Journal of Advances in Medicine and Medical Research

28(12): 1-14, 2018; Article no.JAMMR.47523
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

The Effect of Stress on Cardiovascular Parameters among University Students in Buea during and Out of Examination Periods

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSMB conceived the study, recorded blood pressure and heart rate of some of the students and wrote the manuscript. Authors OSMB and INS designed the study. Authors INS and BAN performed the statistical analysis. Author BAN managed the literature searches, enrolled the students and recorded their cardiovascular parameters including haematological indices. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/v28i1230044
Editor(s):
(1) Dr. Pietro Scicchitano, Cardiology Department, Hospital "F. Perinei" Altamura (Ba), Italy.
Reviewers:
(1) Veeravan Lekskulchai, Srinakharinwirot University, Bangkok. Thailand.
(2) Arthur N. Chuemere, Human Physiology,University of Port Harcourt, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47523

Original Research Article

Received 07 November 2018
Accepted 10 February 2019
Published 06 March 2019

ABSTRACT

Objectives: This study aimed at evaluating the effect of examination stress on the cardiovascular parameters amongst undergraduate students of the University of Buea, out of examination period (OEP) and during examination period (DEP).
Experimental Design: This was a cross-sectional study.
Place and Duration of the Study: The study was carried out at the University of Buea (Cameroon). The field and laboratory work was carried out from January to June 2016 within which are two examination periods and two weeks of first semester break.

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Methodology: A structured questionnaire was used to document student's demographic information and number of hours slept. Blood pressure and heart rate were measured using automatic arm sphygmomanometer. Blood samples were collected for full blood count analysis using a haematology analyser. Stress was measured using Cohen Perceived stress scale (PSS) while salivary alpha amylase (sAA) concentrations were measured by ELISA. Data was collected and analysed from 772 (OEP=378 and DEP= 394) participants. Mann-Witney U-test and students T- test were used for statistical analyses. The significance level was set at P<0.05.
Results: The prevalence of stress was 98.2% (387) DEP vs 97.4% (368) OEP (P=0.372) with PSS of (23.41±4.88 vs. 22.31± 4.53, P=0.002). The sAA concentrations was higher DEP (24.659 ± 16.384) than OEP (18.789±5.865) (P< 0.001). Among the haemodynamic and haematological parameters, SBP and HR were significantly higher DEP than OEP. There was a positive correlation between HR and sAA within both periods. WBC%, Lym%, Mon%, and MCHC were significantly lower (P<0.05) while Gran%, Plt and Pct increased DEP than OEP (P<0.05) with respect to stress.
Conclusions: The prevalence of stress OEP vs DEP implies stress is an important problem in students. This is proven by the increase in PSS and sAA concentrations during examinations and can lead to impairment of cardiovascular parameters.

Keywords: Examination stress; salivary α-amylase; cardiovascular; students; Buea.

1. INTRODUCTION

The term "stress" is used to describe psychological, physiological, and physical stimuli that disturb homeostasis. The examination stress is a condition that creates physiological and emotional stress reactions in the body. Thus, physiological and behavioural responses are enacted to cope with altered homeostatic function [1]. Among the many physiological variables that change in response to stress, Heart rate and blood pressure are the most widely used parameters for mental Stress [2]. Shah and Patel [3] evaluated stress using heart rate and blood pressure and stated they were limiting, hence recommended the use of biological markers, haematological parameters as well psychological based questionnaires as additional measures to evaluate stress. In recent decades, academic stress emerged to be major of psychological influence on physical and mental health [4,5]. Thus, the need arises to understand to what extent examination stress affects cardiovascular parameters among university students.

Taking the case the University of Buea, we hypothesized that psychological stress undergone by students could affect cardiovascular parameters at different levels out of examination (OEP) and during examination periods (DEP). This study aimed at evaluating the effect of stress on cardiovascular parameters OEP and DEP among the University of Buea students.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Buea, South West Region, Cameroon. Buea is situated at the foot of Mount Cameroon and has an estimated population of 150,000 inhabitants, of multi-ethnic groups. Buea has an equatorial climate averaging 18˚C to 28˚C and two seasons: A short dry season (mid-November to mid-March) and a long rainy season (mid-March to mid-November) [6]. The town hosts the University of Buea, the Catholic University Institute and several other higher institutions and vocational training schools. The First semester exams in most of these institutions are scheduled for the month of February and March and the second semesters around June and July.

2.2 Study Population

The study population included undergraduate students of the University of Buea.The study was comprised of 772 participants (394 during examination and 378 out of examination periods) and it included both males and females with ages ≥18 years.

2.3 Study Design

This was a cross sectional study. The field and laboratory work was carried out from January to June 2016 within which we had two examination preparatory periods and two weeks of first semester break in the University of Buea.

Table 1. The different concentrations for standard preparation

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std blank
Maltose solution (std)	0.025	0.10	0.20	0.30	0.40	0.50	1.00	-----
Distilled water	0.98	0.90	0.80	0.70	0.60	0.50	-----	1.00
Colour reagent	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

2.4 Inclusion Criteria

Were included to the study full time undergraduate students with no job, not taking any professional program, and who had not experienced any major stressful event in the past 1-12 months (for example the death of relatives). The participant had also not to be hypertensive nor hypotensive, with no history of cardiovascular or neuronal disease, and not under any antidepressant drug.

2.5 Sample Size

The sample size was calculated using a stress prevalence of 52.9% recorded in a previous study [7]. This was substituted into the formula as described by Cochran [8].

$no = z^2\ p\ (1\text{-}p) / e^2$ at 80% power (i.e. formula accuracy) and a confidence interval of 95%,

Where:

no = sample size,

z = 1.96 for 95% CI

p = prevalence of stress in a previous study (52.9%)

e = tolerable error (5%)

$$n_o = \frac{(1.96)^2(0.53)(1\text{-}0.53)}{(0.05)^2}$$

$n_o = 382.8$

We had total of 785 students who were enrolled in this study: 385 out of examination period (OEP) and 400 during examination period (DEP).

2.6 Questionnaire

A structured questionnaire was used to document demographic information such as sex, age, participant's code, area of residence and socio-economic indicators (such as educational level) and the number of hours slept by each student. The rest of the questionnaire contained elements of Cohan Perceived stress scale. The number of hours slept by the students was classified according to the recommended hours of sleep for the age group of the study population [i.e 0-6hrs (low), 7-9 hrs (normal) and ≥10 hrs (high)] by USA National Sleep Foundation [9]

2.7 Data and Sample Collection, Processing and Examination

2.7.1 Heart rate and Blood Pressure

The haemodynamic parameters were measured by a non-invasive method, using appropriate automatic arm sphygmomanometer (Pic indolor Diagnostic CS410) from which the values of pulse, systolic and diastolic blood pressures were given automatically. Measurements were done on the left arm of each participant rested (10 mins) and seated; with the arm at the level of the heart. As a precaution, individuals with exceptionally high or low readings were allowed to rest for 10mins additional and measurements repeated sometimes up to 3 times to avoid errors.

2.7.2 Stress measurement and analysis

2.7.2.1 Stress measurement using Cohen perceived stress scale (PSS)

The Perceived Stress Scale [10] was designed and used to assess the degree to which situations in life experienced during the previous month are perceived as stressful. Items in the PSS were designed to assess how predictable, uncontrollable and overloading participants consider their lives.
Each participant answered one questionnaire and at the end their responses where scored and grouped into three categories:

i. Normal scores ranging from 1- 13
ii. Moderate scores ranging from 14 -20
iii. Highly stressed scores >20

2.7.2.2 Saliva sample collection and storage for sAA measurements

The saliva samples were obtained in ependoff tubes using sterile straws to facilitate collection into the tubes. The saliva collected was immediately placed into a cooler then frozen at

the end of the collection for subsequent salivary amylase measurement.

2.7.2.3 Salivary alpha amylase measurement

Salivary alpha amylase concentration was measured using the protocol by SIGMA-ALDRICH, The Ezymatic Assay of α-Amylase (EC 3.2.1.1).

On the day of the measurement, the saliva samples were left out of the freezer to defrost. Thereafter, sterile cotton rolls were made and inserted into the tubes, to be just above the saliva. A sterile pipette was used to aspirate through the cotton thereby collecting already filtered saliva just before it was being used. The protocol for the measurement of salivary alpha amylase concentration was strictly followed as prescribed by manufacturer.

Standard solutions were prepared by pipetting (in milliliters) reagents into suitable test tubes (Table 1).

The preparations were then heated in a water bath at 90˚C- 100˚C for 15 minutes and 6 mL of water was added into each test tube after cooling.

The preparations were then pipetted and transferred to a 96 welled sterile microtitre plate. The optical densities (absorbance) were read at a wavelength of 490 nm on an ELISA (SoftMax Pro5) plate reader at the Biotechnology Unit of the University of Buea. The absorbance of each standard was subtracted from the blank as illustrated by the equation below:

$$\Delta A_{490nm}\ \text{Standard} = A_{490nm}\ \text{Std} - A_{490nm}\ \text{Std Blank} \quad (1)$$

Thereafter a plot of ΔA_{490nm} of the Standards vs mL of Maltose released by the standards (standard curve), and the equation of the line was recorded and linear regression (r-square) produced. The plot was made using the statistical software GraphPad instat (GraphPad Software, San Diego, CA, USA).

Starch solution (0.5 mL) was then pipetted and put in labelled test tubes for each sample. It was pre-incubated for 15 minutes at 30˚C to 40˚C. The saliva once filtered using cotton wool, was pipetted and added according to the corresponding participants codes in the corresponding test tubes with pre-incubated starch solution. It was mixed by swirling and incubated for exactly 3 minutes at room temperature. As a precaution test lots were ran one at a time due to the short enzymatic incubation time. After the 3 minutes of incubation, 0.5 mL of colour reagent was added to each tube (A blank was prepared by adding distilled water to the tube containing 0.5 mL of starch solution and 0.5mL of the colour reagent). All the preparations where then placed in a hot water bath at 95˚C to 100˚C for 15 minutes after which, the tubes were removed and allowed to cool and 6 mL of water was then added into each of the test tubes. The process was then repeated for each sample. Using a pipette, 0.2 mL of each sample preparation was pipetted into one well of the sterile microtitre plate and the plate was then read under an ELISA plate reader (SoftMax. Pro5) at 490 nm. The optical densities were then subtracted from the blanks optical like in the equations (2) below. Calculation was done automatically by using the statistical software GraphPad instat (GraphPad Software, San Diego, CA, USA)

$$\Delta A_{490nm}\ \text{Sample} = A_{490nm}\ \text{Test} - A_{490nm}\ \text{Test Blank} \quad (2)$$

2.7.2.4 Blood samples collection and haematological analysis

Under aseptic conditions in the Zoology Research Laboratory of the University of Buea, 3.0 mL of venous blood was collected using a 5 mL syringe and immediately transferred into an ethylenediaminetetraacetic acid (EDTA) vacutainer tube. The gently mixed blood was used for haematological analysis. The blood collected in the EDTA tubes were analysed in the Zoology Research Laboratory of the University of Buea. The blood samples were gently agitated for uniform mixing and to avoid cell lyses. A complete blood count was obtained from blood samples after running the samples using a URIT 3300 haematology analyser that automatically gave values for all the haematological parameters following the manufacturer's instruction: White blood cells (WBC), lymphocytes (Lym), monocytes (Mon), granulocyte (Gran), red blood cell (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haematocrit (Hct), mean corpuscular haemoglobin (MCH), platelet (Plt) and plateletcrit (Pct).

2.8 Statistical Analyses

The data was analysed using the Statistical Package for Social Sciences (SPSS) version 21 (IBM - SPSS, Inc, Chicago, IL, USA) software

and the sAA data was interpolated using the statistical software GraphPad Insta.ink. The student T-test was used to compare the mean salivary amylase concentrations, blood pressure, heart rate, as well as haematological parameters of participants among the two periods of investigation. Proportions between stress with gender, age, level of education and number of hours of slept were evaluated using Pearson Chi-Square (χ^2) test. A correlation analysis was carried out to find the relationship between sAA and Heart rate within the two periods. All analyses were done at 95% confidence interval and $P<0.05$ was level of significance.

3. RESULTS

3.1 Baseline Characteristics of the Study Population

A total of 785 students were enrolled in this study, with 385 students out of examination period (OEP) and 400 during examination period (DEP). Due to incomplete data from some participants, the data from 13 students was excluded and a total of 772 (OEP=378 and DEP= 394) considered. The overall distribution of males (52. 6%) and females (47.4 %) for the two phases of the study was comparable (Table 2). DEP we had a total of 394 students, 188 (47.7%) males and 206 (52.3%) females while OEP we had a total of 378 students, 219 (57.9%) males and 159 (42.1%) females. According to the Cohan perceived stress scale, the overall prevalence of stress in the study population was 97.5% (753), which implies 97.4% (368) OEP vs 98.2% (387) DEP. The overall mean stress score in the study population of 22.87±4.74 shows that averagely all students reported to be stressed as observed in overall stress prevalence of 97.5%.

3.1.1 Prevalence of haematological parameters within the study population

Among the 785 students who took part in this study, only 459 students voluntarily accepted that venous blood should be collected for the full blood count. However to avoid faulty results, only the data for 450 participants (OEP=215 and DEP=235) were used for haematological analysis as observed in Table 3. The overall mean ± Standard deviations of haematological parameters and their ranges within the study population are represented on Table 3, as compared to the physiological ranges.

3.1.2 The number of hours slept by the students DEP and OEP

The number of hours slept by the students among the different periods was significantly (χ^2= 25.801, $P<0.001$) different as represented in Fig. 1. It was observed that students slept less DEP compared to when they were not writing exams.

3.2 Stress among Student out of and During Examination Periods and the Level of Stress within the Different Levels of University Education

3.2.1 Stress DEP and OEP using the perceived stress scale (PSS)

3.2.1.1 The prevalence of stress among students in relation to gender, level of education, age and number of hours slept by the students OEP

Table 4 shows the stress prevalence OEP and the mean stress scores within the groups (gender, age groups, level of education and number of hours slept). There was no significant difference in the stress prevalence within the four groups OEP. On evaluating the mean stress scores with respect to gender, age group and the number of hours slept by the students no significant differences ($P>0.05$) within the groups were observed . However, there was a significant difference ($P=0.013$) between the mean stress score of the students at various levels of education with final year students having the highest mean score (22.96±4.70) and the least being 1st year students (21.23±4.28).

3.1.1.2 The prevalence of stress in the students in relation to gender, level of education, age and the number of hours slept by the students DEP

Evaluating the prevalence of stress and mean stress scores of students DEP in relation to the level of education, age, and number of hours slept by student revealed no significant difference ($P>0.05$) in both the prevalence and the mean stress scores within the groups. However there was a significant difference in mean stress with respect to gender, with female students being more stressed than their male counterparts ($U=16745$, $P=0.02$), although no difference in their stress prevalence was observed (Table 5).

Table 2. Baseline characteristics of the study population

Characteristics		Number (N)	Percentage (%)
Overall		772	100
Gender	Male	407	52.6
	Female	365	47.4
Age group	≤20	361	46.8
	>20	411	53.2
Level of education	1st year	251	32.5
	2nd year	261	34.2
	Final year	257	33.3
Number of hours of sleep	0-6	398	51.6
	7-9	335	43.4
	≥10	39	5.1
Mean Stress score Range	22.87±4.74 4-39		
Mean salivary alpha amylase (U/mL) Range	21.99±13.80 5-98		
Mean systolic blood pressure Range	121.48± 15.06 81-183		
Mean diastolic blood pressure Range	75.64 ±11.35 49-168		
Mean heart rate Range	77.11±12.59 58-116		

Table 3. Means and ranges of haematological parameters

Haematological parameters	N	Mean± SD	Ranges within population	Physiological ranges*
WBC×10^3µl	450	5.567± 3.45	1.40- 34.00	4.0-10
Lym%	450	41.43± 15.33	13.90-83.90	20-40
Mon%	450	12.23±4.95	4.00-24.20	1.0-15
Gran%	450	46.34±14.15	11.50-77.40	50-70
RBC×10^6	450	4.83±0.89	0.5 -9.8	0.6-4.1
Hb(g/dl)	450	13.66±2.41	3.90-25.70	11-15
Hct	450	39.42±7.51	34.91-73.80	36-48
MCV(fl)	450	81.62 ± 8.95	80.0 -98.20	80-99
MCH(pg)	450	28.02 ± 3.73	23.04- 50.30	26-32
MCHC(g/dl)	450	34.28 ± 3.84	29.05 - 58.50	32-36
Plt×10^3µl	450	265.57± 119.00	102-902	100-300
Pct	450	0.31±0.14	0.1-1.06	0.10-0.28

**physiological ranges are automatically given by the haematological analyser*

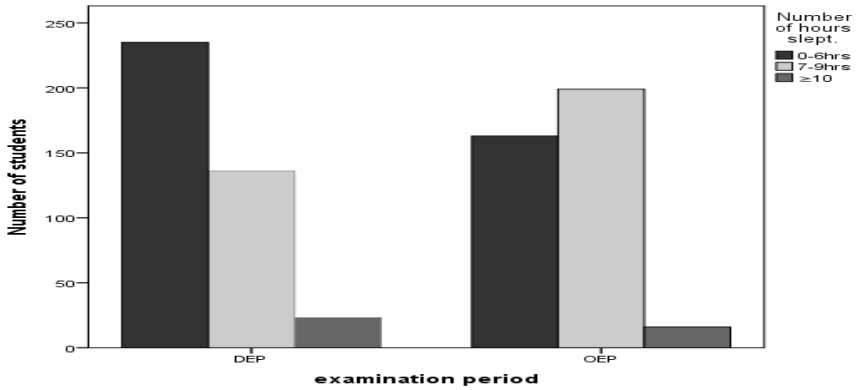

Fig. 1. Number of hours slept by students DEP and OEP

Table 4. Stress prevalence and mean stress scores faced by students OEP in relation to Gender, level of education, age, and hours of sleep of the students

Factor	Category	N	Prevalence (n)	Statistical inference	Mean ±SD	Statistical inference
Gender	Male	159	214(97.7)	χ^2=0.265	22.18±4.514	U=16503.5
	Female	219	154(96.9)	P=0.41	22.49±4.564	P=0.386
Level	1st year	115	112(97.4)	χ^2=0.292	21.23±4.280	H=8.645
	2nd year	139	136(97.8)	P=0.865	22.63±4.458	P=0.013
	Final year	124	120(96.8)		22.96±4.696	
Age groups (years)	≤20	187	182(97.3)	χ^2=0.001	21.88±4.51	H= 17757.50
	>20	190	185(97.4)	P=0.615	22.75±4.53	P= 0.980
Number of hours slept	0-6	163	157(96.3)	χ^2=1.425	22.73±4.701	H= 4.497
	7-9	199	195(98.7)	P=0.490	22.06±4.375	P=0.106
	≥10	16	16(100)		21.25±4.568	

U: Mann-Whitney U test, H= Kruskal Wallis test, χ^2: Pearson's Chi Square

Table 5. Stress prevalence and mean stress scores faced by students DEP in relation to gender, level of education, age, and hours of sleep of the students

Characteristics DEP	Category	N	Prevalence (n)	Statistical inference	Mean ± SD	Statistical inference
Gender	Male	188	184(97.3)	χ^2=1.61	22.84±4.872	U=16745
	Female	206	202(98.2)	P=0.19	23.93±4.849	P=0.020
Level of education	1st year	136	132(97.1)	χ^2=0.15	23.01±5.639	H=1.642
	2nd year	125	124(92.2)	P=0.93	23.93±4.361	P=0.440
	Final year	133	131(98.5)		23.54±4.512	
Age	≤ 20	184	180(97.8)	χ^2=0.02	23.15±5.14	U=19280.0
	>20	210	205(97.6)	P=0.58	23.63±4.64	P=0.891
Number of hours of sleep	0-6	235	230(97.9)	χ^2=0.826	23.28±4.891	H=0.130
	7-9	136	132(97.1)	P=0.662	23.54±4.957	P=0.937
	≥10	23	23(100)		23.91±4.502	

U: Mann-Whitney U test, H= Kruskal Wallis test, χ^2: Pearson's Chi Square

3.2.2 The overall PSS prevalence and perceived mean stress score in students OEP and DEP

3.2.2.1 The perceived stress prevalence DEP and OEP

Comparing the overall stress prevalence OEP and DEP showed a non-significant difference between the two groups with students DEP having higher prevalence (98.2% vs. 97.4%, P=0.372, χ^2= 0.320) (Table 6). However we observed higher mean stress scores (23.41±4.88 vs. 22.31± 4.53, P= 0.002, U=64714.50) DEP compared to those OEP (Figure 2). When the participants were grouped according to the ranges provided in Cohen's PSS scale, it was observed that the difference in the stress prevalence amongst the two periods lies in the degree of stress experienced within the different periods, which could be normal, moderately stressed or highly stressed as represented on Table 6, thus majority of the students OEP found themselves in moderately stress group while DEP they were highly stressed.

3.2.2.2 Changes in mean perceived stress scores and salivary alpha amylase (sAA) DEP and OEP

The mean perceived stress scores were seen to be higher DEP compared to those OEP (23.41±4.88 vs. 22.31± 4.53, U=64714.5, P= 0.0013).

The mean sAA concentrations value DEP (24.659 ± 16.384) was significantly higher than that OEP (18.789±5.865) (U= 60057, P< 0.001).

Table 6. The Perceived stress prevalence DEP and OEP

Category	DEP number (Prevalence)	OEP number (Prevalence)	Statistical inference
Normal	9(2.3)	10(2.6)	χ^2=163.93
Moderately Stressed (S)	85(21.6)	252(66.7)	P<0.001
Highly stressed(HS)	300(76.1)	116(30.7)	χ^2=0.32
Overall stressed (S+HS)	387(98.2)	368(97.4)	P=0.37

3.3 Changes in Cardiovascular Parameters OEP and DEP Due to Stress

3.3.1 Changes in blood pressure and heart rate OEP and DEP

When comparing the haemodynamic parameters, the mean systolic, diastolic blood pressures and the heart rate values were higher DEP than OEP. However, only the difference in mean SBP and HR were significant (P=0.040 and P=0.001 respectively) (Table 7).

3.3.2 Relationship between Heart rate (beats/minute) and sAA (U/mL) DEP and OEP

3.3.2.1 Relationship between heart rate and sAA DEP

The relationship between the heart rate and sAA showed a positive correlation [r =0.5920, r^2= 0.3505, P<0.0001] (Fig. 2).

3.3.2.2 Relationship between heart rate and sAA OEP

The heart rate of the participants OEP was observed to correlate positively with their sAA [r=0.2097, r^2 = 0.04397, P<0.0001] (Fig. 3).

3.3.3 An evaluation of the changes of Haematological parameters DEP vs. OEP

An assessment of haematological parameters showed a significant decrease (P<0.001) in mean WBC ($x10^3$/µL), mean Lym (%) and increase (P<0.001) of mean Gran (%) DEP compared to that OEP.

Except for MCHC (P=0.001) which was higher OEP than DEP, there was no significant change in the red blood cell indices amongst the two periods. The mean Plt /µL and Pct count were observed to be significantly higher DEP compared to those OEP (Table 8).

4. DISCUSSION

This study was carried out to evaluate the effect of stressful conditions on cardiovascular parameters DEP and OEP.

There was no significant difference in perceived stress prevalence but a significant difference in mean perceived stress scores OEP among the different levels of education. The Final year students were the most stressed and the 1st year students being the least stressed OEP. Elias et al. [11] reported a similar observation as they concluded that final year and middle level students experience higher levels of stress compared to first year students. A possible explanation for this could be the fact that final year students in the University of Buea have to write their end of studies research project during which they are each assigned to supervisors. The fear of supervisors, the introduction to research as well as the concerns about life after graduation could be possible sources of stress among students of this level. The 2nd year students experienced higher stress compared to the 1st years. This may be due to the increase difficulties in their courses as they start doing more of their major courses, less of general courses and university requirements during which they find themselves in closer contacts with their lecturers than in the first year. Also this may happen because at the second year often, students are carrying over some of their 1st year courses, which increases their work load and brings about clashing time-tables. Furthermore, the 1st year students being happy to have finished with high school studies are motivated to adapt fast to the new environment, during university life transition described by Towbes and Cohen [12]. This finding however contradicts those of Pancer et al. [13] and Wintre and Yaffe [14] who found first-year students to be at high risk of stress than the other levels.

We observed that DEP the students were similarly stressed since there was no significant difference in prevalence and mean perceived stress scores among the levels of education.

Table 7. Mean values of haemodynamic parameters (OEP and DEP with n=378 and n=394 respectively) of all the students

Parameter	Periods		Statistical inference	
	OEP Mean(SD)	DEP Mean(SD)	t- value	P-value
SBP (mm Hg)	120.51 ± 13.377	122.42±16.476	1.758	0.040
DBP (mm Hg)	75.026 ± 9.957	76.223±12.534	1.465	0.072
HR (beats/min)	75.426 ± 12.467	78.637±12.786	3.533	0.001

t: student T-test

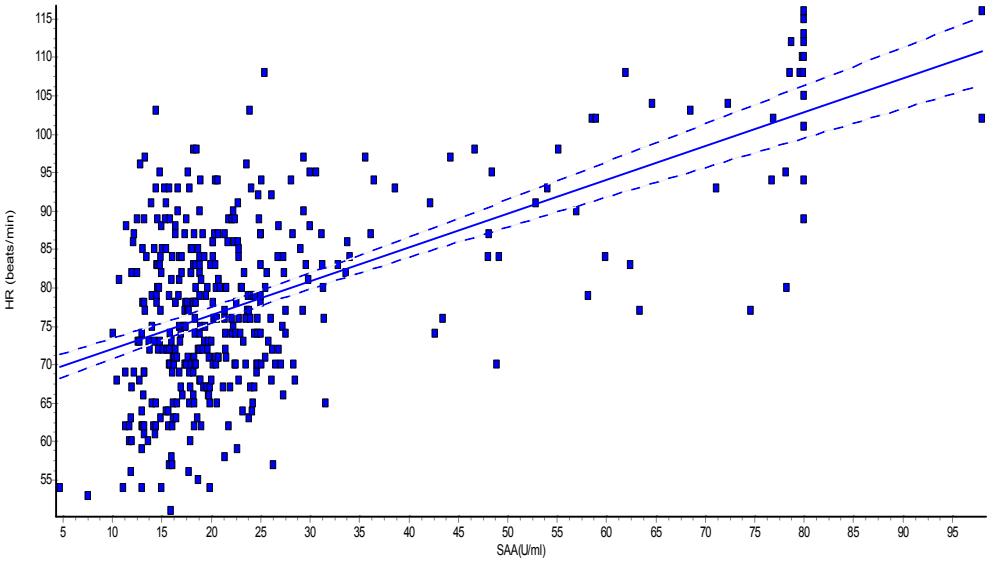

Fig. 2. A correlational trend of heart rate (beats/min) vs. sAA (U/mL) DEP

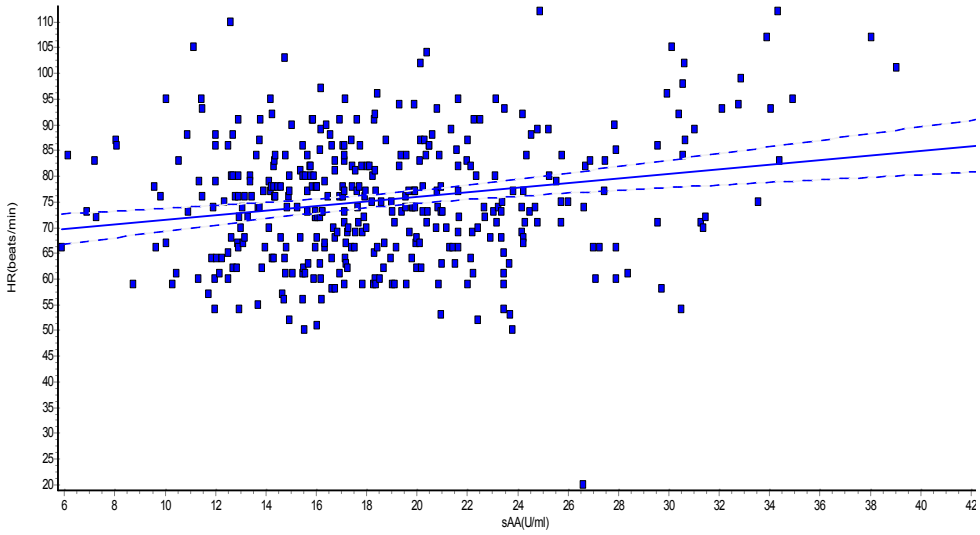

Fig. 3. A correlational trend of heart rate (beats/min) vs. sAA (U/mL) OEP

Table 8. Changes in mean haematological parameter OEP and DEP

Haematological parameter	Period	N	Mean± SD	t-value	P-value
WBC (/µL)	OEP	215	5.76 ± 2.15	1.15	0.009
	DEP	235	5.40± 4.31		
Lym%	OEP	215	43.82 ± 12.62	3.24	<0.001
	DEP	235	39.24 ± 17.19		
Mon %	OEP	215	12.42 ± 4.57	0.80	<0.001
	DEP	235	12.05 ± 5.27		
Gran %	OEP	215	43.75 ± 11.09	-3.82	<0.001
	DEP	235	48.70 ± 16.13		
RBC (/µL)	OEP	215	4.73 ± 0.83	-2.18	0.539
	DEP	235	4.91 ±0.93		
Hb (g/dl)	OEP	215	13.65 ± 2.39	-0.06	0.759
	DEP	235	13.67 ± 2.43		
Hct (%)	OEP	215	38.33 ± 7.242	-2.98	0.806
	DEP	235	40.42 ± 7.62		
Mcv(fl)	OEP	215	81.29 ± 7.70	-0.74	0.995
	DEP	235	81.91 ± 9.96		
MCH(pg)	OEP	215	28.83 ±3.49	4.53	0.228
	DEP	235	27.27 ± 3.79		
MCHC(g/dL)	OEP	215	35.59 ± 3.45	7.36	<0.001
	DEP	235	33.08 ± 3.79		
PLT (/µL)	OEP	215	248.94 ± 94.22	-2.90	0.002
	DEP	235	280.77 ± 136.26		
PDW	OEP	215	11.95 ± 22.28	1.67	0.163
	DEP	235	9.40 ± 2.18		
Pct	OEP	215	0.28 ± 0.11	-2.70	0.004
	DEP	235	0.32 ± 0.161		

t: student T-test

The association between stress and gender OEP revealed that there was no significant difference in both mean and prevalence of perceived stress. Whereas there was a significant difference in mean perceived stress but no significant difference in the stress prevalence DEP with respect to gender. In this period, we observed that female students appeared to be more stressed than their male counterparts. This could be due to higher self-expectations and peer pressure in response to the difficulties experienced by the girls child education in Cameroon [15]. Thus, these students always have a reason to prove that they are worthy of the education offered to them. Another possible explanation for this is the difference in activities of the females and males, added to their studies regardless of the fact they may be preparing for exams, the females culturally and consciously carry some chores at home whether living alone or with family so they need to manage their time more efficiently. Added to these, males are more prone to carry out some relaxation actives like sports (mainly football) which help them cope with the stress they are facing. With this study, we are in line with Salam et al. [16] who reported that female medical students experienced more stress compared to their male counterparts.

On comparing the overall perceived stress experienced by the participants between the two periods (DEP and OEP) we observed no significant difference in the perceived stress prevalence between the two groups. However we noticed a significant difference when the stress experienced by these two groups was categorized (into normal, moderately and highly stressed). From this it was observed that DEP, majority of the students found themselves in the highly stressed categories while OEP they were moderately stressed. These results could be substantiated by the findings that the mean stress scores and sAA concentrations DEP were significantly higher than those OEP. Brand and Schoonheim-Klein [17] stated that Stress among undergraduate and graduate students is multifactorial, arising from both academic and non-academic factors, including socio-cultural, environmental, and psychological attributes. Thus the fact that students are stressed in both periods could be explained by the fact that OEP, students have to carried out other stressful

activities like doing assignments and meeting deadlines for submission, carrying out research and meeting with supervisor for final year students and preparing for life after graduation. In addition, there could also be non-academic sources of stress like changes in family relations and social life, exposure to new people, ideas, and temptations, often for the first time and/or low self-esteem [18], being away from home and assuming responsibilities previously expected from parents, financial pressures, lack of time management skills. Added to these, preparing for exams is just an additional source of stress.

In this study, we were interested in understanding the changes in both haemodynamic and haematological parameters amongst the two periods.

For haemodynamic parameters, our findings revealed that the mean systolic blood pressure, diastolic blood pressure and heart rate (SBP, DBP and HR respectively) were observed to increase DEP compared to those OEP. However, only the differences in SBP and HR were statistically significant. This result is in compliance with those of several authors [5,19, 20] who also reported significant increase in heart rate, systolic and diastolic blood pressures during stressful compared to non-stressful periods in their participants. Understanding the fact that the human body responds to stressful factors by alterations in different biological functions especially autonomic functions like heart rate and blood pressure [21], the increase in blood pressure and heart rate could be explained by the stimulation of the adrenergic nervous system that lead to release of catecholamine in particular noradrenaline at the post-synaptic neuron and adrenaline from adrenal medulla that result in activation of α, β1 and β2 receptors present in the cardiac muscles and blood vessels; consequently bringing an elevation of systolic blood pressure and heart rate [22,23,24].

A correlational trend of sAA against heart rate revealed that as sAA concentrations increases heart rate increases. During stressful condition, a stress response is initiated which increases the activity of the Sympathetic Nervous System (release of adrenaline and noradrenaline) which increases the heart rate in the same line, increases sAA activity [25] under stress that may be due to this sympathetic activation; thus sAA concentrations increase in response to stress.

Also taking note of the fact that, branches of the sympathetic and the parasympathetic nerves are distributed in the salivary glands, stimulation of sympathetic nerves increases salivary protein secretion [26]. However, stimulation of parasympathetic nerves increases the flow of saliva [27]. sAA activity is connected with the sympathetic nervous system's stress response [28]. Thus, unlike most salivary analytes, such as cortisol and testosterone that are transported from the plasma, sAA is an enzyme produced locally in the salivary glands that line the mouth.

More light was put on this when Thoma et al. [29] showed that sAA significantly correlated with noradrenalin during stress. These probably explains why in both periods in our study (OEP and DEP) sAA concentration significantly correlates with heart rate as they are both activated and controlled by the sympathetic nervous system.

Stress was seen to significantly affect several haematological parameters in this study. It was observed that, the mean WBC, Lym%, Mon%, and MCHC were seen to decrease during the most stressful period (DEP) compared to the least stressful (OEP) while Gran%, Plt and Pct. were observed to be higher OEP. Similarly to our observations, Qureshi et al. [30] concluded that academic examinations in medical students are stressful enough to produce changes in blood pressure and blood cells parameters. Just like Qureshi et al. [30] we observed changes in blood pressure as discussed in the previous paragraph as well as some changes in blood cell parameters. The mean WBC count, lymphocytes and monocytes decreased significantly DEP compared to OEP. This finding is consistent with that of some authors [5,30,31]. However, it is not in compliance with those of some others [32,33] who reported an elevation in total leukocyte count during stress.

Lymphocytes and monocytes express receptors for several stress hormones, including norepinephrine and epinephrine as their plasma levels increases in response to stress. These hormones released during stress modulate leukocyte trafficking/decrease and result in the redistribution of leukocytes between the blood and other immune compartments [34] thus causing a drop in free circulating leukocytes. Therefore, physiological response to stressful events could alter immune function [35] which explains the significant decrease of mean

lymphocytes and monocytes percentages in this study. The drop in leukocyte count has been reported also by Kotepui et al. [36] as opposed to other studies which reported an increase [37].

On the other hand, we observed a significant increase in mean granulocyte during examination period. This is in compliance with the studies conducted by Sharma and Gupta [5], Qureshi et al. [30] and Mantur and Murthy [33] who reported significantly higher number of granulocyte (mainly neutrophil count) during the examination period. A possible reason for this increase in granulocytes (neutrophils) during examination period could be sleep deprivation during this period. A study carried out by Ackermann et al. [38] on the effect of acute sleep deprivation in healthy young men revealed a significant increase in granulocyte after long hours without sleep. Participants reported to have slept less while preparing for exams than when they were not preparing for exams and the difference was statistically significant.

Stress of academic examinations also significantly affects the erythron variables [30]. Our study showed a significant decrease in MCHC values which suggest microcytic anaemia probably due to iron deficiency [39] which could be as a result of the feeding habits of the participants.

In this study we also observed a significant increase mean platelet count during examination period. This finding is in compliance with those of Sharma and Gupta [5] and Qureshi et al. [30]. According to Koudouovoh-Tripp and Sperner-Unterweger [40] platelet count increases during stress. This platelet increase and activation can be initiated through different mechanisms such as shear stress, physical and mental stress [41]. Catecholamines, which are the main neurotransmitters in the stress response mechanism, are responsible for this increase and activation via α2 and β2 adrenergic receptors.

5. LIMITATIONS

- Convincing all participants to take part in blood testing.
- Keeping the same participants OEP and DEP.

6. CONCLUSION

In conclusion, majority of students are always provisionally stressed in the University of Buea, but the number of highly stressed increases above double from OEP to DEP. This stress correlates with variations in haemodynamic parameters. Both family relative and education administrators and teachers should take these findings into consideration and take appropriate measures to reduce, in the years ahead, the high prevalence recorded.

CONSENT

Authors declare that written informed consent was obtained from all participants after verbal explanation of the experimental design in the language they understood best.

ETHICAL APPROVAL

The study was administratively approved by the South West Regional Delegation of Public Health (Cameroon) then by the Institutional Review Board of the Faculty of Health Sciences, University of Buea (Ref. 2016-0348-UB-FHS-IRB). On sampling days, participants were recruited and taken to the Zoology research laboratory of the University of Buea. While in the laboratory, the purpose and benefits of the study was explained to the study participants and they were given informed written consent forms for their appraisal and possible signatures if they accepted to participate in the study. Participants were also informed that their participation in the study was completely voluntary and not compelling. Experiments were carried out avoiding any form of stress on participants.

ACKNOWLEDGEMENTS

The authors are very grateful to Professor Fidelis Cho-Ngwa of the Faculty of Science, University of Buea, for the provision of laboratory facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/47523