INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY
Volume: 2: Issue-4: Oct - Dec -2011 IJABPT *ISSN 0976-4550*

STUDY ON MICROALBUMINURIA AND OXIDATIVESTRESS IN DIABETICS

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ABSTRACT

Background

Diabetic nephropathy accounts for about 40% of ESRD. In early stages of diabetic nephropathy there are no clinical signs & symptoms of glomerular changes. The earliest indication of nephropathy is microalbuminuria[1](American diabetes Association).

Advanced Glycation Endproducts in diabetes favorers the Oxidative stress which is implicated in etiology of human diseases.

The present study was undertaken to asses the role of oxidative stress in causing diabetic nephropathy[2] (Josephine M Forbe etal).

Materials & Methods

50 cases of diagnosed diabetic subjects were selected for the present study. Aged 30 – 60 years , both the males & females were included.

Blood samples were collected in fluoride test tubes for estimation of FBS & PPBS. EDTA & heparin blood samples for glutathione & glutathione peroxidase respectively. A fasting urine sample was collected in a sterile container for microalbumin estimation.

Results

Significant increase in the levels of urine microalbumin ($P<0.01$) & Glutathione peroxidase ($P<0.002$) were observed in diabetics compare to healthy controls. Glutathione values were decreased ($P<0.00$).

Conclusion

Lowered glutathione values and elevated glutathione peroxidase values were consistently observed in all the cases indicating the association of oxidative stress in all diabetic patients.

Microalbuminuria is observed in all the patients irrespective of the duration of the illness indicating sub clinical damage of microvasculature probably due to oxidative stress.

Keywords: Diabetes Mellitus, Oxidative stress, Microalbuminuria

INTRODUCTION

Diabetic Nephropathy is a long term microvascular complication of diabetes. The prevalence of complication increases with the duration of the diabetes. It is a major cause of premature death in patients with diabetes. Diabetic nephropathy occurs in about 30% of patients with type 1 diabetes mellitus & 25% of patients type 2diabetes mellitus[3](William J Marshall).

Patients with diabetic nephropathy remains asymptomatic until the GFR falls below 15ml/min or lower. The natural history is of progression to end stage renal failure. The state when conservative measures are no longer sufficient and dialysis or transplantation become necessary to save the patient life[4](William J Marshall). The earliest detectable abnormality is microalbumin. Prediction of diabetic nephropathy can be done by assessing microalbumin which is an earliest clinical evidence of diabetic nephropathy in which repeated appearance of low but above normal levels of albumin(20-200 µg/min or upto 15 mg/L) will be excreted in urine[5](Buranakitjaroen P et al). Hyper glycemia produces increased free radicals like Reactive Oxygen Species & Reactive Nitrogen Species. (Airotdi G et al).

The Reactive Oxygen Species can activate several damaging pathways in diabetes including accelerated formation of AGE products, polyolpathway, hexosmine pathway all of which have been proven to be involved in micro & macro vascular complications. The glycation end products include a variety of protein products whose accumulation alters the structure & function of tissue proteins & stimulates cellular responses. They have been implicated in tissue damage associated with diabetic complications (O.CHAPPEY, C.DOSQUET et al article published online 29th Oct 2003).
Screening of microalbumin and administering antioxidant vitamins can substantially modify the natural history of diabetic renal involvement and possibly reduce the incidence of end stage renal failure[6] (Chiarelli F et al).
The present study was focused on studying the levels of microalbumin and oxidative stress parameters in diabetics which are associated with subsequent development of diabetic nephropathy (Manjunatha goud, Deepa, et.al.

MATERIALS AND METHODS

The study was conducted over a period of six months. The study was done using microalbuminuria test and oxidative stress parameters among the subjects having diabetes. The study includes fifty diabetic subjects admitted in medicine department in Narayana Hospital. They were in the age group of 30 to 60 years. Both the sexes were included.
The data on family history and personal history of diabetes, smoking habits, alcohol consumption and hypertension and treatment history for diabetes were collected through standard questionnaire subjects with renal failure are excluded.
Blood samples were collected after 12 hours of fasting for estimation of FBS and analyzed and blood is collected in EDTA and heparin tubes for the estimation of glutathione and glutathione peroxidase respectively. PPBS sample was collected after two hours of ingestion of food and analyzed.
The blood samples collected in EDTA & heparin tubes were separated by centrifugation and plasma is used for the estimation of glutathione and glutathione peroxidase.
Fasting urine sample is collected in sterilized container and pH of urine is adjusted to 7. Sodium azide preservative 50 µl is added to urine sample for storage upto 1 week. Microalbumin in the urine sample is estimated by using turbidometric method.
25 members working in Narayana Medical College & Hospital having normal fasting blood sugar values & RFT, within the age group of 40 years were taken as control subjects. Both the sexes were included.
The same procedure of sample collection and estimation of FBS, PPBS, microalbuminuria, glutathione, glutathione peroxidase is adopted for control subjects.

1. FBS,PPBS in blood is estimated by GOD, POD method.
2. Microalbumin in urine is estimated by Immunoturbidometry method.
3. Creatinine is estimated by Jaffe's method.
4. Determination of Glutathione is done by DTNB method.
5. Estimation of Glutathione peroxidase is by paglia et al method
6. Estimation of Glucose by Glucose oxidase peroxidase method.

RESULTS

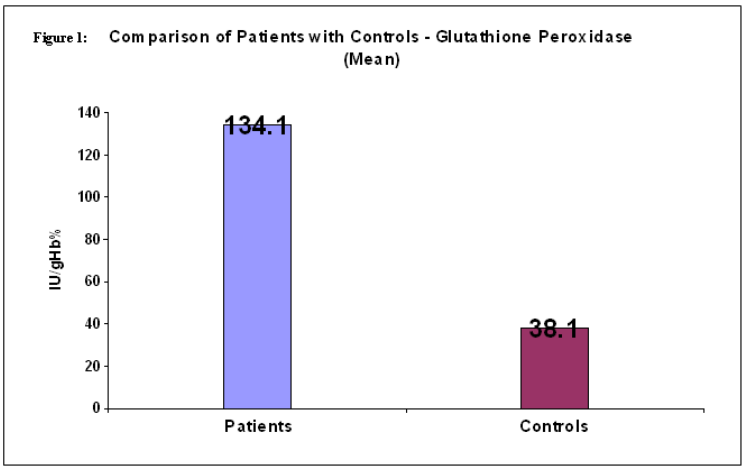
The values obtained on analyzing specimens collected from patients and control groups are tabulated.
The mean values and standard deviation also have been calculated for comparative study of patients and controls.
The values of patients and control groups are also graphically represented for comparison at a glance .
The graphs were plotted using mean values of all the study parameters.

Table 1: Shows the mean, standard deviation and p values of all the study parameters in diabetic and control subjects.

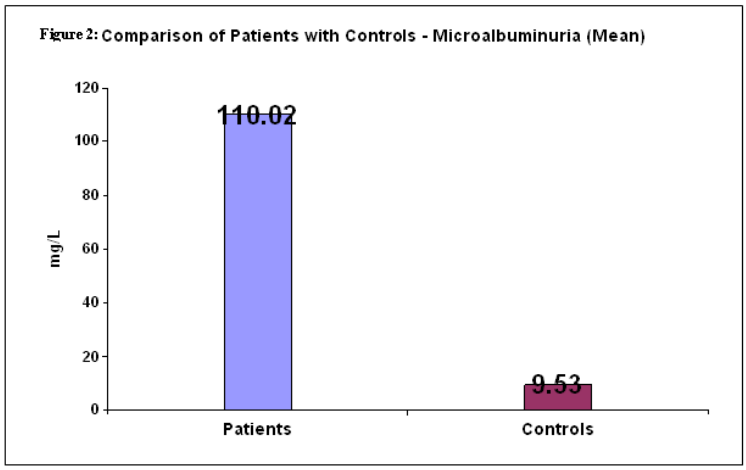
Table 1 showing the comparable values of Patients and Control

Sl. No	Parameter	PATIENTS		CONTROLS		p VALUE	|t| VALUE
		Mean	S.D.	Mean	S.D.		
1	Microalbuminuria	110.2	42.4	9.53	3.63	0.01	16.28
2	Creatinine	2.02	0.43	0.86	0.25	0.01	14.59
3	Glutathione	3.4	1.02	5.98	0.36	0.01	15.8
4	Glutathione Peroxidase	134.1	50.4	38.1	4.8	0.01	13.32
5	PPBS	253.9	41.9	105	11.7	0.01	25.8
6	FBS	203.8	40.8	90.2	9.33	0.01	18.69

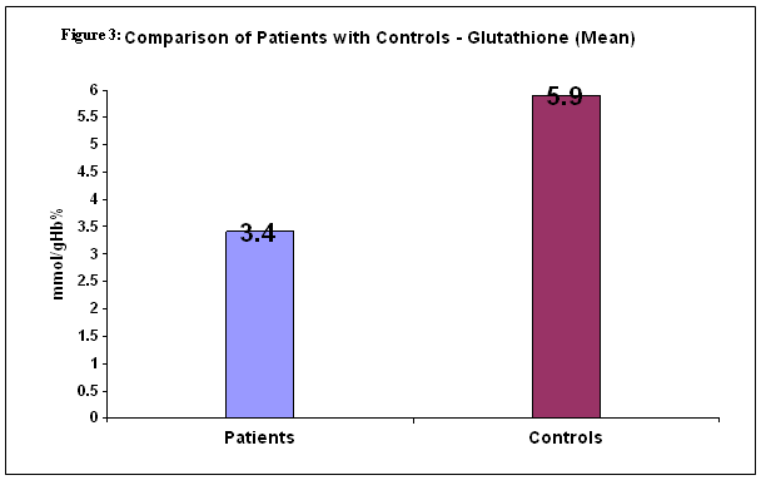
The p value was used to compare cases and controls. P value <0.05 was considered significant.
Levels of reduced glutathione were significantly (<0.00) decreased and glutathione peroxidase values were significantly (<0.02) increased in cases compared to controls and microalbumin levels (<0.001) also shown significant increase in cases.
The values of patients and control groups are also graphically represented for comparison at a glance . The graphs were plotted using mean values of all the study parameters.



Comparision of glutathione peroxidase mean values between patients and controls.
The graph shows Significant elevation of glutathione peroxidase values in diabetic subjects was observed compared to the controls . Increased glutathione peroxidase enzyme activity in diabetic subjects indicates enzyme induction by oxidative stress.



Comparision of microalbumin mean values between patients and controls.
In this graph microalbumin mean value in diabetic subjects is very high compared to the control mean values. The increased value of microalbumin is because of microvascular damage.



Comparision of glutathione mean values between patients and controls.
In this graph Glutathione mean value in diabetic subjects was decreased when compared with the control mean value showing increased utilization of glutathione by the diabetics in clearing of free radicals.

DISCUSSION

This study was undertaken to assess the role of oxidative stress causing nephropathy in diabetes mellitus. The earliest indication of nephropathy is micro albuminuria.
As diabetes advances, especially in ill treated patients, it leads to micro vascular damage causing various complications, one of them being nephropathy (Joseph L et al). In the present study the patients were suffering from DM for the past 5 to 10 years. None of them had clinical manifestations of well developed nephropathy. But almost all of them have shown micro albuminuria varying from 40 to 200 mg/dL (110.2 ± 42.4). It indicates that mild vascular damage starts right from the beginning of diabetes mellitus. As many authors pointed out vascular damage starts with the hyperglycemia and hence it is essential that the patients should be subjected to periodical assessment of renal functions, the best is to test for albumin in urine (micro albuminuria) (Joseph D, kornoz et al).
As per our data microalbumin levels were elevated in all cases compared to the controls. Though micro albuminuria quantitatively seems to raise along with duration of diabetes, it is not proportionate with the duration of the disease. It may be because of so many variables in presentation of the patients, like age, sex, type of management, regularities in treatment, patient's compliance with the treatment etc.
In all the diabetic cases serum creatinine values were not classically elevated to the extent to diagnose renal failure. Though they are elevated in most of the cases to the upper part of normal range, they are not significant as markers for renal function at sub clinical stage Bowers LD, et.al.
Thus it, can be definitely concluded that albumin is excreted in micro quantities in all diabetes patients to some extent. Hence it is a valuable parameter to screen all the diabetic patients periodically for micro albuminuria so that renal damage at the earliest stage can be detected and remedial measures can be instituted.
Reduced glutathione and glutathione peroxidase enzyme activities were measured as markers of oxidative stress. Glutathione is an important antioxidant and its values were reported to be low in diabetes patients with oxidative stress. (Nwose EU et al) Reduced glutathione is a tri peptide with free –SH group and hence it is an antioxidant. In the present study glutathione values were substantially reduced and coincides with earlier reports (3.4 ± 1.2). Sandeep Vijan, Timothy P. Hofer, et.al.

In our study glutathione peroxidase was elevated in all the patients of diabetes mellitus. (Tohru Haminishi , Hiroto Furula et al) It is the enzyme which is glutathione dependant and splits H_2O_2, being the oxidant molecule, to H_2O and oxygen. The elevation of the enzyme is highly significant compared to controls (38.1 ± 4.8). It also correlates well with decreased glutathione values. There are different reports on glutathione peroxidase values and our reports coincide with the earlier reports of elevation of glutathione peroxidase. It is because of the presence of glutathione in invitro analysis of the enzyme. Probably its elevation can be explained by the induction of the activity to combat the effect of oxidative stress caused as evidenced by decreased RBC glutathione levels. Atlit, Keren K et al.

Thus the present study supports the following:

1. All the patients of substantial hyperglycemia are subjected to oxidative stress and various biochemical parameters indicative of oxidative stress are significant.(Ceriello et al)
2. Glutathione levels in RBC were substantially reduced and hence it can be considered as a measure of oxidant damage of vascular endothelium.
3. The extent of oxidant damage can also be assessed by glutathione peroxidase enzyme activity in RBC which is shown to be elevated as a defence molecule against oxidative stress.
4. Vascular endothelium is damaged by oxidative stress which effects kidney and causes renal failure which is a life threatening complication. The damage of vascular endothelium causes albuminuria. Hence test for micro albuminuria periodically in diabetes patients helps to prevent chronic renal failure, if attended at the earliest stage.

Thus it is advisable to measure microalbumin along with creatinine in all diabetes patients & glutathione, glutathione peroxidase as markers of oxidative damage. So that there can be effective monitoring of renal functions & vascular damage.

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