Original Article

Biochemistry

The Role of Biochemical Markers in the Prediction of Microvascular Complications in Type-2 Diabetes Mellitus

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ABSTRACT

The microvasular complications of diabetes encompass long-term complications which affect the small blood vessels. These classically have included retinopathy, neuropathy and nephropathy. The biochemical parameters play a key role in the prediction of microvascular complications in diabetes mellitus. Therefore, the present study was undertaken to investigate the role of biochemical markers in the prediction of microvascular complications in patients with type 2 diabetes mellitus. Fifty type 2 diabetes mellitus patients were studied for their fasting blood sugar, glycosylated haemoglobin, serum creatinine, blood urea nitrogen, lipid profile and microalbuminuria levels and they were also tested for the presence or absence of microvascular complications. The analysis was categorized, based on the presence or absence of the microvascular complications. Thirty non diabetic, healthy subjects were chosen as the control group.

The analysis showed that thirty six subjects had microvascular complications. The incidence and the progression of the microvascular complications increased with hyperglycaemia, a longer duration of diabetes, dyslipidaemia and the presence of microalbuminuria levels in patients with type 2 diabetes mellitus. Therefore, poor glycaemic control, a longer duration of diabetes, dyslipidaemia and the progression of microalbuminuria can predict the microvascular complications in patients with type 2 diabetes mellitus.

Key Words: Type 2 diabetes, Microvascular complications, Glycosylated haemoglobin, Diabetic retinopathy, Diabetic neuropathy, Diabetic nephropathy

INTRODUCTION

Diabetes is caused worldwide due to contributing factors like increasing urbanization, aging populations, increasing obesity, and falling levels of physical activity. In 2000, the number of people with diabetes worldwide was ~ 171 million, with India, China, and the United States having the highest numbers of people with diabetes. [1]. In India, the epidemiological data shows an upward trend in the diabetic population, rising from 33 million in 2000 to 57 million in 2025. [2] Diabetes is a chronic disease which is characterized by hyperglycaemia, with disturbances in carbohydrate, fat, and protein metabolism which results from defects in insulin secretion and/or insulin action. Diabetes can be classified into two major classes : Type 1 diabetes (the classical form of diabetes and these people cannot survive without insulin treatment) and Type 2 diabetes. Like Type 1 diabetes, Type 2 diabetes also involves both genetic susceptibility and environmental factors, although the genetic component may be greater in Type 2 than in Type 1 diabetes. It is caused by a combination of insulin resistance and relative insulin deficiency, with increased hepatic glucose production. It is important to note that some individuals predominantly experience insulin resistance and others experience insulin deficiency. Insulin resistance is generally thought to precede insulin deficiency. [3] Type 2 diabetes is a heterogeneous group of conditions that constitute ~ 90% of the diabetes cases in India. Although Type 2 diabetes is common and tests to screen for and diagnose it are widely available, the disease remains underdiagnosed. [4] Approximately 25% of the people with a new diabetes diagnosis already have microvascular disease, suggesting that they already have had the disease for 4–7 years by the time of the diagnosis. [5-6] In these patients, with earlier disease identification and the intensive treatment of hyperglycaemia, the risk for microvascular complications can be reduced.[7-9] Hence, the present study was undertaken to investigate the role of the biochemical parameters in the prediction of the microvascular complications in Type 2 diabetes patients.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, NRI Medical College and General Hospital, Guntur district, Andhra Pradesh. Fifty Type 2 diabetic patients who visited the out-patient and in-patient departments and 30 healthy individuals who came for general checkup which were considered as the controls were included in the study. The fifty Type 2 diabetic patients were divided into 2 groups based on the duration of diabetes mellitus and the presence and absence of the diabetic complications. The groupwise distribution of the subjects was as follows: Group- I: 30 healthy control subjects; Group-II: 36 Type 2 diabetes mellitus patients with microvascular complications (retinopathy, neuropathy, nephropathy); Group-III: 14 Type 2 diabetes mellitus patients without microvascular complications. All the patients, including the controls, were fully informed about the purpose, the procedures and the hazards of the study. After taking voluntary informed consent, all the subjects were screened for the inclusion criteria (male and female Type 2 diabetes mellitus patients with and without complications, who were aged between 35-65 years). Two types of cases were included in the study, based on the duration of the disease. The duration from the first diagnosis before 5 years and

after 10 years duration from the first diagnosis of diabetes mellitus. The control group included non-diabetic healthy volunteers who were consistent with the patients according to the age and the exclusion criteria (Patients with secondary hyperglycaemic states like hypothyroidism, proteinuric conditions like congestive cardiac failure, renal failure and proven renal diseases, eye disorders before the onset of diabetes mellitus and pregnancy were excluded from the study).

Five millitres of fasting venous blood was collected from all the above-mentioned groups. The samples were centrifuged, separated and stored at 4°C until analysis. The blood samples were analyzed for fasting blood sugar, serum creatinine, blood urea nitrogen and lipid profile. For glycosylated haemoglobin estimation, EDTA blood samples were used.

Fasting blood sugar was investigated by the glucose oxidase method, serum creatinine by Jaffe's method, cholesterol by the oxidase/peroxidase (CHOD–POD) method, triglycerides by the enzymatic GPO–POD method, high density lipoprotein by phosphotungstate precipitation and CHOD–POD, glycosylated haemoglobin (HbA1c) by the cation exchange resin method and microalbumin levels in the urine sample by using the turbilatex method. Retinopathy was tested by using the Direct Fundoscopic Method. Peripheral neuropathy was tested by using the Joint and Position Sense Method.

Statistical analysis was carried out by the Student's 't' test by using microtab-2 software and the *p* values which were < 0.05 were considered as significant.

RESULTS

Diabetes is the leading cause of renal failure and adult blindness in developing countries, which affects the small vessels such as those supplying the retina, nerves and the kidneys. With this background, a case-control study with a total of fifty proven cases of Type 2 diabetes and thirty controls (healthy subjects) were considered to assess the role of biochemical markers in the prediction of the microvascular complications and to describe the correlation between glycosylated haemoglobin and the progression of the complications in Type 2 diabetic patients, with an assessment of the micro-albumin levels in urine. The following findings were observed in this study.

[Table/Fig-1] shows the comparison of the biochemical parameters between the controls (n=30, group I) and the cases with diabetes (n=50, group II and III). There was a significant increase in fasting blood sugar (at the $p<0.001$ level), glycosylated haemoglobin (at the $p<0.001$ level), blood urea nitrogen (at the $p<0.05$ level), microalbumin (at the $p<0.001$ level), total cholesterol (at the $p<0.01$ level) and decreased high density lipoprotein (at the $p<0.05$ level) values in cases with diabetes (group II and III) as compared to those of the controls (group I).

Blood urea nitrogen, serum creatinine and the microalbumin levels were significantly increased in group II as compared to group III [Table/Fig-2]. But there was no significant elevation of the lipid profile levels. [Table/Fig-3] shows the significant difference (at the $p<0.05$ level) between blood urea nitrogen, serum creatinine, total cholesterol and the triglyceride levels in cases with retinopathy as compared to the cases without retinopathy.

Out of the 50 cases (Group II and III) which were studied, 36 cases showed microvascular complications such as retinopathy, neuropathy, and nephropathy. The occurrence of microvascular complications i.e. progression of microalbuminuria, increased in the patients with poor glycaemic control. The glycosylated haemoglobin (HbA1c) levels significantly ($p<0.01$) increased in the microalbuminuric cases as compared to the normoalbuminuric cases [Table/Fig-1].

The incidence and the progression of the microvascular complications increased with a longer duration of diabetes. Twenty five patients with the duration of Type 2 diabetes mellitus from the first

Biochemical parameter	Cases (Group II & III) (n=50)	Controls (Group I) (n=30)	p value
Fasting blood sugar	162.52 ± 68.92	98.43 ± 9.69	<0.001
Glycosylated hemoglobin	8.39 ± 1.59	5.19 ± 0.68	<0.001
Blood urea nitrogen	13.87 ± 4.74	11.49 ± 2.81	<0.05
Serum creatinine	1.16 ± 0.32	1.15 ± 0.23	N.S
Total cholesterol	193.51± 37.75	167.52 ± 28.12	<0.01
Triglycerides	167.92 ± 72.13	159.62 ± 92.15	N.S
High density lipoprotein	38.92 ± 8.12	41.45 ± 7.32	<0.05
Low density lipoprotein	119.50±33.10	107.00±34.80	N.S
Very low density lipoprotein	33.10±14.40	32.20±18.40	N.S
Microalbumin	69.52 ± 59.62	14.97 ± 8.13	<0.001

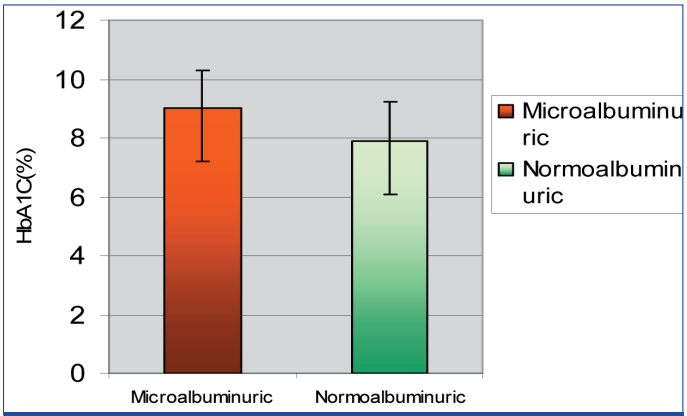
[Table/Fig-1]: Comparison between Biochemical parameters in Cases and Controls.
Values are mean ± SD, values in parenthesis represent sample size, statistical significances between cases with diabetes and controls were evaluated by student's 't' test, $p<0.05$ were considered significant.

Biochemical parameter	Group II (n=36)	Group III (n=14)	p value
Fasting blood sugar	159.32 ± 73.23	170.85 ± 58.12	N.S
Glycosylated hemoglobin	8.37 ± 1.64	8.43 ± 1.61	N.S
Blood urea nitrogen	40.19 ± 8.62	36.64 ± 6.89	<0.001
Serum creatinine	1.21 ± 0.33	1.03 ± 0.17	<0.01
Microalbumin	73.22 ± 59.92	49.93 ± 40.13	<0.01
Total cholesterol	185.0 ± 53.91	167.5 ± 28.12	N.S
Triglycerides	170.12 ± 99.11	132.43 ± 66.63	N.S
High density lipoprotein	36.64 ± 6.89	40.19 ± 8.62	N.S
Low density lipoprotein	109.80±38.70	100.10±21.50	N.S
Very low density lipoprotein	34.40±19.80	26.60±13.40	N.S

[Table/Fig-2]: Comparison of Biochemical parameters between Group II and Group III.
Values are mean ± SD, values in parenthesis represent sample size, statistical significances between cases with diabetes and controls were evaluated by student's 't' test, $p<0.05$ were considered significant.

Biochemical parameter	Cases with retinopathy (n=30)	Cases without retinopathy (n=20)	p value
Blood urea nitrogen	15.25 ± 5.15	12.06 ± 3.70	<0.05
Serum creatinine	1.22 ± 0.34	1.07 ± 0.20	<0.05
Total cholesterol	187.53 ± 57.16	167.79 ± 29.36	<0.05
Triglycerides	174.30 ± 102.79	125.58 ± 71.28	<0.05
High density lipoprotein	37.60±6.56	39.77±9.03	N.S
Low density lipoprotein	112.20±39.30	99.30±25.70	N.S
Very low density lipoprotein	34.60±20.50	28.60±14.50	N.S

[Table/Fig-3]: Comparison of Biochemical parameter between cases with and without retinopathy
Values are mean ± SD, values in parenthesis represent sample size, statistical significances between cases with and without retinopathy were evaluated by student's 't' test, $p<0.05$ were considered significant.



[Table/Fig-4]: The mean Glycosylated hemoglobin (HbA1c) levels in microalbuminuric cases were 9.03 ± 1.31 as compared with normo-albuminuric cases 7.97 ± 1.81. This difference was statistically significant at $p<0.01$.

diagnosis before 5 years and twenty five patients after 10 years of duration from the first diagnosis of diabetes mellitus were studied. Of these, 13 cases had microvascular complications with less than 5 years of diabetes duration, and 23 cases had microvascular complications with more than 10 years of diabetes duration.

DISCUSSION

Diabetes mellitus is a global problem, with approximately 150 million diabetic patients. This chronic condition poses a five times greater risk of developing micro vascular complications, mainly nephropathy and it has become the leading cause of end stage renal disease. NIDDM (Non insulin dependent diabetes mellitus) is a chronic degenerative disease and poses major challenges to the public health [10].

In this study, the role of biochemical markers (such as glycosylated haemoglobin, microalbuminuria, blood urea nitrogen, fasting blood sugar, total cholesterol,triglycerides and high density lipoprotein) were found to be significant in the prediction of the micro vascular complications in diabetic patients. The severity of retinopathy and neuropathy was related to the longer duration of diabetes and the high levels of glycosylated haemoglobin. The incidence of retinopathy was significantly increased with the duration of the diabetes mellitus and it was associated with a poor glycaemic control. Similar results were reported by Gaede et al (1999) [11] and Klein et al (1996) [12].

In the present study, the progression of microalbuminuria was associated with the duration of diabetes and a poor glycaemic control ($p<0.01$). Similar results were observed by Mogensen and Christensen (1984) [13]. In a study by Viberti et al (1982) [14], an increased rate of albumin excretion predicted the impairment of renal function in patients with diabetes. Similar findings were observed by Varghese et al (2001) [15], who reported that the duration of diabetes and retinopathy were the major risk factors for microalbuminuria and that HbA1c was also associated with microalbuminuria, which was consistent with the findings of the present study.

It was observed that the longer duration of diabetes was one of the predictors of the diabetic microvascular complications in the present study. Przegl Lek et al (2002) [16], observed that the most important predictor for all forms of neuropathy was the duration of diabetes. In a study which was carried out by Porta et al (2001) [17] of the EURODLAB Prospective Study Group, the metabolic control and the duration of diabetes were found to be strong indicators of the progression to proliferative retinopathy.

In the present study, elevated levels of total cholesterol and triglycerides and decreased HDL levels were observed in the cases with complications. This was also observed in the studies which were carried out by Tuttle et al (1999) [18] and Ravid et al (1998) [19].

Thus, this study concluded that poor glycaemic control, a longer duration of diabetes, dyslipidaemia and the progression of micro-albuminuria can predict the microvascular complications in patients with Type 2 diabetes mellitus.

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