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Impact of physical activity on the inflammatory biomarkers in type 2 diabetes mellitus

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ABSTRACT

Type 2 diabetes is a silent killer disease and mortality rate is high among the physically inactive middle and old aged people. In this study the impact of physical activity on the inflammatory biomarkers among young, middle and old aged diabetic subjects, living in semi urban area Thanjavur town, Tamil Nadu has been investigated. For the present study, the male subjects of 35-45, 46-55 and 56-65 years were selected and thereafter classified as young, middle and old diabetic subjects. In the present study C reactive protein, homocysteine, fibrinogen and WBC count were analyzed as inflammatory biomarkers in different age group of diabetic subjects. The level of inflammatory biomarkers was found to be very high in elderly diabetic persons when compared to the physically active diabetic subjects. People with diabetes have an elevation in the level of inflammatory biomarkers, because of excess chronic oxidative stress produced in the hyperglycemic state. Everyday physical activity such as walking is associated with the increased insulin sensitivity and decreased oxidative stress. In the present study, physical activity improved the levels of inflammatory biomarkers among the walking diabetics which may be attributed to anti-inflammatory effect of physical activity.

KEYWORDS

Diabetes;
Inflammatory biomarkers;
C-reactive protein;
Homocysteine;
Fibrinogen;
WBC count;
Physical activity.

INTRODUCTION

Diabetes mellitus is a progressive chronic inflammatory disease emerging as a Global epidemic[1]. It has a distinct pathophysiological mechanism that may lead to unique impact on eye, kidney, nerve damage and further complications besides increased risk for cardiovascular disease[2]. Further, diabetes imposes a tremendous burden on health economies mainly because of its devastating nature of complications.

India is currently termed as "*diabetes capital of the world*" due to increasing number of diabetic subjects[3]. According to the diabetic atlas published in 2006 by the International Diabetes Federation, the number of people with diabetes in India is currently around 40 million and this number is expected to rise to 70 million by 2025, unless urgent preventive steps are taken to control this disease[4]. Physical inactivity is an independent risk factor for diabetes in Indians. Researchers looking at levels of physical activity among south Asians

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noted some awareness of its importance but a lack of putting it into practice[5]. The reasons included cultural norms, social expectations, time constraints and health problems. It has also been reported that healthcare professionals perceive South Asians as holding fatalistic beliefs surrounding their health status and are reluctant to provide lifestyle advice because of poor cultural and religious understanding[6], thus exacerbating the problem.

Physicians find pedometers as a useful tool for motivating patients and monitoring their exercise habits. Simple, daily, vigorous walking can significantly improve cardiac risk factors and glucose metabolism. Because walking is accessible and relatively safe it can be easily incorporated into daily routines. It is a form of exercise that is practical and suitable for most diabetic patients, especially elderly persons.

Diabetes is considered as a state of chronic, low-level inflammation[7]. Inflammation is one of the manifestations of oxidative stress and markers of inflammation are all induced by oxidative stress[8]. Inflammation is a key feature of plaque instability and triggers progression of atherosclerosis in diabetic patients[9] and it is associated with a pathway involving cytokine-mediated acute-phase response to infection and other inflammatory processes[10]. Inflammation, endothelial dysfunction and abnormalities of coagulation are associated with insulin resistance and thereby become common antecedents of both diabetes and coronary heart diseases. The novel risk factors for diabetes have been categorized into factors that are derived from adipose tissue, hepatic fat, endothelium and inflammatory markers. Some of these factors are of importance among south Asians than in Europeans[11]. In the present study, C reactive Protein [CRP], Homocysteine[HCy], fibrinogen, White Blood Cells [WBC] were analyzed in the diabetic group as inflammatory biomarkers.

CRP, an acute phase reactant is the most extensively studied biomarker of chronic low grade inflammation[12] and low grade systemic inflammation enhances the risk of type 2 diabetes mellitus [T2DM][13]. HCy is sulphur containing amino acid intermediary in the metabolic pathway of methionine[14]. It has pro-oxidant properties and in high concentrations is a cause of endothelial dysfunction. Plasma fibrinogen level represents a strong cardiovascular risk factor and it is regulated by interplay of genetic and environmental factors. Fibrinogen is an important coagulation protein that is involved in the mesh like network of the common blood clot[15]. The prothrombotic factor fibrinogen and proinflammatory factor HCy play a vital role in the causation of cardiovascular disease among diabetic subjects. Inflammatory response appears to be over activated in diabetes. Leucocytes are major mediators of inflammation. They also contribute to the oxidative stress associated with diabetes.

Reports have pointed out that the actual causes of death among the diabetics are usage of tobacco, physical inactivity, malnutrition, excessive use of alcohol, etc. In the present study the impact of life style intervention (physical activity) on the inflammatory biomarkers among young, middle and older aged diabetic subjects, living in Thanjavur town, Tamil Nadu has been investigated.

EXPERIMENTAL

For the present study, only the male subjects were selected. The age groups selected were 35-45, 46-55 and 56-65 years and categorized as young, middle and old aged diabetic subjects. All the human volunteers were issued with a questionnaire to determine the eligibility for participation in the study. The questions elicited vital information on age, body weight, height, exercise, habits, health status, smoking habit, alcohol intake and the use of dietary supplements. Physically active diabetic subjects with the habit of walking for at least 30 min / day or 2 days once were included for the investigation. Written informed consent was obtained from all the participants of the study after providing sufficient explanation for participation in the study. The blood sample was collected from the subjects using the method described by NCCLS[16]. The blood was collected by venous arm puncture in heparinized and unheparinized tubes after an overnight fasting.

Plasma and serum were separated by centrifugation at 1300 × g for 15 min and stored at 4°C until analysis for inflammatory biomarkers. The CRP was estimated following the method of Singer et al.[17]. When a CRP latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 minutes. The Hcy was determined by ELISA method [18]. Protein-bound Hcy is reduced to free Hcy and enzymatically converted to S-adenosyl-

L-homocysteine [SAH] in a separate procedure prior to the immunoassay. The enzyme is specific for the L-form of Hcy, which is the only form present in the blood.

The Plasma fibrinogen was estimated using the tyrosine method developed by Shaw[19]. Fibrinogen was converted to fibrin and the clot was separated from the plasma. The clot was hydrolyzed by boiling with sodium hydroxide and tyrosine was measured. The blue colour developed was measured in a spectrophotometer at 630 nm. The total leucocyte count was determined by haemocytometry method as described by Becton-Dickinson[20]. The glacial acetic acid lysed the red cells while the gentian violet slightly stained the nuclei of the leukocyte. The blood specimen was diluted to 1:20 in a WBC pipette with the diluting fluid and the cells were counted under low power of the microscope by using a counter chamber. The number of cells in undiluted blood was reported as the number of white cells/ cu.mm of whole blood. The values are expressed as Mean ± Standard Deviation. The values between parameters and age Groups were analyzed by one way analysis of variance (ANOVA) using SPSS version 12.0 for windows.

RESULTS AND DISCUSSION

The levels of CRP in the diabetic subjects and physically active diabetic subjects are shown in TABLE 1. It can be inferred from the table that the levels of CRP is elevated significantly [P<0.01] among the diabetic subjects compared to the walking diabetic subjects. Acute phase responses are associated with T2DM and the mechanism by which atherosclerosis is accelerated in T2DM includes mediation by acute phase proteins themselves. CRP causes expression of endothelial adhesion molecules and chemoattractants and mediates low density lipoprotein [LDL] uptake by macrophages. CRP binds to the LDL particle in atherosclerotic plaques leading to activation of complement thus being proinflammatory and contributing to atherogenesis. CRP may also increase ischemic tissue damage by complement dependent mechanism and tissue factor production by macrophages[21] .Dehghan et al.[9] supported the hypothesis that serum CRP enhances the development of diabetes. Radhika et al.[22] confirmed the presence higher levels of CRP among the diabetic South Indians. Similarly a significant increase in CRP levels was observed in the present study among diabetic subjects. Regular physical activity made an appreciable improvement in insulin sensitivity and hyperglycemia, among diabetic subjects. Regular exercise suppressed the production of CRP. Weight loss reduces CRP levels. Exercise levels are inversely associated with CRP concentrations[23].

It can be seen from the TABLE 2 that the levels of Hcy was significantly elevated [P<0.01] among the old diabetic subjects [32.57±1.60] compared to the physically active diabetic subjects. Walking was found to significantly decline the levels of Hcy among the diabetic subjects, of all age groups [11.50±0.55; 12.29±0.76 and 12.13±0.71 respectively]. Narang et al.[14] suggested that elevated plasma total HCy may be a risk factor for cardiovascular diseases among diabetic subjects. Elevated HCy promotes oxidative damage by ROS and causes smooth muscle proliferation. The increased risk of cardiovascular diseases with hyperhomocysteinemia is independent of other risk factors such as lipid abnormalities. Tawakol et al.[24] reported that putative mechanisms of atherothrombosis in hyperhomocysteinemia include enthothelial cell injury, endothelial dysfunction, increased vascular smooth muscle cell growth, increased platelet adhesiveness, enhanced LDL oxidation and deposition in the arterial wall and direct activation of coagulation cascade. Hyperhomocysteinemia may be directly linked to endothelial dysfunction.An elevated HCy level among T2DM subjects as found in our study predicted the cardiovascular risk and vascular damage, among diabetic subjects. Physically active walking diabetics in our study showed significantly declined levels in the inflammatory biomarkers indicating the anti-inflammatory effect of physical activity.

It can be inferred from TABLE 3 that the levels of fibrinogen were significantly elevated [P<0.01] in all aged diabetic subjects. Walking habit showed significant decline [F value – 26.27; P<0.01] in fibrinogen levels to near normal [275.00±13.78, 310.00±10.00 and 290.42±17.90 respectively]. Lowe[25] cautioned that elevated plasma levels of inflammation biomarker fibrinogen as a strong factor for all causes of death. As for coronary artery disease related death the emerging risk factor, elevated fibrinogen levels show association with cardiovascular mortality. In T2DM, fibrinogen levels increased with age among men[26] as observed in our

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TABLE 1 : Effect of walking on C reactive protein levels among young middle and old aged diabetic subjects

Groups	Age groups	N	Mean±SD	F-value	P Value
Normal	Control	20	4.26±0.18		
	young	16	1.89±0.09		
Diabetic subjects	middle	20	2.23±0.21	1015.38	0.001 [0.01]
	old	14	2.84±0.14		
	young	16	0.96±0.04		
Physically active diabetic subjects	middle	17	1.02±0.12	229.15	0.001 [0.01]
	old	12	4.62±0.26		

Values are means±SD

TABLE 2 : Effect of walking on homocysteine levels among young middle and old aged diabetic subjects

Groups	Age groups	N	Mean±SD	F-value	P Value
Normal	Control	20	12.25±0.97		
	young	16	13.76±0.62		
Diabetic subjects	middle	20	16.51±1.50	216.37	0.001 [0.01]
	old	14	21.79±1.19		
	young	16	11.50±0.55		
Physically active diabetic subjects	middle	17	12.29±0.76	1.37	0.265 [NS]
	old	12	12.13±0.71		

Values are means±SD

TABLE 3 : Effect of walking on fibrinogen levels among young middle and old aged diabetic subjects

Groups	Age groups	N	Mean±SD	F-value	P-value
Normal	Control	20	282.45±29.70		
	young	16	302.44±19.54		
Diabetic subjects	middle	20	382.50±17.73	123.88	0.001 [0.01]
	old	14	402.50±16.26		
	young	16	275.00±13.78		
Physically active diabetic subjects	middle	17	310.00±10.00	26.27	0.001 [0.01]
	old	12	290.42±17.90		

Values are means±SD

TABLE 4 : Effects of walking on WBC count among young middle and old aged diabetic subjects

Groups	Age groups	N	Mean±SD	F-value	P-value
Normal	Control	20	4.26±0.18		
	young	16	5.83±0.42		
Diabetic subjects	middle	20	6.30±0.20	271.96	0.001 [0.01]
	Old	14	6.51±0.21		
	young	16	4.20±0.23		
Physically active diabetic subjects	middle	17	4.41±0.24	8.03	0.001 [0.01]
	old	12	4.62±0.26		

Values are means±SD

study. Hence fibrinogen can be considered clearly as a marker of cardiovascular risk.

It may be seen from TABLE 4 that the WBC count was significantly elevated [P<0.01] among the diabetics of all ages compared to the normal range between 5 and 6. Diabetics with the habit of walking showed near normal of WBC values [4.20±0.23; 4.41±0.24 and 4.62±0.26]. Ruggiero et al.[27] reported that WBC count as a marker of systemic inflammation and an independent risk factor for cardiovascular and cerebrovascular events. Accordingly WBC count is considered as a biomarker of inflammatory processes that actively contribute to vascular injury and atherosclerosis. The close association between WBC count and both micro and macro vascular complication raises the hypothesis that inflammation may be a common linking factor. In support of this notion, the inflammatory process is recognized to be a major component of atherosclerosis[28].

Mononuclear leucocytes are recruited to the site of endothelial injury and form foam cells in the plaque. Activation of neutrophil leads to changes in the rheological properties and adherence to the endothelium, all of which lead to capillary plugging and tissue ischemia. Furthermore, various cytokines and growth factors such as interleukins and tumour necrosis factor are released from activated leucocytes to cause endothelial dysfunction[29]. In addition activated leucocytes release superoxide radicals and proteases, which promote oxidative stress. Taken together, it is plausible that low grade chronic inflammatory response interact with other risk factors, leading to widespread vascular damage, endothelial dysfunction, increased oxidative stress and increased production of growth factors and cytokines to cause micro and macro complication in T2DM patients[30]. The excess chronic oxidative stress produced in the hyperglycemic state by the activated leucocytes explains the mechanism of increased oxidative injury associated with heart disease in diabetes.

Thus, we conclude from the present study, that WBC count is an inflammatory biomarker for cardiovascular risk among T2DM subjects. Physical activity might affect the WBC count via the beneficial effect of exercise on the innate and adaptive immunity. Regular exercise might positively impact the release of anti inflammatory cytokines, T helper 1 / T helper 2 imbalance and neutrophil, natural killer cell and macrophages activity. Even household related physical activity showed a significantly low level of inflammatory biomarkers fibrinogen, CRP, HCy and WBC count. Physical activity was found to be inversely associated

with WBC count. Fonesca et al.[31] suggested that exercise improved endothelial function in diabetic subjects.

CONCLUSION

Diabetes is a chronic disease increasing in explosive pattern in India and Thanjavur is not an exception to it. Changes in socio-economic pattern and life styles may be important in addition to susceptible genes for the causation. Our studies are intended to find out the impact of lifestyle intervention walking on the inflammatory status, among diabetic subjects residing in Thanjavur town. The present study on inflammation markers showed that higher C reactive protein, Homocysteine and fibrinogen besides WBC count in the old diabetic people indicating that inflammation triggers progression of atheroselerosis. The overall study indicated that regular walking augmented normal health and the biomarkers indicated that this kind of lifestyle intervention could help in achieving the management and control of diabetes.

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