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### Effect of stress during exam time on immunity - A Survey based study

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#### KEYWORDS

Exam stress

Stress and health

Stress and immunity

Psychoneuroimmunology

#### ABSTRACT

Recent research indicates an escalating prevalence of stress among students during exam time. Our study aims to explore the correlation between stress induced by exams, its impact on immunity, and the varying effects of stress levels on students' health outcomes. A random online questionnaire survey involving 252 students across three educational levels, school, undergraduate, and postgraduate, have been conducted in this study. This study assessed stress levels, related symptoms experienced during exams, and stress-related health outcomes. The data were analyzed using Venn diagrams and statistically interpreted with Pearson correlation analysis and one-tailed ANOVA. The results revealed that across all three educational levels, females experience higher stress levels than males during exam periods. Additionally, females facing similar stress levels were found to be more susceptible to health issues than their male counterparts. Increased stress levels were correlated with higher incidences of weakness and digestive problems. These findings are consistent with previous research indicating that females are significantly more affected by stress than males and that stress is associated with adverse health outcomes. Our study underscores the need for further investigation into stress and immune response dynamics. Future research could explore blood biomarkers to understand these relationships better.

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#### **1** Introduction

Stress is the body's natural response to any form of physical, mental, or emotional strain or upheaval, serving as a vital mechanism for survival in challenging situations. It comprises a sequence of events: a stressor initiates a reaction in the brain and activates the body's fight-or-flight systems (Mahassni and Eskandar 2019). When under stress, the body perceives itself to be under attack, triggering a cascade of physiological changes, including heightened heart rate, blood pressure, and release of stress hormones. These changes can impact hormones, brain function, behavior, and other bodily systems, which potentially affect immune function (Morey et al. 2015) and other health disorders like depression and anxiety (Shields and Slavich 2017).

Stress comes in various forms distinguished by its duration, i.e., chronic, acute, or episodic acute stress (Cohen et al. 2007). Acute stress is short-lived and may trigger increased levels of proinflammatory cytokines in the blood (Steptoe et al. 2007). In contrast, chronic stress persists for extended periods, ranging from days to years. Like acute stress, chronic stress is associated with elevated levels of proinflammatory cytokines but potentially differing health implications (Gouin et al. 2012). Additionally, chronic stress can activate latent viruses, indicating a loss of immunological control over these pathogens. Frequent activation of latent viruses due to chronic stress can lead to immune system strain and deterioration over time (Pawelec et al. 2005). The impact of stress can differ based on the nature of the stressors encountered, exerting varying effects on the neuroendocrine, autonomic, and central nervous systems, consequently influencing immunological function (Hossain et al. 2006). Exposure to psychological stressors can modulate the significant primary antibody response (Moraska et al. 2002). Academic exams are an example of a brief naturalistic stressor where the subject faces a short-term, real-world problem (Baum et al. 1993).

Given the variability in individual responses to stress, it becomes apparent that immune responses also differ among people. Some studies have explored these inter-individual differences in immune functioning, particularly in response to brief naturalistic stressors (Maydych et al. 2017). For example, engaging in relaxation practices has been associated with increased percentages of T helper cells and higher numbers of T and B lymphocytes during stressful periods, such as academic examinations (Glaser et al. 1986). Conversely, factors like loneliness (Kiecolt-Glaser et al. 1984), emotional instability, and high anxiety have been linked to diminished natural killer cell activity during exams (Borella et al. 1999). Moreover, psychological traits associated with resilience may protect against immune suppression or dysregulation during stressful academic situations (Segerstrom et al. 1998).

Journal of Experimental Biology and Agricultural Sciences http://www.jebas.org The role of cognitive factors in immunological processes, including cognitive states and beliefs, represents a relatively nascent and under-explored area in psychoneuroimmunology. However, limited studies have begun to examine how affective and cognitive factors influence immune responses during brief naturalistic stressors like academic examinations.

Stress related to academic exams has been shown to have a substantial adverse effect on students' well-being and is linked to both physical and mental health problems, including elevated anxiety, depressive symptoms, and immune system dysfunction (Cohen and Herbert 1996). The physiological stress response can become permanently activated in humans due to their capacity to create and experience psychological stresses without external stressors, which frequently have negative consequences (Dhabhar et al. 2012).

Exam stress provides a pertinent framework for investigating the impact of psychological stress on the immune system (Stowell 2003). Confronting academic examinations represents a real-world challenge that elicits varying degrees of stress among individuals. Within the field of psychoneuroimmunology, academic stress is often characterized as a brief naturalistic stressor, encompassing acute moments (immediately before and during exams) as well as prolonged periods (such as during preparation or revision) (Segerstrom and Miller 2004; Preuss et al. 2010). Academic exams thus straddle the spectrum between acute and chronic stress. These designs allow comparing students' immune status before and after exams (Katsuura et al. 2010). Typically, baseline immune status is assessed weeks before and compared with measurements taken shortly before or after exams. However, fewer studies examine immune parameters throughout the exam stress period, including the anticipation and post-exam periods. Various stressful events, including academic examinations, combat tasks, vigilance, and sleep deprivation, have been associated with diminished human immune system functioning (Loft et al. 2007). Psychological or behavioral events such as anger, anxiety, and depression can impact autonomic nervous system activity and hormonal regulation, thereby influencing immune responses. This intricate relationship underscores the brain and immune system's significant influence on each other. Academic exam stress has been shown to affect students' well-being, leading to heightened anxiety profoundly, negative mood shifts, and alterations in immune function (SHAMS et al. 2010). Understanding these dynamics provides valuable insights into the complex interplay between psychological stressors and immune function, offering potential avenues for intervention and support. This study assesses the direct relationship between exam-induced stress and its impact on immune-related outcomes. In this study, we surveyed students across three educational stages, i.e., school level (SL), undergraduate level (UG), and postgraduate level (PG), which represent diverse academic disciplines. The main objective of this study was to analyze the stress levels experienced by students during exam periods and how it affects their health and immunity. During the study, significant variations were observed in stress levels between genders, and different stress levels were investigated to determine how they correlate with various health outcomes, revealing differences in health impacts between males and females.

#### 2 Materials and Methods

#### 2.1 Survey and subject selection

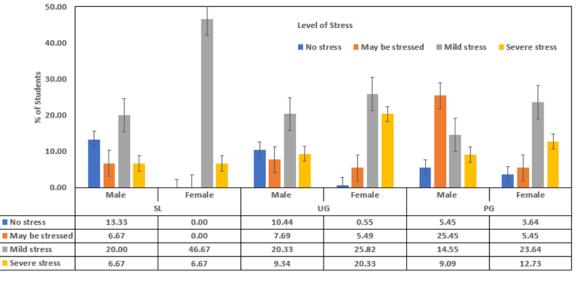
A random questionnaire study was conducted online using Google Forms during the academic year 2021-2022, following the COVID-19 pandemic in April and May 2021-2022. The questionnaire primarily aimed to assess the levels of stress and the related symptoms experienced by students during exam periods. All questionnaires were completed, although some respondents were excluded from the study as the information was inaccurate. Stress levels were categorized into four tiers: no stress, maybe stressed, mild stress, and severe stress, with symptoms also categorized according to stress severity. A total of 252 students responded to the questionnaire. Among them, 15 were SL students, 182 were from UG, and the remaining 55 belonged to PG. All the questionnaires were filled out with prior consent from the participants, including the fact that the identity of the subjects shall remain confidential.

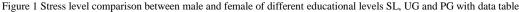
#### 2.2 Study Design

The questionnaire consisted of three main sections. The first part gathered personal data such as gender, age, program of study, and current class levels. In the second part, stress levels were assessed as no stress, maybe stressed, mild stress, and severe stress, along with associated symptoms like increased heart rate, stomach upset, nausea, diarrhea, headaches, lower back pain, menstrual cycle changes, feeling a "lump in the throat," sore or aching muscles, fatigue that does not improve with sleep, feeling uncoordinated and increased or decreased appetite which may be accompanied with weight loss or gain. Additionally, participants rated their level of weakness as slightly weak, moderately weak, very weak, and no weakness at all and indicated cognitive effects such as memory problems/forgetfulness, disorientation (state of mental confusion), confusion, slowness in thinking, analyzing or comprehending (understand) and loss of objectivity (ability to make decisions based on facts rather than on your personal feelings or beliefs). Finally, the third part of the questionnaire asked whether participants experienced several health outcomes during exam time, such as diseases, digestive problems, infections, or inflammation during exam periods, with response options provided as Yes, No, and Maybe.

#### 2.3 Data Interpretation

The survey data was structured into a tabular format to organize responses regarding stress levels for males and females across different educational levels (SL, UG, PG). A graph depicting this information was generated using Excel, and the data has been provided in (Figure 1). Assessment of co-occurrence between health outcomes and stress levels among participants was done by Venn diagrams created using Meta-charts. We utilized the data from the Venn diagrams by converting it into percentage values and organizing it into a tabular format. This method has also analyzed how different health outcomes coincide with varying stress levels. The collected survey data on various health outcomes involved assigning a value of 1 to responses indicating "Yes" or "Maybe." Specifically, "Maybe" was assigned a value of 1 only





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Table 1 Demographic and Education level of the studied respondents		
Studied Characteristics	MALE	FEMALE
E	EDUCATIONAL LEVELS	
School Level (SL)	7	8
Undergraduate (UG)	87	95
Post Graduate (PG)	30	25
	STRESS LEVELS	
No stress	24	3
Maybe stressed	29	14
Mild stress	48	65
Severe stress	23	46
	WEAKNESS LEVELS	
No weakness at all	33	12
Slightly weak	58	52
Moderately weak	26	51
Very weak	7	12
HEALTH OUTCOMES		
Digestive problems	39	58
Infection	18	23
Inflammation	24	22
Disease	36	63

when respondents exhibited at least one symptom of stress. Responses indicating "No" were assigned a value of 0 for all health outcomes except for levels of weakness.

#### 2.4 Statistical Analysis

The data obtained from the survey study was statistically interpreted for correlation among several parameters and the significance of the data. Correlation analysis and one-tailed ANOVA were performed using the data analysis package of MS-Excel 2010 and PAST 4.03. The stress level was marked on a scale of 0-3 based on no, mild, moderate, or severe stress.

#### **3 Results**

#### 3.1 Demographic details

Students under three categories were selected, i.e., SL < age 17 years; UG < 18- 22 years; and PG > 23 years. Within SL, seven subjects were male, and eight subjects were female. Within UG, 87 subjects were male, and 95 subjects were female. Within PG, 30 subjects were male, and 25 subjects were female. Among the total

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#### 3.2 Level of stress among students of various groups

The survey encompassed three educational levels, SL, UG, and PG, with notable findings across each category. For SL students (15 participants), stress responses varied between genders. Among male students, 20% reported mild stress, while 6% reported severe

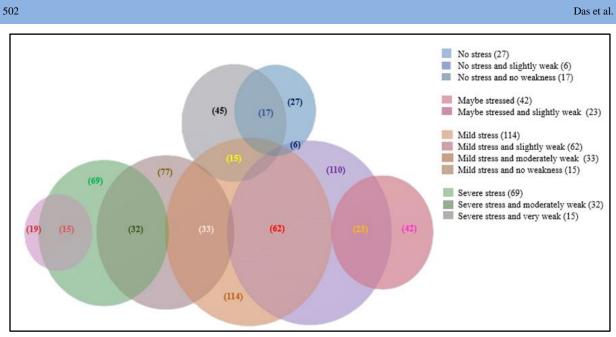


Figure 2 Venn diagrams showing co-occurrence between stress and weakness levels (numbers withinn parentheses indicate no. of respondents)

stress. Conversely, female students exhibited different stress patterns, with no respondents indicating no stress and a significant proportion experiencing mild (46%) and severe (6%) stress. Notably, the highest incidence of stress among female students was observed during exam periods. Similarly, UG students (183 participants) displayed gender-disparate stress responses, and 20% of male students reported mild stress, while 9% reported severe stress. Conversely, only a minimum of 0.55% of female students reported no stress. However, a substantial portion of female students reported experiencing mild (25.8%) and severe (20.3%) stress levels. The data collected from PG students (55 participants) also showed similar stress trends. Among male respondents, 25.4% expressed being may be stressed, while 14.5% and 9% reported mild and severe stress, respectively. In contrast, female postgraduate students exhibited higher stress levels, with 23.64% experiencing mild stress and 12.7% reporting severe stress (Figure 1). Based on the survey results, it is evident that stress among students is a prevalent issue, particularly during exam times. Noticeably, the survey findings highlight a concerning trend of heightened stress levels among female students across all educational levels.

# 3.3 Co-occurrence of various health outcomes with different stress levels

#### 3.3.1 Weakness level

The analysis of the co-occurrence between different stress levels and weakness has been observed using Venn diagrams. These diagrams demonstrate an overlapping percentage of 38% among respondents experiencing "no stress" who also report "no weakness." However, no significant overlap was observed between

Journal of Experimental Biology and Agricultural Sciences http://www.jebas.org "no stress" and other levels of weakness. Furthermore, no cooccurrence was observed between respondents who reported being "may be stressed" and "very weak." However, there were similar percentages of overlap between "may be stressed" and "slightly weak" (21%) and between "may be stressed" and "no weakness" (22%). Among respondents experiencing "mild stress," the majority (56%) were found to be "slightly weak," while only 16% reported being "very weak." In the case of respondents reporting "severe stress," the maximum co-occurrence was observed with the "very weak" level, accounting for 79%. Conversely, there was minimal cooccurrence (7%) with the "no weakness" level (Figure 2).

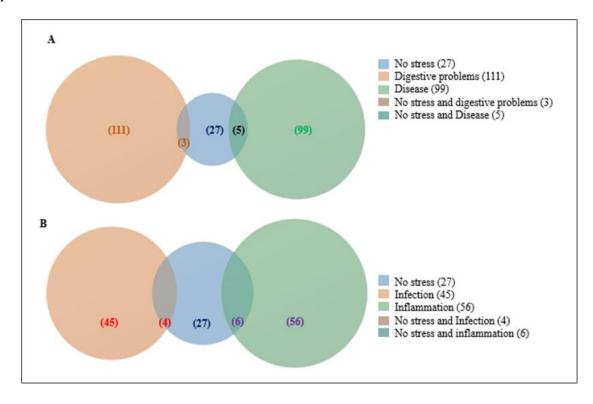
#### 3.3.2 Digestive problems

The analysis of the co-occurrence between different stress levels and digestive problems was observed using Venn diagrams. It was noticed that 49% of respondents experiencing mild stress (Figure 3\_III) and 39% of those facing severe stress encountered digestive problems during the exam period (Figure 3\_IVA). In contrast, only 3% of respondents who reported no stress encountered digestive issues (Figure 3\_IA).

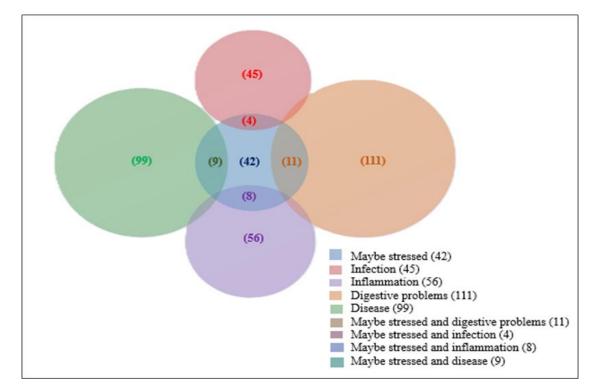
#### 3.3.3 Infection

Venn diagrams were used to analyze the co-occurrence between stress levels and infection. The findings revealed that 49% of respondents experiencing severe stress were susceptible to infection (Figure 3\_IVB). In contrast, 33% of those facing mild stress were prone to infection (Figure 3\_III). While a mere 9% of those experiencing either stress or no stress were similarly affected (Figure 3\_II; 3\_IB).

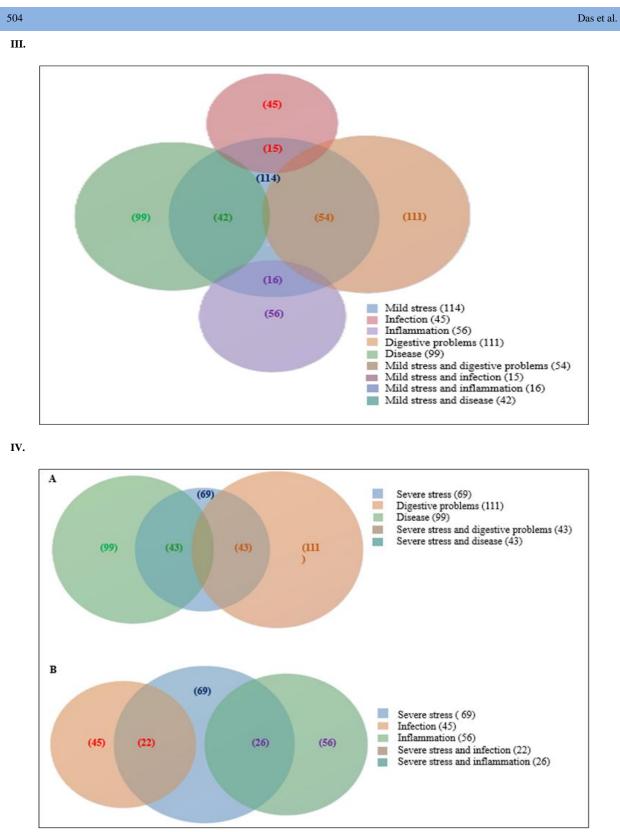


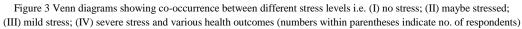


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#### 3.3.4 Inflammation

The co-occurrence between different stress levels and inflammation was examined using Venn diagrams. It was noted that 46% of respondents under severe stress were susceptible to inflammation (Figure 3\_IVB), while only 11% of those experiencing no stress encountered inflammation during the exam period (Figure 3\_IB).

#### 3.3.5 Disease

Examining Venn diagrams, the co-occurrence between various stress levels and prone to diseases was noted. The diagrams revealed that respondents with mild stress (42%) and severe stress (43%) exhibited disease susceptibility (Figure 3\_III; 3\_IV.A). Conversely, only a tiny proportion (5%) of respondents

experiencing no stress suffered from any form of disease during the exam period (Figure 3\_IA).

#### 3.4 Correlation analysis of stress levels and health outcomes

Pearson correlation study among the parameters questioned in the survey showed an overall positive correlation among the level of stress, digestive problems, weakness, prone to disease and infections, and inflammation. Stress level was found to show a maximum positive correlation with weakness (r = 0.461) followed by digestive problems (r = 0.343). A moderate correlation was found with 'prone to disease' (r = 0.314), and a very low positive correlation could be detected with 'prone to infection' and 'inflammation.' The survey data was also subjected to one-tailed ANOVA analysis to understand if the variances of means of the response of each question by 250 subjects varied significantly. The

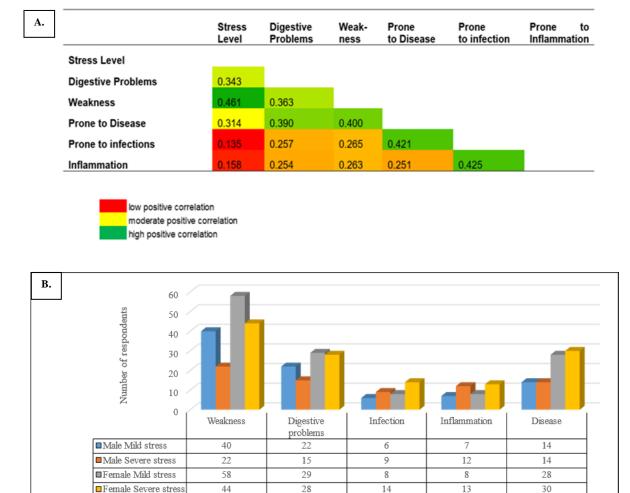




Figure 4 Correlation analysis based on survey response on stress levels and various health outcomes (A); Plot showinghealth outcomes of mild and severe stress in male and female subjects (B)

Journal of Experimental Biology and Agricultural Sciences http://www.jebas.org p-value for one-tailed ANOVA analysis was 0.000, much below the  $\alpha$  of 0.05 with a very high F-value, i.e., 174.31. The analysis indicates that the variances of means of each parameter surveyed are unequal. Hence, the differences are significant (Figure 4A). A correlation between stressed male and female respondents and health outcomes has also been noted. Interestingly, both severe and mild stress appear to affect females more significantly, rendering them more prone to various health issues such as weakness, infection, inflammation, and disease compared to their male counterparts experiencing similar levels of stress. Conversely, mild and severe stress among males correlates more strongly with weakness than other health outcomes (Figure 4B).

#### **4** Discussion

The findings of this study shed light on the significant prevalence of stress among individuals across different educational levels, especially during examination periods. Across SL, UG, and PG, the study's results indicate notable gender-dependent differences in stress responses. Among SL students, females exhibited substantially higher rates of mild and severe stress compared to males, with 46% reporting mild stress and 6% reporting severe stress. Similarly, UG and PG female students showed elevated stress levels, with significant proportions experiencing mild and severe stress compared to their male counterparts (Figure 1). These findings resonate with existing research by Shah et al. (2010) and Eman et al. (2012), which emphasize higher levels of perceived stress among female students, particularly at the PG level. This trend is consistent with previous studies conducted by Dyrbye et al. (2006) and Wadikar et al. (2017), further reinforcing the notion of gender-specific stress responses in academic settings. While Sulaiman et al. (2009) reported lower stress levels among male students compared to females, a body of literature including studies by Hall et al. (1998), Adlaf et al. (2001), Hudd et al. (2000), Kelly et al. (2006), and Kudielka and Kirschbaum (2005) consistently support the notion that female students tend to report greater levels of stress overall. These significant psychological differences between men and women may primarily arise from women's greater inclination to express their concerns and emotions openly. This contrast may be attributed to women's heightened vulnerability to a range of physical and emotional challenges, including depression and fatigue, which are more prevalent among women than men (Basudan et al. 2017; Zinurova and Dehart 2018). Remarkably, our study data revealed a distinct trend between stress levels and perception of weakness during exam time; as stress levels intensify, so does the likelihood of experiencing feelings of weakness. The Venn diagram analysis reveals intriguing patterns between stress levels and weakness. While "no stress" correlates with "no weakness" in 38% of cases, "mild stress" predominantly aligns with "slightly weak" in 56% of respondents, and "severe stress" is strongly associated with "very weak" in 79% of cases (Figure 2). The findings from our data align closely with existing literature, as evidenced by the study conducted by Murphy et al. 2010 and their investigation revealed a notable decrease in secretory immunoglobulin A (S-IgA) levels by 29% during high-stress periods, such as exam weeks, with concurrent increases in cortisol levels (Murphy et al. 2010). Cohen et al. (2001) highlight the indirect influence of stress on immune response through various behavioral changes triggered by stress, including alterations in smoking habits, sleep patterns, and dietary choices (Cohen et al. 2001), which may further exacerbate the immune system's vulnerability during stressful periods. Expanding on these insights, a wealth of literature underscores stress's profound effects on university students' immune function. For instance, research has linked exam stress to reductions in natural killer (NK) cell activity. The recent study also delves into student health, uncovering notable connections between stress levels and health outcomes. Among those experiencing severe stress during exams, a significant 39% reported digestive issues (Figure 3\_IVA), while for those facing mild stress, 49% reported similar problems (Figure\_ 3III). Interestingly, only 3% of respondents experiencing no stress reported digestive issues (Figure IA), underscoring the strong correlation between higher stress levels and gastrointestinal ailments; various studies also support this finding. For instance, Huerta-Franco et al. (2013) highlighted how stress can trigger gastrointestinal health problems, while Knowles et al. (2008) observed spikes in digestive troubles, cortisol levels, and alterations in gut bacteria during exam periods. The link between stress and stomach discomfort, including conditions like irritable bowel syndrome (IBS), has been extensively studied (Moloney et al. 2016). Faixová et al. (2023) found that nearly 60% of students encountered digestive issues during exams, with women being more affected. Our research corroborates this, revealing high rates of digestive problems among both severe and mild stress females (Figure 4B). Our study also found a strong link between severe stress and susceptibility to infection, with 49% of severely stressed respondents (Figure 3\_IVB) affected compared to 33% of those with mild stress (Figure 3III), while only 9% of no-stress respondents are prone to infection (Figure 3\_IB). These findings suggest that stress may compromise immune function, increasing infection vulnerability. These findings were also supported by several studies, where they found that stress can compromise the immune system by reducing the production of key cytokines like IFN-c and IL-2, weakening defense against viral infections, as indicated by Kang and Fox (2001). According to Assaf et al. (2017), medical and health sciences students facing heightened stress during their final exams commonly reported experiencing symptoms such as colds, flu, tonsillitis, and noticeable hair and skin health declines throughout the semester. High stress levels, particularly during exams, are associated with increased cortisol levels, shifting the Th1/Th2 cytokine balance towards Th2 dominance, potentially leading to immune dysregulation rather

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than suppression (Assaf et al. 2017). This study also revealed that 46% of the respondents under severe stress experienced inflammation (Figure 3\_IVB), compared to 11% with no stress (Figure 3\_IB), emphasizing a significant link between stress and inflammation levels. Other research has shown similar results, suggesting that stress can activate inflammation in the body, with an increase in Th2 cytokines like IL-4, IL-5, and IL-13, which can suppress proinflammatory responses. However, this may worsen atopic diseases like asthma or atopic dermatitis. Stressful periods, such as exams, can exacerbate conditions like asthma, as seen with elevated IL-5 levels in students with mild asthma during exams (Wills-Karp 2004; Kamezaki et al. 2012). This study also observed that both mild stress of 42% (Figure 3\_III) and severe stress of 43% were associated with disease susceptibility (Figure 3\_IVA). In contrast, only 5% of no-stress respondents experienced any disease (Figure 3\_IA) during the exam period, suggesting that stress may play a role in disease susceptibility, with higher stress levels correlating with a greater likelihood of experiencing health issues during stressful periods like exams. The findings of this research revealed a relationship between disease and stress levels, and these are reinforced by Trueba et al. (2013), who propose that prolonged stress and depression could result in a reduction in nitric oxide (NO) production, potentially impacting the progression of cardiovascular diseases. Similarly, Barth et al. (2004) and other researchers have also established an association between chronic stress and cardiovascular risks. Researchers studying medical students undergoing exam stress discovered that stress contributes to elevated cholesterol levels. Because blood pressure and serum cholesterol tend to rise during stress, the association between stress and hypertension has long been suspected (Salleh 2008).

Our Pearson correlation analysis revealed that stress exhibits its strongest correlation with weakness (r = 0.461) and digestive problems (r = 0.343), indicating that increased stress levels are closely related to increased reports of weakness and digestive issues among students (Figure 4A). These findings are consistent with research by Faixová et al. (2023), which highlighted that a majority of female students (66.6%) and nearly half of male students (46.7%) experienced higher digestive problems during exam periods. Furthermore, our observations also revealed distinct gender disparities in how stress influences health outcomes, highlighting a more significant impact on females. Specifically, the findings of this study indicate that both severe and mild stress disproportionately affect females, leading to increased susceptibility to health issues such as weakness, infection, inflammation, and disease compared to males experiencing similar levels of stress (Figure 4B). These findings align with research indicating that neuroinflammatory conditions like multiple sclerosis, Alzheimer's disease, and chronic pain are more prevalent among women (Loram et al. 2012). Oertelt-Prigione (2012) and Markle et al. (2013) propose that dysregulated immunocyte Additionally, research indicates that females may be more vulnerable to the effects of peripheral inflammation. In a study where healthy men and women received endotoxin, both groups had increased proinflammatory cytokines. However, only women showed cytokine-induced depressed mood and feelings of social disconnectedness (Moieni et al. 2015).

In the future, this study presents exciting opportunities for further investigation. Research endeavours could explore blood biomarkers to understand the dynamics between stress and immune response, elucidating the physiological mechanisms connecting exam-induced stress and immunity. Furthermore, analyzing the gut microbiome of individuals experiencing digestive issues before and after exams may offer valuable insights into the impact of stress on gut health and overall well-being. These efforts hold the potential to advance our comprehension of the intricate relationship between stress, immunity, and gut health.

#### Conclusion

The study underscores a notable correlation between stress levels and diverse health outcomes among students across various educational levels during exam periods. Female students experience more pronounced stress impacts than their male counterparts at UG and PG levels. The findings indicate that females facing equivalent stress levels are more vulnerable to health issues than males. Elevated stress levels correlate with increased occurrences of weakness and digestive problems.

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