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Research Article

# PHYSIOLOGICAL AND BIOCHEMICAL MARKERS TO ASSESS THE RISK FACTORS FOR DIABETIC NEPHROPATHY: A RETROSPECTIVE RURAL HOSPITAL BASED CASE CONTROL STUDY

Raja Reddy P[1]*, Shashidhar KN[2], Karthiyanee Kutty[3] and Lakshmaiah V[4]

[1]Assistant professor, Department of Physiology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

[2]Professor, Department of Bio-chemistry, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

[3]Professor, Department of Physiology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

[4]Professor, Department of Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

***Correspondence**

Raja Reddy P

Assistant professor, Department of Physiology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka, India

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**ABSTRACT**

Diabetic nephropathy (DN) is a major contributor to chronic kidney diseases globally as well as in India andis associated with increased cardiovascular risk. The aim of this study is to estimate $HbA_1c$, Fasting Insulin, total lipid profile, renal parameters and anthropometric parameters in assessing the most common complications of Diabetic Nephropathy. Two hundred fifty type 2 diabetes mellitus and non-diabetes subjects were studied for their anthropometric and biochemical parameters. Independent paired t-test was used to compare mean differences between parameters and p-value $< 0.05$ was considered significant. Diabetic patients had higher BMI, WHR and higher insulin levels compared to non-diabetics. Significant relation was observed between $HbA_1c$ and HDL-C in diabetic patients. Higher values of fasting insulin, $HbA_1c$, lipid profile with anthropometric measurements and other biochemical parameters were significantly associated with commonest conflict of diabetic nephropathy. It further predicts CVD mortality in patients with diabetic nephropathy. Early screening for incipient diabetic nephropathy and aggressive management of these risk factors is important in optimizing the renal outcome of patients with diabetes mellitus. These results indicate that the risk of developing diabetes is certainly higher in non-diabetes subjects.

**Keywords:** Type 2 diabetes, Diabetic Nephropathy, Fasting Insulin, $HbA_1c$.

## INTRODUCTION

The present era has the most diabetogenic environment seen so far in the human history.[1] From the Indian point of view; there has been an alarming rise in the prevalence of diabetes, which has gone beyond epidemic form to a pandemic one. It was estimated to be 40.9 million in the year 2007 and is expected to increase to 69.9 million by the year 2025.[2] India presently has the largest number of diabetics and is being called the diabetic capital of the World. It is estimated that very soon every fifth person with diabetes will be an Indian. Diabetic nephropathy (DN) is a major contributor to chronic kidney diseases globally as well as in India. However, not all individuals with diabetes develop DN to a similar extent and within a similar duration of time. As was summarized by Ritz and Orth,[3] the risk factors associated with the development of DN in the diabetic population include advanced age, non – Caucasian race, male gender, and poor glycemic, lipid and blood pressure control in the diabetic individuals. Though the risk factors and possible associations have been extensively studied, the pathophysiology of DN is yet to be understood completely. Kidney, being one of the target organs for action of insulin, undergoes flow and pressure changes at glomerular level due to chronic hyperglycemia ultimately resulting in Diabetic Nephropathy.[4] Glycated hemoglobin ($HbA_1c$) is a well-known marker for long-term glycemic control. It indicates mean blood glucose levels and predicts the risk for developing complications in diabetic population.[5] Along with dyslipidemia, elevated $HbA_1c$ was regarded as risk factor for cardiovascular disease (CVD) with or without DM. In diabetic population for every 1 % increase in absolute $HbA_1c$ risk of CVD was increased by 18 %.[6] Hyperlipidemia is common in patients with renal failure. In addition, atherogenic changes in lipoprotein composition occur in most of these patients.[7] It has long been hypothesized that lipoproteins play a role in renal injury similar to their established involvement in atherosclerosis.[8] A number of experimental investigations have provided relevant evidence that lipids may contribute to progressive renal damage.[9] However there are only a few prospective studies that have addressed a possible relationship between dyslipidemia observed in patients with renal failure and the rate of progression in kidney disease.[10] Hence the present study was undertaken to investigate glycated hemoglobin, fasting insulin, total lipid profile, renal anthropometric parameters in the assessment of risk of diabetic nephropathy in type 2 diabetes mellitus.

## MATERIALS AND METHODS

The present study was conducted in RL Jalappa hospital attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India. Randomly selected 250 type 2 diabetes and non-diabetes subjects with the age group of 30-60 years attending medicine outpatient department from March 2012 to January 2013 were included in the study. The study was approved by institutional ethical clearance committee and a written informed consent was obtained from all the participants. Patients suffering from other causes of secondary dyslipidemia, self-reported pregnancy; any chronic infectious diseases and weight loss by > 6 kg during past 6 months were excluded from the study. Weight and height were measured to the nearest 0.1 kg and 0.1 cm and Body mass index (BMI) was calculated as weight divided by height squared in meters ($kg/m^2$). Waist circumference (WC) and Hip circumference (HC) was recorded according to Ash well *et al*[11] as the smallest girth between the rib cage and the top to the lateral border of the iliac crest during the minimal respiration. Waist hip ratio (WHR) was calculated. Systolic blood pressure (SBP) anddiastolic blood pressure (DBP) were measured in all subjects in the supine position, inflating the cuff tied at the level of heart to the left arm after confirming that the patient was in the relaxed state. Biochemical parameters were measured after an overnight fast, and the parameters were estimated using Johnson and Johnson auto analyzer. The blood glucose estimation was done by Glucose Oxidase Peroxidase method (GOD-POD),[12] glycated hemoglobin ($HbA_1c$) was estimated by HPLC, serum creatinine (SCr) was estimated by deproteinisation method[11], uric acid estimation by uricase method,[11] total cholesterol (TC) was estimated by cholesterol oxidase method, triglycerides (TG) estimation is by Enzymatic colorimetric test- GPO PAP, HDL cholesterol (HDL-c) estimation was done by Direct Enzymatic colorimetric method and low density lipoprotein (LDL-c) was calculated.[12] Spot urine albumin (UAE) was estimated by sulfo salicylic acid method. Value of $HbA_1c$ was given as percentage of total hemoglobin and values of all other parameters were given in mg/dl. Dyslipidemia was defined as per American Diabetes Association (ADA) criteria.[13] Statistical analysis was carried out by the Student t-test by using the SPSS version 16. Independent paired t-test (2-tailed) was used to compare mean differences between parameters and p-value < 0.05 was considered significant.

**Table 1: Mean ± SD of Physiological and Biochemical Parameters in Type 2 Diabetes and non-diabetes Subjects**

| Parameters | Diabetes (Mean ± SD) | Non-diabetes (Mean ± SD) | P-value |
|---|---|---|---|
| Body Mass Index ($kg/m^2$) | 25.64 ± 4.74 | 24.57 ± 4.43 | 0.010 |
| Waist hip Ratio | 0.95 ± .10 | 0.96 ± .14 | 0.113 |
| Fasting blood glucose (mg/dl) | 158. 36 ± 81.13 | 85.86 ± 34.90 | 0.000* |
| Post prandial blood glucose (mg/dl) | 256.47 ± 99.37 | 132.27 ± 59.43 | 0.000* |
| Glycosylated hemoglobin (%) | 9.43 ± 5.15 | 6.10 ± 1.22 | 0.000* |
| Fasting insulin (mcU/ml) | 18.45 ± 26.93 | 9.90 ± 11.15 | 0.000* |
| Systolic blood pressure (mmHg) | 126.20 ± 14.80 | 123.06 ± 14.44 | 0.017 |
| Diastolic blood pressure (mmHg) | 79.56 ± 11.18 | 80.02 ± 11.48 | 0.653 |

*significant at $p \leq 0.01$

**Table 2: Mean ±SD of Lipid Profile in type 2 Diabetes and non-diabetes Subjects**

| Parameters | Diabetes (Mean ± SD) | Non-diabetes (Mean ± SD) | P-value |
|---|---|---|---|
| Total cholesterol (mg/dl) | 181.37 ± 104.71 | 173.60 ± 42.23 | 0.277 |
| Triglycerides (mg/dl) | 204.75 ± 110.96 | 164.96 ± 85.47 | 0.000* |
| High-density lipoproteins (mg/dl) | 38.82 ± 27.68 | 38.15 ± 6.08 | 0.706 |
| Low-density lipoproteins (mg/dl) | 97.44 ± 36.73 | 108.22 ± 82.41 | 0.063 |

*Significant at $p \leq 0.01$

**Table 3: Renal Function Parameters for type 2 Diabetes and non-diabetes Subjects**

| Parameters | Diabetes (Mean ± SD) | Non-diabetes (Mean ± SD) | P-value |
|---|---|---|---|
| Serum creatinine (mg/dl) | 1.65 ± 10.94 | .90 ± 1.00 | 0.280 |
| Uric acid (mg/dl) | 4.61 ± 1.44 | 4.64 ± 1.20 | 0.802 |
| Urine albumin excretion | 11.81 ± 362.95 | 22.92 ± 89.08 | 0.000* |

*significant at $p \leq 0.01$

## RESULTS

The observed mean BMI was 25.64 $kg/m^2$ for diabetes and 24.57 $kg/m^2$ for non-diabetes. The mean WHR was 0.95 for diabetes and 0.96 for non-diabetes and was not statistically significant (p = 0.113). The mean SBP in diabetes was 126.20 mmHg and in non-diabetes 123.06 mmHg (Table 1). DBP was 79.56 mmHg in diabetes and in non-diabetes 80.02 mmHg. Fasting blood glucose (FBS) was 158 mg/dl in diabetes and 85 mg/dl in non-diabetes and statistically it was significant (p < 0.000) and $HbA_1c$ was 9.43 % in diabetes and non-diabetes 6.10 % which is statistically significant (p < 0.000) With respect to the Fasting Insulin in diabetes it was 18.45 mcU/ml and in non-diabetes it was 9.90 mcU/ml with the significant p-value of P < 0.000. The mean total cholesterol for diabetes was 181.13 mg/dl and non-diabetes was 164.96 mg/dl and was not significant. HDL-Cholesterol in diabetes was 38.82 mg/dl and in non-diabetes was 38.15 mg/dl. The TG levels were slightly lower in non-diabetes (164 mg/dl) and whereas LDL-c was higher in non-diabetes 108 mg/dl (Table 2). The mean values observed for renal parameters (Table 3) in diabetes (11.81) and non-diabetes (22.92) were significant for urine albumin excretion, whereas similar results were observed for uric acid in both groups (4.61 and 4.64), whereas non-diabetes showed lower serum creatinine (0.90 mg/dl) than diabetes (1.65 mg/dl).

## DISCUSSION

Individuals with type 2 diabetes mellitus ($T_2DM$) are considered on high priority as they are potential targets for rapid evaluation to prevent and halt the progression of complications.[14] There is a high risk of renal disease in people with type 2 diabetes, Diabetic nephropathy occurs in 20-40 % of patients with diabetes and is the single leading cause of end stage renal disease (ESRD) worldwide and is associated with increased cardiovascular risk.[15] In the present study mean BMI of diabetes showed higher than non-diabetes. Controversially, lower WHR was observed in diabetes than non-diabetes. These results indicate that the risk of developing diabetes is certainly higher in non-diabetes subjects.[16] Study conducted by Wannamethee *et al* showed WC and BMI are equal predictors of diabetes.[17] Similar findings were observed by Shah *et al*. This shows that the risk of developing diabetes is higher with WHR > 1.0.[18] the higher systolic and lower diastolic blood pressure were observed in diabetes than non-diabetes. These results are similar to the observations by Wei-Lian Phan *et al*. Lower DBP helps to predict several metabolic Syndrome components in diabetes. One Component is an increase in WC, an obesity indicator, which manifests the strongest independent contribution to elevated SBP.[19] Fasting insulin levels were elevated significantly in both the groups but the levels were found within normal reference range. However, diabetic subjects showed higher insulin levels compared to non-diabetics; this suggests the early risk of insulin resistance. With reference to the mean $HbA_1c$ level of diabetics 9.43 % was more compared to non-diabetics 6.10 %. Most of the type 2 diabetic patients experience poor glycemic control irrespective of their gender. A significant relation between $HbA_1c$ and FBGis in agreement with earlier reports.[20,21] This is consistent with UKPDS (United Kingdom Prospective Diabetes Study)[22] study which demonstrated micro vascular complications were benefited by better control of blood sugar levels and with the fact that diabetic nephropathy and blood pressure have a strong correlation,[23] in our study the incidence of nephropathy increased significantly with rise in blood pressure and dyslipidemia.[24] Serum creatinine levels were increased in diabetics indicating the onset of the micro vascular complications of nephropathy.[25] Similar levels of uric acid in diabetes and non-diabetes were observed which may suggest the presence of risk, andis a consistent feature of the insulin resistance syndrome, which are characterized by high plasma insulin levels, blood glucose and serum triglyceride concentrations and more BMI and WHR.[26] Significantly increased micro albuminuria was observed in diabetes[27] and was associated with generalized vascular disease.[28] We observed significant relation between HbA1c and HDL-Cholesterol in diabetic patients. Several investigators have reported significant correlations between $HbA_1c$ and lipid profiles and suggested the importance of glycemic control in normalizing dyslipidemia.[29] Nephropathy is associated with many potentially modifiable risk factors. In estimating Diabetic nephropathy risk, AER (urinary Albumin Excretion Rate) is most important and should be done frequently but there are gains to be made in predictive precision by considering family history, smoking habits, glycemic, B.P., BMI and lipid levels. Early screening for incipient diabetic nephropathy and aggressive management of these risk factors is important in optimizing the renal outcome of patients with diabetes mellitus.

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