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Full Length Research Paper

Dyslipidemic and atherogenic effects of academic stress

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Evidence suggests that there is strong relationship between stress and development of cardiovascular disorders. Here, the relationship between academic stress and selected traditional markers of cardiovascular disorder such as lipids and lipoprotein profile and apoproteins were investigated. Eighty apparently healthy male and female students participated in the study. Plasma concentration of selected biochemical parameters such as total cholesterol, triglyceride, high density lipoproteins, Apoproteins A and B were determined before academic activities and one hour to an examination. Student's 't' test was used to compare the values before and after stress periods. Results showed significant elevations in total cholesterol, triglyceride, Apo A and B while there was reduction in mean concentration of high density lipoprotein cholesterol (HDL-C) during the intense academic period when compared with when there was no academic activity. These results suggest that intense academic activity may cause stress which may affect plasma lipids and lipoproteins.

Key words: Dyslipidemia, stress, atherosclerosis, apoproteins.

INTRODUCTION

Link between stress and development of cardiovascular disorder has been reported by previous workers (Brent et al., 2010). The signs of stress may be cognitive, emotional, physical or behavioral. There are two (2) types of stress which include the acute and the chronic stress. The acute stress affects an organism or an individual in the short term and the chronic stress affects an organism or an individual over a longer term. The relationship between stress, heart disease and sudden death has been recognized since antiquity. The incidence of heart attacks and sudden death has been shown to increase significantly following the acute stress.

Studies have shown that stressful situations resulted in increased levels of serum lipids that is, total cholesterol, low density lipoproteins, triglyceride and reduction in high density lipoproteins (Feroza et al., 2008; Stoney, et al., 2002). Furthermore, excessive elevations in the serum concentrations of these lipid profiles are traditional predictors of cardiovascular diseases. Therefore, this study aimed at determining the possible effects of academic stress occasioned by end of semester examination on traditional risk factors of cardiovascular disorders among apparently healthy male and female students. In addition, effect of academic stress on apoproteins such as Apoprotein A and B was assessed in this study. The assessment of apoproteins was included based on studies that Apo B in LDL-C particles act as ligand for low density lipoprotein cholesterol (LDL-C) receptors on various cells. Informally, it 'opens doors to cells allowing cholesterol to be deposited into cells. Through a mechanism that is not fully understood, high level of Apo B can lead to plaques that cause vascular disease (atheriosclerosis), leading to heart disease. And most importantly, through series of studies, considerable evidence have emerged that levels of Apo B are a better indicator of heart disease risk than total cholesterol or LDL-C (Walldius and Jungner, 2006; Yusuf et al., 2004).

METHODS

Sample collection and storage

Eighty apparently healthy male and female students (mean age 21 years, mean weight 60.8 kg, mean height 1.70 m) were recruited from the Faculty of Basic Medical Sciences of Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The inclusion

Table 1. Effects of academic stress on risk factors of atherogenicity.

Parameter	Before stress	During stress
Total cholesterol (mg/dl)	157 ± 53.29	263.5 ± 54.02#
Triglyceride (mg/dl)	162.9± 46.18	366.5± 216.57#
HDL-C (mg/dl)	358.8± 216.57	216.57± 144.18#
Apo A (mg/dl)	117.39 ± 37.08	182.55 ± 31.83#
Apo B (mg/dl)	81.6± 41.69	126.44 ± 38.78#
TC/ HDL	0.44± 0.43	1.22 ± 1.26#
Apo B/ Apo A	0.70 ± 0.59	0.69 ± 0.25

Significant at #p<0.05 when compared with values before stress.

criteria are: no history of hypertension, should not be suffering from any cardiovascular disorder such as atherosclerosis, stroke, and hypertension etc. All were normotensive, non-diabetic, without renal or liver dysfunction and with body mass index of less than 30 kg/m^2. Ten milliliters of veinous blood sample was collected from each of the volunteers in the second week of resumption when they have not undergone stress. The blood samples were collected into a heparinized bottle. Physical parameters of the students such as height, weight, hip and waist ratio were measured. Another 10 mm of veinous blood were collected 1 h before the commencement of a first semester examination when they appeared stress. Plasma was separated and used for analysis of total cholesterol; triglyceride, high density lipoprotein cholesterol, Apoproteins A and B. Participants were clearly informed of the procedure in English language since all of them understand and speak English fluently and they gave their consent. The approval of the University Ethical Committee was sought and gotten to be able to carry out this study.

Determination of total cholesterol

Total cholesterol was determined using enzymatic method described by Allain et al. (1974). Cholesterol esterase hydrolyses cholesterol esters to free cholesterol. The free cholesterol produced is oxidized by cholesterol oxidase to cholesten-4-ene-3-one with simultaneous production of hydrogen peroxide which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield chromogen with maximum absorption at wavelength 510 nm, The colour intensity is proportional to the cholesterol concentration.

Determination of triglycerides

Triglyceride was determined using enzymatic method described by Buccolo and David (1973). Triglycerides are hydrolyzed by lipases to yield glycerol and fatty acids. The glycerol produced is oxidized to dihydroxylacetone phosphatase with the production of hydrogen peroxide which oxidatively couples with 4-aminophenazone and 4-chlorophenol to produce a chromogen referred to as quinoneimine. The reaction is catalyzed by peroxidase. The degree of absorbance of the chromogen is directly proportional to the concentration of triglyceride measured at 505 nm.

Determination of high density lipoprotein

The precipitation method by Assmann et al. (1983) was used to determine HDL-cholesterol. The addition of phosphotungistic acid in the presence of magnesium ions precipitates quantitatively low density lipoprotein; very low density lipoprotein and chylomicron fractions from whole plasma, leaving the HDL fraction in the supernatant. The cholesterol in the HDL which remains in the supernatant after centrifugation is estimated using the enzymatic method of Allain et al. (1974).

Determination of Apoproteins A and B

Apoproteins A and B were determined using EasyRID human plasma proteins quantitative determination by radial immunodiffusion kit from Via Scozia, Zona Industriale Roseto d.A. (TE), Italy. This allows the determination of human plasma proteins in radial immunodiffusion. The antigen (protein) inoculated in the well of the plate, diffuses radially in the agarose gel, reacting with specific antibodies incorporated in agarose and forming immune complexes visible as precipitin rings. Diameter of precipitin ring is directly proportional to the concentration of the relevant protein in the sample (Sniderman et al., 1975).

Statistical analysis

Quantitative data were presented as mean ± SD. Triglyceride, total cholesterol, phospholipids, high density lipoprotein and apoproteins between the two periods were compared using student's 't' test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Table 1 shows the effect of stress on the biochemical parameters and atherogenic indices. There were significant ($p<0.05$) elevations in mean serum concentration of total cholesterol, triglyceride, Apo A, Apo B, and total cholesterol-HDL-C ratio during stress when compared with corresponding parameters before stress. There were decreased in mean serum concentrations of high density lipoprotein cholesterol HDL-C and Apo B-Apo A ratio during stress when compared with the corresponding parameters before stress.

DISCUSSION

There were significant increases in the levels of certain lipids (that is, total cholesterol, triglycerides) in the volunteers during the period of stress when compared

with period when there was no stress. A consequent reduction in the level of HDL-cholesterol was observed during the period of stress. There were also significant increases in the levels of Apolipoprotein A and B during the period of stress.

One way that stress might influence lipid concentrations is through stress induced hormonal changes that affect lipid metabolism. For example, stress induces sympathetic nervous system activity leading to concomitant increases in catecholamine (epinephrine and nor-epinephrine) and glucocorticoids (cortisol). These increases have resultant effects on metabolism of the lipoproteins. For example, catecholamines can directly stimulate adipose tissue to release free fatty acids into circulation through the process of lipolysis. Epinephrine induced-increase in free fatty acids may be the result of increased blood flow through adipose tissue or stimulation of adipose-β2 adrenoreceptors. In either of the cases, the accumulation of circulating free fatty acids can trigger the production of triglyceride-rich very low-density lipoprotein (VLDL), which will eventually result in increased concentrations of circulating LDL-cholesterol (Brindley et al., 1993).

Like epinephrine, nor-epinephrine influences lipoprotein metabolism. Increased nor-epinephrine activity stimulates adipose β adrenergic receptors which may result in diminished lipoprotein lipase activity, subsequent decline in triglyceride clearance, lower concentrations of HDL-C and increased level of LDL-C. This may be responsible for elevated triglyceride, total cholesterol, and reduced HDL-C observed in this study. Cortisol is another hormone that may be affected by stress. It also has profound influence on the mobilization of lipids and lipid metabolism through activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Moreover, cortisol and free fatty acids stimulate the secretion of VLDL, increase the synthesis of hepatic triglycerides, inhibit insulin secretion, and increase insulin insensitivity in the tissues (Bjorntop, 2000). These activities may delay LDL clearance from the blood, result of which will be elevated serum lipids level as observed in this study.

A high level of stress promotes an increase in the levels of VLDL-cholesterol and LDL-cholesterol which apolipoprotein B forms a significant part of. It will also lead to the reduction in the levels of HDL-cholesterol (good cholesterol) which contains a significant amount of apolipoprotein A-I or apolipoprotein A-II. This condition is known as dyslipidaemia (Adiels et al., 2006). Hence increase in stress tends to cause an increase in apolipoprotein B concentration and a reduction in the concentration of apolipoprotein A, an observation which is consistent with the findings of this study. Series of studies have shown that Apo B in LDL-C particles act as ligand for LDL-C receptors on various cells. Informally, it 'opens' doors to cells allowing cholesterol to be deposited into cells. Through a mechanism that is not fully understood, high level of ApoB can lead to plaques that cause vascular disease (atheriosclerosis), leading to heart disease. And most importantly, through series of studies, considerable evidence have emerged that level of Apo B is a better indicator of heart disease risk than total cholesterol or LDL-C (Walldius and Jungner, 2006; Yusuf et al., 2004). Results of elevated Apo B concentrations in this study further corroborated the association between stress and risk factors of cardiovascular disorder.

The principal hypothesis to support the relationship between stress and cardiovascular diseases is that the stress-induced activation of hypothalamic and sympatho-hormonal regions occurs repeatedly over time, leading to cardiovascular adjustments that increase hypertension risk. The incidence of heart attacks and sudden death have been shown to increase significantly following the acute stress due to natural disasters like hurricanes, earthquakes and tsunamis. Coronary heart disease is much more common in individuals subjected to chronic stress. The source of stress in this instance was exposure to examination condition. The effect of stress was assessed one hour to the examination. During this period, the students were impatient and have excited pulse. The excited pulse due to examination stress is an indication that there is association between stress and heart beat. Excessive beat of the heart may result in cardiovascular disorder.

This view is also supported by the results of the blood biochemical parameters. There were significant elevations ($P<0.05$) of total cholesterol and triglyceride concentrations during the examination period (stress condition) when compared with the corresponding values before the period. Elevated serum lipids are some of the biochemical markers of cardiovascular diseases such as hypertension, stroke, artheriosclerosis etc. Studies have shown that elevated concentration of triglyceride is a risk factor for cardiovascular disease for both men and women in the general population (John and Melissa, 1996). Several studies detected a trend towards prominent role of lipid levels in the pathogenesis of cardiovascular diseases (Schoofs et al., 2004; Meier et al., 2000; Wang et al., 2000). The pathological background of the artherosclerosis of coronary arteries is the formation of artherosclerotic plaque due to elevated artery cholesterol which additionally induces other cardiovascular diseases (Valentaviciene et al., 2005).

The elevations in the serum concentrations of total cholesterol, triglycerides, total cholesterol- HDL ratio and reduction in serum HDL-C is suggestive of artherogenic tendency of stress. One of the predictive markers of artherogenesis is elevated levels of total cholesterol, triglyceride, LDL-C and reduction in HDL-C. The findings in this study are consistent with this well established theory. Prolonged stress results in inability of the body to convert cholesterol into needed products such as hormones, a situation that may results in accumulation of cholesterol. This may be a reason for the observed

elevation of serum total cholesterol in this study. In a study carried out on female monkeys at Wake forest University by Alice in 2009, he observed that individual monkey suffering from high levels of stress have higher levels of visceral fat in their bodies. This suggests a possible cause and effect link where stress promotes accumulation of visceral fat which in turn causes hormonal and metabolic changes that contribute to heart diseases and other health problems.

In this study, significantly ($P<0.05$) low serum HDL-C concentration was observed during stress when compared with before stress. This may be an indication that stress reduces serum concentration HDL-C that is, that is ability to mop-up excess cholesterol in the blood. In epidemiological studies, high density lipoprotein cholesterol (HDL-C) has also been identified as a strong and independent inverse predictor of cardiovascular events. Higher level of HDL-C predicts longevity (Zuliani et al., 2010). The anti-atherogenic or protective mechanisms of HDL-C are multiple. Most well known is its role in reverse cholesterol transport, by which excess cellular cholesterol is returned to the liver for excretion in the bile (Rothblat and Phillips, 2010). HDL-C has other important roles and these include decreasing inflammation, preventing endothelial cell apoptosis and improving endothelial function. The reduction in HDL-C observed during stress condition in this study may suggest that prolonged and sustained stress may reduce capacity of HDL-C to moderate cholesterol efflux and perform other functions.

REFERENCES

Allain CC, Poon LS, Chan CSG (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Assmann G, Schriewer H, Schmitz G, Hagele EO (1983). Quantification of HDL-C by precipitation with phosphotungstic acid and $MgCl_2$. Clin. Chem., 29(12): 2026-2030.

Brent TM, Susan KR, Micheal GZ, Milos M, Roland VK, Joel ED, Paul JM, Thomas LP, Mathew AA, Sonia A, Igor G (2010). Stress of caring for someone with demential may impair endothelial function. J. Am Coll. Cardiol., 55(23): 2599-2606.

Buccolo G, David H (1973). Quantitative determination of serum triglyceride by the use of enzymes. Clin. Chem., 19: 476-482.

Feroza HW, Muhammad SM, Allah NM, Muhammad HSW, Syed AT, Javel I (2008). Estimation and correlation of stress and cholesterol levels in college teachers and housewives of Hyderabad-Pakistan. J. Pak. Med. Assoc., 15(1): 15-18.

John EH, Melissa AA (1996). Plasma triglyceride is a risk factor for cardiovascular disease. Eur. J. Cardiovasc. Prev. Rehabil., 3(2): 213-219.

Meier CR, Schwinger RG, Kraenzlin ME, Schlegel B, Jickk H (2000). HMB-COA reductase inhibitors and the risk of fractures. JAMA, 283: 3205-3210.

Rothblat GH, Phillips MC (2010). HDE and Cardiovaslar mortality in the elderly. Curr. Opin. Lipodol., 21: 117-126

Schoofs MW, Sturkenboom MC, Vander KM, Hofman A, Pols HA, Sticker BH (2004). HMG COA reeducates inhibitors and the risks of vertebral fracture. J. Bone Miner. Res., 19: 1525-1530.

Sniderman A, Feng B, Jerry M (1975). Determination of B protein of low density lipoprotein directly in plasma. J. Lipid Res., 16: 465-467.

Stoney CM, West SG, Hughes JW, Lentino LM, Finney ML, Falko J, Bausserman L (2002). Acute psychological stress reduces plasma triglyceride clearance. Psychophysiology, 39(1): 80-85.

Valentaviciene G, Paipaliene P, Nedzelskiene I, Zilinskas J, Anuseviciene OV (2005). The relationship between blood serum lipids and periodontal condition. Stomatologiya, 8: 96-100.

Walldius G, Jungner I (2006). The apo B/apo A-I ratio; a new predictor of fatal stroke, myocardial infarction and other ischaemic diseases-stronger than LDL and lipid ratios. Atherosclerosis, 7: 468.

Wang PS, Solomon DH, Mogun H, Avorin J (2000). HMG COA reductase inhibitors and the risk of lip fractures in elderly patients. JAMA, 283(24): 3211-3216.

Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries: case-control study. Lancet, 364: 937-952.

Zuliani G, Cavalieri M, Galvani M, Volpato S, Cherubim A, Bandinelli S, Corsi M, Lauretain I, Guranik J, Fellin R, Ferrucci L (2010). Relationship between low levels of high density impoprotein cholesterol and dementis in the elderly. The inchianti study. J. Garentol. Series A, 65A: 559-564.