



The Internet Journal of Allied Health Sciences and Practice

<http://ijahsp.nova.edu>

A Peer Reviewed Publication of the College of Allied Health & Nursing at Nova Southeastern University

Dedicated to allied health professional practice and education

<http://ijahsp.nova.edu> Vol. 6 No. 4 ISSN 1540-580X

Undergoing Venipuncture in Health-Care Education: The Psycho-Biological Effect on Students

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Israel

CITATION: Sarid, O., Anson, O., Schwartz, D., Yaari, A. Undergoing Venipuncture in Health-Care Education: The Psycho-Biological Effect on Students. *The Internet Journal of Allied Health Sciences and Practice*. October 2008, Volume 6 Number 4.

ABSTRACT

Venipuncture training is an integral part of healthcare educational programs. Despite its extensive use, no previous studies have evaluated the psychobiological impact of this procedure among health-care students. Participants were 26 healthy students, practicing venipuncture on each other as part of their curricular activity. Self reported pencil and paper questionnaires aimed at assessing depression, anxiety and mood states were administered before and after venipuncture and saliva sample collection. Anti heat shock protein (Hsp) 70 and cortisol levels in blood and saliva were collected from the participants. A reduction in anxiety, anger and confusion mood states took place after venipuncture. All participants had high levels of cortisol and anti-Hsp 70 in the serum and saliva. Anti-Hsp 70 and cortisol evaluated in the serum were correlated with some of the mood variables. Routine educational practices such as venipuncture elicit significant psychological stress. If confirmed by additional studies it will have a significant implication on health-care educational practices.

INTRODUCTION

Education of health-care students includes exposure to a variety of stressors such as examinations, interacting with patients in stressful situations, and practicing medical procedures.¹ In past research, academic challenges were considered as natural, transient, predictable, and relatively benign stressors.^{2,3-6} Yet, academic examinations produce immune change and may have a significant impact on students' health.^{2,7} Moreover, academic stress appeared to bring about transient emotions (as measured by self-reported indices) which affected immunological and endocrine markers.^{2,3-6}

Recently, however, it has been argued that stress related to the academic setting, i.e. examinations, do not represent a universal model of natural transient stressor. For example, oral examinations were considered by students to be more difficult and demanding than were written examinations, and objective structured clinical examinations (OSCE) brought about significant mood changes.⁸⁻¹⁰

Venipuncture practice is an integral part of most health-care educational programs. Many of these programs include performing venipuncture on fellow students. The medical risks associated with this procedure are well documented, but no previous studies have evaluated the possible psychobiological impact of this procedure among health-care students. Previous studies showed that blood donation, which involves venipuncture, was regarded by most participants as stressful and triggered a psychological and physiological stress response.^{11,12,13}

Numerous biological markers have been previously used to objectively assess the level of psycho-physiological stress. Additionally, several studies have shown a correlation between an increase in the levels of such markers and patho-psychological and patho-physiological processes.^{14,15} For example, changes in hypothalamic-pituitary-adrenal (HPA) axis activation during prolonged stressful events resulted in a marked increase in the release of the stress-hormone cortisol from the adrenal. Cortisol release from the adrenal is regulated by the adrenocorticotropic releasing hormone (ACTH) from the pituitary, which in turn is primarily regulated by corticotropin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus.¹⁶ Enhanced levels of cortisol were previously reported to be correlated with anxiety disorders, depression¹⁷, and traumatic life events.¹⁸ Thus, measurement of cortisol in serum and saliva can provide important information about hypothalamic-pituitary-adrenal (HPA) axis activity in response to stress and its associations with psychological reactions.^{15,16}

Heat shock proteins (Hsp) are intracellular proteins with prominent cytoprotective factor. They function to limit protein aggregation, facilitate protein refolding and chaperone proteins.¹⁹ During periods of cellular stress, intracellular Hsp levels increase to provide cellular protection.²⁰⁻²³ It has been recognized that Hsp, particularly Hsp 72, are also found extracellularly (eHsp 72), where they exhibit potent immunomodulatory effects on innate and acquired immunity. Circulating eHsp 72 levels also greatly increase during times of stress when an individual is exposed to a physical/psychological stressor or suffers from various pathological conditions.¹⁷ It has been proposed that elevated eHsp 72 have a protective role by facilitating immunological responses during times of increased risk of pathogenic challenge and/or tissue damage.²⁴

Notwithstanding the protective effects of the Hsp 70, other studies indicated the possibility that Hsps can lead, over the long term through the development of autoantibody, to chronic disease^{25,26} such as hypertension²⁷, rheumatoid arthritis^{28,29}, and coronary heart disease.³⁰ Anti-Hsp 70 antibodies were observed among persons exposed to environmental stress and among persons working in noisy, dusty, and hot conditions.^{31,32}

The study aims are twofold: 1. to examine the bio-psychological effects of venipuncture on self reported mood changes, anti-Hsp 70 and cortisol levels in saliva and blood. 2. To assess the correlations between these biological markers and the psychological self report indices.

MATERIAL AND METHODOLOGY

Procedure and Participants

The study population consisted of 26 healthy students, ten males and 16 females, age 24± 3.3 years (range 19 to 29), studying towards their academic degree in emergency medicine. As part of their curricular activity during their first year of training, the students practiced venipuncture on each other. Nineteen students (73.1%) had received and conducted a venipuncture procedure during a high-school first-aid course or during their army service (which is mandatory); for the others, this was the first experience in venipuncture.

Pencil and paper questionnaires were distributed upon entering the practice hall (t1). Venipuncture practice, which was performed on fellow students, lasted for two hours, and was followed by a second application of the self reported scales (t2). All samples were taken between 16:00-18:00 as previously proposed.³³ The institutional Ethics Board approved the study. All participants provided written informed consent.

Measures

1. Biological indicators- blood and saliva samples collection and processing: Each student donated blood and saliva samples simultaneously. Blood was withdrawn by venipuncture into a gel serum separator tube. Immediately after clotting the blood samples were centrifuged at 1000 xg for 10 minutes at 4^o C. Saliva samples were collected asking the participants to spit into a sterile plastic cup. Prior to spitting each student chewed a piece of parafilm for 2 minutes in order to enhance the saliva volume as described by Sarid et al.^{2,3} Saliva samples were centrifuged at 1000 xg for 10 minutes at 4^o C as were the blood samples. Serum and saliva samples were divided into aliquots in appropriate labeled tubes and kept at -70^o C until testing.

Cortisol Testing

- a. Serum cortisol was tested by using the automatic AxSYM system (Abbott Laboratories, Cicago, Illinois, USA), according to the manufacturer's instructions. Values are presented as $\mu\text{g/l}$. Normal range: 1.7-16.6 $\mu\text{g/d}$, between 4-6 pm.
- b. Salivary cortisol was tested undiluted using the Salimetrics salivary cortisol enzyme immunoassay kit (Salimetrics LLC, State College, PA, USA). Values are presented as $\mu\text{g/l}$. (Normal ranges measured between 7-6 pm) are 0.308 $\mu\text{g/dl}$ for males and 0.359 $\mu\text{g/dl}$ for women.

Determination of anti-human Hsp70 antibodies:

Serum and saliva samples were tested for anti-human Hsp 70 using an anti-human Hsp70 (IgG/A/M) ELISA kit, (Stressgen Biotechnologies Corp., Victoria, BC, Canada). Experiments were performed exactly as recommended. The results were detected using a microplate ELISA reader at 450 nm and are expressed as mg/l.

Dilution of samples:

- a. Serum samples: In order to ensure that the result values are within the range of the standard curve and following the manufacturer's instructions the serum samples were diluted 1:700 (v/v) with the diluents provided with the kit. This dilution was found optimal after several prior experiments. After the appropriate dilution was determined the assay was performed following the manufacturer's instructions.
- b. Saliva samples: Following several experiments the kit was adapted for salivary anti-human Hsp70. Optimal results were achieved testing non-diluted saliva and following the manufacturer's instructions.

2. Psychological measurement - Pencil and paper questionnaires were distributed before and after venipuncture and saliva donation. The questionnaire included:

- a. The short form of the Sense of Coherence Scale.^{34,35} The concept of Sense of Coherence (SOC) portrays the degree to which a person perceives the world, and the inevitable stressful events he/she encounters, as comprehensible, manageable and meaningful challenges worth overcoming. Such orientation enables efficient recruitment of available resources to cope with a variety of demanding situations. High SOC facilitates flexibility, and increases the ability to effectively choose the adequate resources for a specific situation. Respondents reported, on a 7-point Likert scale, the degree to which each of the 13 statements represented their attitudes. Possible values ranged from 13-91 with the highest score indicating high SOC. Cronbach's α of 0.79 was observed, similar to past research conducted among students of the health professions.⁵
- b. Depression was estimated by the Beck Depression Inventory (BDI), a 21 item self-report rating inventory measuring characteristic feelings, behaviors and symptoms of depression. The students were asked to indicate, on a four point Likert scale, the degree to which each item had been present or absent during the past month. The final score is the sum of the 21 responses.^{36,37} The range of possible values is 21 to 84, a low score indicating depression. In a previous study among an Israeli population, the scale was found valid and reliable.³⁸ In the current study Cronbach's α was 0.93.
- c. Profile of Mood States (POMS)³⁹ - 58 items which measure tension-anxiety (9 items), depression-dejection (15 items), anger-hostility (12 items), vigor (8 items), fatigue and confusion (7 items each). The students were asked to indicate to what extent each item represented their current mood on a five-point Likert scale. Higher values indicate a higher level of a specific mood. The Hebrew version of the questionnaire was found to be valid and reliable.⁶ Cronbach's α range from 0.73 (confusion subscale at t1) to 0.94 (vigor sub-scale at t2).
- d. Anxiety was assessed by a 10-items symptoms sub-scale taken from the short version of the SCL-90-R.⁴⁰ This inventory is a self-report measure consisting of ten symptom-specific items. Participants rate each symptom on a 5-point Likert scale (e.g., 0 = "not at all," 4 = "extremely"). Scores ranged from 10 to 40, a high score indicating a higher level of anxiety. Cronbach's α range from 0.87 at t1 to 0.75 at t2.
- e. Socio-demographic characteristics, including age, sex, army service, past experience in venipuncture.

Mode of Analysis

First, descriptive statistics were presented for the biological markers. Second, student's *t*-test for paired samples was employed to examine possible changes in psychological variables measured at two time points. Third, partial correlations, controlling for age, were carried out between anti-Hsp 70 and cortisol and separately between psychological variables, followed by partial correlations between biological and psychological variables. Age was controlled for because of the range observed, from young adolescents to young adulthood. The a priori alpha level for analysis was $p < .05$.

RESULTS

The prevalence of the measured biological markers is shown in Table 1

Table 1: Descriptive statistics for anti-Hsp 70 (serum and saliva, mg/l) and cortisol (serum and saliva, µg/l)

	Anti-Hsp 70 serum (mg/l)	Anti-Hsp 70 saliva (mg/l)	Cortisol serum (µg/l)	Cortisol saliva (µg/l)
Mean (SD)	333.5 (207.9)	3.13 (4.52)	87 (30.6)	2.6 (2.3)
Range	128.4-871.1	0.60-17.1	39-158	0.4-7.8

Cortisol was detected in serum and saliva of all participants with a median of 84 µg/l for serum and 1.1 µg/l for salivary cortisol. Values of anti-Hsp 70 in serum were broadly distributed with a median level of 261.4 mg/l. Median value for anti-Hsp 70 in saliva was 1.53 mg/l.

Two male students practiced venipuncture and donated blood samples twice; the second venipuncture was performed one hour after the first. For the first student, serum anti-Hsp70 levels were 160.1 mg/l in the first sample and 200.2 mg/l in the second sample. For the second student, serum anti-Hsp70 levels were 735.1 mg/l in the first sample and 820 mg/l in the second sample

Table 2: Paired sample t-test for transitory mood measurement (t1 and t2) (df=25)

Mood measurement	Mean (SD)	Paired t test	
		t-value	P
Anxiety (t1)	15.53 (5.2)	3.382	0.02*
Anxiety (t2)	12.38 (2.96)		
POMS			
Tension (t1)	6.38 (3.6)	1.72	0.097
Tension (t2)	5.11 (2.4)		
Depression (t1)	4.00 (6.17)	1.88	0.072
Depression (t2)	2.00 (3.41)		
Anger (t1)	4.61 (4.13)	2.58	0.02*
Anger (t2)	3.11 (3.05)		
Vigor (t1)	19.5 (9.22)	2.01	0.056
Vigor (t2)	21.35 (4.47)		
Confusion (t1)	6.26 (3.41)	3.757	0.01*
Confusion (t2)	4.00 (1.78)		
Fatigue (t1)	4.68 (0.91)	1.92	0.065
Fatigue (t2)	4.27 (0.83)		

A statistically significant decline in anxiety, as measured by SCL-90 R, anger and confusion, as measured by POMS, took place between t1 and t2. A decline in tension, depression and fatigue from t1 to t2 had also been observed, though it did not reach statistical significance. Only the scores on the vigor sub-scale increased at t2, that is, after venipuncture ($p=0.056$).

Summing up these findings in light of the first purpose of the study, these observations indicate that all participants had anti-Hsp 70 and cortisol in serum and saliva. Additionally, a change in all psychological indicators between t1 and t2, in the expected direction, took place; though only in three cases did these changes reach statistical significance. It should be noted that no statistically significant associations were observed between any of the above mentioned dependent variables and socio-demographic characteristics.

Correlations between the biological and psychological parameters: First, partial correlations (controlling for age) were calculated between the biological indicators. A statistically significant correlation was observed between anti-Hsp 70 antibodies in serum and anti-Hsp 70 antibodies in the saliva ($r=0.67$, $p \leq 0.001$), and between salivary cortisol and serum cortisol ($r=0.78$, $p \leq 0.001$). Anti-Hsp 70 and cortisol were not statistically significantly correlated in the saliva or in the serum.

Second, partial correlations were calculated between the transitory mood sub-scales, BDI, and SOC (see Table 3).

Table 3: Partial correlation matrix for psychological variables

	SOC	Anxiety (1)	Anxiety (2)	BDI	Tension (1)	Depression (1)	Anger (1)	Vigor (1)	Fatigue (1)	Confusion (1)	Tension (2)	Depression (2)	Anger (2)	Vigor (2)
SOC														
Anxiety (1)	0.26 NS													
Anxiety (2)	-0.41* NS	0.3 NS												
BDI	-0.44* NS	0.05 NS	-0.1 NS											
Tension (1)	-0.52* NS	0.58* NS	0.62** 0.32 NS	0.11 NS										
Depression (1)	-0.53* NS	0.45* NS	0.32 NS	0.5* NS	0.69** NS									
Anger (1)	-0.39 NS	0.55* NS	0.57 NS	0.24 NS	0.79** NS	0.79** NS								
Vigor (1)	0.23 NS	0.01 NS	0.15 NS	-0.66** NS	-0.15 NS	-0.43* NS	-0.26 NS							
Fatigue (1)	-0.64** NS	0.64** NS	0.26 NS	0.46* NS	0.64** NS	0.7** NS	0.51* NS	-0.46* NS						
Confusion (1)	-0.37 NS	0.45* NS	0.39* NS	0.07 NS	0.81** NS	0.7** NS	0.73** NS	-0.19 NS	0.6** NS					
Tension (2)	-0.18 NS	0.21 NS	0.12 NS	0.22 NS	0.14 NS	-0.04 NS	0.13 NS	-0.21 NS	0.2 NS	0.24 NS				
Depression (2)	-0.52* NS	0.27 NS	0.23 NS	0.29 NS	0.3 NS	0.45* NS	0.28 NS	-0.25 NS	0.44 NS	0.13 NS	0.12 NS			
Anger (2)	-0.32 NS	0.27 NS	0.56* NS	0.12 NS	0.53* NS	0.42* NS	0.7** NS	-0.11 NS	0.32 NS	0.39 NS	-0.4 NS	0.38 NS		
Vigor (2)	0.38 NS	0.03 NS	-0.12 NS	-0.52* NS	-0.31 NS	-0.49* NS	-0.28 NS	0.81** NS	-0.54* NS	-0.3 NS	-0.8 NS	-0.16 NS	-0.06 NS	
Fatigue (2)	-0.26 NS	0.26 NS	0.01 NS	0.26 NS	0.05 NS	-0.1 NS	-0.03 NS	-0.36 NS	0.43* NS	0.19 NS	0.86** NS	0.19 NS	-0.08 NS	-0.28 NS
Confusion (2)	-0.16 NS	0.23 NS	0.26 NS	-0.28 NS	0.47* NS	0.14 NS	0.14 NS	0.09 NS	0.17 NS	0.44* NS	0.33 NS	0.27 NS	-0.03 NS	0.05 NS

*p≤0.05; p≤0.001

As observed in previous studies, SOC was negatively correlated with depression indices such as BDI and POMS sub-scale; with anxiety at t2; and with tension and fatigue at t1.

Partial correlations, controlling for age and the level of the psychological markers at t1, were calculated between the biological indicators and the level of each psychological index at t2. Biological markers in serum correlated with a number of the mood variables: anti-Hsp 70 was positively correlated with anxiety at t2 ($r=0.46$, $p\leq 0.05$) and with anger at t2 ($r=0.45$, $p\leq 0.05$). Cortisol was negatively correlated with vigor at both measuring points (t1: $r=-0.44$, t2: $r=-0.48$, $p\leq 0.05$). Biological markers in the saliva did not yield any statistically significant correlation.

DISCUSSION

The current study examined the effect of venipuncture induced stress on biomarkers such as anti-Hsp 70 and cortisol in saliva and serum, and on psychological indices such as depression, anxiety and mood states. Furthermore, links between the biomarkers and psychological self-report indicators were examined.

The results showed high levels of cortisol were detected in the serum and saliva of all participants while they underwent venipuncture performed by their fellow students. Previous studies conducted with students in different academic settings reported high levels of cortisol in saliva⁸ and in serum.⁴¹

A previous study employing similar method of analysis by ELISA detected lower levels of anti-Hsp 70 among the participants than were detected in the current study. It is suggested that antibodies to heat shock proteins are associated with a universal reaction to stressful situation.^{25,42} Yet, previous studies showed such response of auto-antibodies to heat shock proteins can be indicative of chronic conditions.²⁸ Other researchers, however, provided differing results and show no associations between anti-Hsp 70 and chronic states such as coronary disease.^{30,43} Two explanations are therefore provided. The first leans on Pockley's ideas²⁵ which suggest that the presence of both heat shock proteins and antibodies directed against heat shock proteins in normal individuals indicates that these proteins are not just indicative of disease. These stress proteins can interact with cell-surface receptors and elicit a range of biological activities including the downregulation of autoimmune disease and assist in the maintenance of the 'normal' state. Therefore, anti-Hsp 70 antibody reaction can be interpreted as a protective mechanism against stressful situations; a mechanism serving to restore disturbed equilibrium of an organism by switching off or attenuating the stress signal.^{30,44-46}

The second explanation follows from the body of research which found anti-Hsp 70 to be associated with a variety of chronic diseases. Although our participants were young, healthy students studying towards their baccalaureate degree, a university academic setting can be regarded by students as stressful and induce a chronic activation of Hsp 70 and anti Hsp 70. Yet, only future research will be able to entangle the induction pattern of anti-Hsp 70 at various measuring points as it relates to stressful situations.

The findings concerning the psychological indices used suggest that the participants experienced relatively high levels of anxiety, anger and confusion before the procedure of venipuncture and blood withdrawal took place; they also showed relatively low levels after completion of the procedure. Students also expressed an enhancement in the positive mood of vigor from the first to the second measurement. These results are in accordance with former studies which showed that short term stress can induce mood changes.^{10,47} It was previously suggested that mood states such as happiness, anxiety, and anger can also be associated with change in blood pressure via sympathetic activation.⁴⁸ Moods such as anger, anxiety and happiness varied in the intensity of specific physiological reactions namely, blood pressure and heart rate and in their underlying hemodynamics of stroke volume, cardiac output, and total peripheral vascular.⁴⁹ Additionally, specific moods, such as anger, was associated with high signals of skin conductivity and electrocardiogram, an indication for high arousal.⁵⁰ Accordingly, our results support the idea of the specificity of mood states regarding their relationships with adrenomedullary response (cortisol) and chaperone reaction (anti-Hsp 70).

Additionally, the present study examined the relationships between the bio-markers and the psychological self-report indices. Some interesting relationships emerged: levels of anxiety and anger, reported after the procedure of venipuncture, were positively associated with values of anti-Hsp70 in serum. In other words, anti-Hsp 70 in the serum was related to feelings of anxiety and anger, but only at the second measurement; that is, after these feelings declined. Furthermore, in an effort to explain the psycho-biological findings, we suggest that moods such as anger and anxiety, which are known to be related to the activation of the autonomic responses and an increase of the sympathetic system,⁵¹ might have been related to the induction of anti Hsp 70. Moreover, former findings in animal models interpreted the significant increase in Hsp 70 expression after stressful exposure as an important and protective role of Hsp 70 in combating stress. The unregulated expression after stressful exposure was regarded vital to cellular and tissue protection.²³ It can cautiously be suggested that anti-Hsp 70 provided an adaptive and protective mechanisms against negative moods such as anger and anxiety.^{7,25,30,44}

However, in the current study, the time dependent pattern of this biomarker is unknown. Future studies are called to examine it in a prospective way, employing repeated measurements on saliva, blood and self reported psychological indices in an effort to show the pattern of anti-Hsp 70 before, during and after a brief stress situation and the examine the relationships with psychological measurements. Furthermore, our results showed the levels of serum cortisol were inversely related to the levels of self-reported vigor before and after the procedure of venipuncture. Following this finding, a number of suggestions emerge: it would seem that serum cortisol was a more sensitive measure for changes in positive mood than were levels of salivary cortisol. This result is surprising as salivary and serum cortisol correlated positively, though it is possible that the smaller variance in salivary cortisol hampered the correlations with vigor mood state.

Our findings add to the existing body of research which suggested that high levels of positive mood such as vigor are associated with the down regulation of the sympathetic adrenal system. Specifically, higher reports of positive mood were associated with lower levels of cortisol.^{52,53} The relationship between reduced cortisol and positive affect is potentially relevant to health, as cortisol is a key stress hormone related to a range of pathologies.^{54,55} Furthermore, health educators teaching medical procedure are therefore called to keep in mind the possible psychobiological effect of these procedures on their students.

To the best of our knowledge the current study provides a first attempt to relate psychological states with both cell chaperone and cortisol biomarkers in regards to a venipuncture procedure. However, a few limitations should be addressed. Only four out of the sixty-four correlations between the biological and the psychological parameters were statistically significant and there is a possibility of Type-1 error. Another limitation lies in the fact that the biological data was only collected at one time point, limiting the assessment of anti Hsp 70. In the future, it would be advisable to measure biological data at various points throughout the experiment. Yet, two of the participants who provided second samples after an hour had an elevation in the autoantibody level. Future studies are called for to look at the pattern of anti-Hsp 70 along few measuring points before, during, and after a stressful situation.

CONCLUSIONS

Health-care educators need to further evaluate and be aware of the possible psychological and psychobiological effects of venipuncture performed on health-care students in order to maintain their students' well being. Possible short and long-term harmful effects need to be considered as well as methods aimed at reducing such risks.

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