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Research Article

STUDY OF PHYSIOLOGICAL AND BIOCHEMICAL VARIATIONS IN POPULATION GROUPS UNDERGOING ACUTE OR CHRONIC STRESS

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ABSTRACT

Objective: Stress arises from derangements of personal and professional activities. The basic reason is changes in lifestyle and psychosocial adaptations. Life becomes demanding, complicated, mechanical, and devoid of health. In today's modern world, occupational stress has become the major factor for loss of health and peacefulness of mind.

Methods: This is an observational descriptive study which was conducted in a group of 84 people between 25 and 60 years of age to study the association of acute stress on salivary amylase level and effect of chronic stress on lipid profile, glycosylated hemoglobin and certain inflammatory markers in people representing different forms of stress. Three groups of the population were studied. Group 1 (n=20) - Patients undergoing any elective surgery (abdominal/genitourinary) or an invasive diagnostic procedure (upper GI endoscopy, lymph node biopsy) Group 2 (n=32) – High/middle socio-economic male or female working for ≥8 h/day with or without stress. Group 3 (n=32) – Low socioeconomic male or female earning <100 Rs/day with or without stress. Salivary alpha-amylase and chromogranin A (CgA) levels are good markers of stress.

Results: There was increased levels of cortisol ($p<0.0001$), alpha amylase ($p<0.01$), and decreased levels of CgA ($p<0.001$) pre-procedure period as a marker of acute stress. In the chronic stress group of low income as well as high-income status, glycosylated hemoglobin, and blood lipid profile in both males and females were significantly deranged. Plasma malondialdehyde (MDA) levels and interleukin (IL)-1 and IL-2 were significantly raised in both groups. Serum ferritin level was low in the low socioeconomic group but normal in the high socioeconomic group.

Conclusion: Salivary amylase level was raised in acute stress and there is a direct correlation of chronic stress with high low-density lipoprotein/high-density lipoprotein ratio, raised glycosylated Hemoglobin, serum MDA, IL-1, 2 levels irrespective of economic group.

Keywords: Stress, Population groups, Acute, Chronic, Socioeconomic strata.

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INTRODUCTION

qStress involves two-way communication between the brain and the cardiovascular, immune, and other systems via neural and endocrine mechanisms. The HPA systems trigger the production and release of steroid hormones including the primary stress hormone *cortisol.* Cortisol is very important in organizing systems throughout the body including the heart, lungs, circulation, metabolism, immune systems, and skin to deal quickly with the crisis. The HPA system also releases certain neurotransmitters (catecholamines) particularly those known as dopamine, norepinephrine, and epinephrine [5]. The autonomic nervous system controls bodily functions which we are largely unaware of. The part of the autonomic nervous system that is activated during emergencies is the SNS, which speeds up systems needed for survival [6]. The other part, the parasympathetic nervous system, plays an opposing role. It mediates passive activities and promotes growth and energy storage. Parts of this system are also called into play during stress to slow down systems not required for survival. Justin found that stress-induced adrenal cortical hormones starts a chain of reaction in the body which involves each and every organ system and protects the body from noxious stimuli but overuse of this safety measure ultimately leads to permanent changes in the body [7,8].

We are now well aware that emotions and the mind play a critical role in our physiological responses. Dr. Hans Seyle, author of "Stress without distress" says factors such as fear, sorrow, joy, excitement, heat, cold, or drugs elicit certain identical biochemical reactions in the body. Selye and Collip pioneered the concept of biological stress borrowing the word stress from the psychic terminology that defines stress as the interaction between a deforming force and the resistance to it. His initial report in 1936 provided experimental evidence that the adrenal cortex, the immune system, and the gut are commonly altered organs as shown by the hypertrophy of the adrenals, involution of the lymphatic nodes along with the occurrence of gastric erosions in rats exposed to various noxious chemical or physical stimuli [9].

It was recognized that all noxious stimuli resulted in the initiation of a series of metabolic changes, the most prominent being the stimulation of the adrenal cortex [10].

ADAM HEALTH CARE 2002 suggests that stress appears to impair blood cholesterol levels. Freidman (1959), Russek (1967), and Mahendra (1976) established a significant association between emotional stress and hypercholesterolemia. Chronic stress has been associated with the development of insulin resistance [11].

Sustained psychosocial stress, often related to work, is an increasingly important factor in the development of illness, physical as well as mental [12,13]. Several studies are now suggesting that job-related stress is as great a threat to health as smoking or not exercising. At its most extreme, chronic stress places a burden on the heart and circulation that in some cases may be fatal. As the crisis comes closer, the brunt on the cardiovascular system increases instantaneously.

In spite of so many changes in the various fields around the globe, the economic strata of society still remain the same as much as the stress. It is interesting to note and find out whether the sections of society facing different types of stressful situations show any difference in their response to stress which could be reflected in their health status. Therefore this study was undertaken to observe the stress-related physiological and biochemical changes in different population groups.

METHODS

This is an observational descriptive study which was conducted in a group of 84 people between 18 and 60 years of age representing different life situations. The study was conducted in Shadan Institute of Medical Sciences after due permission from the institutional ethics committee. A complete pro forma of all the individuals which included personal, demographic, occupational, and health data was made and details noted after taking due individual consent and counseling. The above population was divided into two groups of those undergoing acute and those under chronic stress. Selection of people undergoing stressful situations was done based on the Holmes and Rahe Stress Scale. A score of 300 plus units were considered as being under stress. Those showing a score below 100 were taken as healthy controls in both the sexes.

The above population is again demarcated according to certain lifestyle parameters like food habits (vegetarians/non-vegetarians), smokers/non-smokers, and alcoholics/non-alcoholics, and the same parameters were noted for any difference.

Study design

Group 1 (n=20) - Patients undergoing any elective surgery (abdominal/genitourinary) or an invasive diagnostic procedure (upper GI endoscopy, lymph node biopsy).

Group 2 (n=32) – High/middle socioeconomic male or female working for ≥8 h/day with or without stress.

Group 3 (n=32) – Low socioeconomic male or female earning <100 Rs/day with or without stress.

Group 1 patients represent people under acute stress and Group 2 and 3 represent people under chronic stress.

Group 1 – Evaluation of Cardiac parameters such as changes in heart rate, systolic and Diastolic blood pressure. Salivary parameters such as salivary cortisol, alpha-amylase and chromogranin A (CgA) levels were evaluated.

Group 2 and 3 – Parameters in blood and plasma-like blood glucose, glycosylated hemoglobin, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), thyroid stimulating hormone (TSH), T3, T4, Plasma malondialdehyde (MDA) levels, serum ferritin, cytokines like interleukin (IL)-1 and 2 were measured.

Laboratory examination

Blood samples were collected for the assessment of a comprehensive chemistry panel, lipid profile, and inflammatory cytokines like IL-1, and IL-2. Venous blood was drawn into tubes containing EDTA, and immediately centrifuged at 1700 rpm at 4°C for 15 min. Plasma was separated and stored in aliquots at −20°C or below for assessment by immunoenzymatic test with spectrophotometric determination. Blood glucose, glycosylated hemoglobin, TSH, total tri-iodothyronine (T3), total thyroxine (T4), prolactin, serum ferritin, and complete lipid profile were analyzed by Thyrocare laboratories.

Collection of saliva

Saliva of all the study subjects was collected 2 h before going for the procedure. Before saliva collection research participants rinsed their mouth with water.

Passive drool was recommended because it is cost-effective and approved for use with almost all analytes. To avoid problems with analyte retention or the introduction of contaminants, the use of only high-quality polypropylene vials for collection, such as 2 mL cryovials (Salimetrics Item No. 5002.01) was done. The vials used were sealed tightly to be able to withstand temperatures as low as −80°C.

Unstimulated saliva was collected from the floor of mouth after 5 min using a sterile disposable cartridge and transferred to a sterile collecting tube. Two milliliters of saliva were kept immediately in the refrigerator for further analysis. Estimation of salivary total protein was done by the modified biuret method. Estimation of salivary amylase was done using the CNP-G_3 method. The procedure was as follows: The samples and the reagent were brought to room temperature before use. About 1 mL of reagent 1 and 1.5 µL of sample were dispensed into a test tube, mixed, and read immediately using a fully automated analyzer. The values displayed on the monitor were recorded and entered in a pro forma. Salivary amylase was estimated using an autoanalyzer (CoBAS Integra; −400 fully-automated biochemistry analyzer, Germany). Materials used are reagents (Roche Cobas Integra; Reagent, a-amylase – CNP-G_3 {2-chloro-4-nitro a maltotrioside). Salivary-free cortisol concentrations were measured using a commercially available chemiluminescence-immuno-assay with a high sensitivity of 0.16 ng/mL (IBL, Hamburg, Germany). Salivary CgA and cortisol were also measured, using enzyme-linked immunosorbent assay with a YK070 kit.

Statistical analysis

All analyses were performed with the SPSS 23 for Windows. Paired t-test was performed to test significance within the groups and unpaired t-test was applied for two different groups.

RESULTS

Acute stress

In individuals of the acute stress group, there was a significant increase in the heart rate calculated from the mean RR interval 3 h before the procedure ($p<0.001$, Table 1).

There was a significant increase in the systolic and diastolic blood pressure also ($p<0.001$ and 0.0001, respectively, Table 1).

Cortisol, alpha-amylase, and CgA levels are good markers of stress. There were an increased levels of cortisol, and alpha amylase ($p<0.0001$, 0.01, Table 1), respectively, and decreased levels of CgA ($p<0.001$, Table 1) pre-procedure period.

Chronic stress

In individuals of chronic stress but of high economic status glycosylated hemoglobin and blood lipid profile in both stressed males and females were significantly deranged.

The plasma MDA levels were raised indicating oxidative stress.

Serum ferritin levels were maintained but inflammatory markers IL-1 and IL-2 were significantly raised (Table 2).

In the low socioeconomic status glycosylated hemoglobin, blood lipid profiles were deranged significantly in females ($p<0.0001$, Table 3).

Plasma MDA levels (raised), serum ferritin (lowered), and inflammatory markers (raised) were deranged in both males and females ($p<0.05–0.01$, Table 3).

Life style

There was a significant change in glycosylated hemoglobin, plasma MDA, and inflammatory markers in smokers and alcoholics ($p<0.05–0.0001$, Table 4).

DISCUSSION

There is strong evidence (p<0.001) to conclude that heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and blood cortisol were significantly increased in participants at the time of acute mental stress. These raised findings were consistent with the results of studies conducted among foreign people, and which were supporting the work showing release of endogenous catecholamines and cortisol secreted by neuroendocrine [14,15]. In general, triglyceride (TG), LDL-C, TC, and HDL-C were found to be increased due to the effect of stress in most of recent past studies [16]. In the present study, although LDL-C and TG were increased by different percentages with different values of positive mean of differences but *p* value for raised observations of both parameters was found the same. The results of LDL-C and TG were consistent with the results of some of previous studies [17,18].

Some of the investigators argued that the increase in concentration of circulating lipoproteins were due to hemoconcentration due to vascular fluid shifts. The changes appeared to be of short duration, along with increased blood viscosity and hematocrit, indicating the occurrences of hemoconcentration under stress [19-21]. Both epinephrine and cortisol were claimed by some authors to be linked in humans to serum cholesterol elevation [22] and the results also indicated the probable hormonal association with said alteration of lipoproteins due to stress. Similarly, the WOLF study was devised to specifically examine stress of routine life, namely "work-stress" and found an adverse relationship in the ratio of low-density to HDL-C only in younger men and women [23]. While, the protective or good lipids for example HDL-C were reduced with 7.94% than and became negatively correlated with increased SBP and DBP responses.

The central nervous system (CNS) innervates adipose tissue through efferent pathways of SNS and the oscillations of plasma free fatty acids and glycerol were completely removed by β-blockade indicating that rapid bursts of lipolysis may be generated by SNS. Whether efferent pathways of CNS including SNS play any role in moment-to-moment regulation of such lipid metabolism under stress remains to be clarified [24]. It is suggested by many authors that the consequences of changes in blood lipids levels may be due to increased mental stress. On one hand, in this study mental stress alters lipids adversely (in atherogenic form) and such changes were found to be correlated with hemodynamic responses during psychological stress, but on the other hand, exact mechanism influencing cholesterol metabolism is not fully

Table 1: Acute stress (Patients undergoing surgery or any diagnostic procedure)

Parameters	Pre-procedure (3 h before)	Post-procedure (After 48 h)	p-value
Cardiac parameters, n=20			
Heart rate/Min (mean RR interval)	81.3±4.1	74±4.3	0.001
Systolic blood pressure	128±6	120.5±5.6	0.001
Diastolic blood pressure	88±4	78±6	0.0001
Salivary parameters			
Salivary cortisol (μ mol/dL)	1.55±0.13	1.22±0.09	0.0001
α-Amylase (μ/mL)	737±160	512±64	0.01
CgA levels Pmol/mL	0.48±0.09	0.61±0.16	0.001

All values are mean±SD: p<0.05 is considered significant, CgA: Chromogranin A

Table 2: Chronic stress (High economic status occupational male/female working ≥8 h a day)

Biochemical parameters	Control males (n=8)	Stressed males (n=8)	p-value	Control Females (n=8)	Stressed females (n=8)	p-value
Glycosylated Hb (units)	5.6±0.7	5.5±1.9	0.05	5.5±0.4	5.9±0.8	0.05
TC (mg/dl)	157±13.7	201±63	0.0001	131±6.8	174±6.3	0.0001
Triglycerides (mg/dl)	94±14	149±27	0.001	82±16	146±28	0.0001
LDL-C (mg/dl)	94±12	117±25	0.001	75±8	110±19	0.0001
TC/HDL-C ratio	3.2±0.5	3.5±0.9	0.001	3.1±0.2	3.6±0.3	0.01
TSH (μIU/mL)	0.45±0.04	0.46±0.04	NS	2.4±0.5	2.3±0.8	NS
T3 (ng/dL)	94±5	84±21	NS	83±11	81±25	NS
T4 (μg/dl)	7.8±2	6.4±2.7	NS	7.3±1	7.7±1.4	NS
Plasma MDA levels (μ mol/l)	2.3±0.3	4.9±1.7	0.0001	2.7±0.3	4.3±0.6	0.0001
Serum ferritin (ng/mL)	153±17	126±48	NS	124±23	114±32	NS
IL-1 (pg/mL, <15)	8.7±1.4	14±5.3	0.0001	11±1.3	16±2.7	0.0001
IL-2 (pg/mL, <31)	24±3	27±9	0.01	21±3	28±4.5	0.001

All values are mean±SD: p<0.05 is considered significant. NS: Non-significant, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, TSH: Thyroid stimulating hormone, MDA: Malondialdehyde, IL: Interleukin, TC: Total cholesterol

Table 3: Chronic stress (Low socio-economic family earning <100 Rs/day with psychosocial problems)

Biochemical parameters	Control males n=8	Stressed males n=8	p-value	Control females n=8	Stressed females n=8	p-value
Glycosylated Hb (units)	5.3±0.9	5.8±0.7	NS	5.3±0.3	6±0.6	0.01
Total cholesterol (mg/dl)	162±10	164±46	NS	129±18	173±14	0.0001
Triglycerides (mg/dl)	92±16	127±39	0.05	91±16	147±28	0.0001
LDL-C (mg/dl)	88±10	121±22	0.0001	90±8	131±17	0.0001
TC/HDL-C ratio	3±0.2	3.2±0.2	0.5	3.1±0.2	3.4±0.3	0.01
TSH (μ IU/mL)	1.9±0.3	1.8±0.5	NS	2.2±0.7	2.6±0.9	NS
T3 (ng/mL)	68±23	74±6	NS	80±10	76±5	NS
T4 (μg/mL)	6.8±1.7	6.6±0.9	NS	6.7±0.8	5.8±0.8	0.05
Plasma MDA levels (μ mol/L)	2.3±0.3	5.1±0.9	0.0001	2.3±0.3	3.5±0.3	0.0001
Serum ferritin (ng/mL)	151±15	125±17	0.01	117±19	111±27	NS
IL-1 (pg/mL)	12±1.8	13±2.3	0.01	12±2.5	14±2.7	0.05
IL-2 (pg/mL)	22±2.3	26±4	0.01	18±3.1	23±3.2	0.01

All values are mean±SD: p<0.05 is considered significant. NS: Non-significant, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, TSH: Thyroid stimulating hormone, MDA: Malondialdehyde, IL: Interleukin, TC: Total cholesterol

Table 4: Bio-chemical parameters in accordance to population lifestyle

Biochemical parameters	Smoker n=17	Non-smoker n=47	p-value	Vegetarian n=15	Non-vegetarian n=49	p-value	Alcoholic n=31	Non-alcoholic n=33	p-value
Glycosylated Hb (units)	6.2±0.7	5.8±1.1	0.01	5.6±0.9	5.5±1.2	0.05	5.1±0.8	5.5±0.7	0.05
Total cholesterol (mg/dL)	218±21	181±12	0.03	157±18	194±21	0.001	186±17	168±22	NS
Triglycerides (mg/dL)	157±24	121±11	0.02	118±34	146±24	0.0001	127±14	132±21	0.05
LDL-C (mg/dL)	98±10	87±9	NS	78±12	111±16	0.001	131±14	91±24	NS
HDL-C (mg/dL)	41±5	52±8	0.01	51±6	48±8	NS	52±8	50±9	NS
TSH (μ IU/mL)	2.17±0.6	5.8±0.7	0.001	1.8±0.3	2.6±0.7	0.05	5.2±0.9	3.1±0.5	0.01
T3 (ng/mL)	105±8	160±10	NS	210±15	185±8	NS	145±14	192±10	NS
T4 (μg/mL)	4.5±0.8	5.9±1	NS	2.8±0.8	3.1±0.9	NS	4.6±1.2	3.9±1.3	NS
Plasma MDA levels (μ mol/L)	7.1±1	2.8±0.3	0.0001	3.6±0.9	3.2±0.7	0.01	5.2±0.8	3.1±0.6	0.0001
Serum ferritin (ng/mL)	159±8	172±11	NS	196±20	224±15	0.01	148±11	172±10	NS
IL-1 (pg/mL)	11±1.5	16±0.5	0.001	9±1	10±2.1	NS	12±0.9	10±1.2	NS
IL-2 (pg/mL)	21±2.5	24±0.5	0.01	19±1	22±0.6	0.01	26±2	28±1.7	NS

All values are mean±SD: $p<0.05$ is considered significant, NS: Non-significant, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, TSH: Thyroid stimulating hormone, MDA: Malondialdehyde, IL: Interleukin

known. The possibility that stress affects plasma lipid concentrations has been the subject of ongoing concern.

The generation of free radicals is dependent on the presence of various transition metal ions. The most important transition metals in vivo are believed to be iron and copper. The most plausible explanation for an effect of iron is the stimulation of oxidation of LDL [25]. Plasma ferritin concentrations are directly proportional to intracellular ferritin concentrations so it is considered to be the best clinical measure of body iron stores and the most feasible one to use in epidemiologic studies [26].

Previous studies have shown that patients on sick leave because of occupational burnout resulting from psychosocial stress, have a disrupted sleep, with more arousals and sleep fragmentation, more wake time, and lower sleep efficiency. Sleep fragmentation is associated with elevated levels of metabolic and cardiovascular risk indicators of stress-related disorders, such as morning cortisol, heart rate, systolic and diastolic blood pressure, TC, HDL- and LDL-C, and LDL/HDL-ratio.

Serum MDA levels, a stable product of lipid peroxidation are an indicator of free radical generation in the human body [27]. Thyroid function slows in stress and T3 and T4 levels tend to fall [28].

There is a lot of evidence that the pro-inflammatory cytokines IL-1 play an important role in neuroendocrine and behavioral stress responses. IL-1 signaling and the resulting activation of the adrenal system and secretion of glucocorticoid secretion mediate the development of depressive symptoms associated with exposure to acute and chronic stressors [29].

The level of cortisol and alpha-amylase in saliva can be considered as one of the major biomarkers of psychosocial stress. Sympathetic activation during psychosocial stress increases plasma norepinephrine with a consequent increase in the production and release of salivary alpha-amylase from the acinar cells of parotid and submandibular salivary glands [30].

Salivary CgA is used as a good marker for catecholamines in psychosocial stress more quickly and sensitively. CgA is a 48-kDa acidic glycoprotein that is stored and co-released with catecholamines by exocytosis [31-33].

CONCLUSION

Salivary amylase level is raised in acute stress and there is a direct corelation of chronic stress with high LDL/HDL ratio, raised glycosylated Hemoglobin, serum MDA, and IL-1, 2 levels irrespective of economic group.

AUTHOR CONTRIBUTION

All Authors Contributed satisfactorily towards the study concept, design, collection of data, analysis of results, scientific knowledge, and publication ethics.

CONFLICTS OF INTEREST

There are no conflicts of interest by the authors.

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