RESEARCH ARTICLE

BIOCHEMICAL MARKERS FOR EARLY DETECTION OF RISK OF NEPHROPATHY AND ATHEROSCLEROSIS IN TYPE 2 DIABETES MELLITUS PATIENTS

Dr Sunita Singh[1], Dr. Sodavadiya Kirankumar B.[2], Dr. Babina singh Bajhaiya[3], Dr. B K Agrawal[4]

1. 3rd Year Resident, Department of Biochemistry, Index medical College, Hospital and Research center, indore. (MD Biochemistry)
2. Assistant Professor, Department of Biochemistry, Index medical College, Hospital and Research center, indore. (MD Biochemistry)
3. 3rd Year Resident, Department of Medicine, Index medical College, Hospital and Research center, indore. (MD Medicine)
4. Professor and Head, Department of Biochemistry, Index medical College, Hospital and Research center, indore. (Phd in Medical Biochemistry)

Corresponding author: Dr Sunita Singh , A-578 Panchsheel nagar, makarwali road, Ajmer, Rajasthan

ABSTRACT:

Title: Biochemical markers for early detection of risk of nephropathy and atherosclerosis in type 2 diabetes mellitus patients. **Introduction** : The major long term complications in patients with Type 2 diabetes are Diabetic nephropathy and Atherosclerosis . It is also a strong indicator of the risk of atherothrombotic diseases and cardiovascular disorders.In Earliest stages of diabetic nephropathy, Microalbuminuria and creatinine are an important marker to diagnose. For Atherosclerosis, Homocysteine and lipid profile are an important marker.Deranged lipid profile, particularly increased total cholesterol and LDL- cholesterol are well known to cause atherosclerosis. According to many recent studies, it has been hypothesized that increased homocysteine level promotes atherosclerosis and is independently associated with increased risk of microalbuminuria in patients of diabetic nephropathy. **Aim and objectives**: This study was intended to establish the role of biochemical markers for early detection of risk of Nephropathy and Atherosclerosis in Type 2 Diabetes Mellitus Patients. To establish the relationship between different markers. **Materials and Methods:** This case-control study was conducted on a total of 200 subjects which were divided into two groups. One Group consisted of 100 diabetic patients & second Group consisted of 100 healthy controls. Baseline investigations and estimation of Creatinine, microalbuminuria, homocysteine, lipid profile, Blood sugar and hsCRP were performed. Statistical analysis of the results was done. **Result and discussion:** The study documented stronger correlation between increased homocysteine levels and the degree of microalbuminuria ($r = +0.401$) in comparison to total cholesterol ($r = +0.131$) and also in comparison to LDL cholesterol ($r = +0.246$). ($p<0.001$).**Conclusions:** Increased homocysteine

, total cholesterol and LDL cholesterol level are of early stage diagnostic indicator of Atherosclerosis and degree of microalbuminuria and increased creatinine level are of early stage diagnostic indicator of nephropathy in case of Type-2 Diabetes.

Keywords: Nephropathy, Atherosclerosis, Type 2 Diabetes Mellitus

INTRODUCTION

Diabetes is one of the most common non-communicable diseases affecting almost 7% of the world's population. Diabetes mellitus prevalence increases continually around the world, it become one of the major global problems for the developing as well as developed countries. It is affecting millions of peoples, about 6-7% of the world population [1].

Diabetes mellitus a metabolic disorder characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action or both.

The most prevalent diabetes form in human is type 1 and type 2 diabetes mellitus. The latest accounts for more than 70% of patients[2]. Many patients with type 2 diabetes are asymptomatic, there is no sharp clinical manifestation and hence patients may remain undiagnosed for many years.

Type 2 DM is characterized by two major defects impaired insulin secretion or decrease in its peripheral action. It is also known as Non Insulin Diabetes Mellitus. The cause of type 2 DM is multifactorial. Genetic susceptibility plays a crucial role in etiology and manifestation of type 2 DM with roots in the interaction of environmental factors, physical activity, obesity, ethnic, drugs and toxic agents, viral infection & location. Individual with a susceptible gene may become diabetic if environment factors modify the expression of these genes. It is evident that environment factors are playing a more increasing role in the cause of diabetes mellitus.

The complication of diabetes include microvascular disease due to damage of small blood vessels that effects eyes, nerves, kidney and macrovascular disease due to damage to the arteries that affect brain, heart & extremities. Strict controlling of blood glucose level and blood pressure play an important role in delaying diabetic complications. On the other hand, less glycemic control, smoking, high blood pressure, elevated cholesterol levels, obesity and lack of regular exercise are considered to be risk factors that accelerate the dexterous of diabetes complication.

Coronary artery disease is one of the most common cause of death among diabetic patient (2-3 high prevalence compared to non-diabetic patients)[3].

Macrovascular disease is a major cause of death in diabetic individuals. Atherosclerosis is multi focal, multi factorial smouldering inflammatory disease that affect the intima of medium sized and large arteries resulting in intimal thickening that may leads to luminal thickening and inadequate blood supply.

Atherosclerosis is progressive disease of arterial wall involving the components of inflammation vascular lipid deposition and re-modeling fibrosis and thrombosis.

Hyperglycemia, insulin resistance, hyperinsulinemia, hyper-lipidemia, hyperhomocysteniemia and increased CRP, represents important pathophysiology components of DM that results in endothelial / vascular dysfunction through several underlying process.

In diabetes; dyslipidemia, obesity, microalbuminuria / macro-albuminuria, elevated serum creatinine, abnormal platelet function is seen[4][5].

So to assess atherosclerosis we need to screen the values of following biochemical markers and they are serum cholesterol, serum triglyceride, serum LDL, serum HDL, serum VLDL, homocysteine and high sensitively C-Reactive Protein and blood glucose level.

Diabetic Nephropathy is a clinical syndrome characterized by the following

- Persistant albuminuria (>300mg/dl or >200µg/min) that is confirmed on atleast 2 occasions, 3-6 months apart[6].

- Progressive decline in the glomerular filteration rate (GFR).

- Elevated arterial blood pressure.

In diabetes following changes occur in the kidney :-

o Alteration in the microcirculation due to glomerular hyperperfusion or hypoperfusion, increased glomerular capillary pressure.

o Change in the structure of glomerulus as there is increase in extracellular matrix, basement membrane thickening, mesangial expansion and fibrosis[7].

o Production of soluble factors like growth factors, angiotensin II, endothelin, AGE

They causes microalbuminuria or macroalbuminuria, hypertension which leads to diabetic nephropathy.

50% will have microalbuminuria at the time of presentation with hypertension, 10-20% with microalbumuiuria will progress to overt nephropathy[8][9].

Risk factors for diabetic nephropathy is age, race, ethnicity, history of microalbumuria, hypertension, poor glyclemic control, increased cholesterol and triglycerides, increased homocysteine and increase in hsCRP, increased in creatinine level, smoking etc[10][11].

The work is undertaken to detect the clinical utility of biochemical parameters like serum level of albumin, creatinine, glucose, cholesterol, triglyceride, LDL, VLDL, HDL, homocysteine, high sensitivity C-reactive protein for early detection of nephropathy and atherosclerosis in type 2 diabetes patients. The study also helps in assessing a correlation between different markers for finding the possibility of nephropathy and atherosclerosis in type 2 diabetes patients.

MATERIALS AND METHODS:
Study Design and Participants:

This study was conducted in the Department of Medical Biochemistry; Index Medical College, Hospital & Research Center, Indore in association with Department of Medicine; Index Hospital, Indore.

200 Patients (100 Diabetic Patients and 100 non-diabetic patients) attending OPD and IPD of Department of Medicine, Index Hospital, Indore were taken. Time period of sample collection was from March 2015 to March 2016.

Study Group- Consist of 200 patients(100 diabetics and 100 non- diabetic patients).

Age Group- Consist of 35 -80 years patients of both genders.

Residence-Consist of Rural and Urban patients

Table 1

Age Distribution

Age Group (Years)	Group A		Group B	
	Number	%	Number	%
Less than 40 years	8	8	9	9
41-60 years	51	51	49	49
Above 60	41	41	42	42
Total	**100**	**100**	**100**	**100**

TABLE-2 SEX DISTRIBUTION:

Sex	Group A (n=100)		Group B (n=100)	
	Number	%	Number	%
Male	55	55	55	55
Female	45	45	45	45
Total	**100**	**100**	**100**	**100**

METHOD:- The following tests were performed in Central Laboratory of Biochemistry, Index Hospital, Indore by ERBA auto Analyzer. We had taken fasting blood sample of patients coming in OPD and IPD of Medicine Department of Index Medical College. The blood sample is collected in plane tube. The sample is then centrifuge in centrifuge machine (REMI). It is then kept in ERBA auto analyzer for the following tests.

Source of materials:
- Sample collection: fasting 4 ml of venous blood was collected from consenting indoor and outdoor patients of Index medical college, Hospital and research center Indore, M.P. Sample was collected in
- supine position under full aseptic precaution after taking written consent and giving complete information regarding to study. Blood was
- collected from Median cubital vein in plain vaccutainer. Dummy identity number was given to each participant involve in study. Same identity number also given on vaccutainer tube.
- All above samples were put at room temperature at 27°C for a period of 1 hr. After 1hr, all above sample was centrifuged at 3000 RPM in R-8C BL Bench top Remi centrifuge for a period of 10 minutes. Aliquot ware prepared from serum separated from above samples.
- Samples were analyzed for Blood Glucose, S. Albumin , S.Creatinine S.Lipid Profile, S.Homocysteine ,High Sensitivity C – Reactive Protein at biochemistry laboratory of Index medical college, Hospital and research center Indore, M.P. in fully auto analyzer ERBA EM- 360, along with quality control sera of normal and abnormal range.
- S.Total cholesterol was measured by Carr-Drekter direct Method , HDL-C by selective apo-B precipitation method, TGs by glycerol phosphate dehydrogenase(GPO)- peroxidase(POD)method.
- Serum VLDL-C and S.LDL-C was calculated by following formula:
- VLDL-C = TGs/5 mg/dl
- LDL-C = Total cholesterol-(HDL-C+VLDL-C) mg/dl
- serum Creatinine was measured by Jaffe's kinetic colorimetric assay method.

> - S.Homocysteine - by CLIA (Chemiluminescent Immunoassay) ,High Sensitivity C – Reactive Protein- done by turbidimetric immunoassay.

RESULTS AND DISCUSSION:

- This study is done to note that - Biochemical   markers for early detection of risk of nephropathy and atherosclerosis in type 2 patients
- Total of 100 controls and 100 diabetic patients were studied. All parameters in controls and diabetic patients are shown in table.

TABLE 3DIFFERENT PARAMETERS IN DIABETIC AND NON DIABETIC PATIENTS

Parameters	Group A (N=100)		Group B (N=100)	
	Mean	SD	Mean	SD
Blood Sugar (mg/dl)	99.32	17.80	217.98	145.67
Serum Creatinine (mg/dl)	1.37	1.79	5.4201	14.85
Serum Albumin (g/dl)	3.59	0.91	2.727	1.09
Serum Cholesterol (mg/dl)	199.22	42.08	272.77	80.97
Triglyceride (mg/dl)	131.84	55.03	177.69	49.42
Serum HDL (mg/dl)	39.85	8.35	29.849	4.22
Serum LDL (mg/dl)	98.22	18.49	142.36	33.22
Serum VLDL (mg/dl)	25.29	12.75	47.174	10.44
hsCRP (mg/L)	0.65	0.47	2.2662	1.17
Homocysteine (μmol/L)	12.86	1.93	15.554	1.44

This table shows different parameters in non diabetic patients (Group A) and diabetic patients (Group B).

The mean value of glucose in non diabetic patients is 99.32 ± 17.80 mg/dl and diabetic patient is 217.98 ± 145.67 mg/dl. Blood glucose level is significantly higher in diabetic patient (r=-8.09, P=<0.001).

The mean value of serum creatinine in non diabetic patients is 1.37 ± 1.79 mg/dl and diabetic patient is 5.42 ± 14.85 mg/dl. This shows that serum creatinine is significantly higher in diabetic patient (r=-2.71, P=<0.001).

The mean value of serum albumin in non diabetic patients is 3.59 ± 0.91 g/dl and diabetic patient is 2.72 ± 1.09 g/dl. This shows that serum albumin is significantly low in diabetic patient (r=6.06, P=<0.001).

The mean value of serum cholesterol in non diabetic patients is 199.28 ± 42.08 mg/dl and diabetic patient is 272.77 ± 80.97 mg/dl. This shows that serum cholesterol is significantly higher in diabetic patient (r=-2.71, P=<0.001).

The mean value of serum triglyceride in non diabetic patients is 131.83 ± 55.03 mg/dl and diabetic patient is 177 ± 49.42 mg/dl. This shows that serum triglyceride is significantly higher in diabetic patient (r=-6.20, P=<0.001).

The mean value of serum HDL in non diabetic patients is 39.85 ± 8.35 mg/dl and diabetic patient is 29.84 ± 4.22 mg/dl. This shows that serum HDL is significantly low in diabetic patient (r=10.07, P=<0.001).

The mean value of serum LDL in non diabetic patients is 98.22 ± 18.49 mg/dl and diabetic patient is 142.36 ± 33.22 mg/dl. This shows that serum LDL is significantly higher in diabetic patient(r=-11.61, P=<0.001).

The mean value of serum VLDL in non diabetic patients is 25.29 ± 12.75 mg/dl and diabetic patient is 47.17 ± 10.44 mg/dl. This shows that serum VLDL is significantly higher in diabetic patient (r=-13.28, P=<0.001).

The mean value of hsCRP in non diabetic patients is 0.65 ± 0.47 mg/L and diabetic patient is 2.26 ± 1.17 mg/L. This shows that hsCRP is significantly higher in diabetic patient (r=-12.78, P=<0.001).

The mean value of homocysteine in non diabetic patients is 12.85 ± 1.93 mol/L and in diabetic patient is 15.55 ± 1.44 mol/L. This shows that homocysteine is significantly higher in diabetic patient (r=-11.19, P=<0.001).

Patients with type 2 diabetes mellitus (n=100) has significantly low serum albumin (P<0.001), serum HDL (P<0.001) and significantly higher serum creatinine (P<0.001), serum cholesterol (P<0.001), serum triglyceride (P<0.001), serum LDL (P<0.001), serum VLDL (P<0.001), hsCRP (P<0.001), homocysteine (P<0.001) than non diabetic patients (n=100).

This table shows hsCRP value in different age groups.

TABLE 4 COMPARISON OF hsCRP AND HOMOCYSTEINE

Age Group (years)	hsCRP		Homocysteine	
	Group A	Group B	Group A	Group B
Less than 40 years	0.42 ± 0.17	0.91 ± 0.18	10.08 ± 0.46	13.32 ± 0.94
41-60 years	0.51 ± 0.31	2.30 ± 1.24	12.47 ± 1.55	15.66 ± 1.33
Above 60	0.87 ± 0.58	2.52 ± 1.03	13.89 ± 1.84	15.90 ± 1.25

In age group <40 years, the mean value of hsCRP in non-diabetic patients is 0.42+0.17 mg/L and the mean value of hsCRP in diabetic patients is 0.91+0.18 mg/L. This shows no significant change in hsCRP level in patients of less than 40 years age group of group A

and group B. In age group of 41-60 years – the mean value of hsCRP in non-diabetic patients (Group A) is 0.51+0.31 mg/L and the mean value of hsCRP in diabetic patients (Group B) is 2.30+1.24 mg/L. This shows that there is significant increase in hsCRP level in diabetic patient as compared to non diabetic patients of age group 41-60 years. In age group of above 60 years – the mean value of hsCRP in non-diabetic patients (Group A) is 0.87+0.58 mg/L and the mean value of hsCRP in diabetic patients (Group B) is 2.52+1.03 mg/L. This shows that there is significant increase in hsCRP level in diabetic patient as compared to non diabetic patients of age group above 60 years.

It signifies that there is increase chances of nephropathy and atherosclerosis in diabetic patients as compared to non diabetic patients of age group 41-60 years and above 60 years of both sexes.

The table shows the mean values of homocysteine in different age groups.

In age group <40 years, the mean value of homocysteine in non-diabetic patients is 10.08+0.46   mol/L and the mean value of hsCRP in diabetic patients is 13.32+0.94   mol/L. This shows no significant change in homocysteine level in patients of less than 40 years age group of group A and group B. In age group of 41-60 years – the mean value of homocysteine in non-diabetic patients (Group A) is 12.47+1.52   mol/L and the mean value of hsCRP in diabetic patients (Group B) is 15.66+1.33   mol/L. This shows that there is significant increase in homocysteine level in diabetic patient as compared to non diabetic patients of age group 41-60 years. In age group of above 60 years – the mean value of homocysteine in non-diabetic patients (Group A) is 13.89+1.84   mol/L and the mean value of homocysteine in diabetic patients (Group B) is 15.90+1.35   mol/L. This shows that there is significant increase in homocysteine level in diabetic patient as compared to non diabetic patients of age group above 60 years.

It signifies that there is increase chances of nephropathy and atherosclerosis in diabetic patients as compared to non diabetic patients of age group 41-60 years and above 60 years of both sexes.
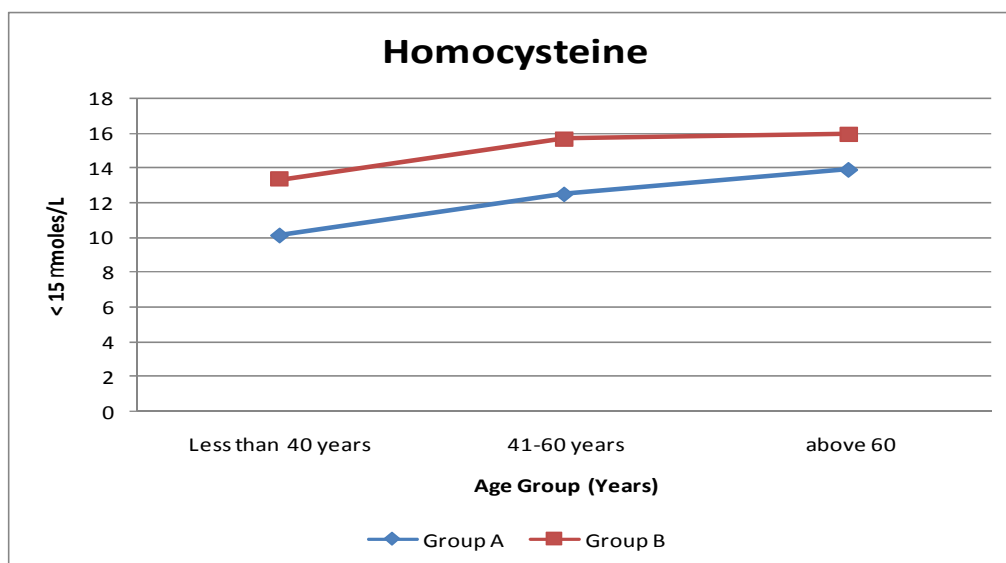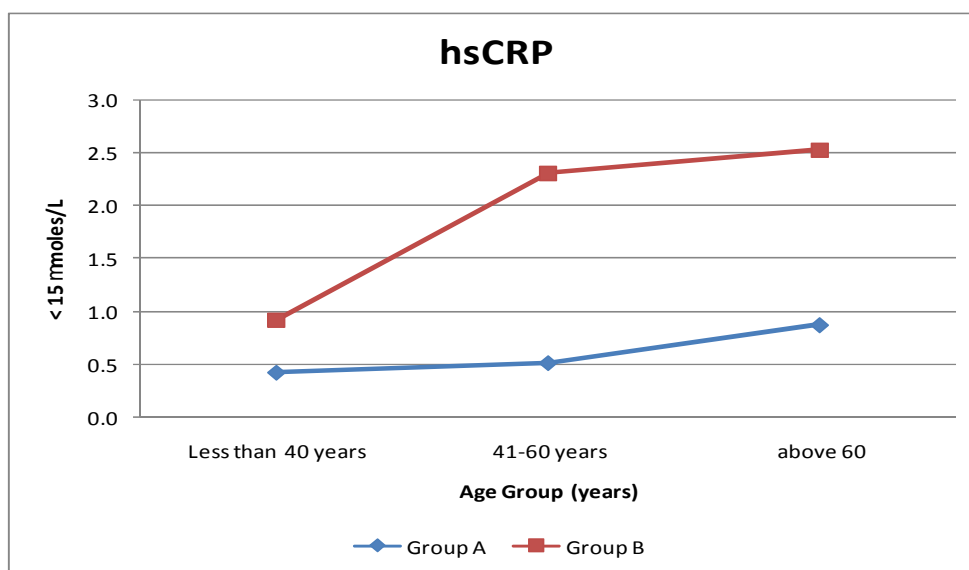
hsCRP

CONCLUSION:

The values of serum creatinine, serum total cholesterol, serum triglyceride, serum LDL, serum VLDL, serum glucose, hsCRP and homocysteine are higher in diabetic patients than non-diabetic patients and the values of serum albumin and serum HDL are lower in diabetic patients than non-diabetic patients. Thus by assessing the biochemical parameters we can assess the risk of atherosclerosis and diabetic nephropathy in type 2 diabetic patients. We conclude that these biochemical tests are early detector of the risk of atherosclerosis and diabetic nephropathy in type 2 diabetic patients.

ABBREVIATION:
• TGs- Trigycerides
• HDL-C- High Density Lipoprotein cholesterol
• LDL-C- Low Density Lipoprotein cholesterol
• VLDL-C-Very Low Density Lipoprotein cholesterol
• IPD – Indoor patient department
• OPD – Out door patient department

REFERENCES:
1. Adeghate, Schattner P, Dunn E (2006) : An update on the etiology & epidemiology of diabetes mellitus. Annual New York Academy of Sciences; 1084: 1-9
2. Abdella NA, Mojiminiyi OA. Association of plasma homocysteine concentration in subjects with type 2 diabetes mellitus. Acta Dibetelol. 2002; 39 : 183-90.
3. Abrass CK: Cellular lipid metabolism and the role of lipids in progressive renal disease. Am J Nephrol 24 : 46-53, 2004.
4. Alan T Ramaley,Nader Rafai :Lipid, lipoproteins, Apo lipoprotenis and other cardiovascular risk factor. Teitz Textbook of Clinical Chemistry and Molecular Diagnostics 2012, 15(27) : 789-790, 794-795.

5. Allain, C.C. Poon, L.S, Chan, C.S.G, Richmond, W. and Fu, P.C. Clin Chem. 1974; 20: 470-475.
6. American Diabetes Association (2004). Nephropathy in diabetes. Clinical Practice Recommendations 2004. Diabetes Care, 27 (Suppl 1) : S79-S83.
7. American Diabetes Association (2006) : Diagnoses and Classification of diabetes mellitus. Diabetes Care 2004; 29(1) : 543-548.
8. American Diabetes Association (2008): Diabetes mellitus and its complication.
9. American Diabetes Association. Economic costs of diabetes in the USA in 2002. Diabetes Care; 2003;26(2) : 917-932.
10. American Diabetes Association. Prevention and Management of diabetes complication. American Diabetes Association, Alexendra, Ya, USA, 2012.
11. American Diabetes Association. Standards of Medical Care in Diabetes, 2010. Diabetes Care, 2010 Jan.; 33 Supp, 1 : S11-16.