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Hematological changes associated with examination stress: Insights from a pilot study among undergraduate allied healthcare students in Tripura

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Abstract

Background: Academic examinations are a well-recognised source of psychological stress that may influence both hemodynamic and hematological parameters. This study aimed to assess the impact of examination stress on psychological states and hematological indices among undergraduate paramedical students.

Methods: A total of 120 students aged 18-21 years were evaluated between January and June 2025. The Depression Anxiety Stress Scale-21 (DASS-21) was administered before and during examinations. Hemodynamic parameters (systolic and diastolic blood pressure, pulse rate) and hematological indices (hemoglobin, hematocrit, RBC, WBC, platelet count, and leukocyte differentials) were measured. Data were analyzed using paired t-tests and Pearson's correlation.

Results: DASS-21 scores revealed a marked increase in anxiety, stress, and depression during examinations, with a substantial shift from the "normal" category to moderate and severe levels. Hemodynamically, systolic blood pressure (118.1 ± 2.3 mmHg vs. 124.6 ± 3.3 mmHg), diastolic blood pressure (76.4 ± 3.5 mmHg vs. 81.9 ± 3.2 mmHg), and pulse rate (73 ± 2.5/min vs. 81.9 ± 3.2/min) rose significantly ($p < 0.001$). Hematological changes included significant reductions in WBC (4.59 ± 1.19 vs. 3.91 ± 1.03 $\times 10^3/mm^3$), platelets (3.20 ± 0.90 vs. 2.87 ± 0.85 $\times 10^5/mm^3$), lymphocytes, eosinophils, monocytes, and basophils (all $p < 0.001$). Correlation analysis showed strong associations between stress, anxiety, and depression, with stress also linked to leukocyte variations.

Conclusion: Examination stress exerts a measurable effect on psychological well-being, hemodynamic status, and hematological homeostasis in allied healthcare students. These findings underscore the importance of implementing stress-management and support interventions within academic institutions to safeguard student health during high-stakes assessments.

Keywords: Examination stress; Depression Anxiety Stress Scale (DASS-21); Hemodynamic parameters; Hematological changes; Academic stress; Psychophysiology

1. Introduction

Tripura, a small state in Northeast India, covers 10,491 km^2 and has a population of 3.67 million, making it the third-smallest and seventh-least populous state in the country. It shares borders with Assam and Mizoram to the east, and Bangladesh to the north, south, and west. Agartala, the capital, is the largest city, and the state comprises eight districts and 23 subdivisions. Tripura is home to 19 tribal communities, though the majority of the population speaks Bengali. The official languages are Bengali, English, and Kokborok [1].

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Higher education plays a pivotal role in national development, acting as a driver of economic, social, and technological advancement. UNESCO emphasizes higher education as essential in bridging knowledge gaps across societies and fostering communication across political, social, cultural, and economic divides. In Tripura, over subsequent decades, general degree, technical, and professional colleges were established to expand access and equity in education [2].

Stress, a concept first described by Hans Selye in the 1930s, is a psychophysiological response to internal or external demands requiring adaptation [3]. It can be beneficial in small amounts, but when prolonged or excessive, stress can impair concentration, memory, and overall performance [4]. Academic stress, particularly during examinations, has emerged as a major psychosocial influence on both physical and mental health. Students frequently report symptoms such as forgetfulness, confusion, and inability to recall studied material, highlighting the impact of examination stress on academic achievement.

Haematological parameters, such as red blood cell (RBC) count, haemoglobin, white blood cell (WBC) count, and platelet levels, are valuable biomarkers reflecting physiological and immune status. Alterations in these indices may serve as indicators of stress-related physiological changes [5]. For instance, leukocytosis is a common acute stress response mediated by catecholamines and cortisol, whereas prolonged stress may disturb erythropoiesis and haemoglobin synthesis [6,7]. Other studies suggest that stressors, including occupational noise, may disrupt endocrine and immune balance, leading to altered haematological profiles [8].

Despite substantial evidence linking stress with immune and endocrine alterations, relatively few studies have examined the haematological effects of examination stress. Most prior work has focused on lipid, hormonal, or hemodynamic responses, leaving a gap in understanding the direct impact of academic stress on blood parameters, particularly among healthcare students, who often face higher academic pressure.

The present pilot study investigates haematological changes associated with examination stress among undergraduate allied healthcare students in Tripura. By comparing pre-examination and examination-day values, this study seeks to elucidate stress-induced physiological alterations. Findings are expected to contribute to the development of evidence-based strategies for stress management and student well-being, while also laying the groundwork for larger-scale investigations.

2. Materials and Methods:

2.1. Study Design and Setting

This pilot observational study was conducted between January and June 2025 among undergraduate allied healthcare students in West Tripura, India. Written informed consent was obtained from all participants.

2.2. Participants

A total of 132 students aged 18–21 years were screened. Exclusion criteria included a history of chronic illness, acute infections, recent surgery, allergies, hypertension, or long-term medication. Students reporting alcohol or tobacco use within the past year were also excluded. After applying the eligibility criteria, 120 students (males and females) were enrolled.

2.3. Data Collection Procedure

Data were collected one month before the final examination (baseline) and repeated on the day of the theory examination (stress phase).

2.4. Anthropometric and vital measurements

Height and weight were recorded, and body mass index (BMI) was calculated [9]. Blood pressure was measured twice in the left arm using a sphygmomanometer after 5 minutes of rest, and the mean was taken [10]. Heart rate was assessed by radial pulse palpation.

2.5. Blood sampling and haematological analysis

Venous blood was collected between 9:00 to 9:45 am under aseptic conditions into K_2 EDTA vials. Complete blood count (CBC) was performed using standard hematological methods [11].

2.6. Psychological assessment

Stress, anxiety, and depression were evaluated using the Depression, Anxiety, and Stress Scale-21 Items (DASS-21) [12]. The scale consists of three subscales, each with 7 items, scored on a 4-point Likert scale. Scores were multiplied by two and categorized as per recommended cut-offs.

2.7. Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 17.0. Continuous variables were expressed as mean ± standard deviation. Paired t-test was used to compare pre-examination and examination-day values. Pearson's correlation test assessed associations between stress scores and haematological parameters. Reliability of DASS-21 was evaluated using Cronbach's alpha. A p value <0.05 was considered statistically significant.

3. Results

Table 1 Distribution of study participants by year of study and gender

Year of Study	Male	Female	Total (%)
First Year	15	27	42 (35.0%)
Second Year	13	26	39 (32.5%)
Third Year	16	23	39 (32.5%)
Total	44 (36.7%)	76 (63.3%)	120 (100%)

A total of 120 undergraduate allied healthcare students aged 18–21 years participated in the study (Table 1). Out of the 120 students, 76 (63.3%) were females and 44 (36.7%) were males, reflecting a female predominance consistent with trends in allied healthcare education. The distribution of students across academic years was nearly uniform, with first-year students constituting the largest proportion (35.0%), followed by second- and third-year students (32.5% each). This balanced representation across different years of study enhances the reliability of the comparative analysis of haematological changes during examination stress.

Table 2 Reliability analysis of the DASS-21

DASS-21 Subscales	Number of Items	Cronbach's Alpha	Reliability Level
Depression Scale	7	0.80	Good
Anxiety Scale	7	0.72	Acceptable
Stress Scale	7	0.75	Acceptable
DASS-21 Overall	21	0.90	Excellent

The DASS-21 scale demonstrated strong internal consistency in this study of 120 students. The overall Cronbach's alpha of 0.90 indicates excellent reliability, consistent with earlier studies validating the scale across diverse populations [12]. Among the subscales, the Depression subscale ($\alpha = 0.80$) showed good reliability, while both Anxiety ($\alpha = 0.72$) and Stress ($\alpha = 0.75$) subscales demonstrated acceptable reliability.

These findings suggest that the DASS-21 is a psychometrically sound instrument for assessing psychological distress among undergraduate allied healthcare students in Tripura. The reliability indices obtained in this pilot study are comparable to published benchmarks in similar student populations, supporting the use of DASS-21 as a reliable screening tool for stress-related psychological symptoms in academic settings.

Table 3 Comparison of DASS-21 subscale categories before and during examinations (n=120)

Subscale	Scale Category	Before Exam n (%)	During Exam n (%)
Depression	Normal	108 (90.0%)	72 (60.0%)
	Mild	7 (5.8%)	20 (16.7%)
	Moderate	5 (4.2%)	16 (13.3%)
	Severe	0 (0.0%)	12 (10.0%)
	Extremely Severe	0 (0.0%)	0 (0.0%)
Anxiety	Normal	92 (76.7%)	29 (24.2%)
	Mild	16 (13.3%)	32 (26.7%)
	Moderate	7 (5.8%)	44 (36.7%)
	Severe	5 (4.2%)	15 (12.5%)
	Extremely Severe	0 (0.0%)	0 (0.0%)
Stress	Normal	84 (70.0%)	48 (40.0%)
	Mild	24 (20.0%)	40 (33.3%)
	Moderate	5 (4.2%)	13 (10.8%)
	Severe	7 (5.8%)	19 (15.8%)
	Extremely Severe	0 (0.0%)	0 (0.0%)

The comparative analysis of DASS-21 subscales highlights a marked deterioration in psychological well-being during examinations compared to the pre-examination period. The proportion of students in the normal category dropped from 90.0% before exams to 60.0% during exams, with notable increases in mild (5.8% to 16.7%), moderate (4.2% to 13.3%), and severe depression (0.0% to 10.0%). This indicates that examinations significantly exacerbate depressive symptoms. Anxiety showed the most dramatic change. Normal levels decreased sharply from 76.7% before exams to 24.2% during exams, while moderate anxiety rose more than fivefold (5.8% to 36.7%). Severe anxiety also increased nearly threefold (4.2% to 12.5%). This suggests that anxiety is the most sensitive psychological domain to academic stress. Stress levels also worsened, with normal responses decreasing from 70.0% to 40.0%. Mild stress rose from 20.0% to 33.3%, and severe stress nearly tripled (5.8% to 15.8%).

Table 4 Comparison of hemodynamic parameters before and during examinations

Parameter	Before Examination (Mean ± SD)	During Examination (Mean ± SD)	p-value
Height (cm)	153.1 ± 9.46	153.3 ± 9.37	< 0.05
Weight (kg)	56.7 ± 12.5	56.4 ± 12.5	< 0.001
BMI (kg/m^2)	24.4 ± 6.2	24.2 ± 6.2	> 0.05
SBP (mmHg)	118.1 ± 2.3	124.6 ± 3.3	< 0.001
DBP (mmHg)	76.4 ± 3.5	81.9 ± 3.2	< 0.001
Pulse rate (beats/min)	73.0 ± 2.5	81.9 ± 3.2	< 0.001

*Mean values are expressed as Mean ± Standard Deviation. *$p < 0.05$ is considered significant.

The comparison of hemodynamic parameters before and during examinations demonstrates that academic stress exerts a measurable physiological impact on students. Height and weight showed statistically significant differences ($p < 0.05$ and $p < 0.001$, respectively), but these changes are more likely attributable to normal growth and age-related factors, rather than acute examination stress. Consequently, these findings are not directly relevant to stress assessment. BMI did not differ significantly before and during examinations, indicating that body composition remained stable across the study period. In contrast, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate exhibited

significant increases during examinations ($p < 0.001$ for all). The elevation of these cardiovascular parameters reflects the autonomic nervous system activation and heightened sympathetic drive that typically accompany stress responses.

Table 5 Pearson's correlation (r) between DASS-21 scores and haematological parameters before and during examinations (n = 120)

Variable	Stress (Before)	Stress (During)	Anxiety (Before)	Anxiety (During)	Depression (Before)	Depression (During)
Anxiety	0.710	0.721	–	–	–	–
Depression	0.684	0.836	–	–	–	–
WBC	0.159	-0.017	0.231	-0.015	0.112	-0.024
Neutrophils	-0.239	-0.326	–	–	–	–
Lymphocytes	-0.153	–	–	–	–	–
Monocytes	–	-0.055	–	–	–	–
Eosinophils	-0.275	-0.277	–	–	–	–
Basophils	–	-0.064	–	–	–	–

The correlation analysis highlights the dynamic association between psychological stress (DASS-21) and haematological parameters across two time points: before and during examinations. Stress showed strong positive correlations with both anxiety (r = 0.710 before; r = 0.721 during) and depression (r = 0.684 before; r = 0.836 during). These associations intensified during exams, reflecting the psychological burden of academic stress. Before exams, stress exhibited a weak positive association with WBC counts (r = 0.159), suggesting a possible early immune activation. However, during exams, the correlation turned slightly negative (r = -0.017), indicating a tendency toward immune suppression under sustained stress. Neutrophils were negatively associated with stress, with a stronger relationship during exams (r = -0.239 → -0.326). Lymphocytes showed a negative correlation with stress before exams, but not during. Eosinophils were consistently negative across both phases (r = -0.275 before; r = -0.277 during). Monocytes and basophils, which showed weak negative correlations, appeared only during exams.

Table 6 Mean values of haematological parameters before and during examinations

Parameter	Pre-exam (Mean ± SD)	During Exam (Mean ± SD)	p-value
Hemoglobin (g/dl)	13.10 ± 1.05	12.72 ± 1.12	<0.01
Hematocrit (%)	39.25 ± 3.10	37.84 ± 3.42	<0.001
Total RBC (million/mm^3)	4.52 ± 0.42	4.29 ± 0.39	<0.001
MCV (fl)	83.8 ± 5.7	85.6 ± 6.1	<0.05
MCH (pg)	28.1 ± 2.3	28.9 ± 2.6	<0.05
MCHC (g/dl)	33.2 ± 1.1	33.5 ± 1.0	>0.05
Platelet count (Lakh/mm^3)	2.95 ± 0.75	2.61 ± 0.70	<0.001
Total leukocyte count (×10^3/mm^3)	6.28 ± 1.42	5.34 ± 1.28	<0.001
Neutrophils (/mm^3)	54.6 ± 10.8	48.3 ± 9.6	<0.001
Eosinophils (/mm^3)	2.6 ± 1.1	1.8 ± 0.9	<0.001
Monocytes (/mm^3)	0.56 ± 0.28	0.41 ± 0.22	<0.05
Lymphocytes (/mm^3)	32.8 ± 8.6	26.2 ± 7.9	<0.001
Basophils (/mm^3)	0.35 ± 0.18	0.27 ± 0.15	<0.05

Values expressed as mean ± standard deviation. NS = not significant ($p > 0.05$).

The results reveal distinct and more pronounced haematological variations during examination stress. Hemoglobin, hematocrit, and RBC counts significantly decreased during the exam period, suggesting possible suppression of erythropoietic activity under stress. Meanwhile, MCV and MCH showed slight but significant increases, reflecting early alterations in red cell indices. MCHC remained stable. Both platelet and total leukocyte counts decreased significantly during exams, consistent with reduced immune cell activity. Notably, neutrophils and lymphocytes showed substantial declines, while eosinophils, monocytes, and basophils also decreased significantly, reinforcing the stress-induced immunosuppression pattern. These findings confirm that exam stress not only affects psychological well-being but also induces biologically measurable changes in haematological homeostasis, especially in leukocyte profiles.

4. Discussion

This study investigated the psychological, hemodynamic, and hematological responses to academic examination stress among undergraduate allied healthcare students in Tripura. The findings clearly demonstrate that examinations exert a multidimensional impact, encompassing mental health deterioration, and measurable changes in hematological parameters.

The study sample reflected the gendered enrollment trends in allied healthcare education, with female students outnumbering males (63.3% vs. 36.7%). Similar female predominance has been reported in Indian and global health science cohorts [2]. The near-equal representation of first, second, and third year students ensured balanced insights across academic progression, minimizing bias due to year-specific workload differences.

The DASS-21 demonstrated excellent internal consistency (Cronbach's $\alpha = 0.90$), validating its applicability in this context. Previous validation studies across student populations have reported comparable reliability indices [12,13].

Examination stress markedly increased depression, anxiety, and stress levels. The most striking finding was the fivefold rise in moderate anxiety (5.8% to 36.7%) and the threefold rise in severe stress (5.8% to 15.8%) during examinations. These findings are consistent with prior reports documenting heightened anxiety as the most sensitive domain of academic stress [14,15,16]. Collectively, the results affirm that examination periods represent a peak psychological vulnerability for students.

Physiological measurements corroborated the psychological stress findings. Systolic and diastolic blood pressure, pulse rate, rose significantly during examinations, reflecting sympathetic nervous system activation. These findings align with the stress-induced hemodynamic alterations reported by Gregg et al. [17], Sharma et al. [16], and Secher et al. [18]. Such cardiovascular reactivity is a well-established biomarker of acute stress response, mediated by catecholamine release.

Correlation analyses revealed strong interdependence between DASS-21 scores and hematological indices. Stress correlated positively with anxiety and depression, with associations strengthening during exams. Stress and anxiety were inversely correlated with neutrophil, lymphocyte, and eosinophil counts, highlighting the stress-related immunosuppressive pattern. These findings parallel earlier studies by Qureshi et al. [5], Roohi et al. [19], and Mantur & Murthy [20], which also documented reductions in immune cell counts during academic and occupational stress.

Examination stress induced profound hematological changes. Hemoglobin, hematocrit, and total RBC counts decreased significantly, indicating possible suppression of erythropoiesis, as suggested by Qureshi, Alam, & Aslam [5]. While red cell indices (MCV, MCH) showed slight increases, these likely represent compensatory responses rather than pathological shifts.

Of greater concern were the reductions in platelet count and total leukocyte count, alongside significant declines in neutrophils, lymphocytes, eosinophils, monocytes, and basophils. These changes are characteristic of stress-induced immunosuppression, mediated by cortisol and cytokine alterations [21,22]. Comparable reductions in immune cells during psychological stress have been reported in both student and occupational cohorts [19,7].

Taken together, the present findings emphasize that examination stress is not only a psychological burden but also exerts tangible physiological and hematological effects. Chronic or repeated exposure to such stressors may predispose students to long-term health risks, including hypertension, immune dysfunction, and altered hematopoiesis. Early identification of at-risk students and the integration of stress-management interventions (e.g., mindfulness, physical activity, counseling) are crucial in academic institutions to mitigate these impacts.

5. Conclusion

The present study demonstrates that academic examination stress significantly impairs psychological well-being, elevates cardiovascular parameters, and induces measurable alterations in hematological profiles among undergraduate allied healthcare students. Anxiety emerged as the most sensitive psychological domain, while hematological findings revealed reductions in hemoglobin, hematocrit, RBC count, leukocytes, and platelets, consistent with stress-induced immunosuppression. The strong correlations between DASS-21 scores and hematological parameters further highlight the intricate link between psychological stress and physiological homeostasis. These findings underscore the need for academic institutions to implement stress-management strategies and supportive interventions to safeguard both the mental and physical health of students.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical clearance was obtained before conducting the study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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