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DIABETIC PATIENTS;
STUDY OF INFLAMMATORY MARKERS

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ABSTRACT… Objectives: Inflammation is the one of the major causes for development of type 2 diabetes mellitus and its complications. In this study, association between inflammation and type 2 diabetes mellitus was studied by measuring various inflammatory markers (soluble vascular cell adhesion molecules type – 1, Interleukin – 6 and C- reactive protein) between healthy and diabetic patients. **Study Design:** A cross sectional comparative study. **Place and Duration of Study:** The study was conducted at Department of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi in collaboration with Combined Military Hospital, Rawalpindi and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi for blood sampling and biochemical assays respectively. **Material and Methods:** The study was performed in 40 human subjects divided into two groups containing 20 subjects each. One group was designated as control while the other was diseased (diabetic) group. Glycemic status was measured to confirm their normal and diabetic state. Inflammatory markers were measured by Enzyme Linked Immunosorbent Assay (ELISA). **Results:** Levels of all inflammatory markers (soluble vascular cell adhesion molecules type – 1, Interleukin – 6 and C-reactive protein) were found to be raised in the experimental diabetic groups; 1991.5 ± 201.97 ng/ml, 24.99 ± 1.366 pg/ml and 2931 ± 168.319 respectively compared to the control group; 570.2 ± 16.526 ng/ml, 6.64 ± 0.3516 pg/ml and 1806.6 ± 183.32 respectively. **Conclusions:** Inflammatory markers were significantly elevated in patients with diabetes mellitus as compared to normal healthy control subjects.

Key words: Diabetes mellitus, inflammation, soluble vascular cell adhesion molecules type – 1, Interleukin – 6 and C- reactive protein

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders, which manifests due to chronic hyperglycemia caused by defects in insulin secretion, insulin action or both. Approximately 285 million people all over the world are a victim of this chronic, insidious disease and it is the fifth leading cause of death all over the world[1,2]. DM is one of the principal cause of amputation, blindness, stroke, heart attacks and renal failure in adults. Type 2 diabetes is the most common i.e. accounts for approximately 90-95% of all the cases of DM, while DM Type 1 is rarer i.e. 5-10 %[3].

The type II DM pathogenesis is of very complex nature and involves relative deficiency of insulin secretion or development of insulin resistance leading to hyperglycemia. Some studies showed the interrelationship between inflammation and non-insulin dependent DM which has proven the fact that inflammation is one of the causative agent for development of type 2 diabetes mellitus and insulin resistance[4,5,6]. The process of inflammation induces hepatic synthesis of various acute phase proteins such as C-reactive protein (CRP), cytokines such as interleukin-6 (IL-6), and various adhesion molecules such as soluble intracellular adhesion molecules type 1 (sICAM-1) and soluble vascular cellular adhesion molecules type 1 (sVCAM-1) which are believed to play a pivotal role in insulin resistance[5,7,8,9].

The mechanism leading to the complications of diabetes are multifactorial, and not yet fully understood to medical science, but involve the direct toxic effects of high glucose levels, along with the impact of deranged lipid levels. It is also clear from a number of studies the risk for cardiovascular disease starts years before the clinical diagnosis of diabetes[10, 11, 12]. Inflammation not only predisposes an individual to develop diabetes mellitus but also tends to cause dyslipidemia, giving rise to increased risk of micro vascular (neuropathy, nephropathy and retinopathy) and macro vascular (peripheral vascular disease, stroke and ischemic heart disease) complications, which can lead to significant morbidity and diminished quality of life[13,14,15].

Based on the above scenario, this cross sectional comparative study was designed in which level of inflammatory markers was compared between diabetic (test) and non-diabetic (Control) groups.

MATERIALS AND METHODS

This cross sectional comparative study was conducted at Department of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi in collaboration with Combined Military Hospital (CMH), Rawalpindi and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi for blood sampling and biochemical assays respectively. Total duration of study was 01 year. This study was performed on 40 human volunteers. Subjects of both sexes, having age between 25 to 55 years and body mass index between 19 – 40 kg/m^2 were inducted in this study. They were divided into two groups containing 20 subjects each. One group was designated as control (healthy) group while the other was diseased (diabetic) group.

GROUP-1 (Control Group)

Control group comprised of 20 normal healthy human subjects having normal fasting blood glucose levels (between 70 – 100 mg/dl). Pregnant women/subjects suffering from any chronic illness or disease or taking any sort of medication / supplements were excluded.

GROUP- 2 (Diabetic Group)

This group consists of 20 type – II diabetic patients having fasting blood glucose in a range of 126 – 400 mg/dl, although taking their usual antidiabetic medication. Patients on insulin therapy, pregnant women, subjects suffering from any other chronic illness or disease or taking any medication other than that of diabetes were excluded out.

Patients of both sexes with known type – II diabetic history and fulfilling all the inclusion criteria were screened by determining their fasting blood glucose by a Glucometer. Similarly, healthy individuals were screened by determining their fasting blood glucose before selection for the study. The subjects selected for the control group belonged to every walk of life i.e. army officers, civil servants, business men, house wives etc. and had no medical history of diabetes or any other disease whereas the diabetic group included subjects visiting the diabetes clinics of Military Hospital (MH) and Combined Military Hospitals (CMH), Rawalpindi. At the end of study, inflammatory markers (sVCAM-1, IL-6 and CRP) were measured for each subject of both the groups, by Enzyme Linked Immunosorbent Assay (ELISA) method.

STATISTICAL ANALYSIS

The data was entered and analyzed using SPSS version 15.0. Mean, Standard error of mean (SEM) & Standard deviation (SD) were calculated. Analysis of Variance (ANOVA) was applied for comparison inflammatory markers between both experimental & control groups. A p-value of = 0.05 and =0.01 was considered significant and highly significant respectively.

RESULTS

Results of inflammatory markers (sVCAM-1, IL-6 and CRP) of group-1 & 2 are mentioned in table I & II respectively. There was a significant difference among the levels of sVCAM-1, IL-6 and CRP between diabetic and control groups. (Table-III, figure 1 and figure 2).

Study group	Parameters	Sample size	Mean	Standard Deviation (SD)
Control Group	sVCAM-1 (ng/ml)	20	570.2	73.9
	IL - 6 (pg/ml)		6.64	1.572
	CRP (ng/ml)		1806.6	819.856

Table-I. Inflammatory markers of control group

Study group	Parameters	Sample size	Mean	Standard Deviation (SD)
Diabetic Group	sVCAM-1 (ng/ml)	20	1991.5	903.27
	IL - 6 (pg/ml)		24.99	6.113
	CRP (ng/ml)		2931	752.74

Table-II. Inflammatory markers of diabetic group. ** p < 0.001 Highly significant*

Inflammatory markers	P-value
sVCAM-1	0.000*
IL - 6	0.000*
CRP	0 000*

Table-III. Analysis of Variance on Inflammatory markers between control and diabetic group.

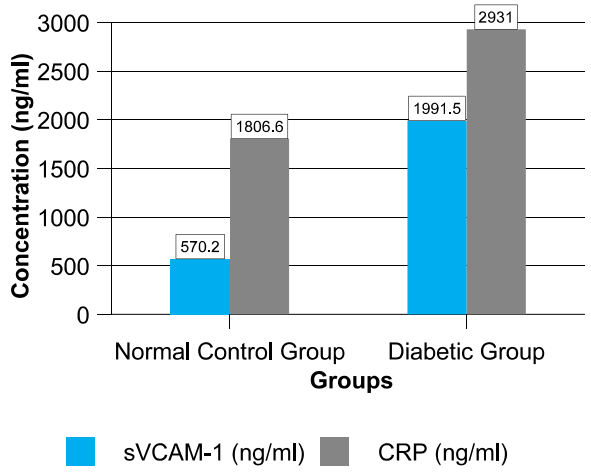

Figure-1. Comparison of inflammatory markers (sVCAM-1 and CRP) between control and diabetic group.

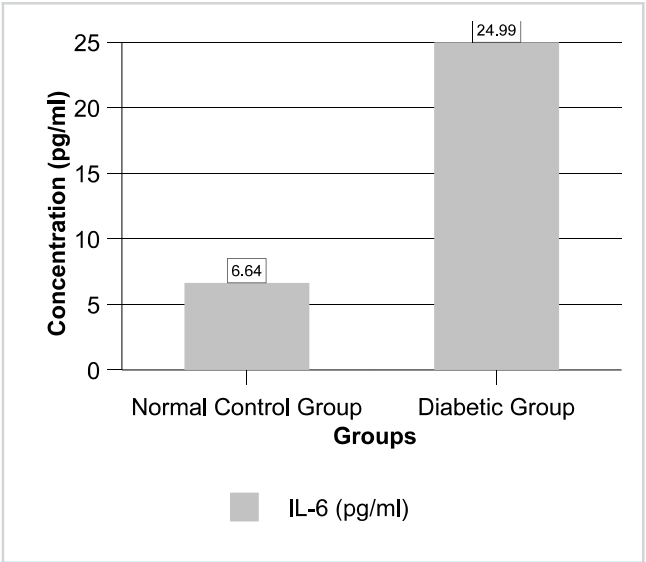

Figure-2. Comparison of inflammatory marker (IL-6) between control and diabetic groups.

DISCUSSION

Ridker et al., and Pradhan et al., demonstrated in a series of multiple studies that CRP is a risk factor for insulin resistance and diabetes mellitus[9, 16, 17]. They concluded that the levels of CRP are associated with type 2 diabetes mellitus, insulin resistance and cardiovascular diseases. In 2006, Rhee et al., studied a positive correlation between long term deranged fasting blood glucose levels and CRP[18]. In conjugation to these findings, studies conducted by pickup et al., and Wang et al supports elevated levels of CRP in type 2 diabetes in association with the presence of inflammation[19, 20].

Our study also supports the above mentioned findings. Blood levels of C-reactive protein (CRP) in the control group were in the range of 840 – 3460 ng/ml with a mean value of 1806.6 ± 183.32 as compared to range of 1680 – 4220 ng/ml with a mean value of 2931 ± 168.319 in the experimental diabetic group. There was a highly significant increase in CRP between both the groups ($p = 0.000$, $p < 0.001$)

Saraheimo et al., 2003 found high levels of interleukin-6 in type 2 diabetic patients[21]. Jones et al., also studied relation of IL-6 and CRP in his studies and explained the underline mechanism[22]. Pepys et al., concluded that liver cells produces CRP in response to IL-6 and therefore have a positive correlation with each other[23]. Levels of circulating IL-6 in the blood were in the range of 4.8 – 9.4 pg/ml with a mean value of 6.64 ± 0.3516 pg/ml for the control group compared to range of 14.8 – 36 pg/ml with a mean value of 24.99 ± 1.366 pg/ml in the experimental diabetic group. Both groups revealed a highly significant difference in the levels of IL-6 ($p = 0.000$, $p < 0.01$).

Boulbou et al., 2005 concluded that type 2 diabetes effects endothelial functions and stimulates adhesion molecules expression[24]. His study aimed that endothelial activation leading to type 2 diabetes through elevated levels of sVCAM-1 molecules. Devaraj et al., 2007 also concluded significantly increased levels of adhesion molecules in diabetes and its progression to complications i.e. diabetic retinopathy[25]. Blood levels of sVCAM-1 were in the range of 466 – 742 ng/ml with a mean value of 570.2 ± 16.526 ng/ml in the control group compared to the range of 630 – 3460 ng/ml with a mean value of 1991.5 ± 201.97 ng/ml for the experimental diabetic group. There is a highly significant increase in the levels of sVCAM-1 from control to diabetic group ($p = 0.000$, $p < 0.001$). These findings are in synchronization with the studies mentioned above though level of significance was quite high in our study.

Above scenario clearly determines that there is an ample data suggesting that long standing inflammation preceding insulin resistance and hyperglycemia leading to raised levels of various inflammatory markers in type 2 diabetes, will have definite role in the pathogenesis of macro and micro complications in diabetes mellitus.

CONCLUSIONS

Present study substantiates the observation that the levels of inflammatory markers (sVCAM-1, IL-6 and CRP) are increased in type 2 diabetics thus having a significant association with glycemic profile.

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