

# Stress Levels and Coping Strategies in Medical Students and its Association with Salivary IL-6 Levels

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## Abstract

**Background:** Medical students face ongoing stress during their training but have developed coping mechanisms. Stress alters various physiological processes, including pro-inflammatory markers like Interleukin-6(IL-6). The present study was conducted to assess stress levels and coping strategies in medical students and their association with salivary IL-6 levels. **Methods:** This descriptive study was conducted after obtaining institutional ethical clearance. A total of 76 consenting undergraduate medical students answered the Cohen's perceived stress scale and BriefCOPE questionnaire. Unstimulated saliva was used to assess salivary IL-6 levels using a Diaclone human IL-6 ELISA kit and the data obtained was analyzed. **Results:** Out of the 76 participants, 59(77.6%) were female and 17(22.4%) were male. Mild stress was reported by 9 students, moderate by 53, and severe stress by 14 students. Based on Kruskal-Wallis p test, most students used approach coping for stress of all levels. This active strategy involves problem-solving and future planning. Approach coping has shown better responses to adversity, physical health, and emotional responsiveness. Mild and moderate stressed students used acceptance, positive-refrain, and planning, while severe stressed students used planning, self-distraction, and self-blame. Despite the perceived stress, there were no significant differences in the salivary IL-6 levels among the three categories. **Conclusion:** 'Approach' coping was commonly used and linked to positive outcomes. Despite this, a number of students have reported to experience stress. Therefore, more effective strategies are needed to handle stress and demands of the profession effectively. Further research with larger samples is recommended to explore salivary IL-6 levels' relation to stress.

## Introduction

Stress is defined as a state of psychological and physiological imbalance resulting from a disparity between situational demands and the individual's ability and motivation to meet those needs. It is the mental and physical response and adaptation by our bodies to actual or perceived changes and challenges in life.<sup>1</sup>

Students face different domains of stress including academic, interpersonal and intrapersonal stress, teaching and learning-related stress, social stress, drive and desire-related stress, and group activity stress. In the case of a medical student, the amount of stress in these domains is so high, making it the second most stressful academic course in India.<sup>1</sup> Acute stress results in decreased cognitive function, impaired attention, problem-solving abilities, and performance. Chronic effects of stress on the students increase the risk of cardiovascular diseases, type 2 diabetes mellitus, metabolic syndrome, depression, as well as suicide. Therefore, addressing stress-related psychological concerns is crucial.<sup>2</sup>

Different students have developed their own coping strategies. Coping with stress can be defined as the process of managing external or internal demands that are perceived as taxing on personal capacities and resources. Many coping strategies like effective time management, social support, and positive reappraisals have been broadly grouped as approach and avoidant strategies.<sup>3</sup>

With the start of the COVID-19 pandemic, there has been increasing levels of stress on the medical students. Being a novice to management and etiquette during a pandemic situation, there were concerns regarding the safety of family, friends, and oneself. Effectively adapting to virtual learning in contrast to the age-old classroom and bedside learning. All these factors were new stressors that had to be efficiently faced. This led the students to search for new coping mechanisms to effectively navigate such stressors and also develop the skill to face any such situation arising suddenly in future.<sup>2,3</sup>

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Stress also has considerable corollary on the biochemical aspects of the body. Interleukin-6 (IL-6) is known to be altered in many diseases with studies associating marked rises in levels of IL-6 with psychological stress and depression.<sup>4</sup> Any stressor that has a negative effect on the central nervous system can stimulate the production of IL-6, initiating various peripheral immunological responses. Different compartments of the immune system can react differently to the stressor stimulus. The most common effect can be seen on endocrine and secretory glands, bringing about changes in the physiological process based on the substance secreted. Salivary IL-6 is a considerably easy marker in terms of sample collection and analysis. Saliva collection is a non – invasive method and an alternative to stressful serum sample collection.<sup>5</sup> The study aims to find the association of salivary IL-6 with stress perceived by medical students. The study also aims to determine the coping strategies used for different categories of perceived stress among medical students.

## Methods

An institution-based descriptive study was conducted from February 2021- January 2022 in the Department of Physiology, Father Muller Medical College, Mangalore- India, after obtaining institutional ethical clearance. The ethical clearance was issued by Father Muller Institutional Ethics Committee (reference no – FMIEC/CCM/113/2021).

The sample size was estimated to be 75 which was determined using the *r* value (correlation coefficient of the reference study) with a confidence interval of 95%, using a reference study.<sup>4</sup> The study population consisted of medical students in their second year onwards to interns and were enlisted by snowball sampling technique. From the students who satisfied the inclusion and exclusion criteria, 76 were randomly chosen to participate in the study. Written informed consents of all the participants were taken with confidentiality assured. Students who smoked cigarettes, consumed alcohol, chronic drug users, and students taking anti-anxiety and anti-depressants medications were excluded. Students suffering from acute or chronic infections were also excluded.

The participants then underwent clinical examination to rule out any systemic illness which was then followed by answering the Cohen's perceived stress scale and BriefCOPE questionnaire on coping strategies provided to the participants as Google Forms. Unstimulated saliva was collected to assess the levels of IL-6.

### Questionnaire

Cohen's Perceived Stress Scale (PSS) - a survey consisting of 10 questions that evaluates the feelings and thoughts in the previous month was used to assess the perceived stress in students, where the participants scored from 0 (never) to 4 (very often) for each question. For questions 4, 5, 7, and 8 reverse scoring was applied. The students were categorized as having mild (score 0-13), moderate (score 14-26), and severe perceived stress (score 27-40) based on the total score obtained.<sup>6</sup> BriefCOPE – a 28 item

questionnaire - was used to assess the coping strategies of the students to handle stress. The participants indicated how frequently they used each coping strategy on a scale, ranging from 0 (*I haven't been doing this at all*) to 4 (*I have been doing this a lot*). The coping strategies were broadly categorized into avoidant and approach, where self-distraction, self-blame, denial, substance abuse, venting, and behavior were subcategories included in avoidant category and active, emotional, refrain, acceptance, planning, and information were subcategories included in approach category with each sub-category having 2 questions (score 2-8). The approach and avoidant categories were scored in the range of 12-48. This questionnaire has been pre-validated in the health-related research field.<sup>7</sup>

### Salivary IL-6

Ten milliliters of unstimulated saliva were collected from each participant without spitting, a minimum of 1 hour after breakfast. The sample was centrifuged at 3400rpm for 7 minutes to get pure saliva in the supernatant. Hundred microliters of supernatant, along with control and diluent standard, and 50µl diluted biotinylated antibody were added into the ELISA wells and then incubated for 1 hour at room temperature. A Diaclone human IL-6 ELISA kit was used for the analysis. The sample was washed 3 times before the addition of 100µl of diluted streptavidin-HRP which was again incubated at room temperature for 30mins, producing blue colored complex. Hundred microliters of TMB substrate and 100µl of stop reagent were added after washing 3 times which arrests further color development, producing a final yellow color. The intensity of the color is proportional to IL-6 levels in the saliva sample. Absorbance was read at 450nm using a Biorad reader. The optical density obtained was used to calculate the levels of IL-6 in each sample. The results were expressed as picogram/milliliter (pg/ml) of saliva. The sensitivity/ minimum detectable dose of the IL-6 kit used was 2 pg/ml.<sup>8</sup>

### Statistical Analysis

Data was collected using Excel and analyzed using SPSS software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, New York, IBM Corp).

Mean ± SD for data following normal distribution and median/ interquartile range was used for skewed values. The PSS and BriefCOPE questionnaires were graded using Likert's Scales. The mean and standard deviation for each question was calculated. Using PSS, the students were categorized as mild (score 0-13), moderate (score 14-26), and severe perceived stress (score 27-40). In BriefCOPE, a score of 2-4 indicated that the coping strategy was least used by the student, a score of 4-6 indicated that the coping strategy was moderately used, and a score of 6-8 indicated the coping strategy was often used by the student to handle stressors. A Kruskal-Wallis test was used to explore the statistical significance of the questionnaire and demographic details. A p-value <0.05 was considered statistically significant.

Results

Table 1. Demographic Characteristics with Lifestyle Data of the Participating Students.

		Count	%	Kruskal-Wallis test p-value
Gender	Female	59	77.6%	0.268
	Male	17	22.4%	
Age	18 - 20 years	25	32.9%	<0.001
	21 - 23 years	51	67.1%	
Currently Studying In	Phase II	8	10.5%	0.001
	Phase III Part I	15	19.7%	
	Phase III Part II	40	52.6%	
	Interns	13	17.1%	
Sleep	1. Disturbed	7	9.2%	0.000
	2 Undisturbed	69	90.8%	
Bowel/Bladder Habits	Regular	69	90.8%	0.017
	Irregular	7	9.2%	
Lifestyle	Sedentary	37	48.7%	0.433
	Active	39	51.3%	
Exercise	Regular	24	31.6%	0.082
	Irregular	52	68.4%	
Are You Aware of The Benefits of Yoga And Breathing Exercises?	Yes	68	89.5%	0.906
	No	2	2.6%	
	Maybe	6	7.9%	
Do You Practice Yoga Or Breathing Exercises?	Yes	13	17.1%	0.264
	No	47	61.8%	
	Maybe	16	21.1%	

A total of 76 medical undergraduate students in their second year to intern year participated in the study. Table 1 shows the demographic details of the participating students. The majority of the participants were females (77.6%) while only 22.4% of students were males.

Based on the response to the Cohen Perceived Stress Scale, students were categorized into mild, moderate, and severe stress as depicted in Figure 1. Nine students perceived mild stress, 53 moderate, and 14 perceived severe stress.

Figure 1. Performance of Respondents on the Cohen's Perceived Stress Scale (PSS).

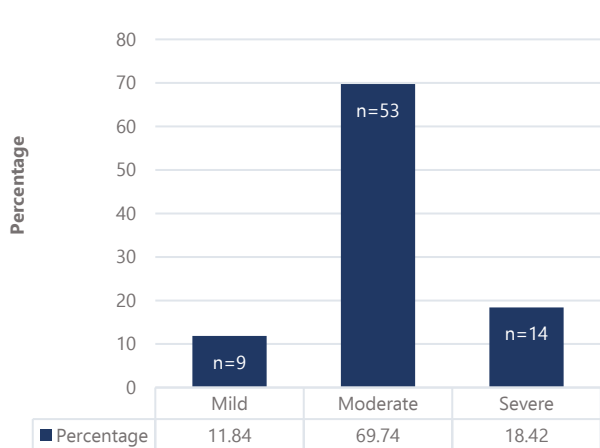


Table 2. Awareness and Practice of Yoga Among the Three Perceived Stress Categories.

		Mild Stress Count (Percentage)	Moderate Stress Count (Percentage)	Severe Stress Count (Percentage)
Are you aware of the benefits of yoga and breathing exercises?	Yes	8(11.8%)	47(69.1%)	13(19.1%)
	No	0(0.0%)	2(100.0%)	0(0.0%)
	Maybe	1(16.7%)	4(66.7%)	1(16.7%)
Do you practice yoga or breathing exercises?	Yes	2(15.4%)	10(76.9%)	1(7.7%)
	No	6(12.8%)	29(61.7%)	12(25.5%)
	Occasion ally	1(6.3%)	14(87.5%)	1(6.3%)

Table 2 compares the awareness and practice of yoga among the three perceived stress categories. Most of the students were aware of the benefits of yoga (89.5%) but only a few actually practiced it (17.1%). Yoga can be used to manage stress; if the students use yoga as a method of coping, we can incorporate yoga into their curriculum. The p-value obtained by the Kruskal-Wallis test were 0.906 and 0.264, hence statistically not significant.

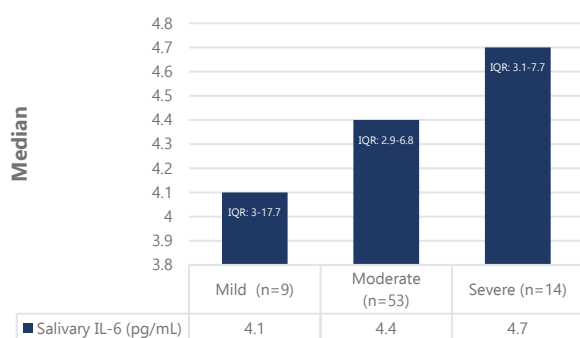
Table 3. Responses to BriefCOPE Questionnaire.

	Mild stress (n=9) mean± SD	Moderate stress (n=53) Mean± SD	Severe stress (n=14) Mean± SD	Kruskal-Wallis test p value
<b>Avoidant</b>	<b>21.11±5.09</b>	<b>23.98±4.57</b>	<b>30.50±4.67</b>	<b>&lt;0.001</b>
a. Denial	4.11±1.05	4.04±0.88	4.79±0.89	0.026
b. Substance abuse	2.67±1.32	2.45±0.91	2.64±1.45	0.759
c. Venting	3.00±1.12	4.26±1.42	5.57±1.50	<0.001
d. Behavior disengagement	3.00±1.41	3.53±1.35	4.86±1.70	0.004
e. Self-distraction	4.89 ± 2.15	5.21±1.41	6.36±1.50	0.029
f. Self-blame	3.44±1.67	4.49±1.59	6.29±2.20	<0.001
<b>Approach</b>	<b>30.22±5.36</b>	<b>30.30±6.01</b>	<b>34.93±5.85</b>	<b>0.035</b>
a. Positive refrain	5.67±1.12	5.70±1.64	5.86±2.03	0.945
b. Planning	5.56±1.51	5.51±1.55	6.50±1.51	0.104
c. Acceptance	6.11±1.54	5.51±1.65	6.21±1.67	0.272
d. Emotional support	4.44±1.01	4.81±1.73	5.07±1.94	0.693
e. Use of instrumental support	4.33±1.50	4.49±1.44	6.07±1.49	0.002
f. Active	4.11±1.36	4.28±1.28	5.21±0.80	0.032
Humor	4.11±1.90	4.51±1.93	6.21±1.76	0.008
Religion	5.11±1.27	4.89±1.87	5.36±2.10	0.689

The responses to the BriefCOPE questionnaire are presented in Table 3. The data has been compared against mild, moderate, and severe stress. Kruskal-Wallis test reveals approach coping strategies to be highly significant with a majority of students using it in all the categories of perceived stress.

Despite of perceived stress, there was no significant difference seen in the salivary IL-6 levels of the students among the three categories. The median salivary IL-6 in mild category (n=9) was 4.1 pg/ml (interquartile range = 3-17.7 pg/ml); 4.4 pg/ml (interquartile range = 2.9-6.8 pg/ml) in moderate category (n=53); and was 4.7 pg/ml (interquartile range = 3.1-7.7 pg/ml) in severe category (n=9) which was not statistically significant (p value=0.823).

**Figure 2. Median Salivary IL-6 Levels with an Interquartile Range of Three Perceived Stress Categories.**



## Discussion

This study aimed to investigate the stress levels and coping strategies in undergraduate medical students and their association with salivary IL-6. A total of 76 students participated in our study, of which 22.4% were males and 77.6% were females. Although stress in medical students has been studied previously, we aimed to assess the stress levels in the COVID era. The various coping strategies used by the students have also been tabulated. Based on Cohen's Perceived Stress Scale, we found that the majority of the students (69.7%) were moderately stressed, 18.4% were severely stressed, and 11.8% were found to be mildly stressed. The lowest score obtained was 5 and the maximum score obtained was 36. This indicates that all students were experiencing some degree of stress. Our study findings are comparable to the study done by Bhavani Nivetha M. et al, who found the prevalence of mild, moderate, and severe stress was 20%, 74%, and 6% respectively.<sup>9</sup> Another study, reported the stress levels to be 51.7% among medical students.<sup>10</sup>

We did not find a significant difference between gender and stress levels. A study by Amr et al also showed similar results, where the level of perceived stress and number of stressors were similar between males and females.<sup>11</sup> A study conducted by Das *et al* had concluded that gender influenced the level of depression rather than the level of stress.<sup>12</sup>

In our study, second-year students reported a higher degree of stress as compared to other years, but as the first-year students were excluded due to ethical considerations, it could be a limitation of the study. A previous study showed that stress was substantially greater in second and third-year students than in first-year students ( $p < 0.05$ ).<sup>13</sup> This shift in the stress category could indicate effective coping strategies employed by the students. It could also be due to adaptation to the stress of medical education as the students pass out each year evincing their preparedness for the future. This is contrary to findings by Supe, Sherin, and Shaikh et al who have reported a higher level of perceived stress among third and fourth-phase students.<sup>14,15,16</sup>

Mental and physical adverse effects have been well-documented side effects of stress.<sup>17</sup> Cognitive decline and learning difficulties are associated with high levels of stress.<sup>18</sup> It is hypothesized that

stress activates the hypothalamic-pituitary axis which causes changes in neurotransmitters serotonin, dopamine, and norepinephrine, which have serious mental effects like anxiety and depression.<sup>2</sup> Thus, there is a need to find ways to manage stress effectively.

The median weight of the students in the mild category was 69 kg (interquartile range 58-82) mean $\pm$ SD=72.94 $\pm$ 19.42, the moderate category was 60 kg (53.5-70) mean $\pm$ SD=61.66 $\pm$ 11.38 and the severe category was 54.5 kg (46.5-64.3) mean $\pm$ SD=55.39 $\pm$ 9.28, which was statistically significant ( $p$  value=0.005). This may be due to unhealthy eating habits that was used as a stress buster, a coping strategy adopted, leading to the students perceiving mild stress but at the same time gaining more weight than their peers.<sup>19</sup>

Although 89.5% of participants were aware of the benefits of yoga, only 10 students in the moderate category were actually practicing it. This result is consistent with the study done by Kathapillai where 89.26% of medical students knew the benefits of yoga but only 30.87% were practicing it.<sup>20</sup> Although students were aware of the benefits of yoga, they did not practice it. Thereby, the incorporation of yoga into the curriculum can be planned for the future.

Using the Brief Cope questionnaire, we studied the coping methods of the students. We found that in all grades of stress, the 'approach' method was the preferred coping strategy amongst respondents. The most commonly used coping strategies in students with mild stress were found to be acceptance, positive refrain, and planning. Students with moderate stress adopted positive refrain more commonly. Whereas in students with severe stress planning, self-distraction, and self-blame were adopted.<sup>3</sup> Previous work has shown coping strategies such as use of the alcohol, tobacco, and drugs were commonly used by medical students in the United Kingdom.<sup>21</sup> In our study, these were reported to be least commonly used. However, under-reporting cannot be definitively ruled out.<sup>22-25</sup> Among the 'avoidant' coping mechanisms, self-distraction, self-blame, and venting had higher scores and there was also a statistical significance between the groups in its usage. The use of avoidant coping mechanisms like denial and behavioral disengagement were also statistically different between the three groups, with the students in the high-stress group scoring higher. "Denial" is an attempt to reject the reality of a stressful event. "Behavioral disengagement" means giving up or withdrawing efforts to attain a goal. These coping strategies help in tackling stress but they are more likely to worsen the stress inclining them to psychological morbidity. Similar results of anger, distraction, and avoidance being the coping strategies of choice for highly stressed students were reported in other studies.<sup>26-28</sup>

Humor was also commonly reported to be used as a coping mechanism, especially by the high-stress group in our study. Humor is considered to be one of the mature defense mechanisms which contributes to one's resilience and well-being.<sup>29</sup> Among 'approach' strategies, active coping and the use of instrumental support were significantly used by the high-stress

group of students. "Active coping" means taking action or exerting efforts to remove or circumvent the stressor.<sup>28,30</sup> Students have cognitively refashioned stress as a daily challenge. They have embraced it and are coming up with ways to positively and effectively handle the stress experienced during medical school to achieve a socially and financially stable life in the future.<sup>31</sup>

In the present study, there was no significant association between stress levels and salivary IL-6 levels which is consistent with a study done by Edan.<sup>32</sup> But in a study done by Vernaza assessing the relationship between perceived stress and serum IL-6 levels, there was a significant association between the two: with an increase in stress levels, there was a statistically significant rise in serum IL-6 levels.<sup>33</sup> This could be due to various factors like genetics and others that affect salivary IL-6 levels more than serum IL-6 levels.<sup>32,33</sup> In a study done by Izawa et al, they found a significant rise in salivary IL-6 levels following acute psychological stress that was created among the young participants following an arithmetic task, which was attributed to sympathetic activity with cortisol secretion stimulated by the acute stress event.<sup>34</sup>

In conclusion, the common coping strategy employed by the students to handle stress was the approach, which is associated with better outcomes and more stable emotional responses. Despite using the approach strategy, a vast number of students have reported to experience stress. Thus, there exists a need to teach more effective coping strategies to efficiently handle the stress and demands of their profession without compromising their service and health. Incorporation of appropriate interventions and support groups in the curriculum and its impact on the stress levels could be a prospective study. At the same time, the salivary IL-6 levels did not show any association with stress levels with no causation proved, which could be attributed to the smaller sample size, providing room for studies addressing larger population.

Limitations of the study – The study involved self-reporting of stress, thus under-reporting or over-reporting cannot be ruled out. The majority of the participants were females and thus external generalizability to the population will be limited. Equal distribution of genders would have enabled better comparison between the groups. A larger sample size across states will help to better generalize the results. The study has used snowball

sampling technique which could lead to sample and anchoring bias with lack of insight for accuracy regarding representativeness of the target population.

## Summary – Accelerating Translation

### Stress Levels and Coping Strategies in Medical Students and its Association with Salivary IL-6 Levels

The medical students experience stress from the time they enter the medical course. Stress alters various physiological processes, including serum and salivary markers and is also linked to the development of various diseases like hypertension, diabetes mellitus, depression etc. Students have developed their own coping mechanism to handle this stress.

**Aim:** The present study was conducted to correlate the stress levels and coping strategies in medical students and the effect of stress on salivary IL-6 levels among medical students.

**Methods:** This descriptive study was conducted in Father Muller Medical College after obtaining institutional ethical clearance. A total of 76 undergraduate medical students participated in the study. After ruling out Systemic illness clinically Cohen's perceived stress scale and BriefCOPE questionnaire were distributed and collected. Unstimulated saliva was collected to assess salivary IL-6 levels. The data was entered into Excel sheet and analyzed.

**Results:** Out of the 76 participants 59(77.6%) were females and 17(22.4%) were males. Based on the Cohen's perceived stress scale score, students were categorized into having mild stress (9 students), 53 participants with moderate stress, and 14 with severe stress. We found that in all grades of stress, approach method of coping had a higher score indicating that this was the method most employed by the students to handle stress. Under the approach method of coping, the most commonly used coping strategies in students with mild stress were found to be acceptance, positive-refrain, and planning. Students with medium stress adopted positive-refrain whereas students with severe stress, adopted planning, self-distraction, and self-blame. Despite the perceived stress, there was no significant difference seen in the salivary IL-6 levels of students among the three categories.

**Conclusion:** The common coping strategies employed by the students to handle stress was approach, which was associated with better outcomes and a more stable emotional response. In spite of this, a vast number of students have reported to experience stress. Therefore, there is a need to teach more effective coping strategies to handle stress and demands of their profession without comprising their service and health.

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### Author Contributions

Conceptualization: MK, ARB. Data Curation: MK, ARB, CN, CR. Formal Analysis: MK, ARB. Funding Acquisition: MK, ARB. Investigation: MK, ARB, CN, CR. Methodology: MK, ARB, CN, CR. Project Administration: ARB, CN. Resources: MK, ARB, CN, CR. Software: MK, ARB. Supervision: ARB, CN, CR. Visualization: MK, ARB, CN, CR. Writing - Original Draft: MK. Writing - Review Editing: ARB, CN, CR.

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