Research article

Role of serum free fatty acids in determining severity of diabetic nephropathy: A case control study

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ABSTRACT

Introduction and Aim: The Diabetic Nephropathy (DN) is a result of impaired renal function in Type 2 diabetes. At the onset of diabetes, microvascular complication increases due to accumulation of free fatty acids (FFA) causing renal damage. A study was conducted to estimate the concentration of serum FFA causing severity of diabetic nephropathy.

Materials and Methods: 90 Type 2 diabetic subjects and 30 study controls (age group 35 to 65 years) were selected from the medicine OPD of S N Medical College and HSK Hospital, Bagalkot. Based on the presence of microalbuminuria, the 90 Type 2 diabetic patients were equally divided in to 3 groups, named as stage I to stage III. The serum FFA was estimated by ELISA method in these three groups and control subjects. The statistical analysis was done using SPSS software version 19 utilizing unpaired "t" test for quantitative data and Pearson's correlation tests.

Results: The estimated serum FFA levels in stage I to III was found to be higher and highly significant as compared to control (p=0.001). We find the best cut off value of serum FFA was 4.75 mmol/L, causing severity of diabetic nephropathy. The area under the curve (AUC) is 0.92 with the specificity of 86%, sensitivity 89% and the diagnostic accuracy was found to be of 87%.

Conclusion: Serum free fatty acid levels were higher in diabetic nephropathy subjects, which could be used as diagnostic marker for the severity of renal damage with cut off value of 4.75 mmol/L.

Keywords: Type 2 diabetes; diabetic nephropathy; serum free fatty acids; microalbuminuria.

INTRODUCTION

Diabetes mellitus is the most common disease worldwide. Insulin resistance and insulin deficiency are the most common causes for Type 2 diabetes (1). Long term diabetes causes impairment and dysfunction of organs like eye, kidney, nerves, heart etc. In diabetes the excess glucose binds to circulating free amino acids and tissue proteins by non-enzymatic reaction, produces early glycation products giving rise to advanced glycation end products (AGE's), which causes micro vascular complications (2).

Diabetic nephropathy is characterized by microalbuminuria (excretion of albumin in urine) and loss of glomerular filtration rate (GFR) due to glomerular lesions. In many cases the terminal stage of life in diabetic subjects with diabetic nephropathy is caused due to complete loss of renal function (3). Therefore, early diagnostic markers for monitoring and predicting the development of diabetic nephropathy are needed to protect the renal function and life of individual. Hyperglycemia mainly causes endothelial dysfunction ultimately leading to albumin loss (4). As the insulin inhibits the hormone sensitive lipase, mobilization of free fatty acids from fat depot takes place in diabetes (5).

Many authors have reported the role of free fatty acids in glucose intolerance causing diabetes (6). The increased serum free fatty acids or sustained hyper-free fatty academia causes insulin resistance (IR) in the liver and muscle (7,8). However, relatively other longitudinal epidemiologic studies have shown the relationship between serum FFA levels and incidents of diabetes (9). Non esterified fatty acids (NEFA) also called as Free Fatty Acids (FFA) corresponds to IR and Type 2 diabetes. Insulin level regulates release of free fatty by breakdown of TAG. Insensitivity to insulin by adipose tissue leads to lipid overload in liver and pancreas due to excess FFA, causing development of Type 2 diabetes by impaired functioning of islets of β-cells of pancreas (10).

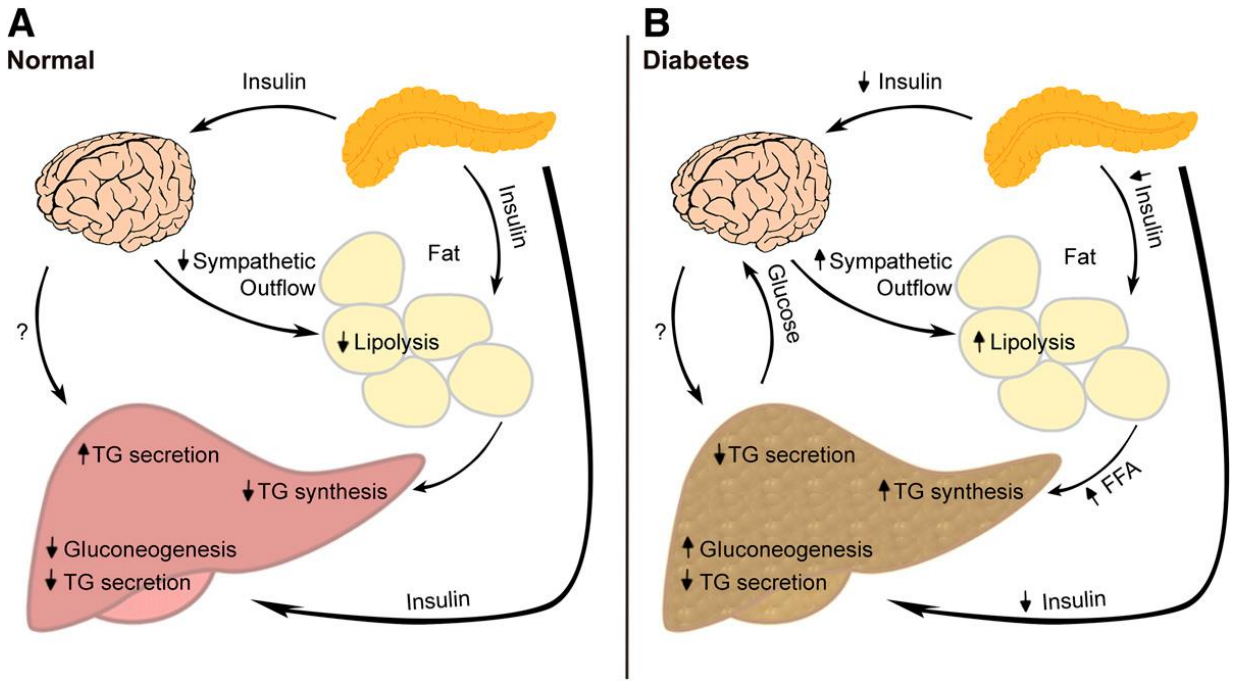

Fig. 1: Showing increased formation of free fatty acids and insulin resistance in diabetes (11)

To correlate the serum free fatty acids (FFA) levels and severity of DN, we estimated serum FFA levels in 3 stages of DN and in the study control subjects, in the present study. With the data analysis, we also tried to find the cut off value of serum FFA concentration responsible to cause severity of diabetic nephropathy.

MATERIALS AND METHODS

The present study was conducted in Medicine and Biochemistry department of S. N. Medical College and HSK Hospital and Research Centre, a tertiary care hospital in Bagalkot, Karnataka, India. Institutional ethics committee approval was taken for the study. Informed consent was obtained from all the study participants.

Ninety type 2 diabetic subjects with the onset of disease for more than 5 years, within the age group of 35-65 years, were divided 30 each and classified in to mild, moderate, and severe (stage I, II and III) diabetic nephropathy based on the presence of microalbuminuria, were selected for the study. 30 Subjects with the same age group not having diabetes were considered as healthy control group. Subjects with the age < 35 years or > 65 years, systemic diseases (hypothyroidism or hyperthyroidism), cardiovascular diseases, pregnancy, malignancy and systemic drug or alcohol abuse were excluded from the study.

To separate the serum and plasma, 5 ml of fasting blood sample was drawn under aseptic conditions and transferred into plane (3 ml) and EDTA coated vacutainer tubes (2 ml), mixed gently and then centrifuged at 3000 rpm for 20 minutes. The separated serum and plasma samples were stored at -20^0 C until assayed for serum glucose, plasma $HbA1_C$ and serum FFA. At the same time, 10 ml of urine sample were collected from the same subjects in a sterile container and assayed within 2 hours for microalbuminuria (12). Serum glucose, (Ba 400 Biosystem), plasma HbA1C (D10 Biorad machine) was estimated using Biosystem kits (13, 14). The serum FFA was estimated by ELISA method (Robonic) using kits of Bioassay Technologies (15).

Sample size calculation was done by open Epi software version 2.3:1, retrospectively with 90% power of the study; the sample size calculated was 28-33. Hence, 30 cases in each group (stage I, II and III) of DN and 30 healthy controls were taken for the study.

The data was analyzed by taking mean$\pm$ SD for age (years), microalbuminuria (mg%), FBS (mg%) $HbA1_C$ (%) and FFA (mmol/L). Statistical analysis was done by using ANOVA, unpaired "t" test for quantitative data and Pearson's correlation tests. The SPSS software version 19 was used for ROC curve analysis, Tests of validity, viz.- sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of serum FFA to find optimum cut off value for severity of Diabetic nephropathy.

RESULTS

The data given in Table 1 shows mean $\pm$ SD of age, microalbuminuria, FBS, $HbA1_C$ and ANOVA f value.

Table 1: Demographic characteristics of cases and controls

	Controls (Mean ± SD)	Cases (Mean ± SD)			ANOVA	
		Stage-I	Stage-II	Stage- III	F value	p value
Age (in years)	51.54 ± 8.79	53.32 ± 8.61	53.93± 8.02	58.85± 5.06	2.378	0.079
Microalbuminuria (mg/dl)	23.31 ± 5.17	34.41 ± 5.92	137.93 ±54.55	118.28 ± 11.67	90.298	**0.001**
FBS (mg%)	98 ±5.89	120.53± 15.64	152.81 ± 18.54	163.96 ±18.65	76.394	**0.001**
HbA1$_C$ (%)	5.09 ± 0.48	6.92 ± 0.68	6.79 ± 0.52	8.32 ± 0.60	97.933	**0.001**

The data given in Table 1 doesn't show any statistical significance for age (p=0.079). Microalbuminuria, FBS, HbA1$_C$ were found greater in all the stages of DN as compared to healthy controls and it was found to be highly significant (p=0.001)

Table 2 shows serum FFA (mmol/L) in control and all the stages of DN, suggests highly significant (p=0.001) when compared to healthy controls.

Table 2: Serum free fatty acid in cases and controls

	Controls (Mean ± SD)	Cases (Mean ± SD)			ANOVA	p value
		Stage-I	Stage- II	Stage-III	F value	
SerumFFA (mmol/L)	0.59 ± 0.27	6.16 ± 1.85	6.68 ± 1.75	7.10 ± 1.40	99.631	**0.001**

The best cut off value for serum FFA (4.75mmol/L) was obtained from ROC curve given in fig. 2 and the sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and AUC of serum FFA is given in Table 3.

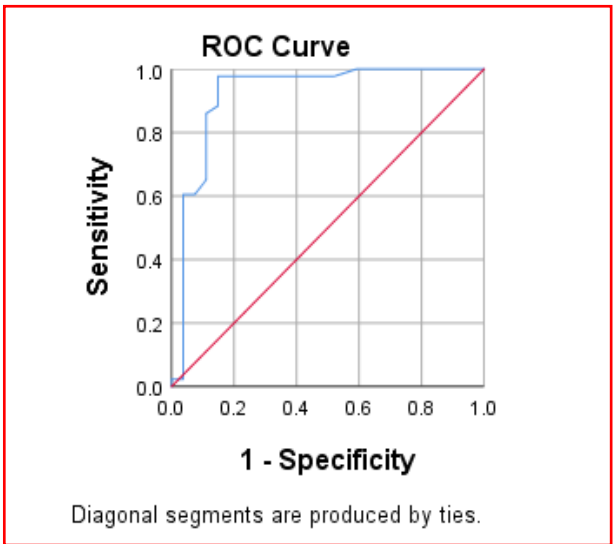

Fig. 2: The best cutoff value of serum free fatty acids for diabetic nephropathy by ROC curve.

Table 3: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy of serum free fatty acids in diabetic nephropathy stages

Serum free fatty acid (mmol/L)	
Sensitivity	89%
Specificity	86%
PPV	80%
NPV	92%
Diagnostic Accuracy	87%
AUC	92%

DISCUSSION

Triglycerides on hydrolysis produce FFA. Many biological processes require FFA as important intermediary metabolite. Free Fatty acids act as important key component of glycolipid and phospholipid in cell structure and function. Energy for cell is provided by fatty acids in between the meals and during starvation. Fatty acid metabolism, when abnormal, leads to conditions like hyperthyroidism, obesity, severe liver dysfunction, insulin resistance and Type 2 DM. The serum lipid and lipoprotein abnormality occur in nephrotic syndrome due to impaired clearance and biosynthetic alterations (16).

In our study, the statistical difference in age group was not found to be significant between cases and healthy controls (p = 0.079), whereas the cases with diabetic nephropathy showed higher levels of microalbuminuria, FBS, HbA1c and serum FFA, when compared to healthy controls (p=0.001) was found to be highly significant.

In previous study carried out by Xin *et al.*, the process of glucose induced insulin secretion by free fatty acid is explained. Free fatty acid levels when elevated, offset of insulin resistance compensates for acutely elevated insulin secretion. So, function of insulin is not only reducing blood sugar but also inhibit breakdown of fat and promotes fat synthesis (16).

In our study there were low levels of serum free fatty acids in healthy controls as compared to DN subjects, the study with similar findings carried out by Zhang *et al.*, showed association of increased levels of FFA and proteinuria that increases the risk for kidney damage, inflammation, oxidative stress, activated

RAS and impairs insulin signal transduction. The effects of nitric oxide synthesis and endothelial programmed cell death are some mechanisms, affecting endothelial dysfunction carried due to increased accumulation of free fatty acids causing renal injury (17).

Xin *et al.*, reported that diabetic person with microalbuminuria had significant increase in fasting blood glucose levels and AGE's. Microalbuminuria is associated with progression end stage renal disease and CVD indicating early clinical marker for diabetic nephropathy (16). In a study by Ninomiya *et al.*, patients with advanced diabetic nephropathy (macroalbuminuric diabetic patients) had higher significant value of serum FFA than fasting blood glucose (18).

In our study we find that the best cut off value of serum FFA (4.75 mmol/L) for severity of diabetic nephropathy. The sensitivity, specificity and diagnostic accuracy was found to be at par with the study reported by Zhang *et al.*, (17). Further studies need to be done with larger sample size for better understanding the role of FFA in early diagnosis of diabetic nephropathy.

CONCLUSION

Serum FFA was found to be increased in all the stages of diabetic nephropathy. Hence, it can be used as an early diagnostic marker to prevent the severity of diabetic nephropathy with the cut off value of 4.75 mmol/L.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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