ORIGINAL ARTICLE

Assessment of Circulating Blood Lipid Profile Markers in medical students during and after examination stress

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ABSTRACT

Background: Stress of examinations is a factor that affects many organ systems of our body. A relation exists between stress and Physiological and Biochemical markers.
Aim: Assessment of the level of blood Lipid profile in medical students during and after examinations.
Methods: This cross sectional study was conducted at the department of Physiology, The university of Lahore from January 2013 – February 2013. Sixty healthy medical students planning to take their professional exam were recruited. Students with health behaviors or health problems that may alter immune oxidative stress measures were excluded from the study. Study participants reached at the laboratory in morning hours (7-11 am) in fasting state in the week preceding annual examination. Then the second visit was scheduled a month or more (4-7 weeks) after the examination. Fasting blood samples were obtained to be used in evaluation of Lipid profile.
Results: Out of these sixty subjects, 23 were males and 37 were females. Their age ranged from19 to 21 years. Significant difference was observed in the values of total cholesterol(TCh), triglycerides(Tg), low density lipoproteins(LDL) and high density lipoproteins(HDL)during stressed(Phase-I) and relaxed phase(Phase-II). Levels of TCh, Tg and LDL were raised during the stress state while the level of HDL during the Phase-I was decreased as compare to the Phase-II. The differences between the stressed and relaxed state were significant
Conclusion: Stress of examination affects the levels of Lipid profile markers significantly.
Key words: Oxidative Stress, Lipid profile, Reactive oxygen species (ROS)

INTRODUCTION

In everyday life, all organisms are faced with various types of stresses. In medical profession, stress is common and it can lead to physical tiredness and mental fatigue[1]. Examination stress is one of the well-known etiological event that can disturb the human chemistry and affect the balance between pro-oxidants and anti-oxidants, producing a wide range of detrimental effects on physiological and psychological homeostasis[2].

Dyslipidemia is a universal phenomenon in the world[3]. Although genetic factor is the main cause of dyslipidemia but in the last decade researchers had worked on the risk factors of dyslipidemia[3]. Hypercholesterolemia, hypertriglyceridemia and relevant lipid disorders are too much prevalent and their occurrences are between 20% and 50% in variety ofpopulations[3]. At present there are less studies available that informed us the role of environmental risk factors for dyslipidemia[4]. Psychological stress and examinations stress had impact on our body with special effects on few particular organs and physiological parameters. This examination stress can affect lipid profile also. Physical stresses too can affect lipid profile.

One study showed the impact of stress on low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides[5]. There is a connection between stress and dyslipidemia like total cholesterol and LDL and decreased level of HDL[6]. There are strong evidences that the stress may cause indirectly an increase in the bad cholesterol. Another study tells that stress is positively connected to having less healthy dietary habits and increased body weight. These bad dietary habits along with obesity are well known risk factors for increasing the level of cholesterol.

In our body, radicals & reactive oxygen species (ROS) are continuously formed in high amounts. Reactive oxygen species (ROS) include peroxides, superoxide anion, hydroxyl radicals, hydrogen peroxide, nitric oxide and singlet oxygen.

Under normal conditions, a delicate balance exists between the generation of ROS and antioxidant[7]. Various factors can be readily upset this delicate balance resulting in a state known as oxidative stress. Oxidative stress has been associated with the pathogenesis of several diseases[8].

In stress the level of these ROS is increased. They lead to a series of reactions that attack cell membrane phospholipids and induce lipid peroxidation. Lipid peroxidation is the result of oxidative deterioration of polyunsaturated fatty acids (PUFA). Very low density lipoprotein (VLDL), low density lipoproteins (LDL) and high density lipoprotein(HDL) all are used for peroxidation, but LDL is being used maximally. Foam cells are formed by Ox datively modified LDL (Ox-LDL). Foam cells are the main factor for atherosclerosis formation.

The mitochondrial and plasma membrane, consists of lipids are vulnerable by the damage caused by free radicals and ROS induced lipid peroxidation leads to cells dysfunction and damage of adipose tissue resulting in the formation of lipid degradation products like malondialdehyde (MDA)[9].

In stress Hypothalamus releases corticotrophin releasing hormone(CRH).Corticotrophin-releasing hormone is the key element that drives our body's response to

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stress.CRH acts on Ant. Pituitary to release Adrenocorticotropic hormone (ACTH). This ACTH act on adrenal cortex to release Cortisol which acts on adrenal medulla and adipose tissues. Nor-adrenaline is released from adrenal medulla also act on the adipose tissues. Action of both Cortisol and nor adrenaline on adipose tissue may results in the release free fatty acid which leads to dyslipidemia.

Increased levels of cortisol due to long-term stress too can be a mechanism behind how stress may raise the level ofcholesterol. Catecholamines may be released at the same time, and these hormones trigger a "fight or flight" response in order to tackle with stress. Then this response will trigger triglycerides, that may boost "bad" cholesterol in human body.

Stress hormones mobilize the lipids into circulation to fuel the "fight or flight" response[10]. Lipids are oxidized for energy, and glycerol can be used for gluconeogenesis. The mobilization of lipids fuel takes place in part because catecholamines boost expression of adipose triglyceride lipase and hormone sensitive lipase[11]. Catecholamines too phosphorylate and activate hormone-sensitive lipase, and phosphorylate perilipin proteins, that result in conformational changes which allow lipases to contact with droplets of lipids.

Increased level of cortisol leads to the increased amount of serum free fatty acid(FFA), glycerol, and whole-body lipolysis. Also, cortisol, catecholamines, and glucagon stimulate phosphatidate phosphohydrolase, whichincreases the hepatic TG formation[12]. Likewise, glucocorticoids and FFA also increasethe activity of HMG-CoA reductase in liver, thus stimulating the cholesterol synthesis[13]. Norepinephrine promotes lipolysis by stimulating beta receptors present at the adipose tissue, and by decreasing the level of insulin. Further, norepinephrine declines the hepatic lipase activity, that could enhance plasma levels of VLDL and LDL[13]. In short, during stress, liver secretion of VLDL increases, due to increased stock of fatty acids.

Many studies showed a positive correlation among high stress and high cholesterol. Although there are many other factors that may contribute to raise level of cholesterol, it seems that stress may be one, also.

MATERIALS & METHODS

This cross sectional study was conducted at physiology department of the university of Lahore from January 2013 – February 2013. A total of sixty healthy medical students, both male and female who were planning to take their professional exam were recruited from the medical college. Students with health behaviors or health problems that may alter immune oxidative stress measures were excluded from the study like regular use of tobacco, excess consumption of alcohol, illegal drug use, or diagnosis of severe asthma, arthritis, diabetes, cancer, heart disease, hypertension, autoimmune disorders, major depression/ anxiety disorder or major psychiatric disorders. In addition, participants were screened on the day of arrival for the presence of an infectious illness.

Participating students arrived at the lab in morning hours (7-11 am) in the week preceding annual exam. Upon arrival at lab, written informed consent was taken from each study participant. They were fasting overnight(12hours). Demographic details were noted and parameters like height, weight and blood pressure were recorded. Their fasting blood samples were obtained using aseptic technique to be used in evaluation of Lipid profile [Total cholesterol (TCh), triglycerides(Tg), low density lipoprotein(LDL), high density lipoprotein(HDL)].

The second visit was scheduled a month or more (4-7 weeks) after the exam, when anxiety about the exam was expected to have dissipated. Fasting blood samples were obtained again to be used in evaluation of Lipid profile.

Estimation of lipid profile:

CHOLESTEROL:

Principle: Enzymatic color test for the quantitative determination of cholesterol in human serum was performed on Beckman Coulter AU analyzer(AU 480).

Cholesterol is synthesized in body and is important part of cell membranes & lipoproteins as well as a precursor for the formation of steroid hormones and bile acids[14].

Chemical reaction scheme:

CHE

2 Cholesterol esters + 2 H_2O→2 Cholesterol + 2 Fatty acids

CHO

2 Cholesterol + 2 O_2 2→Cholestene-3-one + 2 H_2O_2

POD

2 H_2O_2 + 4-Aminoantipyrine + Phenol→ Quinoneimine + 4 H_2O

Type of specimen:

Serum. Calibration:

Prior to test calibration was done.

Calculations:

The Beckman Coulter analyzer automatically compute the cholesterol concentration of each sample. If anyone taking plasma samples then total cholesterol levels in plasma should be corrected by multiplying the result obtained by 1.03 to be equivalent to serum levels of total cholesterol[15].

2.TRIGLYCERIDES:

Principle:

Enzymatic color test for the quantitative determination of triglyceride in human serum was performed on Beckman Coulter AU analyzer (AU480).

Method:

In this procedure there are chains of coupled enzymatic reactions. Triglycerides present in the givensample are first hydrolyzedto fatty acids and glycerol. Phosphorylation of glycerol is taken place by adenosine triphosphate (ATP) during the presence of glycerol kinase (GK) and then there is formation of glycerol-3-phosphate. In the presence of GPO (glycerol phosphate oxidase) molecular oxygenoxidized the glycerol-3-phosphateso that the dihydroxyacetone phosphate plus hydrogen peroxide (H_2O_2) are formed. In the presence of peroxidase (POD), H_2O_2 reacts with N,N-bis(4-sulfobutyl)-3,5-dimethylaniline and 4-aminophenazone, disodium salt (MADB) and a chromophore is formed, This chromophore is interpreted at 660/800nm. The level of increased absorbance at 660/800nm is proportional to the triglyceride level in the given sample[16,17,18].

Chemical reaction scheme:

Lipase

Triglycerides + 3 H2O→ Glycerol + 3 Fatty acids
GK, Mg2+
Glycerol + ATP→ Glycerol-3-phosphate + ADP
GPO
Glycerol-3-phosphate + O2-→ Dihydroxyacetone phosphate + H2O2
POD
H2O2 + 4-AAP + MADB-→ Blue Dye + OH- + 3 H2O
Type of specimen:
Serum
Calibration:
Calibration was done prior to test.
Calculations:
The Beckman Coulter analyzer automatically compute the triglyceride concentration of each sample.

3. LOW DENSITY LIPOPROTEIN:
Principle:
Enzymatic color test for the quantitative determination of LDL-cholesterol in human serum was performed on Beckman Coulter AU analyzer (AU480).
Chemical reaction scheme:
CHE and CHO
2 LDL-cholesterol + 2 H2O + 2 O2→ 2Cholest-4-en-3 one+2Fattyacids+2H2O2
POD
2 H2O2 + 4–AA + HDAOS→Blue Dye+ + OH- + 3 H2O
Type of specimen:
Serum
Calibration:
Calibration was done prior to test.
Calculations:
The Beckman Coulter analyzer automatically compute the LDL-cholesterol concentration of each sample.

4.HIGH DENSITY LIPOPROTEINS:
Principle:
Enzymatic color test for the quantitative determination of HDL-cholesterol in human serum was done on Beckman Coulter analyzer (AU480).
Methodology:
Anti-human-β-lipoprotein antibody in R1 binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons). The antigen-antibody complexes formed block enzyme reactions when R2 is added. HDL-cholesterol is quantified by the presence of an enzyme chromogen system.
Chemical reaction scheme:
Anti human - β lipoprotein antibody
LDL,VLDL and chylomicrons→Antigen–Antibody complexes
CHE and CHO
HDL-cholesterol + H2O + O2→ Cholest-4-en-3-one + Fatty acids + H2O2
POD
H2O2 + 4 – AA + F–DAOS→ Blue dye+ + F- + 2H2O
Type of specimen:
Serum
Calibration:
Calibration was done prior to the test.
Calculations:
The Beckman Coulter analyzer automatically compute the HDL-cholesterol concentration of each sample.

Results were expressed as mean±SD. Statistical significance was determined by Wilcoxon test. One-way analysis of variance and spearman correlation(two-tail) was used to correlate the different variables. P-value of<0.05 was considered as statistically significant.

RESULTS

Out of the 60 subjects, 23 were males& 37 were females. Age ranged from 19 to 21years. Levels of lipid profile[Total cholesterol(TCh), triglycerides(Tg), low density lipoprotein (LDL), high density lipoprotein(HDL)] were checked.
Reference Intervals:
1.Cholesterol:
National Cholesterol Education Program Adult Treatment Panel III recommendations: [19]
< 5.2 mmol/L (200 mg/dL) Desirable
5.2 – 6.2 mmol/L (200 – 239 mg/dL) Borderline High
≥ 6.2 mmol/L (240 mg/dL) High
2.Triglycerides: [19]
< 1.70 mmol/L (150 mg/dL) Normal
1.70 – 2.25 mmol/L (150 – 199 mg/dL) Borderline high
2.26 – 5.64 mmol/L (200 – 499 mg/dL) High
≥ 5.65 mmol/L (500 mg/dL)Very high
3.Low density lipoprotein: [19]
< 2.6 mmol/L (100 mg/dL) Optimal 2.6 – 3.3 mmol/L (100 – 129 mg/dL) Near Optimal 3.4 – 4.1 mmol/L (130 – 159 mg/dL) Borderline High
4.1 – 4.9 mmol/L (160 – 189 mg/dL) High ≥ 4.9 mmol/L (190 mg/dL) Very High
4.High density lipoprotein: [19]
< 1.03 mmol/L (< 40 mg/dL) Low HDL-cholesterol
≥ 1.55 mmol/L (≥ 60 mg/dL) High HDL-cholesterol

The level of Total cholesterol(T.Ch) was increased during stress than the relax state. The value T.Ch. at Phase-I (stress state) was 5.87 and at phase-II (relaxed state)was 4.40.The difference of these values was significant.
The values of triglycerides(Tg) was raised during the stress phase as compared to the relaxed phase. During the stress phase, the mean value of Tg was 1.38 and during the relaxed phase was 1.24 showing significant difference among them.

Phase–I showed higher value of low density lipoprotein(LDL) as compare to phase-II, which were 2.80and 2.29 respectively. High density lipoprotein(HDL) concentration determined during Phase-I(stress state) was 1.42, which was high during the Phase-II (relaxed state) andwas 1.69 depicting a significant difference between the two. Table 2 shows the variation of above discussed parameters with respect to gender. The result show non significance indicating that gender has no significant role in stressful conditions.

Table 1: Assessment of circulating lipid profile in medical students during examinations (phase-i) and after examinations (Phase-II)

Variables	PHASE-I±SD (n=60)	PHASE-II±SD (n=60)	Sig (P≤0.05)
TCh	5.87±0.68	4.40±0.38	0.000
Tg	1.38±0.13	1.24±0.13	0.001
LDL	2.80±0.58	2.29±0.15	0.000
HDL	1.42±0.12	1.69±0.16	0.000

Table 2: Assessment of circulating lipid profile in medical students during examinations (phase-i) and after examinations (phase-ii)

Variables	Males±SD (n=23)	Females±SD (n=37)	Sig (p≤0.05)
T.Ch	4.96±0.88	5.37±0.95	0.086
Tg	1.30±0.13	1.32±0.16	0.628
LDL	2.58±0.61	2.53±0.35	0.679
HDL	1.58±0.23	1.53±0.15	0.299

DISCUSSION

This research was intended to gauge the effect of stress on lipid profile. Hyperlipidemia is a state which refers to the raised levels of plasma lipid above the normal range [20]. It is characterized by elevation in the plasma cholesterol and triglycerides levels. Hyperlipidemia is also present in the people of Pakistan [21].

In our study the level of Cholesterol, triglyceride and LDL was raised along with decreased level of HDL during examination stress and these parameters returned to normal range during relaxed state. The results show that there is no significance regarding gender.

Stressful situations are hazardous for lipid profiles. These hazards include physical and psychological stress. [22,23]

Cortisol released during stress act on adrenal medulla and adipose tissues. Nor-adrenaline is released from adrenal medulla also act on the adipose tissues. Action of these Cortisol and nor adrenaline on adipose tissue contribute towards the release of free fatty acid which leads to dyslipidemia.

Lipid peroxidation is the result of oxidative damage of polyunsaturated fatty acid. Very low-density lipoprotein (VLDL), LDL along with HDLare involved inperoxidation, butinvolvement of LDL is maximum. Ox datively modified LDL cause the formation of foam cells which is important factor for atherosclerosis.

CONCLUSION

Our findings suggest that examination stress affects the levels of Lipid profile. Examination stress has an effect on deranging thetotal cholesterol(TCh), triglycerides (Tg), low density lipoprotein(LDL) and high density lipoprotein(HDL).

REFERENCES

1. Supe AN. A study of stress in medical students at Seth G.S. Medical College. J Postgrad Med 1998; 44: 1-6.
2. Malarkey WB, Pearl DK, Demers LM, Kiecolt-Glaser JK, Glaser R. Influence of academic stress and season on 24-hour mean concentration of ACTH, cortisol, and β-endorphin. Psychoneuroendocrinol 1995; 20: 499-508.
3. World Health Organization, World Health Statistics. 2013:10–30. Available at: http://www.who.int. Accessed June 1, 2014. Few of the risk factors can be modified like mental and physical stresses.
4. Elsevier, Fine LJ, Rosenstock L. Rosenstock L, editor. Cardiovascular Disorders. Clinical Occupational and Environmental Medicine. Vol. 2 2005;549–64.
5. Djindjić N, Jovanović J, Djindjić B, et al. Work stress related lipid disorders and arterial hypertension in professional drivers—a cross-sectional study. Vojnosanit Pregl 2013;70:561–8. [PubMed]
6. Catalina-Romero C, Calvo E, Sánchez-Chaparro MA, et al. The relationship between job stress and dyslipidemia. Scand J Public Health 2013;41:142–9. [PubMed]
7. McCord JM. Human disease, free radicals, and the oxidant/antioxidant balance. Clin Biochem 1993; 26: 351-57.
8. Aruoma OI. free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998; 75: 199-212..
9. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology 2000; 7: 153-163.
10. Gropper S, Smith J, Groff J. Advanced nutrition and human metabolism. Fifth ed.Belmont, CA: Wadsworth, Cengage Learning; 2009.
11. Rosenfeld M. Lipid Digestion and Absorption, Fatty Acid Synthesis and Oxidation, Triglyceride Synthesis, Adipose Tissue Lipoprotein Metabolism, Cholesterol, Lipid Metabolism in Pregnancy and Lactation. Presented at Nutrition and Metabolism II,University of Washington, Seattle WA 2012.
12. Brindley DN, McCann BS, Niaura R, et al. Stress and lipoprotein metabolism: modulators and mechanisms. Metabolism 1993;42(9 Suppl 1):3-15.
13. Niaura R, Stoney CM, Herbert PN. Lipids in psychological research: the last decade. Biol Psychol. Netherlands, 1992:1-43.
14. Riesen WF. Lipid Metabolism. In: T homas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:167-169.
15. Current status of blood cholesterol measurement in clinical laboratories in the United States: a report from the LaboratoryStandardizationPaneloftheNationalCholesterolEducationProgram. ClinChem1988;34:193-201.
16. Jacobs NJ, Van Denmark PJ. Arch Biochem Biophys 1960;88:250-255.
17. Koditschek LK, Umbreit WW. Alpha-glycerophosphate oxidase in streptococcus faecium F 24. J Bacteriol 1969; 98:1063-1068.
18. Trinder P. Ann Clin Biochem, 1969; 6:24-27
19. National cholesterol education program expert panel. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.
20. Saadiq, F.,(2013).Hyperlipidemia---Update & Review. 35:1 (online) Available at:<http//www.wfprofessional.com/documents/Hyperlipidemia-Update%20&%20Review-1.13pdf> [Accessed 25 June 2013]
21. Chaudhry, M.A., Waseem, M.,Ahmad,F.,ashraf,M.Z. and Bhatti,A.,2012.frequency of Coronary Heart Disease:Risk Factors among Doctors of CMH Lahore Medical and Dental College,Lahore-Pakistan.A.P.M.C.,6:2.
22. Dochi M, Suwazono Y, Sakata K, et al. Shift work is a risk factor for increased total cholesterol level: a 14-year prospective cohort study in 6886 male workers. Occup Environ Med 2009;66:592–7. [PubMed]
23. Mosendane T, Mosendane T, Raal FJ. Shift work and its effects on the cardiovascular system. Cardiovasc J Afr 2008;19:210–5. [PMC free article] [PubMed].