0.001$. FB= Fasting blood glucose, PG = postparendial blood glucose.

Table 2
Correlation between Serum fructosamine and other risk factors in type 2 diabetic patients with and without CKD.

Parameters	T2DM	T2DM + CKD
BMI	$P = 0.358$ $R = -0.133$	$P = 0.292$ $R = -0.152$
S. creatinine	$P = 0.077$ $R = 0.252$	$P = 0.00000508$ $R = 0.595$
Sys. BP	$P = 0.678$ $R = 0.060$	$P = 0.608$ $R = 0.0744$
Dys BP	$P = 0.956$ $R = -0.0079$	$P = 0.518$ $R = 0.0936$
GFR	$P = 0.245$ $R = -0.168$	$P = 0.503$ $R = 0.097$
HbA1C	$P = 0.0000314$ $R = 0.553$	$P = 6.49$ $R = 0.900$
HDL	$P = 0.030$ $R = -0.306$	$P = 0.055$ $R = -0.273$
LDL	$P = 0.087$ $R = 0.244$	$P = 0.0003$ $R = 0.488$
TC	$P = 0.325$ $R = 0.142$	$P = 0.035$ $R = 0.298$
TG	$P = 0.0892$ $R = 0.243$	$P = 0.06$ $R = 0.265$

P is significant when $p < 0.001$.
HDL= High density lipoprotein; LDL = Low density lipoprotein; TC = Tricholestrol; TG = Triglyceride; GFR = Glomerular filtration rate.

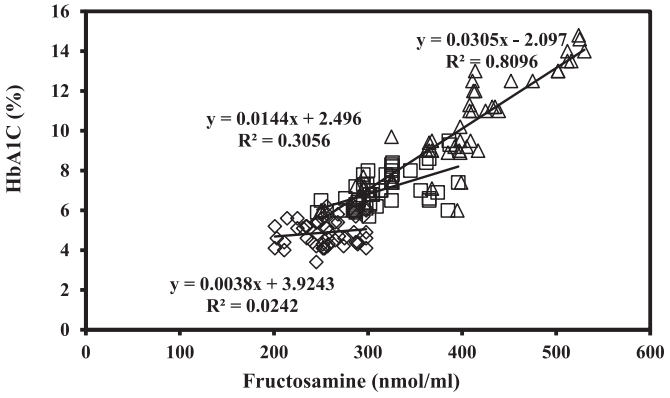

Fig. 2. Correlation between serum fructosamine and A1C in diabetic patients with CKD ( ), without CKD ( ) and healthy subjects ( ).

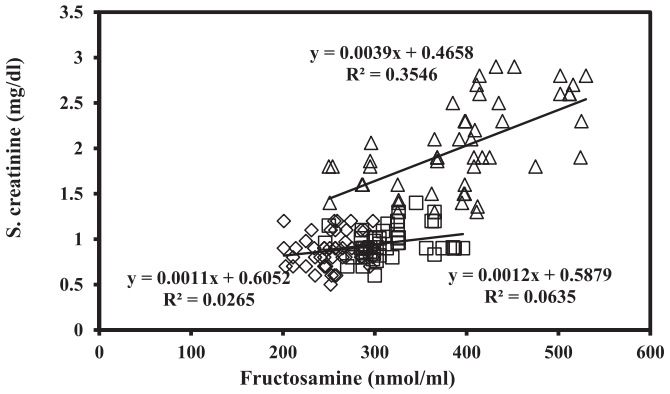

Fig. 3. Correlation between serum fructosamine and Serum creatinine in diabetic patients with CKD ( ), without CKD ( ) and healthy subjects ( ).

5. Discussion

This study revealed the increase in serum fructosamine in diabetic patients with the progression of complications such as nephropathy. We also observed that serum fructosamine

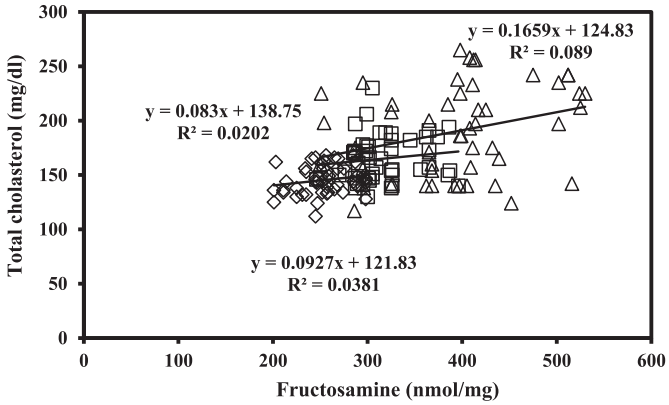

Fig. 4. Correlation between serum fructosamine and total cholesterol in diabetic patients with CKD ( ), without CKD ( ) and healthy subjects ( ).

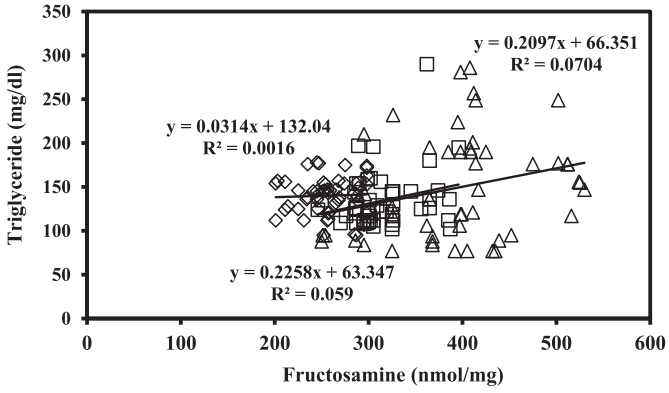

Fig. 5. Correlation between serum fructosamine and triglyceride in diabetic patients with CKD ( ), without CKD ( ) and healthy subjects ( ).

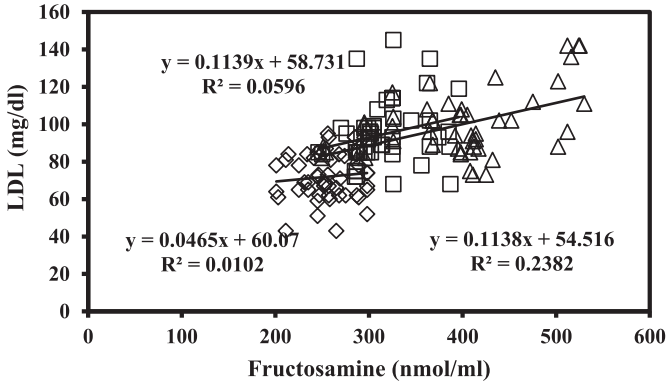

Fig. 6. Correlation between serum fructosamine and LDL in diabetic patients with CKD ( ), without CKD ( ) and healthy subjects ( ).

concentrations were significantly associated with several known diabetic nephropathy related risk factors such as glycemic control (A1C), renal dysfunction (S. creatinine), hypertension for the development of diabetic micro and macrovascular complications. Our results are consistent in accordance with recent studies in the same area [12,13]. The need for early predictors of diabetic vascular complications, such as nephropathy, has recently been reviewed [14]. If serum fructosamine increases before microangiopathy develops, it may be an early signal of processes such as hypercytokinemia that cause, or drastically increase, the risk of renal

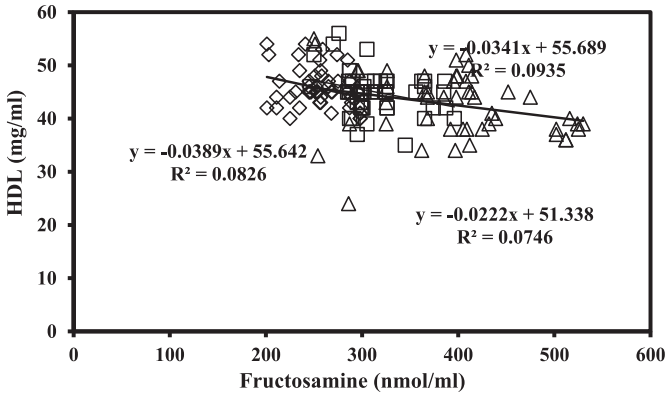

Fig. 7. Correlation between serum fructosamine and HDL in diabetic patients with CKD ( ), without CKD ( ) and healthy subject ( ).

failure. In conclusion, the present study suggests that increased serum fructosamine levels are strongly associated with the development of diabetic nephropathy. The markers of glycemic control (blood glucose and A1C) and renal insufficiency (serum urea and creatinine), hypertension and obesity are clinically correlated with increasing concentration of fructosamine. Diabetic nephropathy is also associated with high blood pressure, which is known to damage the renal function [15]. We have also found the high blood pressure in our nephropathy patients. Another important independent risk factor is hypercholesterolaemia for diabetic nephropathy which deterioration of renal function and mortality in diabetic patients [16]. We found the higher cholesterol and triglycerides levels in diabetic nephropathy patients with respect to control. It has been reported that increase level of plasma triglyceride and cholesterol significantly elevated plasma peroxide levels which may play a causative role in the generation of ROS in diabetes and its complications [17]. This correlation based study have provided adequate evidence that development and progression of diabetic nephropathy can be reduced and prevented through improvement in diabetic control optimization of blood pressure, good glycemic control, reduced protein intake etc. this study is also shown that serum fructosamine was also frequently increases in nephropathy group than the control. So it may be independent risk factor associated with nephropathy with type 2 diabetes. However, fructosamine may simply be a marker at high risk and modification of the degree of serum fructosamine may not influence risk at all. In addition, the presence of other parameters at increasing level may indicate a higher risk of renal disease in type 2 diabetes.

These findings strengthen the hypothesis that an increase in circulating serum fructosamine is an early manifestation of diabetic renal disease. Further research would be of help to clarify the role of fructosamine in the development of diabetic nephropathy. Limitations of our study include a relative inadequate sample size. In future researches are needed to determine fructosamine worth as a robust monitor of glycaemic control and its role in diabetic nephropathy.

Disclosures

The authors have no conflicts of interest related to financial, personal relationships and affiliations related to the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dsx.2018.08.007.

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