A STUDY OF RELATIONSHIP BETWEEN C - REACTIVE PROTEIN AND MICROALBUMINURIA IN DIABETIC PATIENTS

Palvasha Waheed, Abdul Khaliq Naveed*

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Islamic International Medical College/Riphah International University Islamabad Pakistan

ABSTRACT

Objective: To see the relationship between C-reactive protein (CRP) and microalbuminuria in type 2 diabetic patients.

Study Design: Cross sectional comparative study.

Place and Duration of Study: Department of Biochemistry and Molecular Biology and Centre for Research in Experimental and Applied Medicine, Army Medical College Rawalpindi over a period of 2 years from January 2009 to December 2010.

Material and Methods: Non-probability convenience sampling was done. A total of 80 (n=80) subjects were recruited in the study and were divided into four groups. Each group consisted of 20 patients. Group I consisted of normal healthy individuals, group II comprised normoalbuminuric type II diabetics, group III had microalbuminuric type II diabetics, and group IV consisted of overt albuminuric type II diabetic subjects. The albuminuria status was defined as (i) normoalbuminuria: albumin excretion rate (AER) less than 30 mg / 24h (ii) microalbuminuria: (AER) between 30-300 mg / 24h (iii) overt albuminuria: (AER) greater than 300 mg / 24h. Samples of 10 ml venous blood and 24 hour urine were collected. Plasma CRP was measured by turbidimetric method using Electa. Albuminuria was determined using Microlab semi-analyser (Randox, UK). Data were analysed using SPSS version 17 and Microsoft Excel worksheet 2010 with add on Statistical Package. Mean and standard error of mean (SEM) were used to describe numeric variables among different groups. Minimum and maximum values were also obtained to evaluate variations in the collected data. Analysis of variance (ANOVA) was applied to find out significant differences among groups. ANOVA was followed by Post Hoc Tuckey's test for multiple comparisons among groups. A *p*-value of less than 0.05 was considered significant and a *p*-value of less than 0.01 was considered highly significant. Pearson's correlation coefficient (r) was applied to find out the correlation of various parameters in different groups. A positive and negative value near 1 represented strong direct and inverse relationship respectively.

Results: The mean ± SEM value of urinary albumin in group I was 27.91 ± 2.26 mg/day. In group II it was 27.37 ± 2.21 mg/day, in group III it was 220.52 ± 17.83 mg/day, in group IV it was 330.78 ± 26.75 mg/day. There was significant difference between all the groups ($p<0.001$). In group I, the mean ± SEM value of CRP was 1806.6 ± 183.32 ng/mL. In groups II, III and IV, the mean ± SEM value of CRP was 1987.26 ± 201.66 ng/mL, 2384.712 ± 241.99 ng/mL and 3338.60 ± 338.79 ng/mL respectively. There was significant difference between all the groups ($p<0.001$).

Conclusion: The levels of CRP are higher in diabetics as compared to normal healthy controls and there is a progressive increase in the levels of CRP with increase in microalbuminuria in type 2 diabetics.

Keywords: C-reactiveProtein, Diabetes mellitus, Microalbuminuria.

INTRODUCTION

Diabetes mellitus (DM) is defined a group of metabolic disorders in which there are elevated levels of blood glucose due to abnormal insulin secretion, abnormal activity of insulin action or a combination of both[1]. A patient of diabetes mellitus may present with a range of symptoms characterized by polyphagia, polyuria, polydipsia, weight loss and blurring of vision to more serious conditions like stupor, coma and even death caused by ketoacidosis or non-ketotic hyperosmotic states. The chronic complications of

Correspondence: Dr Palvasha Waheed, Dept of Biochemistry, AM College Rawalpindi Pakistan (*Email: p_waheed@hotmail.com*)
Received: 08 Dec 2015; revised received: 14 Dec 2015; accepted: 06 Jan 2016

diabetes mellitus include nephropathy leading to renal failure and end stage renal disease (ESRD), retinopathy causing potential blindness, and neuropathy leading to foot ulcers and amputations. Studies show that the patients suffering from DM have increased chances of suffering from cardiovascular, cerebrovascular and peripheral vascular diseases[1].

A person is labelled as a diabetic if the fasting level of plasma glucose is equal to or more than 7.0mmol/L or 2 hours post prandial level is equal to or more than 11.1 mmol/L[2]. However, the glycosylated hemoglobin (HbA1C) assays are believed to be better tools of diagnosis of diabetes mellitus. The consensus is that HBA1c ≥6.5% is taken as diabetic[3].

One of the hall marks of diabetic complications is diabetic nephropathy. The increased incidence of diabetic nephropathy not only increases the economic burden of a community but renal insufficiency itself plays an important role in cardiovascular mortality[4]. The impaired kidney function is caused by alteration in the normal endothelial cell lining which is in turn caused by the accumulation of plasma macromolecules in the interstitial compartment of the body. Therefore, prevention of micro-albuminuria is believed to be an important factor for development of better treatment modalities of diabetic vascular complications[5]. Micro-albuminuria is defined as a subclinical condition characterized by increased urinary albumin excretion (UAE) of more than 30 mg / 24 hours[6]. Studies show that by the time microalbuminuria is detected, typical diabetic nephropathy changes are already established in the glomerular tissue[7]. Increase in glomerular size, thickening of the glomerular basement membrane (GBM), mesangial expansion and widening of podocyte foot processes are the characteristics of early diabetic changes[7].

The most typical features of decreased renal function are tubulo-interstitial inflammation and fibrosis caused by an influx of immune cells like monocytes and macrophages[8]. Studies have demonstrated the role of inflammation in renal

Table-I: Mean difference in levels of albuminuria in different groups.

Group comparison	Albuminuria	
	p- value	Mean difference
Groups I & II	.55 NS	1.000
Groups I & III	-192.61***	.000
Groups I & IV	-302.87***	.000
Groups II & III	-193.15***	.000
Groups II & IV	-303.41***	.000
Groups III & IV	-110.26***	.000

NS $p > 0.05$ = not significant
** $p \leq 0.05$ = significant
*** $p \leq 0.001$ = highly significant

Table-II: Mean difference in levels of CRP in different groups.

Group comparison	CRP	
	Mean difference	*p*-value
Groups I& II	-180.66NS	.956
Groups I &III	-578.11 NS	.361
Groups I &IV	-1532.00***	.000
Groups II & III	-397.45 NS	.673
Groups II & IV	-1351.34***	.001
Groups III & IV	-953.88**	.040

NS $p > 0.05$ = not significant
** $p \leq 0.05$ = significant
*** $p \leq 0.001$ = highly significant

disease linked with microalbuminuria[9]. Navarro also established that local inflammatory activity in the kidney results in microalbuminuria[10]. Stehouwer found that as the level of CRP rises in diabetic patients so does the degree of microalbuminuria[11]. This data provides evidence of inflammation playing a pivotal role in the pathogenesis of early nephropathy in type 2 diabetes. A marker of inflammation is C-reactive protein (CRP). The aim of our study was to see the correlation between inflammation and diabetic nephropathy by studying and comparing the levels of CRP with varying degrees of microalbuminuria in type 2 diabetics in order to further elaborate the link between these two conditions in our local population.

MATERIAL AND METHODS

This study was a cross sectional comparative study conducted at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi and Centre for Research in Experimental and Applied Medicine Rawalpindi. The duration of the study was 2 years from January 2009 to December 2010. Non-probability convenience sampling was done. In this study inflammatory status was compared in patients with varying ranges of albuminuria in type 2 diabetics and non-diabetic healthy controls. Type 2 diabetic patients were recruited from the diabetic clinics of Military Hospital and Holy Family Hospital, Rawalpindi. Normal healthy controls were recruited from colleagues and family members of the researchers. After obtaining study approval from ethical committee, written informed consent of subjects was taken. The subjects were divided into four groups. Each group consisted of 20 patients. Group I consisted of normal healthy individuals, group II comprised normoalbuminuric type II diabetics, group III had microalbuminuric type II diabetics, and group IV consisted of overt albuminuric type II diabetic subjects. The albuminuria status was defined as (i) normoalbuminuria: albumin excretion rate (AER) less than 30 mg / 24h (ii) microalbuminuria: (AER) between 30-300 mg / 24h (iii) overt albuminuria: (AER) greater than 300 mg / 24h. Patients with type-2 diabetes mellitus of 5 years or more duration between the ages of 18 to 65 years with body mass index between 19-40 kg / m2 and HbA1c less than 10 % were included in the study. While patients with significant co-morbidities like chronic liver disease, known cases of ischemic heart disease, cardiomyopathies, patients with ESRD (creatinine clearance less than 10 ml/min), patients taking medications for chronic painful conditions like osteoarthritis and pregnant and lactating mothers were excluded from the study.

Samples of 10ml venous blood and 24 hour urine were collected. Plasma CRP was measured by turbidimetric method using Electa. Albuminuria was determined using Microlab semi-analyser (Randox, UK).

For the purpose of statistical analysis the data were analysed by statistical package for the social sciences (SPSS) version 17 and Microsoft Excel worksheet 2010 with Add on Statistical Package. Mean and standard error of mean (SEM) were used to describe numeric variables among different groups. Minimum and maximum values were also obtained to evaluate variations in the collected data. Analysis of variance (ANOVA) was applied to find out significant differences among groups. ANOVA was followed by Post Hoc Tuckey's test for multiple comparisons among groups. A p value of less than 0.05 was considered significant and a p value of less than 0.01 was considered highly significant. Pearson's correlation coefficient (r) was applied to find out the correlation of various parameters in different groups. A positive and negative value near 1 represented strong direct and inverse relationship respectively. Percentage differences between mean values of all the parameters among groups were determined.

RESULTS

The (mean ± SD) age in group I was 49.25 years ± 6.26 while in groups II, III and IV it was 49.15 ± 5.98, 49.5 ± 5.63, 49.45 ± 6.41 years respectively. The p-value between groups was found to be non-significant. There were 12 males

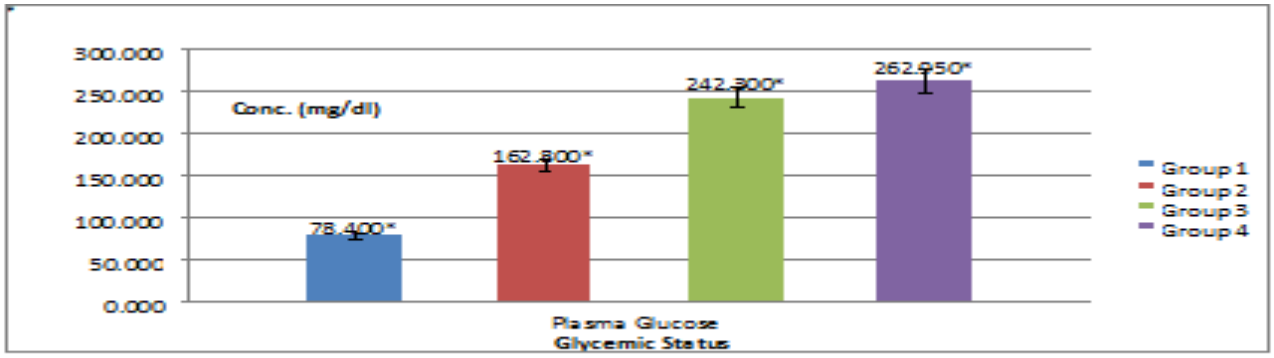
and 8 females in group I while group II had 7 males and 13 females. In groups III and IV there were 10 males and 10 females and 7 males and 13 females respectively. The gender difference between groups was found to be non-significant. The levels plasma glucose in different groups are depicted in fig. (These findings have already been published in authors' 2 original articles12, 13).

The mean ± SEM value of urinary albumin in group I was 27.91 ± 2.26 mg/day (range 11.36 to 49.51 mg/day). In group II it was 27.37 ± 2.21 mg/day (11.13 to 48.53 mg/day), in group III it was 220.52 ± 17.83 mg/day (89.74 to 391.12 mg/day), in group IV it was 330.78 ± 26.75 mg/day (134.61 to 586.69 mg/day). There was significant difference between all the groups (p<0.001). Post Hoc comparisons are shown in table-I.

In group I, the mean ± SEM value of CRP was 1806.6 ± 183.32 ng/mL (840 ng/mL - 3460 ng/mL). In groups II, III and IV, the mean ± SEM value of CRP was 1987.26 ± 201.66 ng/mL (924 ng/mL – 3806 ng/mL), 2384.712 ± 241.99 ng/mL (1108.8 ng/mL - 4567.2 ng/mL) and 3338.60 ± 338.79 ng/mL (1552.32 ng/mL to 6394.08 ng/mL) respectively (figure-2). There was significant difference between all the groups (p<0.001). Post Hoc comparisons are shown in table-II. The percentage difference in CRP levels between group I and II was 9.10 %, between I and III was 24.24 % and between I and IV was 45.89 %, being lowest in group I and highest in group IV. It was 16.67% lower in group II as compared to group III and 40.48 % lower in group II as compared to group IV. In group III it was 28.57% lower than in group IV.

DISCUSSION

Our study demonstrated two imperative points: firstly, the levels of CRP were considerably higher in the diabetic patients as compared to the normal control group; secondly, this difference was more pronounced in patients with increased albuminuria. These findings are consistent with our own previous investigations as well as other studies on correlation of inflammation and diabetes[14,15,16] where an association was seen between high levels of CRP and diabetes. The development of diabetes and its consequent microvascular complications can be predicted by measuring low grade inflammation taking place in the body. Inflammation is closely linked to obesity and insulin resistance. This is due to the fact that adipocytes secrete certain pro-inflammatory cytokines[17]. These inflammatory cytokines can inhibit insulin secretion and signaling. Furthermore insulin itself has numerous anti-inflammatory effects[18]. This leads to the conclusion that diabetes is not only characterized by impaired glucose metabolism but is also a state in which the ability to fight inflammatory signals is compromised[19]. Inflammation has also



*p<0.001 = highly significant

Figure: Comparison of plasma glucose levels in all the groups. Data are means ± SE. The * denotes significance with respect to control normoalbuminuric diabetics, microalbuminuric diabetics and microalbuminuric diabetics.

been found to have close links with microalbuminuria and therefore higher levels of serum CRP are anticipated in cases of increased albumin excretion[20]. This correlation between elevated serum CRP levels and microalbuminuria are not only seen in diabetics but also in the general population[21,22]. Mojadedi et al suggested that raised levels of CRP implicate an important role of activated inflammatory pathways in the commencement and advancement of renal and cardiovascular diseases[23]. However, it is yet to be established whether CRP is a cause or a consequence of vascular damage associated with microalbuminuria[24]. More recent studies show that inflammation is related to the onset of microalbuminuria rather than predicting the risk of its development[25].

CONCLUSION

The levels of CRP are higher in diabetics as compared to normal healthy controls and there is a progressive increase in the levels of CRP with increase in microalbuminuria in type 2 diabetics.

Considering the role of microalbuminuria in aggravating the microvascular complications of diabetes, steps must be taken to reduce the onset of early nephropathy and the process of inflammation must be kept under check and scrutinized strictly. The anti-inflammatory status of the diabetic patients can be assessed and any rise in inflammatory markers must be addressed immediately.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2011; 34(1).
2. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997; 20: 1183-97.
3. International Expert Committee. International Expert Committee report on the role of A1C assay in the diagnosis of diabetes. Diabetes Care. 2009; 32 (7): 1327-34.
4. Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, et al. Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study. Kidney Int. 2002; 62 (4): 1402-07.
5. Basi S, Lewis JB. Microalbuminuria as a target to improve cardiovascular and renal outcomes. Am J Kidney Dis. 2006; 47 (6): 927-46.
6. Viberti G, Hill R, Jarret R, Argyropoulos A, Mahmud U, Keen H. MA is a predictor of clinical nephropathy in type 1 diabetes. Lancet. 1982; 1: 1430-32.
7. Vestra DM, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P. Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes. 2003; 52 (4): 1031-35.
8. Rodriguez-Iturbe B, Pons H, Herrera-Acosta J, Johnson RJ. Role of immunocompetent cells in nonimmune renal diseases. Kidney Int. Suppl. 1991; 59(5): 1626-40.
9. Festa A, D'Agostino R, Howard G, Mykkänen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. Kidney Int. Suppl. 2000; 58 (4):1703-10.
10. Navarro JF, Mora C, Maca M and Garca J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. Am J Kid Dis. 2003; 42 (1):53-61.
11. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes. 2002; 51 (4): 1157-65.
12. Waheed P, Naveed AK, Ahmed T. Thiamine deficiency and its correlation with dyslipidemia in diabetics with micro-albuminuria. JPMA 2013; 63(3): 340-345.
13. Waheed P, Naveed AK, Ahmed T. The effect of plasma thiamine deficiency on the antioxidant status of type 2 diabetics having diabetic nephropathy. Pak Armed Forces Med J. 2012; 62 (2): 255-60.
14. Waheed P, Naveed AK, Farooq F. Levels of inflammatory markers and their correlation with dyslipidemia in diabetics. J Coll Physicians Surg Pak. 2009; 19 (4): 207-10.
15. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001; 286 (3): 327-34.
16. Han T, Sattar N, Williams K, Gonzalez-Villalpando C, Lean ME, Haffner SM. Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. Diabetes Care. 2002; 25 (11): 2016-21.
17. Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc. 2001; 60 (3): 329-39.
18. Dandona P, Aljada A, Mohanty P. The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm. Diabetologia. 2002; 45 (6): 924-30.
19. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet. 2002; 360 (9326): 57-8.
20. Hogan D, Lurbe E, Salabat MR, Redon J, Batlle D. Circadian changes in blood pressure and their relationships to the development of microalbuminuria in type 1 diabetic patients. Curr Diab Rep. 2002; 2 (6): 539-44.
21. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. C-reactive protein and soluble vascular cell adhesion molecule-1 are associated with elevated urinary albumin excretion but do not explain its link with cardiovascular risk. Arterioscler Thromb Vasc Biol. 2002; 22 (4): 593-8.

22. Brownlee M, Aiello L, Friedman E, Vinik A, Nesto R, Boulton A. Complications of diabetes mellitus. Endocrinology. 10th ed. Philadelphia: WB Saunders. 2003; 1509-40.
23. Mojahedi MJ, Bonakdaran S, Hami M, Sheikhian MR, Shakeri MT, Aiatollahi H. Elevated serum C-reactive protein level and microalbuminuria in patients with type 2 diabetes mellitus. Iran J Kidney Dis. 2009; 3 (1): 12-6.
24. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. Diabetologia. 2003; 46 (10): 1402-7.
25. Marcovecchio ML, Giannini C, Widmer B, Dalton RN, Martinott, S, Chiarelli F, et al. C-reactive protein in relation to the development of microalbuminuria in type 1 diabetes: the Oxford Regional Prospective Study. Diabetes Care. 2008; 31 (5): 974-6

.....