ORIGINAL PROF-788

BLOOD CHOLESTEROL IN RELATION TO HAEMO-DYNAMIC REACTIVITY UNDER EXAMINATION STRESS

DR. GHULAM MUJADID QURESHI, MBBS, Ph.D
Senior Demonstrator Physiology
Chandka Medical College Larkana

DR GHULAM MUSTAFA SEEHAR, MBBS, Ph.D
Chairman, Physiology Department
University of Sindh, Jamshoro

DR. SAEED AHMED SANGI, MBBS, MS
Associate Prof and Head of Cardiology
Chandka Medical College Larkana

Dr. Zafar Ali Pirzado, **Ph.D**
Professor and Head of Biochemistry Department
Chandka Medical College Larkana

ABSTRACT ... gmqlrk@yahoo.com.pk **Objective:** This study was a part of our major work, which was being conducted on haematological variations and haemodynamic changes among different study groups ,selected from medical students (2001-2003) in order to interpret and establish a base line for understanding the physiological facts related to such variations and their co-relationship under stress. **Design:** In this study the participants were subjected to mental stress during degree examination for the analysis of changes in blood cholesterol, namely Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), and High Density Lipoprotein Cholesterol (HDLC) in relation with heart rate (HR), pulse rate (PR) , systolic & diastolic blood pressure (SBP & DBP) responses. Venous blood samples were collected from the students in fasting condition along with estimation of all those cardiovascular responses during both, the stress period of examination & non-stress period of one and half month rest given to them, when there was no such serious academic activity. **Results:** The means and standard deviations of differences of stress and non stress conditions for all cholesterols and cardiovascular observations were evaluated by method of paired sample design. For the level of significance those results were analyzed by student's -test and 95% confidence interval. Conclusion: It is concluded that the variations in TC, LDLC and HLD-C may be due to haemoconcentration under acute mental stress given to the subjects. As the findings were observed in the fasting group of the subjects, so such related facts regarding mechanisms affecting the variables of the cholesterols in relation with haemodynamic under stress changes have been discussed. However further studies may also be needed.

Key words: Cholesterol, Heart rate, Pulse rate, Systolic-BP, Diastolic-BP, and Mental stress.

INTRODUCTION

There is substantial evidence that psychological stress enhances risk for coronary artery disease[1-2]. It is well established that acute stress elevates blood pressure[3] and chronic stress may give rise to hypertension[4-5], which is a major risk factor for coronary heart disease. Mental stress may also increase serum lipids[6-

[7], but some observations are on the record that significant co-relationship of the acute and chronic stress was not apparent for TC, LDLC and HDLC[8]. It has also been seen that postprandial changes in the serum cholesterols were not significantly effected by mental stress[9] and even there is no influence of negative life changes on serum cholesterol in such situations[10]. Although acute and chronic stress have been shown to raise serum lipids and are also associated with clinical coronary disease but the mechanisms by which stress contributes to alterations in lipid levels are not fully known. Only various pathways involving hormones and diet--etc have been implicated[11].

The possibility that the stress affects plasma lipid concentrations has been the subject of recent investigation[12]. In this regard, for elucidating of possible relation in between psychological stress and cardiovascular disease, further studies about the effect of mental stress on haematological factors, like lipid profile, plasma coagulation, fibrinolytic system, peripheral blood cells and haemodynamic changes" are needed[13-14]. In this connection to determine whether psychological stress contributes to variability in serum cholesterols like, TC, LDLC and HDLC in relation with haemodynamic activities for concomitant changes in health behaviors, the effects of acute stress during degree examination on these parameters were examined in medical students.

MATERIALS & METHODS

Male medical students (n= 58) were included in this study and they were selected from 1st and 2nd year MBBS at CMC-Larkana. They were divided into groups according to the examination schedule. The purpose of study was explained to all participants before the examination started. They were instructed to avoid heavy exercise and also they were restricted to take any medication on one day before and on the day of sampling. All were placed in fasting condition up to the end of sampling.

The selection of subjects was based on their well being and consent was also taken from them for collecting blood samples at both times, at the commence of their annual examination for stress study and after the rest given to them for the control study. Before collecting the samples, all-important information related to the study were recorded in the proforma and the questionnaires were also filled by them designed for the purpose. The modified version of the questionnaires of Speilberger, the State Trait Anxiety Inventory (STAI) (Gruber & Beauchamp, 1978) to determine pre-task anxiety levels was applied. The subjects had also completed post-exam questionnaire packet contained another STAI to determine post-exam levels of anxiety during the study of non-stress period after a resting period of one and half month given to them. The participants were examined for their physical well being. The systolic and diastolic blood pressures, the heart and pulse rates were estimated at the time of both sampling. In addition to the filling of questionnaire, the estimated changes in the haemodynamic parameters among the medical students were also used for assessment of stress level. Thus three stress assessment tools were used to assess the stress level. The students, showing presence of stress as per result of any two of all those three used tools, were included in the study.

The blood samples collected in labeled centrifuge tubes (without addition of anti-coagulant) were left for half-hour to allow the formation of clot. The centrifugation was carried out to separate serum from blood and then the serum samples were drawn carefully & were transferred into sterilized bottles already labeled. The LDLand HDL cholesterol experiments were performed by Kit CHOD-PAP methods, using Diagnostica Merk KGaA-64271 Darmstadt, Germany, while TC by Merckotest of E.Merck, Posttach 4119,D-6100 Darmstadt.

The spectrophotometer machine made by Interlabs instruments of Bausch and Lomb USA, namely the spectron-21 was used. The standard containing standard concentration of parameter-chemical and other necessary reagents were available in the kits. Three solutions, namely the sample, the standard and the blank were prepared from serum, standard chemical and reagent, respectively as per Kit method

applied.

Zero level on spectron-21 was adjusted to measure each cholesterol by using its blank solution separately under specific wavelength of light intensity of that cholesterol according to the kit method. Then the absorbance levels of sample and standard solutions were determined separately and concerned parameter's level was calculated by the formula, given below:

$$\text{Parameter} = \frac{\text{Sample Absorbance} \times \text{concentration of standard}}{\text{Standard Absorbance}}$$

RESULTS & OBSERVATIONS

The findings of all three cholesterols (TC, LDLC and HDLC) and all four haemodynamic parameters (HR, PR, SBP and DBP) were evaluated during both conditions of mental stress and non-stress periods as given in Table I.

Table-I. Variables of cholesterol and haemodynamic changes expressed as mean$\pm t_{05}$ during stress and control periods in male medical students. Numbers of observations are given in parenthesis*

Variable	Stress Period (n = 58)	Control Period (n = 58)
Heart Rate (beats/minute)	82.465 ± 2.276	71.482 ± 1.446
Systolic –BP (mm Hg)	136.982 ± 2.989	123.448 ± 2.189
Diastolic-BP (mm Hg)	85.00 ± 1.704	75.948 ± 1.676
Total Cholesterol (mg / dl)	162.602 ± 4.148	161.206 ± 3.996
LDL-Cholesterol (mg / dl)	82.499 ± 3.956	81.482 ± 3.716
HDL-Cholesterol (mg / dl)	43.033 ± 2.562	42.12 ± 2.454

* "Mean ± t.05Se" of each variable is calculated as 95% confidence interval of mean in both conditions, the stress and rest respectively.

Table-II. Showing statistical analysis of differences in between stress* and control observations for cholesterol with paired student's t-test and P-level significance in male students (n = 58).

Statistical analysis	Cholesterol		
	TC	LDL-C	HDL-C
Mean of difference	1.386 mg/dl		
Stand: deviation of difference	4.941 mg/dl	3.40 mg/dl	0.301 mg/dl
Test statistic of difference	2.154	2.28	2.311
P level significance	P<0.05	P<0.05	P<0.05

* Percentage increments during stress for:

1. Total Cholesterol (TC) = 0.87%.
2. LDL-Cholesterol (LDL-C) = 0.25%. 3. HDL- Cholesterol (HDL-C) = 2.15%.

There was equal significant evidence (P<0.05) for all three cholesterols to say that all cholesterols were increased, with different percentage increments in TC (0.87%), in LDL-C(1.25%), and in HDL-C(2.15%) at the time of mental stress of degree examination (Table-II) . The means and standard deviations of differences in between the stress and non stress conditions for TC, LDLC, and HDLC observations were statistically evaluated by paired sample design. Their respective means were used for

the comparison of stress and non stress samples under the student's paired t-test and **95%** confidence interval for level of significance (Table: II).

Table-III. Statistical analysis of differences in between stress* & and control observations for the haemodynamic changes with paired student's t-test and P-level significance in male students (n = 58).

Statistical analysis	Haemodynamical changes		
	HR	**SBP**	**DBP**
Mean of difference	10.983 beats/min	13.534 mmHg	9.052 mmHg
Stand: deviation of difference	6.228 beats/min	8.786 mmHg	6.718 mmHg
Test statistic of difference	13.442	11.738	10.261
P level significance	P<0.001	P<0.001	P<0.001

* Percentage increments during stress in:

1. Heart Rate (HR) =15.36%
2. Systolic-BP (SBP) =10.96%
3. Diastolic-BP (DBP) =11.88%

Table-IV. Statistical analysis for co-relationship in between cholesterols and haemodynamic parameters with student's t-test and p-level significance in male students (n=58).

Parameters		Statistical analysis		
		Co-relation co-efficient(r)	**t-statistic Value (ts)**	**P level Significance (P)**
TC	HR	0.497	6.79	P<0.0001
	SBP	0.304	3.25	P<0.001
	DBP	0.196	1.94	P<0.042
LDL-C	HR	0.563	8,86	P<0.0001
	SBP	0.323	3.34	P<0.001
	DBP	0.201	2.47	P<0.49
HDL-C	HR	0.502	7.98	P<0.0001
	SBP	0.376	3.68	P<0.001
	DBP	0.289	2.57	P<0.05

Similar processes of the statistics were applied for HR, SBP and DBP findings. It was observed that all those three haemodynamic parameters were increased during mental stress. The p-values for level of significance and percentage increments of HR (15.36%), SBP (10.96%), and DBP (11.88%) during mental stress along with their standard deviations and t-values were also evaluated as shown in Table III.

While the Table-IV shows the co-relationship of cholesterol variations with haemodynamic changes. There is evidence to conclude that the correlation of TC, LDL-C and HDL-C with HR is highly significant and respective P-value detected was less than 0.0001 (P< 0.0001), while correlation of all cholesterols with SBP and DBP was significant (P< 0.0001) and nearly significant, respectively. The correlation co-efficient

of those co-relative data are given in the Table IV.

DISCUSSION

Various studies have been conducted to examine TC, LDLC and HDLC under different stresses. Some of those studies have shown the increase in TC, LDL-C and HDL-C[15-17], while no change or even decrease in the lipoproteins were observed by some other studies[9,10,18]. In case of increments the changes have been attributed to the effect of epinephrine on lipoprotein lipase, hepatic lipase and hormone sensitive activities[19-21]. Which in turn increase fatty acid efflux from adipose tissue[22], providing the liver with substrate for triacylglycerol synthesis and VLDL production[23].

In the present study all the three cholesterols were increased highly significantly ($P<0.05$) during stress. Our findings were in agreement with previous works[16,24], in which TC, LDLC and HDLC were also observed at higher level (significantly) during stress. In those studies the blood samples were also taken in morning under fasting condition from students during stress and non stress periods as we had it in our present study. Both stress and epinephrine infusion raised not only the TC, LDLC and HDLC but also raise Very Low Density Lipoprotein Cholesterol (VLDLC) , Very High Density Lipoprotein Cholesterol (VHDLC) and Apoprotein-B concentration, while the comparable increases during control session were not observed[25].

The association between serum lipid levels and the cardiovascular reactivity to laboratory stressors has been reported[26], but such findings were not fully discussed. So those were not applicable of interpretations and evaluation or the mechanism analysis. Probably due to the reason that, the investigators have conducted most stress related studies in the presence of acute exposure to experimental stress in the laboratories[27]. It has also been seen that associations were completely absent in young age group during acute mental stress but indeed it existed for stronger cardiac responsitivity with cholesterol in relatively older males, suggested age & sex dependency association[26].

Actually no studies have compared the effects of acute and chronic stressors on lipid responsitivity in the same individuals[8], and the stress induced lipid changes in blood have not been clearly elucidated by physiological and behavioral mechanisms[6]. Stresses not only increase the blood pressure but also increase the blood viscosity and raise the haematocrit value of the blood in humans[28-29]. The stressed subjects showed significant reduction in plasma volume and increase in plasma viscosity compared with controls[1], probably due to increased peripheral capillary filtration, which in turn may be due to the vasoconstriction as a part of hamodynamic reactivity to emotional stress[30-31]. Some of investigators argued that the increase in concentration of circulating lipoproteins were because of this haemoconcentration due to vascular fluid shifts[1,30,32].

CONCLUSION

The TC, LDL-C and HDL-C in present study were significantly increased during stress period, which may be due to haemoconcentration under acute (short-term) stress given to the subjects. The co-relationship of variations in TC, LDLC and HDLC had been observed with haemodynamic changes in the present study. As the correlation of cholesterols with HR was highly significant , so the raised findings of the cholesterols may be due to the inotropic effects and increased force of contraction of the heart pumping under acute stress in fasting condition. All such factors under stress in turn may cause increase in cardiac out put leading to the haemo concentration, which needs further evaluation. However, it is difficult to evaluate the exact mechanism regarding such changes due to the non availability of hormonal observations in our present study. Also, a thorough comparative study of the lipoprotein cholesterol in serum under acute and chronic stress may be needed in this regard.

REFERENCES

1. Muldoon.MF, Herbert.TB, and et al. **"Effects of acute psychological stress on serum lipid levels, haemoconcentration and blood viscosity".** Arch. Intern. Med (1995), 155 (6): 615-620.

2. Vrijkotte.TG, Van Doormen. LJ and DeGeus.EJ. **"Work stress and metabolic and homeostatic risk factors".** Psychosom. Med, 1999, 61 (6):796-805.

3. Lovallo.WR. **"Stress, Health, Biological and psychological interactions thousand oaks,** stage publications (1997): California.

4. Alloy.LB, Acocella. J and Bootzin. RR. **"Abnormal psychology current perspectives",** Mc Graw. Hill (1996), Ed-7th : NewYork.

5. Carlson. NR. **"Foundations of physiological psychology".** Allyn and Bacon (1995), Ed-3rd: Bostan.

6. Niaura.R, Stoney.CM and Herbert, PN, **"Lipids in psychological research: the last decade".** Bio. Psycho. (1992), 34 (1): 1-43.

7. Magil.NF, **"International Encyclopedia of psychology".** Fitzroy Dearborn (1996), Vol.-II: London.

8. Stoney. CM, Niaura. R, Bausserman. Land Matacin. M. **"Lipid reactivity to stress: I. Comparison of chronic and acute stress responses in middle aged airline pilots".** Health psycho. 1999, 18 (3) : 241-50.

9. Le Fur. C, Romon M. Lebel. P and at al, **"Influence of mental stress and circadian cycle on postprandial lipemia".** American journal of clinical Nutrition (1999), 70 (2): 213-220.

10. Helminen. A, Rankinen. T, Halonen. P. and Rauramaa. R. **"Positive and negative life changes and LDL-cholesterol".** Biososci (1999), 31(2):269-277.

11. Calderon.RJ, Schneider. RH, Alexander. CN and Myers. HF **"Stress, Stress reduction and Hypercholesterolemia in African Americans: a review".** Ethan.Dis (1999), 9 (3): 451-462.

12. Mc Cann.BS, Benjamin. G.A, Wil Kinson. CW and Retzlaff BM, **"Plasma lipid concentration during episodic occupational stress".** Ann.Behave. Med (1999), 21 (2): 103-110.

13. Jern.C, Wadenvik. H, Mark.H Hallgren. T and Jern.s, **"Haematological changes during acute mental stress".** British journal of haematology (1989), 71: 153-156.

14. Memon. MA **"Haematological changes during acute mental stress".** Thesis. JPMC (1991): Karachi.

15. Peter.R , Alfredsson. L, Hammar. N and et al, **"High effort, low reward and cardiovascular risk factors in employed Swedish men and women: base line results from the wolf study".** Epidemiol community. Health (1999), 52 (9): 540-547.

16. Scheuch. K Pietruschka. WP, Eckhardt. G & at al, **"HDL and LDL-cholesterol changes in psychological stress in relation to stress experience".** Z. Gesmate. Inn. Med. (1984), 39 (12): 273-277.

17. Arnetz, BB, Brenner. SO Levil. L and Hjelm. R,. **"Neuroendocrine and immunologic effects of unemployment and job insecurity".** Psychother.Psychosom (1991), 5 (2-4) : 76-80.

18. Tsopanakis.C and Tsopanakis. A, **"Stress hormonal factors, fatigue and antioxidant responses to prolonged speed driven".** Pharmacol, Biochem.Behav. (1998), 60 (3): 747-751.

19. Yukht.A, Davis.RC, Ong.JM and at al, **"Regulation of lipoprotein lipase translation by epinephrine in 3T3-L. I cells, Importance of the 3-untranslated region".** J. Clin. Invest (1995), 96: 2438-2444.

20. Ong.JM, Saffari.B, Simsolo.RB, and kern. PA. **"Epinephrine inhibits lipoprotein lipase gene expression in rat adipocytes through multiple steps in post transcription processing"** Mol. Endocrinol (1992), 6: 61-69.

21. Neve.BP, Verhoeven.AJ and Jan Sen. H, **"Acute effects of adrenaline on hepatic lipase secretion by rat hepatocytes":** Metabolism (1997), 46:76-82.

22. Samra.JS, Simpson.EJ and Clark. ML, **"Effects of epinephrine infusion on adipose tissue: Interactions between blood flow and lipid metabolism."** Am. J. Physiol, (1996), 271: E 834-839.

23. Murray.RK, Granner.DK, Mayes. PA and Rodwell. VW, **"Harper's Biochemistry."** Appleton and Lange, California (1999), Ed. 25th.

24. Kaasik. AT, Jurimae. T and et al, **"Influence of four week examination session stress on hypokinesia and serum lipoprotein pattern in students".** Canadian Journal of Cardiology (1997), 13: 320.

25. Mc Cann. BS. Magee.MS. and Broyles. FC. **"Acute psychological stress and epinephrine infusion in normolipidemic and hyperlipidemic men: effects on plasma lipid and apoprotein concentrations".**

Psychosom. Med (1995) 75(2): 165-176.

26. Van Doreen. LJ, Senieder.H and Boomsma. DI, **"Serum lipidsa and cardiovascular reactivity to stress".** Biol. Psychol. (1998), 47 (3): 279-297.

27. Fukuda.S **"Effect of the Hanshin-Awaji Earthquake on post-traumatic stress, life style changes and cortical levels of victims"** Helder publication, Japan (2000).

28. Benton.JG, and Rusk AH, **"The relation of physical activity and occupation to coronary artery heart disease"** Ann.Int.Med, (1954),41:910-917.

29. Russek HI **"Role of Heredity, diet and emotional stress in coronary heart disease"** JAMA (1959), 171:503 -508.

30. Patterson.SM, Goltdiener.JS and Hecht.G. **"Effects of acute mental stress on serum lipids: mediating effects of plasma volume".** PsychosomMed. (1993), 55 (6): 525-532.

31. Seyle.H **"The physiology and pathology of exposure to stress. A treatise based on concepts of general adoption-syndrome and diseases of adaption.** Montreat 1950 (cited in Jern.C, 1989-Birtish.J.haematology, 71:153-156).

32. Muldoon M.F. Bachen. EA, Manuck SB and et al. **"Acute cholesterol responses to mental stress and change in posture".** Arch. Intern. Med,(1992), 152 (4): 775-80.