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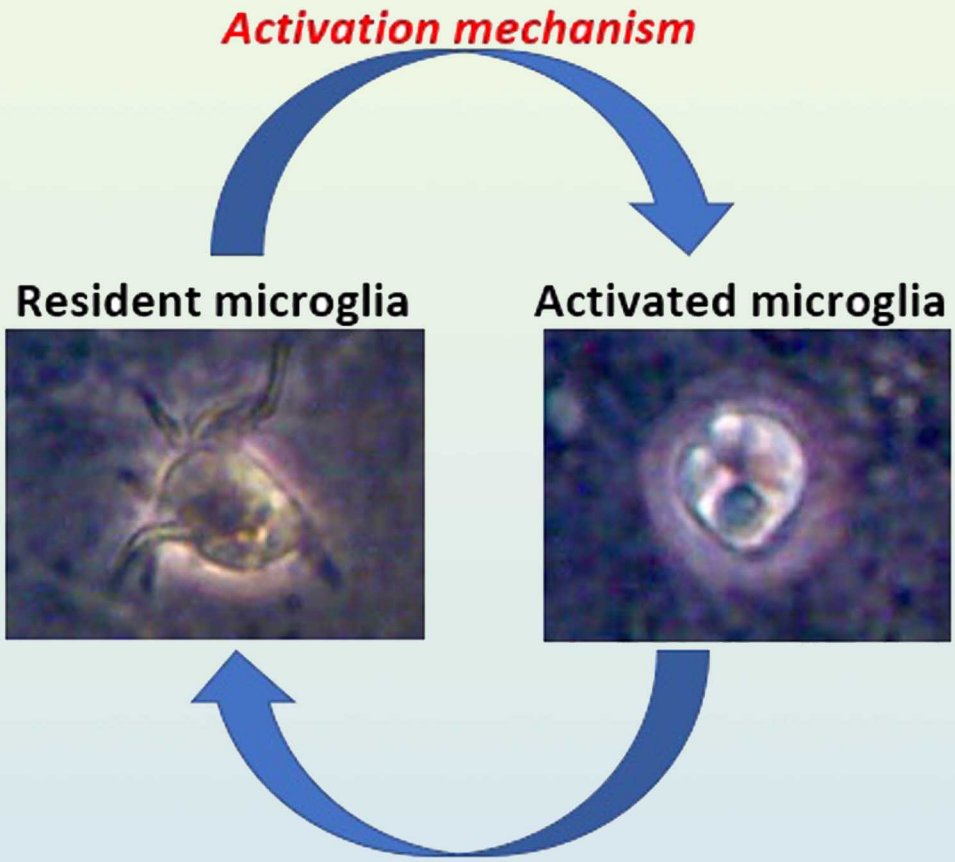

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

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(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

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RESEARCH ARTICLE

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Effect of stress on alteration of haematological parameters: A preliminary study on preclinical medical students in Malaysia

Mohammad Ahmed Issa AL-HATAMLEH[1], Imilia ISMAIL[2], Omar Mahmoud AL-SHAJRAWI[1], Tengku Mohammad ARIFF[1*]

[1]*Faculty of Medicine, Sultan Zainal Abidin University, Terengganu, Malaysia.*

[2]*School of Biomedicine, Faculty of Health Sciences, Sultan Zainal Abidin University, Terengganu, Malaysia.*

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Abstract

The present study aimed to evaluate the effect of stress on haematological parameters among preclinical medical students. A cross-sectional study has been conducted on a total of 105 preclinical medical students at Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia. The validated depression anxiety stress scales-21 (DASS-21) questionnaire was distributed and blood samples were collected from the subjects on the same day to perform full blood count (FBC) test. There was no significant association between levels of stress with red blood cells (RBCs) count and indices. However, a significant negative association was identified between stress level and white blood cells (WBCs) count ($r = -0.204$, $p \leq 0.05$). Furthermore, no significant association was found between levels of stress and platelets count and indices, except the mean platelets volume (MPV); a significant positive association between students measured MPV and their perceived stress ($r = 0.195$, $p \leq 0.05$) has been noted. Mean WBCs count was decreased, while mean MPV was increased with increasing stress levels. Furthermore, the structural equation model predicted that some parameters were near to be significantly associated with stress, which needs further investigation. This study provided novel insights about the potential effects of stress on blood cells and platelets. The results will help the researchers to uncover the critical areas of increasing production of reactive oxygen species caused by chronic life stress.

Keywords: Stress, Oxidative stress, Haematological parameters, Medical students, Malaysia.

Address for correspondence:

Prof. Dr. Tengku Mohammad Ariff,

Faculty of Medicine, Sultan Zainal Abidin University,

20400, Kuala Terengganu, Terengganu, Malaysia.

***Tel:** +60 199 131 415*

***E-mail:** tg_mariff@unisza.edu.my*

List of Abbreviations;

BC, bias-corrected; **DASS-21**, depression anxiety stress scales-21; **FBC**, full blood count; **HPAA**, hypothalamus–pituitary–adrenal axis; **MPV**, mean platelets volume; **RBC**, reactive oxygen species; **RBC**, red blood cell; **WBC**, white blood cells

Introduction

The description of stress was quoted from the field of physics through one of the fathers of stress, Hans Selye (Viner, 1999). He coined the conception of stress, who defined it as “the non-specific response of the body to any

request for change" (Selye, 2013). Stress can lead to many health disorders via many of the behavioral and physiological pathways due to some essential biochemical, metabolic, and hormonal pathways (Epel, 2009). The dramatic elevation of the chemical mediators which have been release by body during stressful event produced many physiological effects (McEwen, 1998). To add on, stress reaction will lead to neuroendocrine response which involved the sympathetic nervous system, and the hypothalamus–pituitary–adrenal axis (HPAA) stimulation that will result in the secretion of glucocorticoids and catecholamine. Subsequently, this response will be followed by a sharp increase in oxidative stress (Chrousos, 2009).

This biochemical changes that have been set in motion and induced due to the stress will accelerate the cell ageing mechanisms; through the telomerase inhibition and the shortening of telomere length, hence, increase the cells senescence (Hewitt et al., 2012). Oxidative stress describes the imbalance status between free radicals and antioxidants in the body (Halliwell, 2012), which resulting in the damage of important biomolecules and cells that consequently will give impact on the whole body (Rahal et al., 2014). Meanwhile, free radicals are unstable molecules, or atoms having an unpaired valence electron that are eligible to stay in independent existence status (Rima et al., 2011). These free radicals may cause damage of fatty acids and proteins in the cell membrane, which lead to the destruction of cells due to its characteristics of having an odd number of electrons, thus make it short-lived and highly reactive (Birben et al., 2012). Noteworthily, reactive oxygen species (ROS) are classified as the most common and harmful types of free radicals (Rahal et al., 2014).

In addition to the well-established mechanisms of initial coordination of pro-inflammatory cytokine production, signals provided by ROS also play a significant role in increasing complexity (Martinon et al., 2009). Furthermore, slightly elevated white blood cells (WBCs) count has been reported to be significantly associated with stressful lifestyles (Nakanishi et al., 2003; Nishitani and Sakakibara, 2007). On the other hand, the red blood cells (RBCs) are continuously facing ROS from exogenous and endogenous sources, which can lead to insufficient RBCs functions and destruction (Rifkind et al., 1997; Nagababu and Rifkind, 1998; Mohanty et al., 2014). Stress by its correlation with oxidative stress, can result to malabsorption of hematinic factors such as iron, vitamin B_{12}, and folic acid, which in turn, may lead to RBC integrity and erythropoiesis disorders (Thakur et al., 2016; Al-Hatamleh et al., 2017). Although there is some considerable numbers of previous studies reported the effects of stress on platelets formation and activity, and the major source of platelet's ROS was NADPH oxidase (Pignatelli et al., 2004; Violi and Pignatelli, 2012). However, it appears that no attempt was made to investigate the effects of stress and/or oxidative stress on platelets count and indices to date. So, the above limitations have motivated the present study in investigating the potential effects of stress on RBCs, WBCs, and platelets among preclinical medical students living in a stressful life style, through exploring their stress levels and haematological parameters.

Materials and Methods

Experimental design

A cross-sectional study was conducted among undergraduate preclinical medical students at Universiti Sultan Zainal Abidin (UniSZA), Kuala Terengganu, Terengganu, Malaysia. A total of 122 students (66 first year and 56 second year, male= 39 and female= 83) were invited to participate in the study in November 21st, 2017. This study was approved by the UniSZA Human Research Ethics Committee (UHREC), reference number: UHREC/2017/3/003. Samples were collected from subjects after obtaining informed consent in accordance with the Declaration of Helsinki.

Inclusion/exclusion criteria

Healthy, preclinical medical students, non-smokers, not under any form of medications, do not have family history with any hereditary anaemia, without previous history of alcohol abuse, non-pregnant, and who were not exposed in last 3 months to diseases, surgery, blood donation, accident, or any cause leading blood loss.

Questionnaire distribution and blood samples collection

The questionnaire was distributed, and blood samples were collected at UniSZA Medical Clinic. Both validated English and Bahasa Melayu versions of the DASS-21 questionnaire were distributed with a consent form to preclinical medical students. Then, a total of 5 ml

EDTA blood sample was collected from each subject.

The Depression Anxiety Stress Scales-21 (DASS-21) has been used as one of the most common and widely accepted instruments for assessing the severity of stress in clinical and non-clinical samples. The DASS-21 questionnaire working on the basis of three self-report scales designed to measure levels of the negative states of stress (seven items; 1, 6, 8, 11, 12, 14, 18), depression (seven items; 3, 5, 10, 13, 16, 17, 21), and anxiety (seven items; 2, 4, 7, 9, 15, 19, 20). The participants respond to the items on a 4-point scale, including; 0= did not apply to me at all, 1= applied to me to some degree, or some of the time, 2= applied to me to a considerable degree, or a good part of time, and 3= applied to me very much, or most of the time. The total scores were calculated by summing up the scores for each subscale then multiply the result by two, subsequently transferring the final score to the DASS-21 profile sheet and compare it with special table to know the severity of stress, anxiety, and depression (Lovibond and Lovibond, 1995).

Full blood count (FBC)

FBC test was performed by using automated haematology analyser (Sysmex, Japan). The parameters that were included in the study were WBCs count, neutrophils count and percent, lymphocytes count and percent, MXD (monocytes, eosinophils and basophils) count and percent, RBCs count, haemoglobin (Hb) concentration, haematocrit (Hct) percent, platelets count, neutrophils/lymphocytes ration (NLR), as well as the following auto-calculated parameters.

- Mean corpuscular volume

$$(\text{MCV}) = \frac{\text{Haematocrit (\%) x 10}}{\text{RBC (x } 10^{12}\text{/ L)}}$$

- Mean corpuscular haemoglobin

$$(\text{MCH}) = \frac{\text{Hb (g/dl) x 10}}{\text{RBC (x } 10^{12}\text{/ L)}}$$

- Mean corpuscular haemoglobin concentration

$$(\text{MCHC}) = \frac{\text{Hb (g/dl) x 100}}{\text{Haematocrit (\%)}}$$

- Mean platelet volume

$$(\text{MPV}) = \frac{\text{Plateletcrit (\%)}}{\text{Platelet count (x } 10^{9}\text{/ L)}} \text{ x } 10^{5}$$

- Red cell distribution width-standard deviation (RDW-SD) = an actual measurement of the width of the MCV histogram at the 20% height level.
- Platelet distribution width (PDW) = an actual measurement of the width of the MPV histogram at the 20% height level.
- Platelet large cell ratio (P-LCR) = the percentage of the platelets with a size of more than 12 fL.

Statistical analysis

Data was analysed by using the Statistical Package for the Social Sciences (SPSS), version 21.0 (IBM Corporation, Armonk, NY). Means and standard deviations were used to describe the continuous variables, and the Pearson's correlation test was employed to assess the bivariate association between metric continuous variables. The relative importance index (RII) was used for the indicators of stress, anxiety and depression, and the Cronbach's alpha test of internal consistency was used to assess the reliability of the DASS-21 inventory.

The SPSS AMOS program was used to explore the multivariate association between the students' demographic data, stress, anxiety and depression, with the main haematological parameters (RBCs count, WBCs count, PLTs count, RDW and P-LCR). The haematological parameters were set as a dependent variable, while regressing the students perceived stress level against these parameters but by accounting for the gender differences (direct effect for gender on the students' haematological parameters due to the physiologic differences between males and females). The depression and anxiety were set as precursors for background stress while maintaining their correlated nature at the same time. The goodness of fit (GOF) statistics, the direct and indirect standardised regression effects for the proposed model, were assessed by evaluating the fit between the proposed model, the reproduced model and the measured data following the standards devised by Barbara Byrne (2013) and Joseph Hair et al. (2006).

Results

Demographic data

At the begin of study, out of 122 preclinical medical students, a total of 105 students were enrolled in this study, including 57 first year students and 48 second year students (Table 1).

Table 1. The total number of preclinical medical students who participated in the present study.

Year of study	Gender		Total
	Male	Female	
First year	20	37	57 (54.3%)
Second year	15	33	48 (45.7%)
Total	35 (33.34%)	70 (66.66%)	105 (100%)

Determining stress levels among preclinical students via DASS-21

The Cronbach's alpha test of internal consistency was used to assess the reliability of DASS-21. The test suggested that DASS-21 was overall reliable to measure levels of students' stress, anxiety and depression; Cronbach's alpha was equal to (0.87), which is above (0.70) as a general cut-off limit. The sub-concepts measured with seven indicators were also reliable according to Cronbach's alpha results as follow: stress= 0.72, depression= 0.78 and anxiety= 0.67 (Table 2).

Table 2. Reliability analysis of the DASS-21.

DASS-21 subscales	Number of items	Cronbach's alpha	Decision
Stress scale	7	0.72	Good
Depression scale	7	0.78	Good
Anxiety scale	7	0.67	Acceptable
DASS-21 overall	21	0.87	Very Good

The results of DASS-21 reflected that students were categorised under five groups (normal, mild, moderate, sever, and extremely severe) for each DASS-21 subscale. The stress scale reported 72 (68.6%) students under normal category, 14 (13.3%) in mild category, 15 (14.3%) in moderate category, and 4 (3.8%) in severe category. The other scales' results are available in Table 3.

Quantifying of haematological parameters for preclinical students

The present study showed that overall results of haematological parameters were within the normal ranges in preclinical medical students as summarised in Table 4.

Table 4. Descriptive statistics for the haematological parameters of preclinical students.

Haematological parameters	Mean (SD)	Normal ranges
Hct %	40.50 (4)	M: 40.1-50.6 , F: 35.1-44.9
Hb (g/dL)	12.87 (1.66)	M: 13.5-17.4 , F: 11.6-15.1
Lymphocytes %	34.84 (7.4)	20-40
Lymphocytes x 10^3/µL	2.45 (0.67)	1,847-4.807
MCH (pg)/cell	26.45 (2.9)	26.9-32.3
MCHC (g/dL)	31.72 (1.4	31.9-35.3
MCV (fL)	82.72 (8.2)	80.6-95.5
MPV (fL)	10.21 (0.93)	8.6-15.5
MXD %	10.59 (4.0)	3.5-13.0
MXD x 10^3/µL	0.72 (0.28)	0.385-2.063
Neutrophils %	54.39 (9.0)	40-70
Neutrophils x 10^3/µL	3.93 (1.34)	3.929-7.147
NLR %	1.68 (0.66)	1.2-4.4
PDW (fL)	12.65 (2)	8.3-25.0
Platelets x 10^3/µL	316.81 (70.4)	M: 142-350 , F: 171-399
P-LCR %	26.55 (7.4)	11.9-66.9
RBCs x 10^6/µL	4.90 (0.47)	M: 4.53-5.95 , F: 3.87-5.21
RDW (fL)	44.14 (2.82)	37.5-48.1
WBCs x 10^3/µL	7.1 (1.7)	4.078-11.370

Note. M: male, F: female.

Table 3. The frequency of scale categories for DASS-21 subscale

DASS-21 subscales	Scale categories				
	Normal	Mild	Moderate	Severe	Extremely severe
Stress	72 (68.6%)	14 (13.3%)	15 (14.3%)	4 (3.8%)	0 (0.0%)
Anxiety	24 (22.9%)	11 (10.5%)	35 (33.3%)	17 (16.2%)	18 (17.1%)
Depression	62 (59.0%)	19 (18.1%)	18 (17.1%)	1 (0.95%)	5 (4.8%)

The Association between DASS-21 results and haematological parameters

According to the bivariate Pearson's correlation test (r), the results revealed a statistically significant positive correlation between students' perceived stress levels, their perceived anxiety (r= 0.695) and depression (r= 0.687) levels, each respectively ($p \leq 0.01$). A statistically significant negative direct association between students' perceived stress levels and their measured WBCs count (r= - 0.204, $p \leq 0.05$) has also been noted. The WBCs count was also found to be negatively indirect associated with students' perceived stress levels through their depression (r= - 0.218) and anxiety (r= - 0.195) levels, each respectively ($p \leq 0.05$). Further, the students' perceived stress levels has been shown to have a negative indirect association with lymphocytes count through their perceived depression levels (r= - 0.206, $p \leq 0.05$). Moreover, there was a significant positive association between students' perceived stress levels and their measured MPV (r= 0.195, $p \leq 0.05$). However, it is interesting to be noted that a direct negative association between students' perceived stress levels and their measured neutrophils, lymphocytes, platelets counts and Hb has been found, but it was a non-significant association (Table 5).

Table 5: Pearson's correlation (r-value) between the students stress, anxiety, depression and haematological parameters

	Stress	Anxiety	Depression	WBCs	RBCs	Hb	HCT	MCV	MCH	MCHC	PLTs	LYM %	MXD%	Neutr%	LYM	MXD	NEU	RDW	PDW	MPV	P-LCR
Anxiety	**.695****																				
Depression	**.687****	.547**																			
WBCs	**-.204***	**-.195***	**-.218***																		
RBCs	.096	.135	.081	-.019																	
Hb	-.117	.154	.161	-.152	.488**																
HCT %	.134	.159	.166	-.112	.629**	.959**															
MCV	.039	.036	.113	-.130	-.304**	.478**	.379**														
MCH	.034	.045	.098	-.147	-.263**	.706**	.546**	.779**													
MCHC	.012	.080	.084	-.168	.018	.779**	.579**	.559**	.856**												
Platelets	-.175	-.091	-.073	.404**	-.118	-.465**	-.408**	-.258**	-.416**	-.453**											
Lymphocytes %	.000	-.003	-.016	-.332**	-.074	-.077	-.095	-.039	-.020	.016	-.050										
MXD %	.106	.086	.173	-.387**	.101	.171	.176	.112	.098	.079	-.330*	.027									
Neutrophils %	-.049	-.031	-.064	.476**	.023	-.024	-.012	-.041	-.045	-.054	.228*	-.833**	-.559**								
Lymphocytes	-.177	-.191	**-.206***	.604**	-.116	-.228*	-.206*	-.144	-.150	-.157	.330**	.492**	-.315**	-.256**							
MXD %	.038	-.038	.085	.171	.087	.042	.088	.024	-.039	-.096	-.063	-.142	.803**	-.319**	.067						
Neutrophils	-.165	-.130	-.171	.893**	-.037	-.143	-.118	-.109	-.127	-.147	.396**	-.610**	-.523**	.788**	.293**	-.048					
RDW	-.003	-.159	-.064	-.006	-.465**	-.275**	-.244*	.234*	.057	-.290**	.163	.025	.044	-.051	.021	.075	-.025				
PDW	.153	.007	.149	.049	.152	-.019	.031	-.164	-.139	-.131	-.357**	.017	.055	-.037	.033	.133	-.005	-.068			
MPV	**.195***	.057	.139	.012	.015	-.012	.007	-.047	-.029	-.035	-.419**	.061	.054	-.076	.035	.117	-.049	-.032	.875**		
P-LCR %	.191	.038	.128	.007	.042	-.009	.012	-.073	-.047	-.041	-.429**	.062	.055	-.076	.029	.107	-.049	-.046	.892**	.994**	
NLR %	-.065	-.031	-.038	.396**	.021	.012	.020	.015	-.007	-.026	.120	-.894**	-.318**	.922**	-.407**	-.144	.716**	-.069	-.037	-.071	-.069

Note. **Correlation is highly significant at (p value ≤ 0.01); *Correlation is significant at (p value ≤ 0.05); (-) negative association; **Highlighted values:** the main significant values.

Structural equation model (SEM)

The maximum likelihood regression estimator was employed in this analysis since it is the default estimation in the AMOS program, and there was no violations to the normality assumptions in the students measured stress and haematological parameters as evidenced with lack of multivariate outliers and normal distribution of the observed variables. The multivariate Mardia's statistic kurtosis (= 1.67) and skewness (= 0.53) indices were within expected values, as such the maximum likelihood estimation was acceptable. The standardised Z-score was computed for the haematological parameters and used for the structural equation model to unify the unit of measurement of these scales in standardised points. The Z-score is a score with a mean= 0 and a standard deviation= 1 is computed by misusing the true score from

the overall mean score and dividing the result by the standard deviation of the measured variable. The Z-score was admitted in the path model instead of the observed scores of the blood parameters for the ease of interpretation.

The initial analysis suggested that the path model was a good fit with the data, denoting that the specified path models and the one reproduced by the program did not differ significantly. As such, the model fit suggested an overall satisfactory fit between the specified path model and the data.

The standardised and unstandardised regression coefficients (effects) from the path model were summarised in Table 6 and Table 7, respectively. The path model suggested that both the students perceived anxiety and depression levels were predicted with a significantly higher stress levels ($p < 0.001$), respectively. Moreover, the gender of students differed statistically on their measured RBCs ($p < 0.001$), platelets ($p < 0.001$), and WBCs ($p = 0.005$). However, the two genders did not differ statistically on their measured P-LCR ($p = 0.935$) and RDW ($p = 0.052$) (Table 6).

Table 6. The unstandardised direct regression coefficients for the estimated relationships from the path model.

Predictor	Dependent variable	Estimated coefficient	SE	CR	p value	Label
Anxiety	Stress	0.82	0.129	6.383	<0.001	Significant
Depression	Stress	0.851	0.122	6.951	<0.001	Significant
Sex	Z-PLTs	0.772	0.189	4.076	<0.001	Significant
Stress	Z-PLTs	- 0.024	0.014	-1.672	0.095	Not significant
Sex	Z-P-LCR	0.016	0.203	0.081	0.935	Not significant
Stress	Z-P-LCR	0.03	0.015	1.978	0.048	Significant
Sex	Z-RBCs	-1.102	0.176	-6.279	<0.001	Significant
Stress	Z-RBCs	0.01	0.013	0.737	0.461	Not significant
Sex	Z-RDW	0.395	0.203	1.94	0.052	Not significant
Stress	Z-RDW	0.001	0.015	0.098	0.922	Not significant
Sex	Z-WBCs	0.546	0.195	2.794	0.005	Significant
Stress	Z-WBCs	- 0.029	0.015	-2.006	0.045	Significant

Note. Z: Z-score; SE: standard error of estimate; CR: the critical ratio for t-test.

Table 7. The standardised indirect regression coefficient estimates of the path model

Predictor	Dependent Variable	Estimate
Anxiety	Stress	0.425
Depression	Stress	0.463
Sex	Z-PLTs	0.367
Stress	Z-PLTs	- 0.15
Sex	Z-P-LCR	0.008
Stress	Z-P-LCR	0.19
Sex	Z-RBCs	- 0.523
Stress	Z-RBCs	0.061
Sex	Z-RDW	0.187
Stress	Z-RDW	0.009
Sex	Z-WBCs	0.26
Stress	Z-WBCs	- 0.186

Interestingly, by considering the effect of the gender differences as accounted, the students perceived stress level was associated significantly with their measured WBCs count, the standardised regression coefficient was equal to - 0.19 ($p= 0.045$), as well as associated significantly with their measured P-LCR, the standardised regression coefficient was equal to 0.19 ($p = 0.048$). However, students perceived stress level was weakly associated (not significant) with their measured platelets count, the standardised regression coefficient was equal to 0.367 ($p = 0.095$) (Table 6 and Table 7).

A bootstrap 95% bias-corrected (BC) confidence interval for the indirect effects for each of the students' race, depression, and anxiety was performed on the modelled haematological parameters model and was estimated with 2000 bootstraps. The results suggested a

significant but low (in magnitude) negative indirect effect for each of these predictors on the students measured WBCs count (Table 8). The results was similar on the students race indirect effect on PLTs (p= 0.049), the students perceived depression had positive indirect but significant effect on their measured P-LCR (p= 0.049), and the students race had a significant indirect effect on their measured P-LCR via their measured stress (p= 0.030) (Table 8).

Table 8. The standardised indirect effects for the predictors in the path analysis.

	Race	Depression	Anxiety
Stress	b= 0.065, NS	-	-
Z-P-LCR	**b= 0.012 p= 0.030**	**b= 0.088 p= 0.049**	b= 0.081, NS
Z-RDW	b= 0.001, NS	b= 0.004, NS	b= 0.004, NS
Z-PLTs	**b= - 0.01 p= 0.049**	b= 0.07, NS	b= - 0.064, NS
Z-WBCs	**b= - 0.012 p= 0.014**	**b= - 0.086 p= 0.021**	**b= - 0.079 p= 0.017**
Z-RBCs	b= 0.004, NS	b= 0.028, NS	b= 0.026, NS

Note. The significant indirect effects at an alpha significance level of (p= 0.05) are marked with **bold and underlined** face in the above table. NS: not significant; b: beta coefficient, it is the regression coefficient, or correlation coefficient between a predictor and an outcome dependent variable.

Discussion

The present study revealed that there was a statistically significant negative association between stress levels with both WBCs count and lymphocytes count (directly/indirectly), as well as a significant positive association between stress levels and MPV (Table 5). Furthermore, the SEM analysis showed some interesting results; the students perceived stress level converged significantly on their measured WBCs count with standardised regression coefficient (- 0.19), denoting that as students perceived higher stress, their measured WBCs count tend to decline significantly (p= 0.045). Both the students perceived anxiety and depression levels were predicted with a significantly higher stress levels (p <0.001). The students perceived stress predicted a significant higher P-LCR (standardised regression coefficient= 0.19) (p= 0.048).

Moreover, the model also showed that students' race had a significant indirect effect on their measured P-LCR via their measured stress (p= 0.030), denoting that Malay students had a significantly greater impact of their perceived stress on their P-LCR. This indicates that Malay students perceived stress level may lead to higher P-LCR compared to Indian, or the interaction between race and stress may have an influence on the P-LCR reaction to stress. Also, in a similar way, the students' perceived depression had positive indirect but significant with the effect on their measured P-LCR (p= 0.049).

In contrast to the evidences by previous studies, there were no significant differences in RBC count and indices. A study has been conducted to correlate the prevalence of anaemia and psychological stress among 200 females aged 20 to 40 years old suffering mainly with deterioration in the quality of life due to their general circumstances at Al-Hawija district, Iraq (Hassan et al., 2016). This study has reported a significant decrease in both Hb and Hct of participated females, which was indicated a mild symptomatic anaemia (Hassan et al., 2016). Although the subjects in present study were facing academic-based psychological stress, they supposed to be in a better situation compared to the subjects of previous study mentioned above. Another investigation has been conducted to explore the impacts of psychological stress on serum iron level and erythropoiesis in rats (Wei et al., 2008). According to this study, the psychological stress was significantly associated with reduced Hb, RBCs count and MCV, together with an increase of RDW (Wei et al., 2008). However, it is noticeably impossible to control stress levels among human subjects (as in the present study), while in animal-based stress studies it is possible by several lab techniques.

On the other hand, the major finding of studies that investigated the effect of stress on WBCs was a positive association between each of them (Maes et al., 1999; Nishitani and Sakakibara, 2014). However, the present study shows a reversed finding compared to these studies. In a study conducted to examine the effects of academic examination stress on WBCs subset distribution in university students, students who respond to academic examination stress with a strong psychological reaction showed a significant increase of WBCs count (Maes et al., 1999). Moreover, another experimental investigation conducted to reveal the association between work-related psychological stress responses in Japanese workers (Nishitani and Sakakibara, 2014). This study

demonstrated that stress levels were significantly related with the fatigue score and increased WBCs count, which was hypothesised to be attributable to increased neutrophils count (Nishitani and Sakakibara, 2014). The same study also reported a non-significant association between stress and lymphocytes count (Nishitani and Sakakibara, 2014), which is inconsistent with the findings of present study, while another study revealed a consistency with the present study in this regard (Ge et al., 2017). However, Shafiee and his colleagues have reported that a significant positive association between both depression and anxiety symptoms with increased mean WBCs count among healthy men (Shafiee et al., 2017).

It is also interesting to be noted that the present study is the first attempt in investigating the potential effect of stress on platelets count and indices, while the previous studies explored only the effect of stress on platelets formation and suggested that stress enhances platelet activity, reactivity and immune-modulatory capacities (Hamer et al., 2006; Steptoe et al., 2003).

This study demonstrated that the alteration of haematological parameters among a group of people facing same stressful events (preclinical medical students) provide new insights about the potential effects of stress responses on blood cells and platelets. The results of this experiment will help researchers to uncover the critical areas of increasing ROS and oxidative stress in human body caused by chronic life stress. Thus, a new theory on the vague alterations of some haematological parameters among healthy individuals could be confirmed by further study in the future, based on hypotheses of the present study.

Lastly, it has been noted that the sample size of the present study was comparatively small, and stress levels might have not been enough to cause the expected effects on haematological parameters. Therefore, authors recommend a larger sample size to be used in the future studies to investigate the association between stress levels and alteration of haematological parameters.

The present study found that the overall prevalence of stress among preclinical medical students was acceptably within normal levels, and their haematological parameters were within the normal ranges. Therefore, there were only limited associations have been reported. The findings of this study are essential to build a model of causation for the alteration of haematological parameters among healthy individuals based on their stress levels. To the best of knowledge, this is the first study investigated the effect of stress on all FBC results.

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Authors' contribution

MAIA was responsible for conceptualisation, methodology, validation, data analysis, investigation and writing the original draft. II was responsible for conceptualisation, supervision, reviewing and editing. OMA was responsible for methodology and writing the revised draft. TMA was responsible for resources, supervision, reviewing and editing. All authors listed have made a direct contribution to the work and approved it for the publication.

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