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ASSOCIATION OF BLOOD IL-6, CORTISOL AND HAEMODYNAMICS IN HEALTHY ADULTS WITH STRESS: A POSSIBLE ROLE IN EARLY ATHEROGENESIS

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Abstract

The stressful events might provoke immune alteration in human, which lead towards serious health disorders including ischemic heart diseases. Many studies demonstrated the association of IL-6 with cardiac problems particularly in middle age population. The stress linked changes in physiological parameters in young adults with no apparent heart disease is substantial. Therefore objective of study was to explore the association of altered IL-6, blood cortisol, and changes in haemodynamic variables during stressful life event. In this cross-sectional study, 114 subject participants were studied twice**:** during examination event as "stress study" and after two months period of conditioned rest they were examined again and taken as "control study". Present results indicate that IL-6 values increased in stress significantly. Comparatively the haemodynamics and cortisol level in the subjects with 'stress' were found different from that of 'non-stress' controls, at significant level ($p<0.001$). Correlation of IL-6 with SBP and DBP was significant but interestingly non-significant with HR findings in stress, which need further studies. Conclusively this work paves a way for better understanding of the stress association with process of atherosclerosis in young age males, suggesting future studies on gender differences.

Keywords: Psychological stress, IL-6, Cortisol, Haemodynamics, Atherosclerosis

1. Introduction

In early 1980s, the researches began to consider that stressful events might provoke immune alteration in human, and a number of studies demonstrated that the 'time limited stressors' have immunological consequences (Janice, K. *et al*., 2002). Many studies were conducted to evaluate the relation of IL-6 and cardiac problems among middle age population including heart patients or patients of risk factors like hypertension and diabetes. Very scanty work has been carried out on association of stress with life events, work stress, including job strain to early atherogenesis in subjects since their earlier life (Brunner, *et al*., 2004). It was further assumed that hemodynamic reactivity may affect microvascular permeability during stress (Von Kanel, *et al*., 2009). Hence there is lack of studies conducted on young adults under the age of forty with no apparent IHD (Hemmingsson. T. 2006). However, to determine the possible physiological and/or pathological mechanism of stress-induced changes in blood contents is important that may lead to early atherogenesis.

The anxiety, a most common component of daily life stresses, mostly occurs during stressful events in humans' life, for instances in the students during exam-event (Rasof, 1998 and Shireen, 2008). Little is known about the hidden 'after-effects' of psychological stress in the people who faces the stressful situation, which may reason the atherogenesis by affecting blood constituents e.g. blood cells, coagulation factors, blood lipid and interleukins. No such work on stress-related consequences has been carried out among the young adults. Therefore this study was aimed to explore the association of altered IL-6 level, blood cortisol, and haemodynamic variables during and after stressful life event.

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2. Methodology

Study population: In this cross-sectional study male medical students belonging to 1st and 2nd year MBBS of Chandka Medical College, Larkana, were included during examination years 2004 to 2006. Before processing the haemodynamic tests and blood sample collection; the importance, nature and purpose of study were explained to participants in detail. Selection of subjects was finalized as per 'criterion' approved by scientific committee constituted on the members from Biochemistry Department CMC, Larkana, Larkana Institute Nuclear medicine and Radiotherapy (LINAR-Larkana) and department of Physiology University of Sindh, Jamshoro. Successfully selected participating students (n=114) were between 18 and 23 years of age (mean 19.45 years).

Experimental: Initially haemodynamic tests were applied; Pulse rate (PR) examined according to procedure of Swash and Mason before determination of Heart rate (HR), both were confirmed by ECG findings. Systolic blood pressures (SBP) and Diastolic blood pressure (DBP) were recorded by auscultatory method as per recommendations of American Heart Association. Blood samples were taken during 9.30 -12.30 AM and investigated for cortisol level by using Radio-immune assay method on gamma counter at LINAR-Larkana according to protocol provided in RIA kits. Enzyme Linked Immunosorbent Assay (ELISA) method was applied to estimate blood level of IL-6 by using 'IL-6 Assay Kit' (R and D system Minneapolis) on E-LIZA-3000 machine made by D.R.G International Inc. Laboratory work was completed at Biochemistry Department, CMC Larkana and data were analyzed statistically by computer software SPSS version 14.

3. Results

Initially 297 students approached were fully explained about the criteria for participating in study. During whole process 143 student participants failed to resume at different stages, therefore were excluded. The number and percentage of excluded student participants were according to 'exclusion criterion' as given in (**Table-1)**. The successfully selected participants (n=114) were examined twice; during 'examination event' considered as 'subjects' and after taking conditioned complete rest for two months were considered as 'controls'.

Table-1.The number and percentage of participating students not excluded according to exclusion criterion.

S. No.	Exclusion Criterion	Exclusion No.	Exclusion % age
1	Without stress	41	13.80
2	Non-respondents	18	06.06
3	Absent in 2nd phase	22	07.40
4	Taken medicines	08	02.69
5	With ill health	05	01.68
6	With weakness	03	01.00
7	The smokers	46	15.45
	Total	**143**	**48.14**

Outcome of present study after statistical analysis shows increased values of test parameters in stress. Level of blood IL-6 during examination stress was increased and different from that of 'after stress (control)'. Paired sample test indicate 'mean of difference' value 0.421pg/dl for IL-6, which is statistically significant **(Table 2)**.

Table 2. IL-6 and haemodynamics during stress and after-stress sessions expressed as Mean ± $t_{.05}$ SE in 114 male subjects.

Parameters	Stress	Control	Mean of Differences
Interleukin (IL-6) (pg / dl)	1.743 ± 0.096	1.322 ± 0.188	0.421*
Blood Cortisol (ng /ml)l	179.55 ± 2.680	156.77 ± 2.210	22.780**
Heart Rate (HR) (beats/min)	83.360 ± 0.776	71.263 ± 0.618	12.097**
Systolic Blood Pressure (SBP) (mm Hg)	135.960 ± 1.080	122.37 ± 0.790	13.590**
Diastolic Blood Pressure (DBP) (mm Hg)	86.930 ± 0.792	75.921 ± 0.796	11.009**
Heart Rate (HR) (beats/min)	83.360 ± 0.776	71.263 ± 0.618	12.097**

****** Highly significant level (P<0.001).
***** Significant level (P<0.01).

The values of haemodynamic variables; HR, SBP and DBP were increased in stress event by positive mean of differences in order of 12.097 beats/minute, 13.590 mmHg and 11.009 mmHg respectively. Paired sample analysis for these variables is highly significant at 0.01 level of confidence intervals.

Table 3 shows correlation coefficient of IL-6 with other study parameters. This analysis results correlation coefficient of increased IL-6 with raised HR, by value of r=0.15, which is statistically non-significant (N.S) in stress event. While, positive correlation of IL-6 with SBP and DBP observed with positive correlation coefficient value of r = 0**.**22, and r =0**.** 286 both are seen at significant correlation coefficient. (P<0.05). Table 3 further shows correlation coefficient with blood cortisol also at significant level (P<0.042)

4. Discussion

Results of present study reveal the stress effects by means of the changes in hemodynamic and blood parameters in the subjects under investigation. It is already reported that the individual during days of examination period is more likely under threat of 'exam-stress' (Byrd, 1966). Persons who remain under stress of various health conditions have been found with associated alterations in level of blood IL-6 (Daniela, L. *et al*., 2002). Level of blood IL-6 after analysis was significantly increased (Table 2) during 'stress session'. This increased IL-6 level was consistent to earlier reported work on students (Lin, Y.L. *et al*., 2002). However, stress may affect different types of cytokines in human' body by different physiological mechanisms (Thompson, A. 1998, Nicolas. R *et al*., 2002). In this way each cytokine was being regulated by its own path way (Susan, K.L. *et al*., 2004, Sansanee. N *et al*., 1998, Khalid ZM 2003). The mechanisms for increased level of IL-6 under mental stress as proposed were by sympatho-adrenal-medullary (SMA) and hypothalamus-pituitary-adrenal (HPA) axis (Gregory. E M. 2002, Sansanee.N *et al*., 1998). Many experiments were documented for the role of IL-6 to stimulate release of cortisol by directly acting on the adrenal gland (Franchimont, D. *et al*., 2000. Path, G. *et al*., 1996) and through HPA axis (Mastorakos, G. *et al*., 1993, Zhou, D. *et al*., 1993) in blood during stressful situation (Susan, K.L. *et al*., 2004, Adam, T. C. *et al*., 2007). In this work the blood cortisol was increased during stress at highly significant level (P<0.001), when statistically analyzed this raised level of cortisol and increased IL-6 were positively correlated at significant level (P<0.042) with correlation co-efficient value of r=0**.**286 (Table 2 and 3). Whereas, suggesting the active role of IL-6 in the bidirectional approach between the interleukins and cortisol mode of response to stress.

The association of increase in haemodynamic variables was in agreement with the reports supporting the release of endogenous catecholamines and cortisol by neuro-endocrines pathways at stress time (Gregg, M.E. *et al*., 1999, Sternberg EM. 2001). Therefore, occurrence of HPA and SAM axis activity during stress might play the role in alterations of the haemodynamic variables. Table 2 indicate the increased values of haemodynamic parameters (HR, SBP, DBP) along with blood cortisol and IL-6 at highly significant level in participants at the time of 'exam-stress' as compared to the 'controls' who returned back after enjoying full rest, which may be specified to the associated role of parameters included in this study. Though, the haemodynamic parameters (HR, SBP and DBP) were increased with highly significant level, in 'stress event' but it is observable that the increased IL-6 became positively correlated only with raised SBP and DBP significantly (P< 0.02 & P<0**.**05) **(Table 3)**.

Table 3. Correlation coefficient of interleukin-6 with blood cortisol, HR, SBP and DBP among 114 male participants

Parameters		**Statistical Analysis**	
		Co-Relation Co-efficient	Signific-ant level
Interleukin -6 (pg / dl)	Cortisol	+ 0 .286	P < 0.042
	HR	+ 0. 15	NS
	SBP	+ 0. 22	P <0.02
	DBP	+ 0 .194	P < 0.05

P<0.05

This study determined the effect of stressful task on IL-6 among 114 apparently healthy and young male subjects. According to reported findings the healthy individuals who were most reactive to stress (high, H.R reactors) have had a greater increase in interleukin production in response to stress task than the individuals who were less reactive (low, H.R reactors) to the same stress (Mark, R.L 2001). But in this work the raised IL-6 was not found in association with raised level of HR at the time of stress, the correlation co-efficient for correlation of IL-6 and HR observed was non-significant with correlation co-efficient value of r=0.15 only (Table-3). Among the probable reasons, haemodynamic associated reactivity and microvascular permeability has been taken to understand the changes in blood bio-concentration during stress (Jet, J. Zanten, *et al*., 2009). Consequently, the increase in IL-6 values may not be claimed as per criterion of high or low, H.R reactors. Furthermore, in present study only those participants were included who were found with positive stress-perception established on 'selection protocol' and volunteers without stress were excluded. Therefore, increase of blood IL-6 might be due to higher stress perception. Irrespective of probable role and mode of alteration in level of IL-6 during stress, it is concluded that "the increased blood IL-6 may depend on type and severity of stress" for this further studies are suggested.

Majority of previous studies carried out on the association of biological factors with psychological stress were on a small number of known patients above 40 years age (Strike, P.C 2003). But very scanty references are available to address this issue of hidden development of atherosclerosis in youth around thirty under stress. Findings of this study indicate the association of interleukins, cortisol and haemodynamics in the young student subjects, which can be as a 'base line idea' for future studies.

5. Conclusion

In conclusion, present findings of IL-6 with haemodynamics and cortisol in the young population were significantly associated with stress. Which need regular follow-up and coherent broad base study to prove the development of atherosclerosis a very frequent reason of cardiovascular diseases.

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