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Research Article

INFLAMMATORY MARKERS FOR EARLY DIAGNOSIS OF PERIPHERAL VASCULAR DISEASE IN DIABETIC PATIENTS

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ABSTRACT

Peripheral vascular disease (PVD) is a major cause of morbidity in diabetic patients. Inflammatory markers allow earlier detection of PVD. To identify inflammatory markers like hsCRP, apo-A, apo-B, homocystine levels in early detection of peripheral vascular disease in asymptomatic diabetic patients. This study is a cross sectional study. Diabetic patients for more than 3 years without other comorbid illness were included in study. The selected subjects after screening ,hCRP, apoA, apoB, homocystiene,doppler study of lower limb vessels were done. Of the 88 patients only 18 patients had elevated level of apoA (20.5%) apoB levels were elevated in 62 patients. (70.5%). Highly sensitive CRP was elevated in 57 patients. (64.5%) The mean age of the study population was 50 years and the mean duration of diabetes was 7 years.13.5% of diabetic patients had normal HbA1C and did not have elevated biomarker levels. Patients were followed up for 3 years .Of patients who had elevated biomarkers 42%developed PVD. This study shows that inflammatory markers like hCRP, apo-A and apo-B are sensitive markers for detection of peripheral vascular disease early .Tight control of diabetes is important to prevent occurance of complications of diabetes.

Key words- PVD(peripheral vascular disease), hsCRP(high sensitive c- reactive protein), apo-A(apolipoprotein A), apo-B(apolipoprotein B)

INTRODUCTION

Peripheral vascular disease (PVD) is a major cause of morbidity and mortality especially affecting the elderly population. The prevalence of PVD is multifold higher in patients with diabetes compared with age- and sex matched non diabetic subjects, and this may be because of hyperglycemia, hypertension, hyperlipidemia, platelet factors, and other factors that are increased in diabetic subjects. , the overall prevalence of PVD estimated in the whole population was 3.2%, and it was 6.3% in the diabetic population. Thus although prevalence of PVD is low among south Indian population, it is relatively high among those with diabetes Conventional risk factors known to contribute to markers in pathways of inflammation, thrombosis, lipoprotein metabolism, and oxidative stress in determining susceptibility to PAD is not fully defined. Validation of novel risk markers for PAD may allow earlier detection, an improved understanding of disease etiology and progression, and the development of new therapies. Since PAD may have varying clinical presentations, a valuable tool for investigating novel markers for this disease is the ankle–brachial index (ABI), an objective, reproducible, non-invasive measure that correlates with PAD severity. ABI = 0.90 is 95% sensitive and 90% specific for the presence of a = 50% narrowing of a lower extremity artery and is used in the clinical setting to establish a diagnosis of PAD. ABI over time provides a measure of PAD progression and ABI also

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provides prognostic information. Patients with severe PAD (ABI = 0.40) have a significantly decreased survival, with only 24% of patients alive at 12 years. In recent years, several 'novel' circulating markers, including C-reactive protein (CRP), fibrinogen, lipoprotein(a) (Lp(a)), and homocysteine have been examined as potential risk factors for atherothrombotic vascular disease. This review will focus on role of inflammatory markers in predicting the onset of PAD before the manifestation of clinical features.

AIM:

To identify whether inflammatory markers are useful in early detection of peripheral vascular disease in chronic asymptomatic type 2 Diabetes Mellitus patients.

MATERIALS & METHODS:

This study was conducted in the Department of Medicine of SreeBalaji Medical College, Chennai, Tamil Nadu during the period of August 2010 to August 2011.Total number of patients included in this study were 88. There were 31 males, 57 female patients ranging from 30 years to 70 years, who were diagnosed to have type 2 diabetes mellitus of more than 3years of duration.This study is a cross sectional study. This study is aimed to assess the role of inflammatory markers like high sensitivity CRP, apo-A, apo-B, homocystine level in asymptomatic type 2 diabetic patients as a predictor for peripheral vascular disease.

INCLUSION CRITERIA:

1.Male and female patients of age > 30 years were selected.
2.Subjects who are diabetic for > 3 years with or without treatment were included.
3.Subjects Blood Pressure should be between 110-140/70-90mm Hg with or without treatment.
4.Subjects with no history of heart disease or complaints related to cardiac disease like history of CAD, moderate to severe valvular disease, atrial fibrillation, or other severe arrhythmias and congenital heart disease were enrolled in to the study.
5.Subject with no history of End Stage Renal Failure
6.Subjects with normal Doppler study

EXCLUSION CRITERIA:

1.Subjects with presence of pathological Q waves, LV hypertrophy on voltage criteria, or ST/T wave abnormalities in ECG.
2.Subjects with history of peripheral vascular disease
3.History of Congestive Heart Failure.
4.History of End Stage Renal Failure.
5.History of Smoking and alcohol intake.
6.Subject with uncontrolled hypertension
7.Clinically significant abnormal laboratory results at screening
8.History of autoimmune disorders and connective tissue disorders.

The selected subjects were given a screening number and subjected to screening procedure to find out eligible candidates for enrollment. The screening procedure includes obtaining subject's demographic data and medical history, physical examination, baseline symptomatology, laboratory investigations like fasting blood glucose, HbA1C, Lipid profile, urea, creatinine, Liver function test and ECG.The selected subjects after the screening procedure were given a Subject Number. They were asked to come on next day for next sets of investigation like hCRP, apo-A, apo-B, Homocystiene ,Doppler .

RESULTS:

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Of the 88 patients only 18 patients had elevated level of apo –A (20.5%) whereasapo-B levels were elevated in 62 patients. (70.5%). Highly sensitive CRP was found to be elevated in 57 patients. (64.5%) The mean age of the study population was about 50 years and the mean duration of diabetes was 7 years. The renal function tests and liver function tests' mean were within normal limits. The mean apo-A level was 96 mg/dl (with 110mg/ dl being the upper limit for normal) with a range from 0.97 to 139 mg/dl. The mean apo-B level was 91.8 mg/dl with 90mg/dl being the upper limit for normal. The mean homocysteine level was 2.8mg/dl with 1 mg/dl being the upper limit for normal. Our study population consisted of predominantly female population, due to regular outpatient visits and compliance with follow up protocols by females compared to males in our study group.

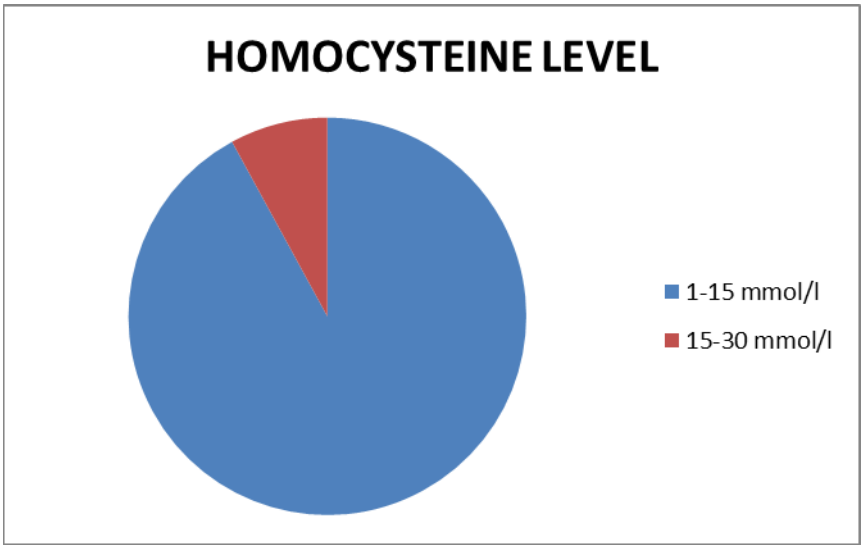
APO A Level	Frequency	Percent
Normal	70	79.5
Abnormal	18	20.5
Total	88	100.0

APO B Level	Frequency	Percent
Normal	26	29.5
Abnormal	62	70.5
Total	88	100.0

Apo-A levels were only elevated in a minority of the study group (20.5%) whereas Apo-B levels were elevated in about (70.5 %) of the study group.

HCRP Level	Frequency	Percent
Normal	31	35.2
Abnormal	57	64.8
Total	88	100.0

Highly sensitive CRP levels were elevated in about 64.8% of the study population One parameter was elevated in almost 40 % of the study population and two parameters were elevated in 35 % of study population thus adding up to a significant percentage of those studied.



92.05% had normal homocysteine levels and 7.95% had mild hyperhomocysteinemia.

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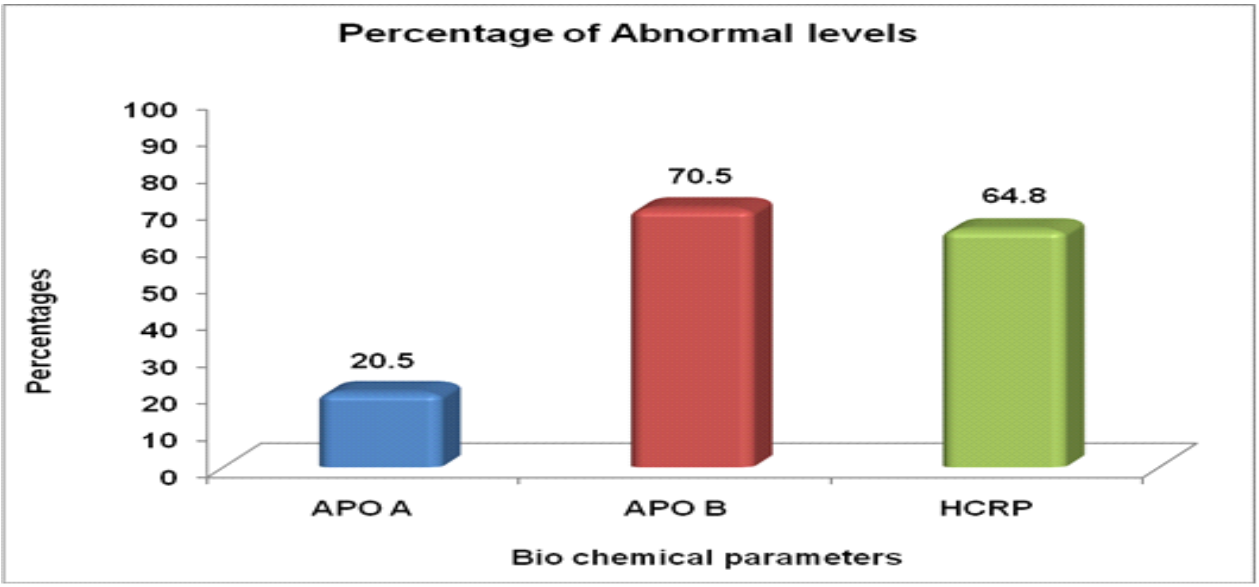
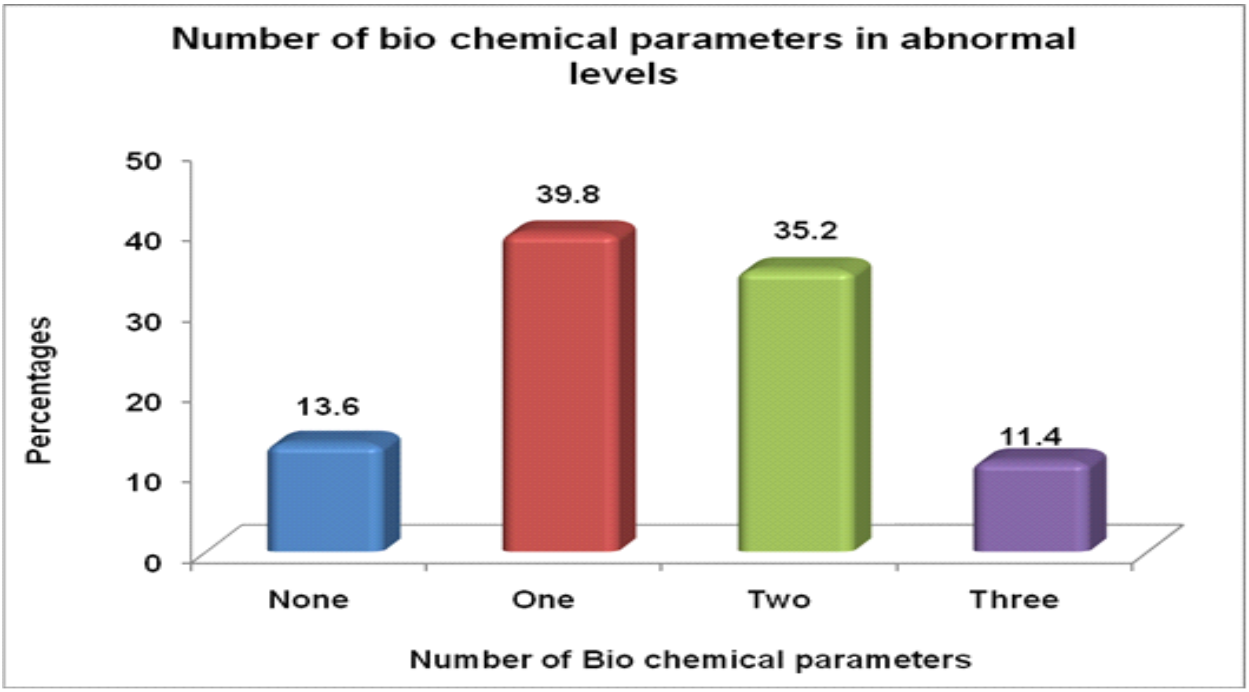
Number of parameters abnormal	Frequency	Percent
None	12	13.6
One	35	39.8
Two	31	35.2
Three	10	11.4
Total	88	100.0





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The above chart shows that one parameter was alone elevated in 39.8% of the study group and two parameters were elevated in 35.2 % of the study group and three parameters were elevated in 11.4 % of the study group and only 13.6% of the study population had all parameters within normal range.

DISCUSSION

Although coronary and peripheral arterial diseases are macrovascular complications of diabetes, the clinical manifestations of peripheral vascular disease occur almost a decade later than coronary artery disease. Majority of patients with peripheral vascular disease have associated coronary artery disease, however the opposite isn't true. Although atherosclerosis in patients with diabetes is similar to that seen in non-diabetic patients , it is generalized, occurs prematurely and progresses at an accelerated pace. Peripheral vascular disease in diabetics differs from that in non diabetics in many aspects.

In non diabetics the sites of occlusion are usually the infra-renal aorta, iliac and superficial femoral arteries, with sparing of distal vessels. Whereas, in diabetics occlusive lesions occur in crural arteries, namely tibials and peroneals, with sparing of the arteries of the foot. This characteristic vascular involvement in diabetics had made it possible to carry out vascular reconstruction, where proximal vessel like popliteal is anastomosed to foot vessels like dorsalispedis thus bypassing the obstructed tibial and peroneal vessels., This pedal artery bypass technique has lead to a significant decline in the incidence of all levels of limb amputations .Hence the need to diagnose peripheral vascular disease in diabetics at an early stage so that prevention of complications by early intervention is possible .

The overall prevalence of PVD among Indians is considerably low as compared to the Western patients. Mohan et al have reported the prevalence of PVD in South Indian diabetics to be 3.9%. in Western series the prevalence ranges between 22 – 45% . The prevalence of PVD in diabetics increases with age increasing from 3.2% in those below 50 yrs of age to 33% in those above 80 yrs. of age. The prevalence of PVD in diabetics also increases with the duration of diabetes from 15% to 45% at 10 to 20 years respectively after the diagnosis of diabetes . In India, the number of diabetic patients above the age of 80 years or with duration of diabetes more than 30 years is extremely low, thus explaining the low prevalence of PVD in diabetics. In the coming years with better disease care, longevity of our diabetics would significantly increase and it will not be surprising to see an alarming increasing prevalence of PVD in Indian diabetics.

Indian diabetics also present with peripheral vascular disease at an advanced stage with foot ulcers and advanced vascular disease where even limb salvage is not possible. The existing investigations like Doppler, duplex Doppler, CT angiogram, MR Angiogram all detect vascular disease after onset and there are no standard tests to diagnose peripheral vascular disease before the onset.We generally investigate for peripheral vascular disease only in a symptomatic patient with positive history for claudication pain. In such symptomatic patients we also preformed serum inflammatory markers along with Doppler study to find out the association between them. We found serum pro-inflammatory markers were significantly elevated in peripheral vascular disease patients. This was the basis for our study to be performed in chronic asymptomatic diabetic patient, as a predictor of peripheral vascular disease. Atherosclerosis is by far the most common cause (>90%) of arterial problems in the leg .[1] PAD affects about 5% of the elderly

population over 55 years in the western world[2] . Several studies have demonstrated that inflammation is also involved in the development of PAD [3,4].Previous studies have demonstrated that PAD is closely associated with elevated plasma levels of the inflammatory markers C-reactive protein (CRP) and fibrinogen [3,4] .Moreover, other studies have focused on the association between such pro-inflammatory markers and PAD in patients with diabetes and the arterial consequences of diabetes, such as PAD. Accordingly, diabetes shows a high prevalence in the general population and is a crucial risk factor for cardiac and arterial disease [5,6,7,8.]The pivotal and crucial role played by inflammation in the pathophysiology of PAD has received much attention. Based on our current knowledge, we propose that inflammatory mediators may be involved in PAD development. A substantial body of evidence has accumulated to support a key role for inflammation in the development and progression of atherosclerosis. Several studies have investigated the association of various markers of vascular inflammation including acute phase reactants (CRP), cytokines (interleukin-6(IL-6)), cellular adhesion molecules (CAMs), white blood cell (WBC) count, and beta2-microglobulin, with manifestations of atherosclerotic vascular disease. Novel circulating markers that have been implicated in PAD.

CRP:

CRP has also been studied in PAD patients as a marker of disease progression, functional activity, and adverse cardiovascular events such as myocardial infarction and stroke. Plasma levels of CRP predicted progression of PAD (as determined by a decrease in ABI over time) in two large population based longitudinal cohort studies: the Rotterdam Study (n = 7987, mostly white men and women in The Netherlands; age ≥ 55; follow-up 6.5 years)[9] and the Edinburgh Artery Study (n = 1592, mostly white men and women in the UK; ages 55–74;follow-up 5–12 years)[10] . Inflammation also appears to be associated with a decline in function and physical activity often seen in patients with PAD and resulting in a significant impact on quality of life. Higher levels of CRP were correlated with impaired functional capacity, decreased physical activity, and future functional decline in several cohort studies[11] .

IL-6 is an inflammatory cytokine produced by hepatocytes, lymphocytes, and endothelial cells.53Whether IL-6 is associated with incident PAD, PAD severity, or cardiovascular events in PAD patients are not known. However, in the Edinburgh Artery Study, IL-6 was the strongest predictor of PAD progression over a 12-year period, and was the only biomarker to remain independently associated with disease progression after all 'novel' biomarkers and conventional risk factors were included in the analysis.

APO A/ APO B

The authors, led by Dr Allan Sniderman (Mike Rosen bloom Laboratory for Cardiovascular Research, Montreal, QC), report that four large prospective studies have shown that apolipoprotein B is superior to total cholesterol or LDL cholesterol to predict the risk of vascular disease. They also reported that the ratio of apolipoprotein B/apolipoprotein A-1 was superior to the conventional LDL/HDL cholesterol ratio as an overall index of vascular disease risk. However, data now suggest that values of apolipoprotein B and the apolipoprotein B/apolipoprotein A-1 ratio are more sensitive indices of risk of vascular disease and are more robust predictors of future vascular events for patients on statin therapy than concentrations of LDL cholesterol, non-HDL cholesterol, or the LDL/HDL cholesterol ratio. LDL cholesterol is an established risk factor for atherosclerotic

vascular disease and a major therapeutic goal in PAD patients is the reduction of LDL cholesterol levels

The association between Lp (a) levels and PAD have yielded conflicting results.The Physicians' Health Study[12] did not find Lp(a) levels to be predictive of symptomatic PAD. However, several cross-sectional, case–control studies have found an association between elevated Lp(a) levels and PAD in various populations including those with premature PAD (men aged < 45), [13]those with known PAD, [14]and those referred for lower extremity revascularization.

HOMOCYSTEINE

Homocysteine is an amino acid intermediate produced during the metabolism of methionine. [15]Its role in atherogenesis was proposed subsequent to the observation that children and young adults with cystathione beta-synthase deficiency and markedly elevated homocysteine levels developed premature atherosclerosis. Homocysteine may promote atherosclerosis through several mechanisms, including increased oxidant stress and adverse effects on endothelial function. [15]Increased homocysteine levels may result from genetic polymorphisms in enzymes related to its metabolism, as well as aging, menopause, hypothyroidism, low B vitamin and folic acid levels, and chronic kidney disease. [16] .Several studies found homocysteine levels to be associated with PAD, the Physicians' Health Study being a notable exception. [12] .In a cross-sectional population-based study, higher homocysteine levels were associated with a reduced ABI (<0.90) [17] .Elevated homocysteine levels were also associated with a lower ABI in a multiethnic cohort of predominantly hypertensive adults [18] with symptomatic PAD in a referral population.

This study shows that inflammatory markers like hCRP, apo-A and apo-B which are sensitive markers for detection of peripheral vascular disease are also elevated in diabetics who do not have overt vascular disease. Highly sensitive CRP was elevated in 64.8% of the population,20.5% of them had elevated apo-A and apo-B was around 70.5% of the total population. Among all these bio-markers apo-B was significantly found to be elevated in our study. Also it demonstrates that only 13.5% of the diabetic patients remain to be unaffected, rest all the diabetic patients showed significant elevation in the serum biomarkers .The main aim of our study is to identify the diabetic patients prior to this development of macrovascular complications using a simple screening tool. Hence these simple blood tests can be used to follow up this subset of diabetic population who are at high risk of development of peripheral vascular disease so that early intervention is possible.

LIMITATIONS OF THE STUDY:

Since this study was done in a small subset of a population it cannot be generalized for the entire population. Further prospective studies are required involving larger patient population to identify whether inflammatory markers can be used as a screening tool at primary health care level to identify peripheral vascular disease in diabetic patients.

CONCLUSION:

Inflammatory markers have been proven to have a role in diagnosing cardiovascular disease at an early stage before the onset of symptoms. They have also been studied extensively in peripheral vascular disease and found to have a role in the diagnosis of peripheral vascular disease at an early stage. In this study we have studied the levels of inflammatory markers in chronic diabetic population (>3 years) and found out that 2 inflammatory markers i.e. hCRP and apo-B were elevated in the study population. This subset of population is being monitored for

the onset of peripheral vascular disease by using ABI and Doppler imaging. Thus inflammatory markers serve as a sensitive tool to identify the subset of chronic diabetics who are at a high risk of developing peripheral vascular disease.

CONFLICT OF INTEREST :

Conflict of interest declared none

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