ORIGINAL RESEARCH PAPER

Biochemistry

KEY WORDS: Case Control Study, Inflammatory Biomarkers, Cimt, Cardiovascular Risk Factors

ASSOCIATION OF INFLAMMATORY BIOMARKERS IN NEWLY DIAGNOSED TYPE 2 DIABETICS WITH ATHEROSCLEROTIC CHANGES.

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ABSTRACT

Increased risk of atherosclerosis in type 2 diabetics has been attributed to high prevalence of multiple atherosclerotic risk factors. A hospital based case-control study was conducted for 2 years to compare the serum levels of inflammatory biomarkers in newly diagnosed type 2 diabetics with healthy controls, to find out the correlation of these biomarkers with cardiovascular risk factors, and to observe the association of these biomarkers with atherosclerotic changes evidenced by Carotid Intimo-Medial Thickness (CIMT). Out of total 80 subjects, 40 healthy individuals were taken as control group and 40 cases of newly diagnosed patients with type 2 diabetes mellitus were taken as test group. The inflammatory biomarkers analysed were IL6, IL18, fibrinogen and hsCRP. ApoB and lipid profile were taken as cardiovascular risk factors. CIMT was measured by Doppler ultrasonography. The mean serum FBS, PPBS, HbA1C, lipid profile, ApoB and inflammatory biomarkers were significantly raised in test group compared to the control group. Significant positive correlation have been found between cardiovascular risk factors (ApoB, Total cholesterol, LDL-C) and inflammatory biomarkers. The inflammatory biomarkers are also positively correlated with CIMT. The study concluded that inflammatory biomarkers are associated with type 2 diabetes mellitus and lead to increased intimo-medial thickening of the carotid arteries.

INTRODUCTION

Atherosclerosis involves a chronic inflammatory process comprising an interaction of immune mechanism with metabolic risk factors that manifests as vascular events. Plasma levels of circulating biomarkers of inflammation are believed to reflect the severity of inflammation and extent of underlying atherosclerosis. This process is triggered by some pro-inflammatory biomarkers like cytokines (Interleukins), acute phase protein (hsCRP), pre thrombotic protein (fibrinogen) etc. These new findings provide important risk factors and the mechanism of atherosclerosis.[1,2]

Studies suggest that various conditions like oxidized lipoproteins, dyslipidaemia, hypertension, diabetes mellitus, obesity etc, are related to the inflammatory process. Among these, type 2 diabetes mellitus is one of the important conditions associated with chronic inflammations. It is a metabolic disorder associated with hyperglycemia either due to deficiency of insulin secretion or reduction of biological effectiveness of insulin. Type 2 diabetes melltius combined with dyslipidemia is associated with elevated risk of cardiovascular disease (CVD).[3,4] Although the main physiological abnormality of this disease is associated with insulin resistance or impaired insulin secretion, some underlying determinants of this metabolic defect is not clear as yet.[5] Hyperglycemia associated with diabetes mellitus can lead to modification of macromolecules like "Advanced Glycation End products" (AGE). The AGE modified proteins can augment the production of proinflammatory cytokines and other inflammatory pathway in vascular endothelial cells.[6]

Proinflammatory markers like IL-6, IL-18, fibrinogen along with hsCRP and Apo B are thought to play major role in inflammations as well as atherogenesis in diabetes. Interleukin-6 is a multifunctional cytokine regulating both humoral and cellular response and play a central role in inflammation and tissue injury. Presence of higher level of IL-6 has been observed in the patients suffering from atherosclerotic cardiovascular disease.[7]

Interleukin-18, a proinflammatory marker of cytokine family is associated with poor glycemic control of type 2 diabetics and found to play a role in progress of coronary artery disease.[8]

Fibrinogen, a hemostatic protein and also considered as prothrombotic factor is associated with development and progression of atherosclerotic changes. Fibrinogen and non HDL cholesterol synergistically effect as factor for accelerating the progression of carotid atherosclerosis in diabetic patients.[9]

C-reactive protein, as a positive acute phase protein is a marker of systemic inflammation that increases in response to some other inflammatory agents like IL-6 and plays an important role in assessing the cardiovascular risk associated with the chronic vascular inflammation of atherosclerosis.[10]

Apo lipoprotein B-100 is the major Apo lipoprotein of LDL-C and synthesized in the liver. Though it is well documented that elevated level of LDL-C and other Apo B containing lipoproteins lead to atherosclerosis, the molecular and cellular mechanisms are still poorly understood. Several hypotheses suggest that sub-endothelial retention of lipoproteins is the initial step leading to oxidation and inflammation along with endothelial dysfunction.[11]

The present study is designed to study the role of different inflammatory biomarkers in development of atherosclerosis in type 2 diabetics, by observing carotid intima media thickening. Though the cardiovascular complications among type 2 diabetics are not uncommon in this region there is very little information about the inflammatory biomarkers in development of adverse effects in type 2 diabetics. Hence this study is expected to provide information about alterations of the inflammatory biomarkers in type 2 diabetics and their role in development of atherosclerotic changes.

OBJECTIVES :

- To study inflammatory biomarkers (IL-6, IL-18, Fibrinogen, and hsCRP) in newly diagnosed type 2 diabetics and to compare the serum values of these biomarkers with healthy individuals.
- To find out the correlation of these biomarkers with cardiovascular risk factors (ApoB-100 and fasting lipid profile).
- To observe the association of these biomarkers with atherosclerotic changes evidenced by Carotid Intimo-Medial Thickness (CIMT).

METHODS AND MATERIALS:

This is a case-control hospital based study which includes newly diagnosed type 2 diabetic patients without cardiovascular signs & symptoms, attending Department of Medicine of an apex medical college & hospital, both as indoor and outdoor patients during the time period from 01-03-2016 to 28-02-2018. About 40 subjects within the age group of 25-50 years of both sexes are randomly selected. Equal numbers of age and sex matched healthy individuals are selected as control.

***Selection of patients*:**

The type 2 diabetic patients diagnosed within last six (6) months not under antidiabetic treatment and without cardiovascular signs & symptoms are selected as subjects. The diagnosis of diabetes is done on the basis of guidelines of American Diabetic Association: Fasting Plasma Glucose (FPG)≥126 mg/dl (7.0 mmol/L) Or a 2 hr Post prandial Plasma Glucose≥200 mg/dl (11.1 mmol/L) Or a Random Plasma Glucose≥200 mg/dl (11.1 mmol/L) with patient with classic symptoms of hyper glycemia or hyperglycemic crisis. Hb A1C level of 6.5% or higher should be primary diagnostic criterion.

***Exclusion criteria*:**

- Known type 2 diabetic patients with treatment
- Patients having other inflammatory diseases
- Type 1 diabetes mellitus
- Patients on statin or other hypolipidemic therapy for more than 6 months

Laboratory investigations carried out are as follows:

(a) Plasma glucose and Fasting lipid profile by colorimetric method.
(b) HbA1C by HPLC based method.
(c) Interleukin-6, hsCRP, Interleukin-18 and Apo B-100 by immunoassay method
(d) Fibrinogen by coagulation based method.
(e) Carotid intima-media thickening by Doppler Ultrasonography.

All statistics were analysed by using SPSS software. The results were presented in number, percentage, mean and standard deviation. Intergroup comparison was done by Student 't' test. The Pearson correlation was used for correlation analysis. Simple linear regression was performed to study the determinants of mean CIMT and inflammatory markers. Two sided $p<0.05$ was considered statistically significant.

RESULTS:

Age and sex distribution of subjects: In the control group, the age of the subjects ranged from 25 to 50 years, with a mean of 38.55 years and a standard deviation of 8.33. The majority of them belonged to fifth decade constituting 45% of the total. Out of a total of 40 controls 28 were male (70%) and 12 were female (30%). The age of the patients ranged from 25 years to 50 years, with a mean of 39.3 years and a standard deviation of 7.4. The pick incidence of the disease was observed in the age group of 40 to 50 years (55%) followed by 30 to 39 years (32.5%) and 20 to 29 years (12.5%). Out of a total of 40 cases 29 were male (72.5%) and 11 were female (27.5%).

TABLE 1 Age and sex distribution of subjects

Variables		Group			
		Control (n=40)		Case (n=40)	
		Number of subjects	Percentage	Number of subjects	Percentage
Age in years	20 – 29	6	15	5	12.5
	30 – 39	16	40	13	32.5
	40 – 50	18	45	22	55
Sex	Male	28	70	29	72.5
	Female	12	30	11	27.5

Demographic and baseline characteristics:

TABLE 2 Demographic profile of diabetic and control group

Variables	Controls (n=40)	Cases (n=40)	t	p
Age (Years)	38.55 ± 8.33	39.3 ± 7.4	0.425	0.672
Sex (Male/Female)	28/12	29/11		
Systolic B.P. (mm Hg)	121.7 ± 9.27	126.3 ± 13.1	1.805	0.07
Diastolic B.P. (mm Hg)	77.35 ± 7.24	80 ± 8.5	1.475	0.144
BMI (Kg/m^2)	25.3 ± 2.1	26.1 ± 3.3	1.303	0.196
Smoking	5	12		
Alcoholism	2	5		
Family history of diabetes	2	11		
FBS (mg/dl)	85.75 ± 9.24	188.13 ± 86.77	7.420	<0.0001
PPBS (mg/dl)	119.1 ± 24.65	280.45 ± 121.66	8.221	<0.001
HbA1C (%)	5.06 ± 0.77	8.36 ± 3	6.741	<0.001
Total Cholesterol(mg/dl)	134.43 ± 40.56	161.43 ± 38.87	3.040	0.003
LDL cholesterol(mg/dl)	71.72 ± 34.32	81.67 ± 31.4	1.353	0.180
HDL cholesterol(mg/dl)	42.83 ± 18.78	39.2 ± 9.86	1.081	0.283
Triacylglycerol (mg/dl)	99.38 ± 58.36	163.38 ± 105.24	3.364	0.001

Table 2 shows subject characteristics in diabetic patients and healthy controls. Control subjects have lower systolic and diastolic blood pressure and BMI but it is not significant. 7(Seven) patients (17.5%) in the diabetic group had evidence of coronary artery disease in the form of atheromatous plaque in the coronary artery. Twelve diabetic patients were smokers. Among control group there were 5 subjects who were smokers. Five diabetic patients were alcoholic in comparison to 2 alcoholics in control group. 11 diabetic patients have family history of diabetes while only 2 control subjects have relevant history.

Diabetic patients had significantly higher total serum cholesterol and triglyceride. The serum LDL-cholesterol level is high and HDL-cholesterol is low in diabetic subjects as compared to control subjects but which is not significant.

Inflammatory biomarkers:

TABLE 3 Inflammatory markers in diabetic and control groups:

Variables	Controls	Cases	t	p
Fibrinogen	134.1 ± 76	260.8 ± 113.4	5.871	<0.001
IL6	5.04 ± 4.40	36.76 ± 28.66	6.915	<0.001
IL18	67.65 ± 19.48	376.18 ± 211.67	9.180	<0.001
ApoB	64.4 ± 18.3	107.1 ± 46.1	5.456	<0.001
HsCRP	0.93 ± 0.62	4.18 ± 3.85	5.268	<0.001
CIMT	0.52 ± 0.1	0.7 ± 0.2	4.924	<0.001

Table 3 shows that all the inflammatory markers are raised significantly in type 2 diabetic subjects when compared to normal controls

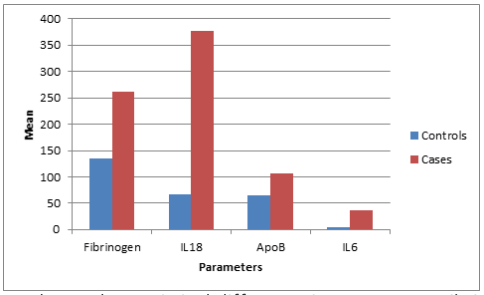

Figure 1 shows the statistical difference in parameters Fibrinogen, IL18, ApoB and IL6 between control and diabetic group.

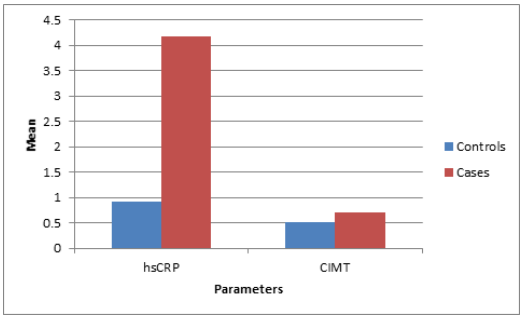

FIGURE 2 shows the statistical difference in parameters hsCRP and CIMT between control and diabetic group.

Carotid Intimo-medial Thickness (CIMT) in diabetic patients and control group:

The mean carotid IMT was significantly higher in diabetic patients than in healthy controls. The mean carotid IMT in diabetic subjects was 0.7+0.2 mm versus 0.52±0.1 mm in controls (p<0.01). The range of carotid IMT and diabetic and control group was 0.45-1.5 mm and 0.3-0.64 mm, respectively. None (0%) of healthy control had evidence of carotid artery atherosclerosis while 17.5% of diabetic subjects (7/40) showed features of carotid artery atherosclerosis in the form of diffuse thickening, atheroma formation, stenosis and calcified plaque formation (p<0.01).

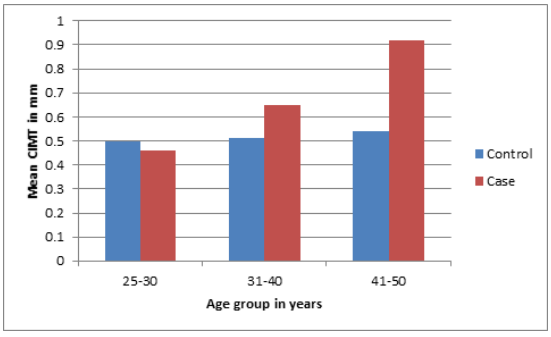

FIGURE 3 Mean CIMT in different age groups in controls and cases

Figure 3 shows that the mean carotid IMT was higher among the diabetic subjects in the higher age groups and slightly lower among the lower age groups when compared with the control group. At age 25-30 years, mean IMT in diabetic group was 0.46±0.01 mm while in the control group it was 0.5±0.09 mm (not significant, P=0.4), in the 31-40 year group 0.65±0.09 mm, in the diabetic group and 0.51±0.09 mm in the control group (significant, p<001), and at 41-50 years 0.92±0.29 mm in diabetic group and 0.54±0.07 mm in control group (significant, p<001). The difference in carotid IMT levels was significant in all age groups (p<0.01).

Correlation studies:

Correlation between Cardiovascular risk factors and inflammatory biomarkers:

Linear regression was performed between the parameters of cardiovascular risk factors (Cholesterol, LDL-C, TGL, HDL-C and ApoB) and inflammatory biomarkers (Fibrinogen, hsCRP, IL6 and Il18). Significant positive correlation was found between Cholesterol and fibrinogen (r=0.64, p<0.001), Cholesterol and hsCRP (r=0.62, p<0.001), Cholesterol and IL 18 (r=0.591, p<0.001), Cholesterol and IL6 (r=0.374, p<0.05), LDL-C and fibrinogen (r=0.898, p<0.001), LDL-C and hsCRP (r=0.616, p<0.001), LDL-C and Il18 (r=0.834, p<0.001), LDL-C and IL6 (r=0.485, p<0.01), ApoB and fibrinogen (r=0.985, p<0.001), ApoB and hsCRP (r=0.768, p<0.001), ApoB and IL18 (r=0.901, p<0.001), ApoB and IL6 (r=0.523, p<0.01), TGL and hsCRP (r=0.456, p=0.003). There is no significant correlation between HDL-C and fibrinogen (r=0.277, p=0.083), HDL-C and hsCRP (r=0.276, p=0.084), HDL-C and Il18 (r=0.238, p=0.14), TGL and Il18 (r=0.234, p=0.147), TGL and fibrinogen (r=0.137, p=0.401), TGL and IL6 (r=0.115, p=0.481), HDL-C and IL6 (r=0.085, p=0.603).

TABLE 4 Correlation between cardiovascular risk factors and inflammatory biomarkers:

	Fibrinogen		hsCRP		Il6		Il18	
	R	p	R	p	r	p	R	P
ApoB	0.985	<0.001	0.768	<0.001	0.523	<0.01	0.901	<0.001
Total Cholesterol	0.64	<0.001	0.62	<0.001	0.374	<0.05	0.591	<0.001
LDL-C	0.898	<0.001	0.616	<0.001	0.485	<0.01	0.834	<0.001
TGL	0.137	0.401	0.456	<0.01	0.115	0.481	0.234	0.147
HDL-C	0.277	0.083	0.276	0.084	0.085	0.603	0.238	0.14

Correlation between inflammatory biomarkers and CIMT:

a) Correlation between IL18 and CIMT:

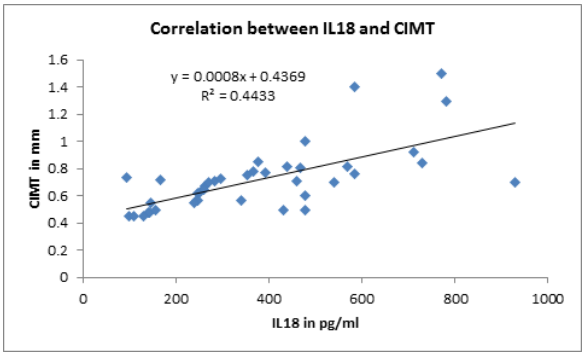

Figure 4 shows that there is positive correlation between IL18 and CIMT in cases with correlation coefficient r = 0.666 which is significant with p<0.001.

b) Correlation between Fibrinogen and CIMT:

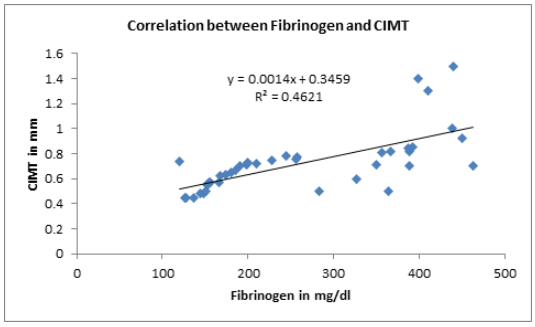

Figure 5 shows that there is positive correlation between Fibrinogen and CIMT in cases with correlation coefficient r = 0.680 which is significant with p<0.001.

c) Correlation between IL6 and CIMT:

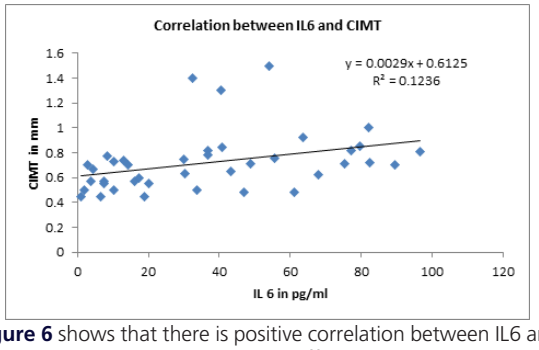

Figure 6 shows that there is positive correlation between IL6 and CIMT in cases with correlation coefficient r = 0.352 which is significant with p<0.05.

d) Correlation between hsCRP and CIMT:

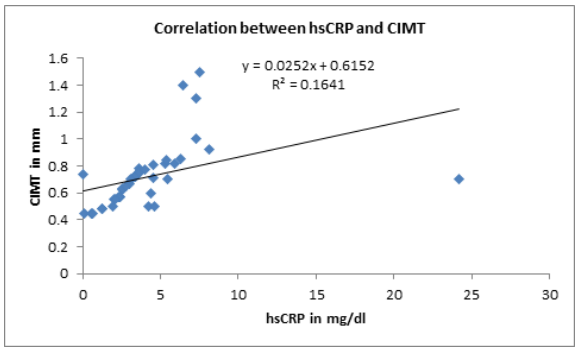

FIGURE 7 shows that there is positive correlation between hsCRP and CIMT in cases with correlation coefficient r = 0.405 which is significant with p<0.05.

TABLE 5 Correlation between other factors and CIMT:

	Age		BMI		FBS		PPBS		HbA1C	
	r	p	r	p	r	p	r	p	r	P
CIMT	0.753	<0.001	-0.041	0.797	0.642	<0.001	0.591	<0.001	0.204	0.207

Table 5 shows that Age, FBS and PPBS are significantly positively correlated with CIMT while BMI and HbA1c has no correlation with CIMT.

DISCUSSION:

We have studied the association of inflammatory markers (IL6, IL18, hsCRP, fibrinogen) in Type 2 diabetic subjects and non-diabetic control. Concentration of hsCRP, IL6, IL18 and fibrinogen were significantly increased in diabetic patients when compared with non-diabetic controls. One study found significant increase of inflammatory biomarkers fibrinogen, CRP and TNF-α in diabetic patients.[12] Another study demonstrated that there is significant rise of serum IL18 in diabetic subjects compared to normal subjects.[8] The study by other researchers also showed that plasma IL18 concentrations were significantly higher in type 2 diabetic patients than in age-matched control subjects.[13] One study found that the majority of markers of low grade inflammation and endothelial activation except IL6 were significantly higher in the diabetic cohort than in healthy controls.[14]

The significant correlation between inflammatory markers (hsCRP, IL18, IL6 and fibrinogen) and traditional risk factors (ApoB and lipid profile) may be a reflection of its dependence on these factors. Although further studies are required to confirm these observations, the findings raise questions on the mechanisms of increased inflammatory markers in diabetic patients.[12]

The present study shows that the mean carotid IMT is increased in diabetic patients compared with non-diabetic subjects both in men and women (p<0.01). Thus diabetes per se augments the process promoting IMT. Our data are complementary to previous studies showing that carotid IMT is thicker in diabetic patients than healthy controls.[15]

Moreover, the mean carotid IMT was higher among the diabetic subjects in the higher age groups and slightly lower among the lower age groups (but not significant) when compared with the control group. None (0%) of healthy control had evidence of carotid artery atherosclerosis while 17.5% of diabetic subjects (7/40) showed features of carotid artery atherosclerosis in the form of diffuse thickening, atheroma formation, stenosis and calcified plaque formation (p<0.01). There is significant correlation between age and CIMT in diabetic subjects (r=0.753, p<0.001).

Mean IMT was significantly correlated with plasma glucose fasting and postprandial plasma glucose but not with HbA1 C (p<0.001). No correlation was found between BMI and CIMT in both diabetic and control group. Mean IMT was also correlated with hsCRP (r=0.405, p<0.05), IL18 (r=0.666, p<0.001), fibrinogen (r=0.680, p<0.001) and IL6 (r=0.352, p<0.05) in diabetic population.

Hypothetically an association between diabetes and atherosclerosis might be explained with the inflammation. Diabetes has been linked to several inflammatory markers such as CRP and interleukin-6.[16,17] Furthermore, inflammation is also seen in patients with accelerated atherosclerosis.[18,19]

While many mechanisms are undoubtedly involved in the development of vascular disease in diabetes, insulin resistance is thought to play a major role in acceleration of atherosclerosis. The biological mechanisms underlying the association between insulin resistance and haemostatic variables are not yet completely clear. It is now recognized that impaired fibrinolytic potential is major feature of insulin resistance.[20]

The study has some limitations. Although the patients were carefully selected to exclude those who may have disease associated with acute phase response we cannot absolutely exclude the presence of occult subclinical state that could increase serum markers of inflammation.[12] This is a case control study that has evaluated an association of inflammatory markers and carotid IMT in diabetics and the results should be interpreted with the limitations of such an observational study.

CONCLUSION: From this study it can be concluded that patients with newly diagnosed type 2 diabetes mellitus had increased inflammation and carotid intimo-medial thickness than in the normal subjects. Inflammatory biomarkers are associated with several risk factors for the development of cardiovascular disease in Type 2 diabetic patients. Moreover, inflammation is also associated with probable atherosclerosis in Type 2 diabetes and has severe consequences at the level of carotid wall. However, it is not yet clear that whether inflammation is a primary phenomenon in patients of Type 2 diabetes leading to accelerated atherosclerosis. More detailed studies are needed to understand the relationship between inflammation and type 2 diabetes mellitus. Our results have strongly supported the inclusion of inflammatory markers in the risk assessment of diabetic patients. Those with elevated levels of these inflammatory biomarkers should be managed aggressively to prevent the development or progression of cardiovascular disease.

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