Original Article

C- Reactive Protein As a Low Grade Inflammatory Marker in Type 2 Diabetic Nephropathy

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Objective: To determine the association of C-reactive protein as low grade inflammatory marker in type 2 diabetic nephropathy.

Study design; Cross sectional comparative study

Place and duration: Study was conducted at diabetic clinic of general medicine in Pakistan institute of medical sciences over a period of 12 months

Materials and methods: Type 2 diabetic patients coming to diabetic clinic in Pakistan institute of medical sciences were included and after informed consent were divided into two groups A and B on the basis of absence or presence of diabetic nephropathy by measuring 24 hour urinary proteins. The association of low grade inflammation was done by measuring CRP levels

Result: Out of 108 type 2 diabetic patients 38(35.2%) were male and 70 (64.8%) were females with mean age of 47.8 $\pm$10.22 years. They were divided in to two groups on the basis of 24 hour urinary proteins. C reactive proteins were found to be raised in 52 (96.2%) patients with diabetic nephropathy as compare to 7(12.9%) diabetic patients without nephropathy with statistical significance of .000. Considering other risk factors age, gender, raised sugars and hypertension were found to be similar in both the groups meaning having no major influence on the propagation of type 2 DN however deranged renal functions, anemia were found to be statistically significant in the type 2 DN patients with p value of 0.000,0.000 respectively

Conclusion: The c-reactive proteins are raised in patients with type 2 diabetic nephropathy hence suggesting that low grade inflammation is the cause of development and progression of the renal disease in type 2 diabetic patients.

Key Words *Diabetes type 2, Diabetic Nephropathy, C - reactive protein,*

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Introduction

Type 2 diabetes mellitus a leading cause of morbidity and mortality worldwide.[1] Several mechanisms have been proposed for the understanding of its pathogenesis. Type 2 diabetes is frequently associated with an acute-phase reaction, suggestive of a low-grade inflammatory status[2] In the last few years numerous studies have shown that low-grade inflammation is associated with the risk of developing type 2 diabetes.[3-5] As far as nephropathy is concerned, several studies have examined the relationships with inflammation, leading to conflicting results.[6-10] Overall, however, most studies have reported an increase in acute-phase markers in patients with nephropathy and also in patients with micro albuminuria.[11]

The objectives of the study are to confirm inflammation as underlying cause of diabetic nephropathy by measuring plasma level of C reactive protein in patients with type 2 diabetes.

Material and Methods

The type 2 diabetic patients were recruited for the study from Diabetic clinic held in the General Medicine OPD at Pakistan Institute of Medical Sciences. Pakistan. The study was a cross sectional comparative study in which convenient sampling was done. This study was conducted for a period of twelve months from 4th August 2007 to 4th August 2008.

Inclusion criteria:

1. Both male and female patients with in age group of 35 years to 60 years
2. Type 2 diabetic patients

Exclusion criteria:

1. Acute illness on course
2. Diabetic patients with recent myocardial

infarction
3. Other factors interfering with CRP levels e.g. infection, other inflammatory conditions.
4. Patients with age below 35 and above 60 years.

A detailed history regarding duration of diabetes, current medical treatment(whether using oral hypoglycemic agents (OHA), OHA and insulin or insulin alone),compliance of the patients, control of diabetes, eyesight changes, symptoms of lower extremity arterial insufficiency and sensory & autonomic neuropathy, swelling of the body or feet and difficulty in breathing were taken.

Pulse rate was recorded in one minute. Blood pressure was recorded using mercury sphygmomanometer. General physical examination was done to look for anemia or sallow complexion, raised jvp, pedal and sacral edema. Chest was examined for signs of pulmonary congestion. Examination of eyes was done using fundoscope to look for different stages of retinopathy. Examination of peripheral arterial system with palpation of dorsalis pedis, posterior tibial, popliteal and femoral artery for determination of normal, diminished or absent pulses below a certain level was done. Examination of sensory system of lower limb for determination of normal or diminished or absent sensation up to a certain level was carried out using cotton wool for light touch, tuning fork of 128cps(cycles per second) for vibration perception, pin prick for pain, 10gm Weinstein monofilament for light pressure and hot and cold metal object for temperature sensations. Blood sample was taken and send to the pathology laboratory for estimation of fasting and random blood Sugar, Renal function tests and electrolytes, hemoglobin in mg/dl and C- reactive protein. 24hr urine was collected by the patients. They were advised to exclude first early morning urine and collect the rest of the days urine till the next morning. The urine samples were sent to pathology laboratory for estimation of 24 hour urinary protein in mg/24hr. 24hr urinary proteins was measured by using a spectrophotometer. C-reactive proteins were measured via agglutination method. Patients were divided into two groups depending on the presence or absence of protienuria. Group A included type 2 diabetic patients without microalbuminuria. And group B included type 2 diabetic patients with microalbuminuria.

All the information collected by history, examination, and lab investigations was entered in the Performa. Data was entered in SPSS version 10.0 for analysis. Mean $\pm$ S.D was calculated for C-reactive protein, microalbuminuria and other continuous variables. Frequencies and percentages were presented for the categorical variables. The chi-square test was used to compare categorical variables. An odds ratio of > 2 was taken significant. The one way ANOVA was applied to compare group A (patients without diabetic nephropathy) and Group B (patients with diabetic nephropathy) for CRP levels and other continuous numerical variables. A P value was calculated to find out statistical significance.

Results

Out off 108 there were 38(35.2%) male and 70 (64.8%) females. There were 20 (37%) male and 34(62.9) females in non nephropathy group i-e group A. Similarly there were 18 (33.3%) male and 36 (66.6%) female in nephropathy group i-e group B.

Mean age was 47.8 $\pm$ 10.22 years. The patients were further divided into 5 sub groups on the basis of the ages.35-40 years, 41-45 years, 46-50 years, 51-55 years, and 56-60 years.

In case of non nephropathy group, patients 12 (22.2%) , 14 (25.9%) , 9(16.6%), 5(9.25%), and 14 (25.9%) were in the age groups of 35-40yrs, 41-45yrs,46-50yrs, 51-55yrs and 56-60yrs respectively. Similarly in case of the nephropathy group, patients in the age groups of 35-40 yrs, 41-45 yrs, 46-50 yrs, 51-55 yrs and 56-60 yrs were 12 (22.2%) ,9 (16.6%) , 10 (18.5%) ,5 (9.25%) , 18 (33.3) respectively.

The mean duration of diabetes in all the patients was 8.9 $\pm$ 5.38 years. The patients in each group i-e group A and group B were further divided into 5 sub groups on the basis of duration of diabetes which were < 5 years, 6-10 years,11-15 years, 16-20 years,> 20 years.

In non nephropathy group 13(24%) patient had diabetes for < 5 years, 1(1.8%) patient had diabetes for > 20 years. While 15(27.7%), 23(42.5%) and 2(3.7%)patients had diabetes for 6-10 yrs,11-15 yrs and 16-20 yrs.25(46.2%) nephropathy patients had diabetes for 6-10 yrs and 19(35.1%) had diabetes for < 5 years , while 7(12.9%),2(3.7%),1(1.8%) patient had diabetes for duration of 11-15 yrs,16-20 yrs and > 20 yrs respectively.

The mean systolic and diastolic blood pressure in the patients was 138.2 $\pm$ 11.33 mm/hg, 88.5 $\pm$ 8.66 mm/hg respectively. Patients were divided in to subgroups for both systolic and diastolic blood pressures. Systolic blood pressure in 6 sub groups <120 mmhg,120-130 mmhg,131-140 mmhg,141-150 mmhg,151-160 mmhg,>160 mmhg and 5 for Diastolic blood pressure <70 mmhg, 70-80 mmhg, 81-90 mmhg, 91-100 mmhg, >100 mmhg.

In non nephropathy group there were 3(5.5%), 10(18.5%), 31(57.4%), 7(12.9%), 2(3.7%), 1(1.8%) patients and nephropathy patients were 1(1.8%),5(9.2%),35(64.8%),8(14.8%),4(7.4%), in systolic blood pressure groups of <120 mmhg,120-130 mmhg,131-140 mmhg,141-150 mmhg,151-160 mmhg, >160 mmhg groups respectively.

In case of diastolic blood pressure the no of patients in group of <70 mmhg,70-80 mmhg,81-90 mmhg,91-100 mmhg,>100 mmhg in non nephropathy

patient was 0(0%), 21(38.8%), 29(53.7%), 3(55.5%),1(1.8%) and nephropathy patient was 1(1.8%), 5(9.2%),41(75.9%), 5(9.2%), 2(3.7%).

The mean blood sugars of the patients were 206.1± 77.94 mg/dl. Patients were divided into 4 groups on the basis of blood sugar levels. ≤ 100 mg/dl, 101 - 150 mg/dl, 151 – 200 mg/dl,> 200 mg/dl.

In non nephropathy patients 5(9.2%) patients had sugars less than 100 mg/dl and 27 (50%) patient had sugars more than 200 mg/dl. while 8(14.8%) and 14(24.5%) patients had sugar in the groups of 101-150 mg/dl and 151-200 mg/dl respectively. In case of nephropathy patients there were 3(5.55%) patients with blood sugars less than 100 mg/dl and 28(51.8%) patients with sugars more than 200 mg/dl. 6 (11.1%) and 17 (31.4%) patients with sugars in ranges of 101-150 mg/dl and 151-200 mg/dl respectively.

Renal function test were considered normal when serum creatinine was 1.1 mg/dl and serum urea was 42 mg/dl. It was seen that 57.4% of the diabetic patients had normal renal function test and 42.6% patient had abnormal renal function. 8(14.8%) patients without diabetic nephropathy had deranged renal functions while 38(70.3%) of patients with diabetic nephropathy had deranged renal functions.

The mean hemoglobin of the patients was 11.1 ± 2.36. 14 (25%) non nephropathy patients had hemoglobin less the 11 mg/dl and 40 (74.0%) patients had normal hemoglobin I-e between 12-16 mg/dl. In nephropathy patient group 36 (66.6%) patient had Hb less than 11 mg/dl and 18 (33.3%) patient had Hb between 12-16 mg/dl

C-reactive protein was positive in 54.6% of the patients and negative in 45.4% of the patients. It was present in 7 (12.9%) non nephropathy patients while in 52(96.2%) of the nephropathy patients. ANOVA was applied and p value was calculated between the diabetic nephropathy group and C-reactive proteins and was found to be highly significant $P < 0.000$.

Discussion

Diabetes mellitus is the most common metabolic disorder [12] and leading cause of morbidity and mortality world wide [2] secondary to several micro vascular and macro vascular complications. A diabetes epidemic is underway. Type 2 diabetes mellitus also known as non insulin diabetes mellitus is due to insulin resistance and now several studies have shown clear association of type 2 diabetes with inflammation. In 1998, a hypothesis was proposed suggesting that long-term innate immune system activation resulting in chronic inflammation, elicited disease instead of repair, leading to type 2 diabetes.[13] Recent epidemiological data demonstrates a dramatic increase in the incidence of end stage renal disease in patients with non insulin dependent diabetes mellitus. Several recent studies have also shown that patients with type 2 DM and overt nephropathy exhibit high levels of diverse acute-phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A, fibrinogen, and IL-6. [2,14] Our study describes an association between C-reactive proteins as acute phase marker of inflammation and albumin excretion rate in patients with type 2 diabetes. Several risk factors like duration of diabetes, blood sugar levels, systolic and diastolic blood pressures, serum urea and creatinine levels and anemia were evaluated. Along with this the demographic data like gender and age was collected and chi square was applied to see the association of different variables.

Gender affects the prevalence of NIDDM-related nephropathy. Data from the 1988 United States renal data system reported that the relative risk of diabetic ESRD for females compared with males was 1.20 in blacks and .85 in white[15] Similar results were seen in our study that nephropathy was more prevalent in females two times then the male patients of our study population including the non nephropathy group and all the type 2 diabetic patients included in our study. In our study when we compared the other factors we found out that factors like age , duration of diabetes , systolic and diastolic blood pressures and blood sugar levels were comparable in both the groups with p values of 0.81, 0.014,0.616,0.007,0.786 respectively but nephropathy patients had higher levels of the CRP along with deranged renal functions and anemia (table I). Demographic factors were also not found to be significant in other different studies like Choudhary et al[16] found that these factors were not even significant between a control group and diabetic patients. In case of deranged renal functions similar patterns were seen in study done by Berger[17] who stated that, long term DM significantly increase serum creatinine meaning there is a measurable renal impairment.

In our study 66.6% of diabetic nephropathy patient had hemoglobin less then 11 g/dl while just 25% non nephropathy patients had Hb below 11g/dl. Bosmen et al [18] compared patients who had diabetic nephropathy with matched controls suffering from glomerunephritis. Despite similar levels of protienuria and serum creatinine in the two groups, nearly half of the patients with diabetic nephropathy were anemic compared with none of the patents in control group. Early onset anemia in diabetic nephropathy results from the kidneys inability to increase EPO concentration appropriately.

So if the factors like gender, age , duration of diabetes, systolic and diastolic blood pressures and duration of diabetes are comparable in both the nephropathy and non nephropathy patients then the question here is what is the reason that one group developed type 2 diabetic nephropathy and the other has not. Can it be inflammation responsible for early development of protienuria in type 2 diabetic patients?

Table I: Comparison of Categories of Type 2 Diabetic Patients with Normoalbuminuria (NA) and Microalbuminuria (MA)

Categories	Diabetics	NA	MA	P value
Age	47.8± 10.22	45.9± 11.08	49.7± 8.98	P value .81
Duration of diabetes	8.9 ± 5.38	10.2± 5.50	7.6 ± 4.97	P value .014
Systolic blood pressure	138.2 ± 11.33	136± 11.82	139±10.75	P value .616
Diastolic blood pressures	88.5 ± 8.66	84.3± 7.97	88.6±8.85	P value .007
Blood sugar levels	206.1± 77.94	205± 79.39	206± 77.2	P value .786
Renal function tests	Normal 57.4%	Normal 85.2%	Normal 29.6%	P value .000
	Deranged 42.6%	Deranged 14.8%	Deranged 70.4%	
Anemia	11.1 ± 2.36	12.0± 2.06	10.2± 2.33	P value .000
C-reactive proteins	>3.33 45.4%	>3.33 13%	>3.33 96.3%	P value .000
	<3.33 54.6%	<3.33 87%	< 3.33 3.7%	

Three very recent papers illustrate the idea that inflammation plays a significant role in the pathogenesis of diabetic nephropathy. Dalla Vestra and colleagues[2] showed that patients with type 2 diabetes and overt nephropathy exhibit the highest levels of diverse acute-phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and IL-6. Furthermore, levels of CRP, SAA and IL-6 were higher in subjects with increased glomerular basement membrane (GBM) width. The Casale Monferrato Study [19] demonstrated that fibrinogen is an independent predictor of progression to overt nephropathy in white patients with type 2 diabetes. Chow et al[20] showed that db/db mice, a model of type 2 diabetes and DN, exhibited an increased expression of intracellular adhesion molecule-1 (ICAM-1), which promotes inflammation by increasing leukocyte infiltration and adherence, in glomeruli and tubules, along with a marked increase in macrophage infiltration.

CRP is a plasma protein, an acute phase protein produced by the liver and by adipocytes. The level of CRP rises when there is body-wide (systemic) inflammation. The CRP test is considered a general test, not a specific one. CRP is produced in response to various cytokines, including interleukin IL-6, IL-1 and tumor necrosis factor (TNF)-alpha during acute injury, infections, inflammatory stimuli, and malignant disease. Currently CRP is being used in prediction of the coronary artery disease and flare up of Inflammations in diseases like lupus, rheumatoid arthritis. Therefore we excluded patients with acute illness on course, diabetic patients with recent myocardial infarction and other factors interfering with CRP levels e.g. infection, other inflammatory conditions

In our study we see that 96.2% of the type 2 diabetic nephropathy patients have raised C- reactive proteins levels as compare to only 7 non nephropathy patients.

Similar results have been reported by Chaudhary et al [15] who explained the finding of association of markers of inflammation with protienuria of diabetic nephropathy by three mechanisms. First, elevated levels of inflammatory markers may be the result of pre-existing atherosclerosis in patients with microalbuminuria. In no diabetic individuals as well as patients with type 2 DM, microalbuminuria is associated with increased cardiovascular morbidity and mortality, suggesting that in individuals with albuminuria, atherosclerotic disease prevails. Second, elevations of acute-phase reactants and/or inflammatory cytokines may directly alter glomerular function and thus be causally involved in the development of albuminuria. Third, there is a potential link between inflammatory cytokines and glomerular function.

Conclusion

In conclusion, the pathogenetic vision of diabetes mellitus has changed in the last few years, with inflammatory pathways playing pivotal roles in the development and progression of diabetic complications which in our study shown by increased levels C-reactive proteins in type 2 diabetic nephropathy patients. These new pathogenetic factors lead to a consideration of new therapeutic approaches. Modulation of inflammatory processes in the setting of diabetes is nowadays a matter of great interest. It is possible that in the coming years the hope of new therapeutic strategies based on inflammatory properties with beneficial actions on diabetic complications can be translated into real clinical treatment.

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