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Impact of Academic Stress on Oral Microbiota and Medical Student's Immunity

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Impact of Academic Stress on Oral Microbiota and Medical Student's Immunity

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Abstract

Background: Academic stress (AS) is recognized as a reproducible model of psychological stress that can affect immune system regulation. rare reports focus on the correlation between AS and oral microbial composition. Objective: This study aims to investigate the effect of academic examinations on immune function and oral microbial colonization among third-year medical students at the University of Sabratha. Methods: A descriptive cross-sectional study, questionnaires and saliva samples were collected from the students before, and after the end-term examination. A total of 84 students were initially registered, of whom 68 (61 females, 7 males aged 21–25 years) completed a questionnaire survey, and 69 oral samples were collected before examinations.

Result: The finding demonstrated an increase in microbial colonization during the examination period compared to pre-examination samples. Elevated levels of psychological stress were also reflected in students' responses, indicating impaired immunity and a higher prevalence of stress-related symptoms. The association

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between elevated academic stress and reduced host defenses supports the hypothesis that stress-induced immune modulation can create favorable conditions for opportunistic microbial overgrowth. Moreover, during the examination, some genera of the oral-beneficial bacteria, markedly decreased in students with AS. On the other hand, the potential pathogenic genera, such as *Streptococcus mutans Streptococcus pneumoniae*, *Candida* sp significantly increased in the students with AS. And it effects on oral microbes displayed a gender difference among students. Conclusion: A high ratio of AS existed in the students during their examination period, and could significantly alter the oral microbial composition, decrease beneficial microbes, and promote potential pathogenic oral microbes.

Keyword
Psychological stress, Microbial colonization, cortisol, immunity, academic examinations, University of Sabratha.

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تأثير الضغط الأكاديمي على ميكروبات الفم ومناعة طالب الطب

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الملخص

الخلفية العلمية: – يُعدّ الإجهاد الأكاديمي نموذجًا معترفًا به للإجهاد النفسي القابل للتكرار، والذي يمكن أن يؤثر في تنظيم الجهاز المناعي. هدفت هذه الدراسة إلى تقصي تأثير الامتحانات الأكاديمية على وظيفة الجهاز المناعي واستعمار الميكروبات الفموية لدى طلبة السنة الثالثة بكلية الطب في جامعة صبراتة الهدف: تهدف هذه الدراسة الى دراسة تأثير الضغط الأكاديمي على ميكروبات الفم ومناعة طالب الطب. تم تسجيل 84 طالبًا في البداية، أتمّ منهم 68 طالبًا (61 من الإناث و7 من الذكور، تتراوح أعمارهم بين 21–25 عامًا) استبيانًا مُنظّمًا. كما جُمعت 69 عينة فموية قبل فترة الامتحانات، و63 عينة خلال فترة الامتحانات. الطريقة: شملت عملية جمع البيانات استبيانًا ذاتي حيث استُخدمت مسحات فموية (Oral swabs) لتقييم المستعمرات الميكروبية، بينما جرى تقييم التغيرات المناعية المرتبطة بالإجهاد بشكل غير مباشر من خلال الأعراض المُبلّغ عنها ومؤشرات اللعاب.

فقد وُجدت نسبة عالية من التهابات لدى الطلاب خلال فترة الامتحانات، ويمكن أن يُغير هذا الالتهاب بشكل كبير تركيبة ميكروبات الفم، ويُقلل من البكتيريا المفيدة، ويُعزز نمو البكتيريا الممرضة المحتملة. النتائج: أظهرت النتائج انخفاض بعض أنواع البكتيريا المفيدة للفم بشكل ملحوظ لدى الطلاب كما لوحظ زيادة في المستعمرات الميكروبية الضارة مثل المكورات العقدية الطافرة، والمكورات الرئوية، وفطريات المبيضات، بشكل ملحوظ لدى

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هؤلاء الطلاب. كما لوحظ ارتفاع في مستويات الإجهاد النفسي لدى الطلبة، كما انعكس في استجاباتهم، مما يشير إلى تراجع كفاءة الجهاز المناعي وزيادة في انتشار الأعراض المرتبطة بالإجهاد. الخلاصة: – تدعم هذه النتائج الفرضية القائلة بأن الإجهاد الأكاديمي يمكن أن يُقلل الاستجابة المناعية، مما يهيئ ظروفًا ملائمة لنمو الميكروبات الانتهازية.
الكلمات المفتاحية: الإجهاد النفسي، الميكروبات الفموية، الكورتيزول، المناعة، الامتحانات الأكاديمية، جامعة صبراتة

Introduction

Medical education is widely recognized as one of the most demanding academic disciplines. Medical students are exposed to continuous academic pressure resulting from extensive curricula, frequent examinations, prolonged study hours, and high expectations from faculty members and families.[1]. Such stressors may adversely affect both mental and physical health. Academic stress has been associated with various psychological consequences, including anxiety, depression, emotional exhaustion, and burnout. Beyond its psychological impact, chronic stress may also influence physiological systems, particularly immune function. Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to increased cortisol secretion, which can suppress immune responses and alter host defense mechanisms. [2][3][4]

In addition to its effects on systemic immunity, academic stress may influence the composition of the oral microbiota. The oral cavity harbors a complex microbial community that plays a crucial role in maintaining both oral and systemic health. Psychological and physiological stress may disrupt microbial homeostasis through immune suppression, hormonal changes, and alterations in salivary composition, thereby increasing susceptibility to opportunistic microorganisms.[5][6] Among these, Candida species, particularly *Candida albicans*, and some species of bacteria are considered common opportunistic fungi capable of colonizing the oral cavity under conditions of immune imbalance. Increased oral candida and some bacterial sp. has been associated with immunosuppression, stress and changes in host defense mechanism [5] [7].

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Medical students may be particularly vulnerable to stress-induced immune alterations, which can manifest as fatigue, recurrent infections, headaches, gastrointestinal disturbances, and sleep disorders. Despite the growing recognition of the relationship between academic stress, immune function, and oral microbial changes, limited studies in Libya have investigated this association among medical students. Therefore, this study aims to evaluate the effect of academic stress on immune-related health and oral microbiota, including oral some bacterial and Candida colonization, among third-year medical students at the Faculty of Medicine, Sabratha University.

Psychological stressors can alter human immune function, which might predispose one to an increased susceptibility to infections [8]. Activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system during stress results in increased cortisol secretion and other mediators. While such responses are adaptive in acute stress, prolonged or recurrent stressors such as academic examinations can disrupt immune regulation and impair host defenses. [4][9]. Among university students, examination periods represent a reproducible model of psychological stress. Previous research has demonstrated that academic stress is associated with measurable alterations in salivary biomarkers of immunity, which have been reported during examination periods, while increases in salivary cortisol reflect HPA axis activation and stress-related immune modulation. [4] [5]. The WHO defines oral health as the absence of the following indicators: extended discomfort in the maxillofacial area, neoplasia of this area and throat, infections and ulcerative lesions of the oral mucosa, inflammatory-dystrophic changes in the periodontal tissues, demineralization of hard dental tissues, their loss, and a number of other diseases that cause disruptions in the mechanical processing of food lumps, articulation, and a person's ability to smile; additionally, a person must be in good psychological and social health.[10]. The composition of the oral microbiota includes over 700 types of microorganisms [11], dependent on, among other things, diet or environmental conditions. Alterations in the oral microenvironment can lead to shifts in the biofilm's microbial

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landscape, allowing specific bacterial strains to proliferate, increase their virulence, and become opportunistic pathogens. [12]
The oral cavity, which is a gateway to our internal body, deserves specific attention as the colonization of pathogens may lead not only to the loss of implants through the immune system but also to serious life-threatening diseases [13] [14]. These immune alterations may create favorable conditions for the proliferation of opportunistic microorganisms in the oral cavity. [15][16]. Candida species and other oral bacterial species, which normally exist as commensals, can become opportunistic pathogens when host immunity is suppressed, leading to increased colonization and possible infection. Evidence suggests that psychological stress may facilitate this microbe overgrowth by weakening mucosal immunity [17][18]. In this study, changes in oral microbe colonization were investigated among third-year medical students at the University of Sabratha. A total of 68 saliva samples were collected before the examination period, and 63 samples were collected after the start of the examinations in the microbiology course. This study aims to investigate the relationship between academic stress and the diversity of the oral microbiome among students, through the analysis of self-reported questionnaires and microbiological examinations of saliva samples, and to evaluate the sensitivity of the isolated microorganisms to antibacterial and antifungal agents.

Methodology

A descriptive cross-sectional study.

Study Setting

This study was conducted at the Faculty of Medicine, Sabratha University, Libya. Third-year medical students who are enrolled in the academic year.

Sample Size

All available third-year medical students who agreed to participate in the study were included. Out of the 84 distributed questionnaires, 16 were empty or incomplete. Thus, the final sample consisted of 68 third-year students (61 females, 7 males), aged 21-25 years. Regarding the oral sample, 15 students refused to cooperate;

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therefore, 69 samples were taken from a class of 84 students for the first time. For the second time, 63 samples were collected.

Materials

The supplies needed to conduct this experiment are gloves, lab coats, face masks, normal saline, permanent marker, 75% rubbing alcohol, buccal swabs, plastic inoculation tubes, nutrient agar, blood agar, antibiotic discs (Ciprofloxacin, Imipenem, and Ampicillin), Nystatin ointment, plant extracts, pipette tips, thumb forceps, ruler, and an incubator.

Procedure

The third-year students completed the psychosocial questionnaires and collected saliva samples for oral microbic analysis for the first time two months before exams.

Isolation and identification of oral microbiota.

Swabs were collected from each Students for qualitative assessment of microbiota. in vitro culture techniques were applied for bacteria and fungi specific identification [19][20]. The swab material was grown aerobically on bacteriological agar . The samples were then cultured on nutrient and blood agar plates and incubated at 37 °C for 24 hours.

Antibiotic test:

A sterile forceps was used to place the antibiotic disks on the surface of the inoculated MHA plate. Each of the disks was carefully placed and slightly pressed on the MHA plates using the forceps. The placement of the disks close to the edges of the plate was avoided to ensure zone measurement errors. The MHA plates were incubated at 37°C for 24 hours. Resistance antibiotic testing follows, using Ciprofloxacin, Imipenem, and Ampicillin discs to differentiate between bacterial and fungal growth. Nystatin ointment is also applied to the agar plates using a diffusion-based assay known as the "agar-well diffusion method". The agar plates are then incubated at 37°C for an additional 24 hours. [21]

Statistical Analysis

Data were computed and analyzed by using Statistical Package for Social Sciences (SPSS) software (version 22) to check frequency, mean, and standard deviation. All samples were tested in triplicate.

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Result

In the first group of oral samples collected from third-year medical students, 9 samples showed positive findings for microbial growth. However, the second group of oral samples taken during exam season revealed that 46 samples contained pathogenic growth Fig (1).

Fig. (1). Isolated bacteria from students' saliva samples

The microbial composition of the biofilm may change as a result of changes in the oral environment, which may allow some bacterial species to multiply, become more pathogenic, and develop opportunistic traits. Number and percentage of oral microbe isolated shown in Table (1). Gram-positive and gram-negative, motile, anaerobic bacteria, which are also known to be common in the oral environment, appear at the beginning. Numerous microorganisms, such as Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Helicobacter pylori, Pseudomonas species, and Candida species, have been identified in periimplantitis patients [22] [23].

In orthopedic talks, *S. aureus* is frequently highlighted as the main source of implant-related infections and subsequent bone infections or osteomyelitis due to its notable adaptability. [24].

Understanding the characteristics of normal oral microbiota and how it affects the immune system is crucial medical students are under more academic pressure, which causes an imbalance in bacteria that

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Table 1: - Number and percentage of oral microbe isolated.

NO.	Isolates Bacterial Strains	Total bacterial count CFU/ml	Percentage %
1.	*Staphylococcus aureus*	11	16.17%
2.	*Streptococcus pneumoniae*	24	35.29%
3.	Candida sp.	15	22.05%
4.	unknown	18	26.47%
Total		68	

live in the oral cavity. This imbalance then adds to a rise in immune and digestive system diseases, which can show up as inflammation.[24]

If harmful bacteria get into the bloodstream, oral biofilms may also cause systemic issues that could lead to illnesses in other parts of the body. Therefore, managing and controlling biofilms is essential to dental hygiene and general oral health. Biofilms can elude the immune system by creating physical barriers that hinder the penetrate of immune cells and the effectiveness of antimicrobial agents.[24]

Personal Data- :

This study surveyed 68 medical students from third year. The third-year students completed the psychosocial questionnaire they were excluded from the respondent criteria. The data on respondent characteristics is presented in Table 2.

Accompanying figures (2,3,4,5, 6,7, 8, 9) that represent data from self-reported questionnaires about stress levels, chronic conditions, and symptoms could further clarify the relationship between perceived

Table 2:- Distribution of Respondent Characteristics

Variables	Frequency	Percentage
Gender - Male - Female	 07 61	 10.29% 89.70%
Age - 20 – 22 years	 35 33	 50.70% 49.30%

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- 23 – 25 years		
Academic Stress:- - Very High - High - Intermediate - Low	 24 37 6 1	 35.8% 53.7% 9% 1.5%
Stressful study period - Study Period - Exam Period - Both	 1 48 19	 1.5% 70.1% 28.4%
Sick more during exam Periods - Yes - No	 44 24	 64.1% 35.9%
Presence of oral symptoms during exam season - White Spots - Abnormal Taste - Burning Sensation - Dry Mouth - Mild Bleeding - Other	 9 7 6 20 18 8	 14.3% 11.9% 7.1% 10.7% 29.8% 26.2%

stress and microbiological outcomes.Health Conditions: The correlation between stress levels and pre-existing health conditions reported by participants could be illustrated to add depth to the analysis.The findings emphasize the role of chronic stress in altering salivary composition, potentially leading to xerostomia (dry mouth), which diminishes the natural defense against oral pathogens.Implications for health strategies such as stress management programs for students could be highlighted as a way to mitigate these effects on oral health.

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Personal Data:-

1. Gender .

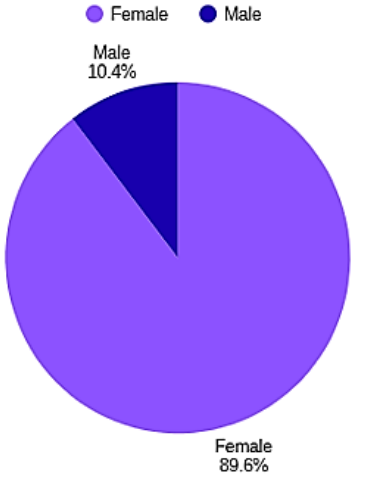

Fig (2):- Distribution of Respondent Characteristics (Gender).

2. Age

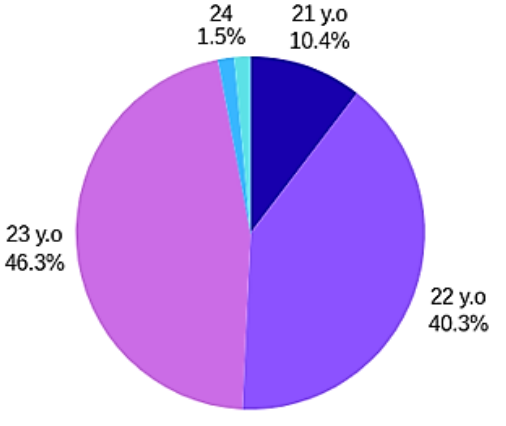

Fig (3):- Distribution of Respondent Characteristics (Age).

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3.Health Conditions; -1. Do you suffer from any chronic diseases?

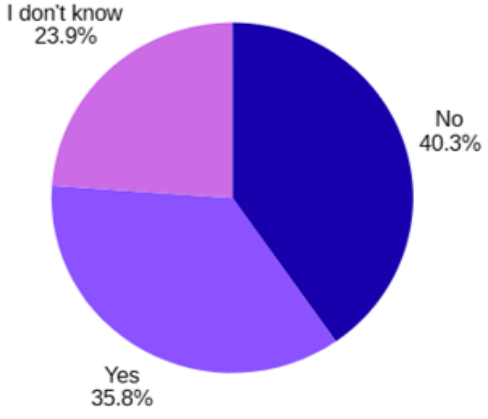

Fig (4):- Distribution of Respondent Characteristics (Chronic diseases).

2. Do you take medications regularly?

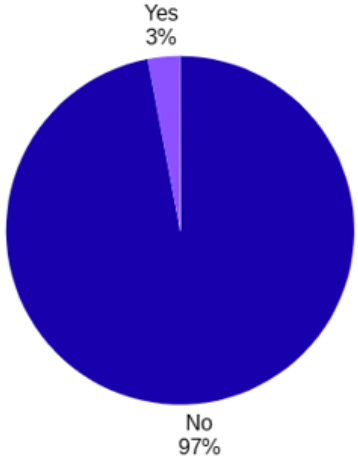

Fig (5): - Distribution of Respondent Characteristics (medications regularly).

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4. Academic Stress: - 1. How stressed are you about studying?

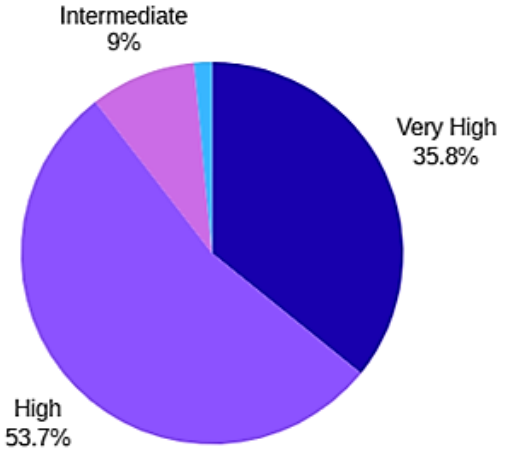

Fig (6):- Distribution of Respondent Characteristics (Academic Stress).

2. What is the most stressful study period?

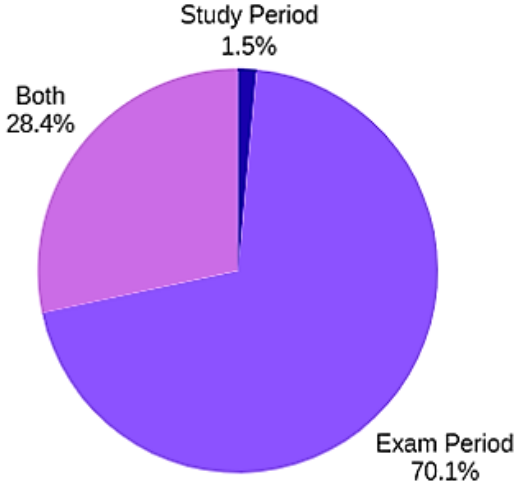

Fig (7): - Distribution of Respondent Characteristics (Stressful study period).

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3. What are the main causes of stress for you?

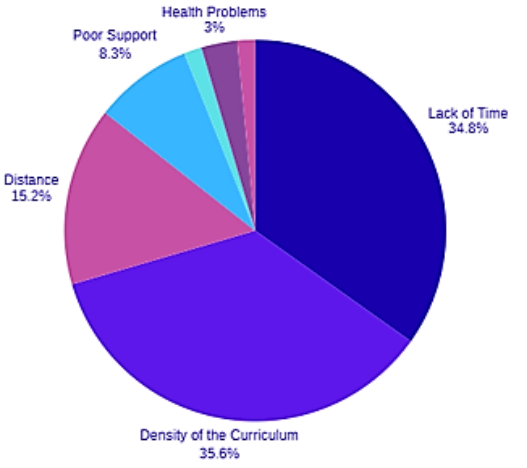

Fig (8): - Distribution of Respondent Characteristics (the main causes of stress).

4. Physical and Immune Effects

- Chronic psychological stress has been shown fig (9, 10, 11) to suppress cell-mediated immune responses critical for controlling oral pathogens. The findings correlate with evidence indicating that stress-induced immunosuppression diminishes the host's capacity to manage microbial colonization, thus facilitating conditions for infections such as oral candidiasis and periodontal disease (Robles et al., 2023).
- Implication: Addressing stress management among students could potentially mitigate this microbial overgrowth and its consequent health impacts.

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1. Do you get sick more during the exam periods than during normal periods?

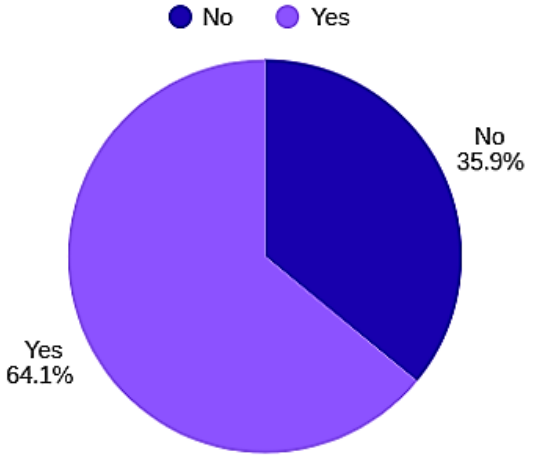

Fig (9): - Distribution of Respondent Characteristics (Getting sick during exam periods).

2. What types of symptoms do you experience during periods of stress?

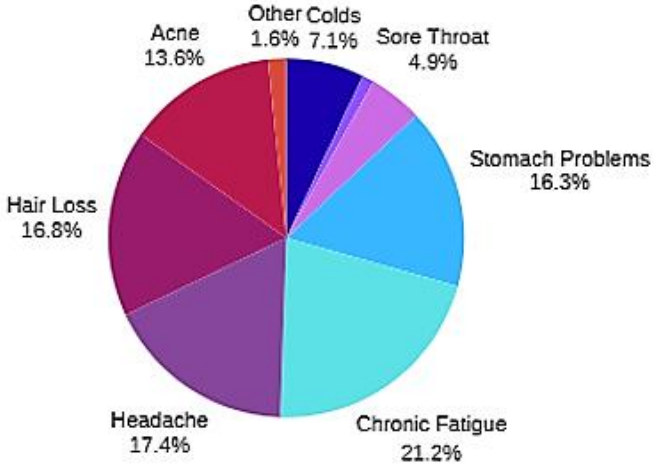

Fig (10): - Distribution of Respondent Characteristics (Symptoms during stress).

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3. Psychological effects of academic stress

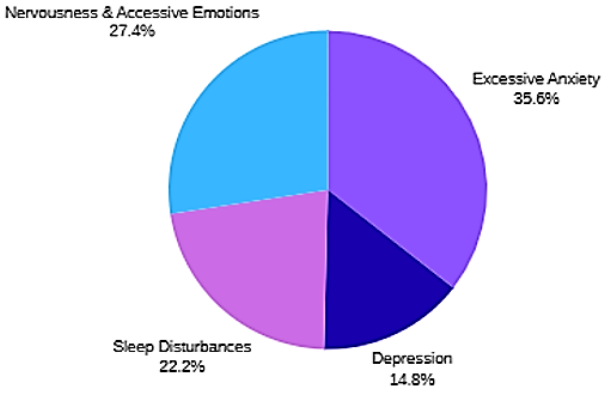

Fig (11): - Distribution of Respondent Characteristics (Psychological effects).

6. Oral symptoms associated with pathogenic infection

According to fig (12,13,14) Stress can decrease salivary flow (xerostomia) and alter the composition of saliva, including reductions in immunoglobulin A (IgA) and antimicrobial peptides.[26]

1. **During the last week, have you had any of the following symptoms?**

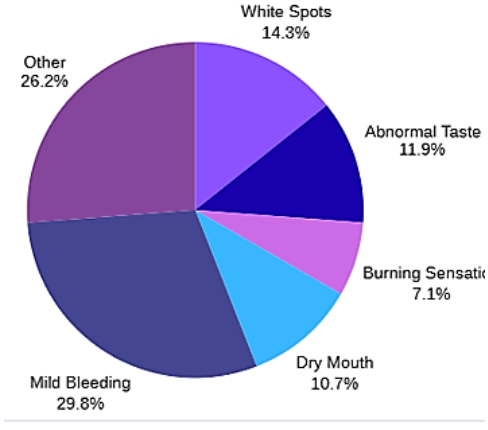

Fig (12): - Distribution of Respondent Characteristics (Oral symptoms).

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Saliva is enriched with anti-candidal peptides, considered to be part of the host's innate immunity [27], and plays a critical role in maintaining oral microbial homeostasis; thus, reduced salivary defense mechanisms increase the risk of opportunistic infections.

2. Have you taken an antibiotic in the last month?

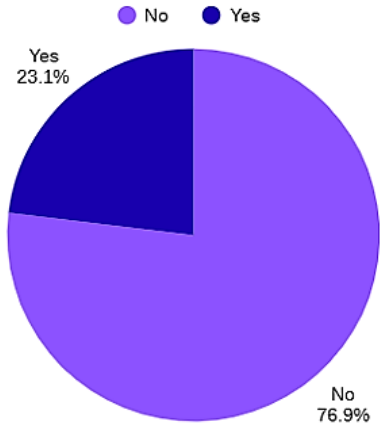

Fig (13):- Distribution of Respondent Characteristics (Taking an Antibiotic).

3.Do you use an antifungal mouthwash?

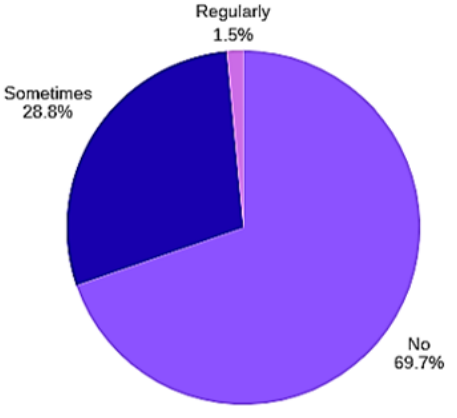

Fig (14):- Distribution of Respondent Characteristics (use a mouthwash).

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Discussion

Chronic stress triggers the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated levels of cortisol and catecholamines. Cortisol exerts immunosuppressive effects by reducing the activity and proliferation of lymphocytes, suppressing pro-inflammatory cytokine production, and impairing neutrophil function. Psychological stress has been found to suppress cell-mediated immune responses that are important for limiting the proliferation of oral pathogenic microorganism [4][6]. This systemic immunosuppression diminishes the host's capacity to control microbial colonization in the oral cavity, facilitating infections such as oral candidiasis or periodontal disease.[25]

Individuals experiencing psychological stress often exhibit behaviors that further predispose them to oral infections, such as poor oral hygiene, increased consumption of sugary foods, tobacco use, or parafunctional habits (e.g., bruxism or lip/cheek biting), which can disrupt mucosal integrity and promote microbial invasion. [28][29] Stress-induced neuroendocrine changes can promote a chronic low-grade inflammatory state, leading to alterations in mucosal immunity. This can exacerbate susceptibility to both bacterial and fungal colonization in the oral cavity.[30]

Conclusion

This study revealed an association between academic stress and increased oral microbial colonization among third-year medical students. Stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis and the resulting immune modulation may contribute to conditions favorable for opportunistic fungal colonization, particularly Candida species and some bacterial species. These findings highlight the importance of implementing stress-reduction interventions and promoting healthy coping strategies among medical students to support both oral and general health. Furthermore, monitoring changes in oral microbiota may provide a potential non-invasive indicator of stress-related immune alterations. Additional studies with larger sample sizes are recommended to further clarify the relationship between academic stress, immune function, and oral microbial changes.

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Ethics approval and consent to participate:
Ethical approval was granted by the Biosafety and Bioethics Committee of the Libyan Medical Research Center (NBC:018.H.26.120). Written informed consent was obtained from all participants

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