The Assessment of Stress Indicators of Mobile Brigades Using the Periodontal Parameter and Cortisol Titer

Rike Rayanti[1], Ariadna A. Djais[2]*, Sri Lelyati C Masulili[3], Rinny Wowor[4]

1. Doctoral Program, Dentistry Faculty, Universitas Indonesia, Salemba, Jakarta Indonesia.
2. Oral Biology Department, Dentistry Faculty, Universitas Indonesia, Salemba, Jakarta Indonesia.
3. Periodontic Department, Dentistry Faculty, Universitas Indonesia, Salemba, Jakarta Indonesia.
4. Department of the state intelligence agency, Personnel Psychological Development and Maintenance, Police of Republic Indonesia, Jakarta, Indonesia.

Abstract

When dispatched to conflict areas, stress is a problem for mobile brigades. The hormonal assessment of stress and changes in oral biology can provide clues to clarify the questionnaire stress evaluation. This study evaluated the periodontal index and cortisol titers of the police mobile brigade corps as the parameter of stress assessment that brought to the conflict zone in Indonesia.

The stress was assessed by Police stress questionnaire (PSQ), saliva collected and periodontal index assessment, blood and saliva cortisol by ELISA, and periodontal bacterial identified by qRT-PCR.

Categories stress of subjects were identified, little stress (18:46%), mild stress (6:15%), moderate stress (13:33%), and severe stress (2: 5%). All of subject contained normal cortisol levels (saliva) below 2.16g/dL and (serum) below 27.23g/dL. Plaque index in 39 subjects, good (63%) and moderate (37%), calculus index is good (24%), and moderate (76%). Periodontal bacteria were Treponema denticola (54%), Tannerella forsythia (25%), and Porphyromonas gingivalis (21%). Serum cortisol levels and oral biological status were positively associated with the PSQ. It linked positively with Tannerella forsynthia, plaque index, calculus index, and PSQ but negatively with T Treponema denticola and PSQ.

The PSQ-stress values correlated with periodontal index, periodontal bacteria, and serum cortisol levels.

Clinical article (J Int Dent Med Res 2022; 15(3): 1202-1210)

Keywords: Cortisol, periodontal index, periodontal bacteria, Police stress questionary.

Received date: 28 April 2022 **Accept date:** 13 June 2022

Introduction

Stress is a state of emotional or physical tension caused by any event or thought that causes you to be frustrated, angry, or nervous. The impact of stress tends to affect survival and has been identified as a barrier to work[1]. Selye defines stress as a non-specific response to the environment[2]. Stress reactions occur when demands, pressures, or professional aspects must be met, affecting a person physically and psychologically[3].

Corresponding author:
Prof. Ariadna A. Djais, M.Biomed., Ph.D. Jl. Salemba Raya IV No.2, RW.5, Kenari, Kecamatan Senen, Jakarta Pusat, Daerah Khusus Ibukota Jakarta 10430.
E-mail: ariadna.adisattya@ui.ac.id

The police in the mobile brigade unit have the same physical focus as the military (army) at all levels. The work-related to war conflicts make these officers tend to experience stress. Hartley (2013) reported that military experience positively influenced stress[4].In addition, the impact of stress can affect the immune system, which causes many health disorders, including changes in oral health.

Stress indicators in military activities were evaluated psychologically, mentally, and in terms of soldiers' adaptation responses. Psychosocially often use the PSQ as a standard evaluation, but evaluation using this principle has shortcomings in response to assessment by soldiers, including the stress evaluation process and psychological and hormonal indicators[5]. The hormone cortisol is a standard indicator for evaluating psychological stress. Cortisol, the primary stress hormone that increases glucose in the bloodstream, increases the brain's use of

glucose to increase the availability of tissue-reducing substances. Cortisol also limits functions that are not essential or harmful in fight-or-flight situations[6].

In general, stress conditions can cause changes in the body's immune system that can affect oral health, including changes in the quality of oral health, including the development of periodontal bacteria. Akcali (2014) reported that cortisol affects the growth of *Porphyromona gingivalis*[7]. In addition, it also influences the development of *Treponema denticola*, *T medium*, *Tannerell*a *forsythia*, *P endodontalis,* and *Filifactor alocis*[8]. Duran-Pinedo reports that stress has been shown to cause microbiome imbalances[9].

This study was reported to evaluate the relationship of cortisol hormone titer content in saliva and blood serum of mobile brigade in the face of war associated with assessing periodontal index as a stress parameter. In addition, the study found indicators of the relationship between the development of periodontal bacteria with cortisol hormone titers and stress before facing war.

Materials and methods

As many as 39 subjects are in the mobile brigade Kelapa Dua, Indonesia, assigned to conflict areas in Irian Jaya, Indonesia. Stress parameters were assessed based on PSQ analyzed and confirmed by cortisol titers from saliva and serum, then confirmed with periodontal index and periodontal bacteria.

Subject Selection

The research subjects are members of the Indonesian National Police Mobile Brigade from the Pioneer Troop I Regiment, Jakarta, Indonesia, who will carry out OMB and operate Nemangkawi in Papua, Indonesia, from September 2019 to March 2020. All research subjects are participants of the Periodic Rikkes, namely health checks that are carried out regularly once a year, which meet the inclusion criteria, namely the subject is willing to participate in this study, age 20-40 years, good general health as seen from blood pressure checks, laboratory examinations, and chest x-ray examinations, and willing to participate in all research activities by signing informed consent. In addition, exclusion criteria were also assessed. Namely, the subject was not taking antibiotics, and the subject was not taking antidepressants, antipsychotics, and anti-anxiety agents.

Informed Consent and Medical Record assessment

Participants in this study were required to sign an informed consent form. Subjects who met the inclusion criteria were informed about the study's benefits, risks, and stages. Furthermore, demographic data such as the subject's name, age, gender, and address were evaluated using the identity sheet completed by the patient. Furthermore, the medical record data was analyzed to determine the cortisol from serum and saliva of the subject to identify the stress indicator also correlated with police stress questionary.

Collect the Saliva and Blood serum

Jo adopted the collected saliva used in the oral rinse approach (2019)[10]**.** The patient was instructed to rinse his mouth for 60 seconds with 10 ml of sterile PBS (phosphate-buffered saline) and then pour the rinse into a 15 ml sterile plastic tube. After that, the plastic tube is sealed and transported to the laboratory. For 10 minutes, the oral rinse was centrifuged at 3500 rpm to separate the supernatant and pellet. The supernatant was discarded, and only the pellet was kept. Afterward, the pellets were homogenized in 1 ml of phosphate buffer saline (PBS) 5 mL of blood was taken from the subject's vein and then collected in a vacuum tube. Then let it sit until the blood clots. Then centrifuged at 300 rpm for 10 minutes, the supernatant was collected as blood serum[11].

Periodontal Index Assessment

The *Oral Hygiene* Index Simplified *(*OHI-S) performance was adopted by Al-Jasser (2021)[12]. Debris examination is carried out using the Debris Index. Measurements were made with a periodontal probe. The tooth surface is divided into three parts: cervical 1/3, middle 1/3, and incisal 1/3. The examination was carried out on the index teeth, namely teeth 11, 16, 26, 31, 36, and 46. The analysis was started on 16 buccal teeth, 11 facial teeth, 26 buccal teeth, 36 lingual teeth, 31 labial teeth, and 46 lingual. The tooth's periodontal probe was placed on the incisal 1/3 and moved towards the cervical 1/3 of 46 teeth.

The assessment of *OHI-S involved the* calculus Index, and the examination began with 16 buccal teeth, 11 facial teeth, 26 buccal teeth, 36 lingual teeth, 31 labial teeth, and 46 lingual teeth. The gingival margin was swabbed with the

periodontal probe both above and below the gingival margin. Direct observation of supragingival calculus is also possible. Furthermore, measured the Pocket depth. The distance from the gingival margin to the bottom of the pocket is called the pocket depth, measured in millimeters. Pocket depth, 0 (very good), 0.1-0.6 (Good), 0.7-1.8 (Medium), 1.9-3 (Poor)[13].

Other assessments include Gingival recession with grading limits, mild gingivitis: 0.1 - 1.0, Moderate gingivitis: 1.1 - 2.0, Severe gingivitis: 2.0-3.0[14]. Whereas the loss of attachment measured from the cementum enamel boundary to the periodontal sulcus or pocket base with category attachment loss, 1-2 (Slight), 3-4 (Moderate), 5 mm (Severe)[15]. Moreover, plaque index was examined tooth using a mouth mirror and a periodontal probe on four surfaces: mesiobuccal, mid buccal, distobuccal, and palatal/lingual, category 0 = 1 (Light) and 1= >1 (Heavy)[16]. Papillary Bleeding Index is another assessment to describe Gingival. The number of surfaces examined. 0 (no bleeding), 1 (bleeding in the form of dots), 2 (bleeding in the form of lines), 3 (bleeding in the form of triangles), 4 (spreading bleeding) with categories 0 = 1 (mild) and 1 = >1 (severe)[17].

Periodontal Bacteria Culture

Bacterial culture was started by taking subgingival fluid from the subject, which was planted on Tryptic soy agar (TSA) media with a zigzag scratch method in the area of the medium plate. It was then incubated for 48 h at 37 ^{0}C under anaerobic conditions. One colony that grew was then made into a suspension-cultured on Tryptic Soy Broth (TSB) medium for 48 h at 37 ^{0}C. The bacterial suspension was then used to examine the periodontal bacterial species[18].

ELISA Assay for Cortisol Titer

Examining cortisol levels using the ELISA principle was adopted by Restituto (2008)[19]. Saliva and serum samples from the patient were centrifuged at 3000 rpm for 15 min at 4 ^{0}C. The supernatant was taken and transferred to a 1.5 mL Eppendorf tube, then used for running ELISA based on the working principle of Cusabio kits (Cusabio Technology LLC, Houston, USA) in stages, placing all reagents, samples, and standards at room temperature before use, then 50 µL of standard and samples were added to all wells. Then 50 µL of antibody (1x) was added to all wells. Then the sample was incubated at 37 °C for 40 min and washed three times with wash buffer. Then 100 µL of HRP-conjugate was added to all wells, set at 37 ^{0}C for 30 minutes, and washed 5 times with wash buffer. In the dark, a total of 90 µL of TMB substrate was added to all wells incubated for 20 min at 37 ^{0}C. Then 50 µL of stop solution was added to all wells and read at a wavelength of 450 nm.

qRT-PCR of Periodontal Bacteria species

Periodontal bacterial species identification was used qRT-PCR based on bacterial primer sequences (Table 1)[20,21,22]. This technique was used for the SYBR Green kits (Sigma-Aldrich, Darmstadt, Germany), previously done by Bachtiar (2021)[23]. In the first stage, bacterial DNA was extracted from saliva. The 1 mL sample was centrifuged for a minute at 10,000-12,000 rpm. The supernatant was discarded, 200 uL of Instagene matrix (Biorad brand) was added to the pellet, then incubated at 56 ^{0}C for 15-30 min. Vortexed for 10 sec and placed the tube in a water bath (thermoblock) at 100 ^{0}C for 8 min, vortexed for 10 sec, centrifuged at 10,000-12,000 rpm for 2-3 mins. The supernatant as bacterial DNA was used for RT PCR.

The RT-PCR stage begins with a mix of reagents for running RT PCR with a composition of 10 µL per well consisting of 5 ul SYBR mix Hi Rox (Bioline), Primer Forward (target DNA): 0.5 µL, primer reverse (target DNA): 0.5 µL, nuclease-free water: 1 µL, and DNA template (which has been extracted): 3 µL. Each sample is run in duplicate. Then the sample was put into the well and centrifuged at 3000 rpm for 3-5 min. The well plate is inserted into the RT PCR machine. Running sample with pre denaturation temperature of 95 ^{0}C, 2 min, denaturation 95 ^{0}C 5 sec, annealing 60 ^{0}C 10 sec, extension 72 ^{0}C 15 sec with 40 cycles. Primers and sequences of periodontal bacteria of gene target are 16S rRNA; *T. forsynthia* (F. 5'-ATCCTGGCTCAGGAT-3'; R. 5'-TACGCATACCCATCCGCAA-3'. *T. denticola* (F. 5′ AGAGCAAGCTCTCCCTTACCGT-3′; R. 5′-TAAGGGCGGCTTGAAATAATGA-3′. *P. gingivalis* (F. 5′-TACCCATCGTCGCCTTGGT-3′; R. 5′- CGGA CTAAAACCGCATACACTTG-3′. Total Bacteria (F. 5'-TTAAACTCAAAGGAATTGACGG-3'; R. 5'-CTCACGRCACGAGCTGACGAC-3'.

Statistical Analysis

Stress category data based on PSQ parameter and cortisol titer from saliva and

serum sources were analyzed by Kruskal-Wallis. Meanwhile, Spearman's rho analyzed the relationship between salivary and serum cortisol titers with oral biology status and PSQ indicators with a strong correlation limit (r = 1).

Results

As shown in Table 1, the PSQ device was used to assess stress in 139 participants. Thirty-nine subjects met the inclusion criteria following the assessment, and 100 subjects completed the exclusion criteria. Based on the results of a study of 39 respondents, the following categories of stress were identified: little stress (18:46%), mild stress (6:15%), moderate stress (13:33%), and severe stress (2: 5%). It is explained by individuals sent to war zones in Indonesia generally experiencing little stress, even though 67 percent of respondents have never been to a conflict area. The cortisol hormone titer did not increase, indicating that all participants maintained normal levels (100%).On all criteria, the periodontal indices were outstanding. Only the calculus and plaque indices changed somewhat but remained non-dangerous. Additionally, all participants possessed a variety of periodontal microorganisms.

This study reports the number of cortisol titers examined from blood and saliva with several indicators of the periodontal index and their association with psychological stress evaluation with the PSQ. Table 2 reported that all study subjects showed normal salivary cortisol levels and blood serum. Each subject ranged from 1-5, and some were above 5%. The number of subjects with the most dominant saliva and serum cortisol levels was 1-2% (Table 4), followed by 3-4% and 5% groups, which also explains the distribution of cortisol titer values from both sources (saliva and serum) has high reliability (0.81). There is a significant difference between the three concentration variables ($p<0.05$). Total serum and saliva cortisol levels refer to the criteria for normal values for serum cortisol levels, which are 3.95-27.23g/dL, while normal salivary cortisol levels are 0.5-2.16g/dL.

In Figure 2B, it is reported that salivary cortisol has a positive relationship with *T. forsynthia*, plaque index, calculus index. In contrast, serum cortisol negatively correlates with *T. denticola*. The rest have a positive relationship direction. Cortisol titers in saliva and serum have a weak relationship with periodontal bacterial variables, oral biologic indicators, and PSQ. In addition, there is no significant relationship in all the variables tested.

Figure 3 indicates that 39 subjects had a good (63%) or moderate plaque index (37%). Calculus indexes were classified as good (24%) and moderate (24 percent) (76%). Meanwhile, there was no change in the bleeding index, pocket depth, gingival recession, or attachment loss. Because the bleeding index, pocket depth, and attachment loss did not change (normally), only the plaque index and calculus index were analyzed statistically because both subjects were not changed. Spearman's rho analysis revealed that both variables were significantly associated with the development of subject stress ($r=0.72$). There was a statistically significant difference between the groups ($p<0.05$; 0.00).

Table 3 shows that the stress value possessed by the subject is dominated by a minimum scale (18 people) and moderate (13 people), the rest on a mild scale (6 people), and severe (2 subjects). Data based on the police stress questionnaire (PSQ) showed high reliability, with a Cronbach alpha (α) of 0.84. Its means that every statement in the questionnaire can be declared reliable. In addition, the stress response experienced by PSQ has a significant difference between subjects (<0.05), with a moderate influence relationship, meaning that the subject's activity influences the environment and activity as a parameter of the degree of stress between subjects.

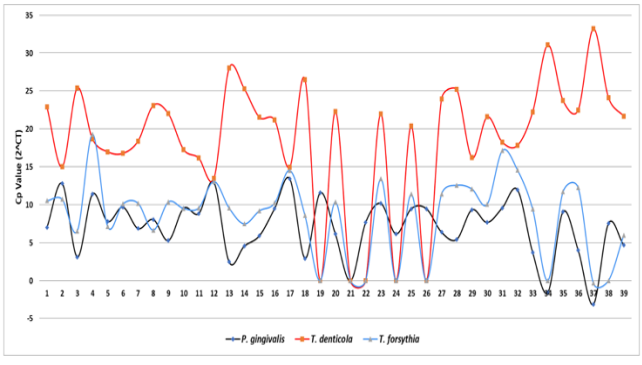

Figure 1. RT-PCR evaluation of the population of periodontal bacteria in mobile brigade subjects before carrying out military activities in conflict areas. The critical point value of the periodontal bacterial population showed that *T. denticola* bacteria had a higher Ct delta value than *T. forsythia* and *P. gingivalis*. Reading at 48 cycles.

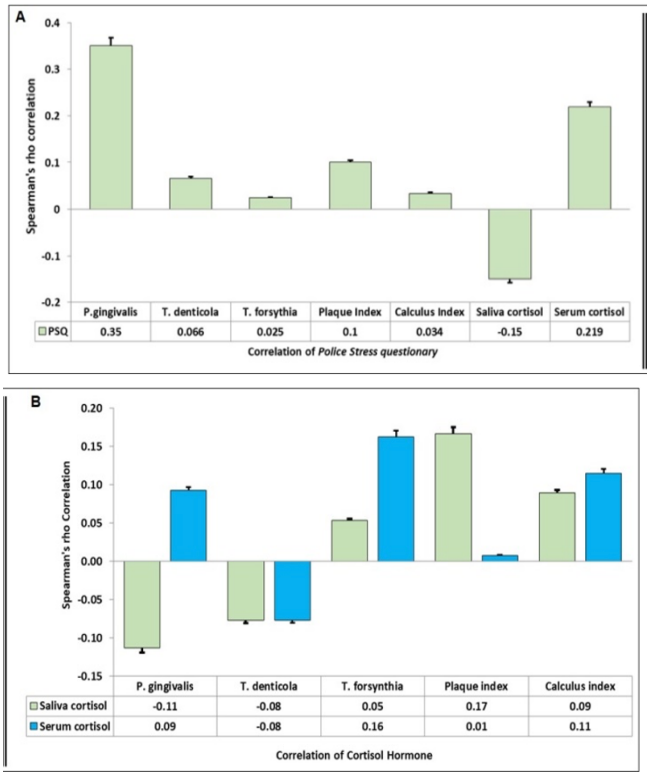

Figure 2. Correlation exists between variables. Correlation between salivary and serum cortisol titers in the PSQ (A) and the periodontal index and periodontal bacteria in the PSQ (B). The PSQ correlates positively with periodontal bacteria and the periodontal index. Cortisol, on the other hand, has a positive correlation with *T. forsynthia* and the periodontal index. PSQ is negatively correlated with salivary cortisol, and saliva cortisol is negatively correlated with *P. gingivali* and *T. denticola*.

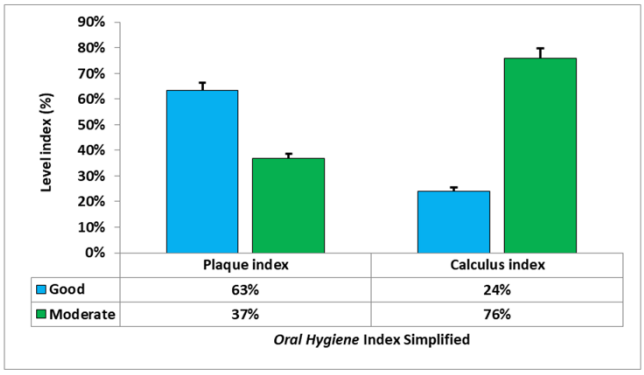

Figure 3. Oral Hygiene Index Simplified of research subjects. In general, the subjects had an excellent plaque index status compared to the calculus index. These two indices are interrelated in evaluating oral health status. Both include good levels in all research subjects. Bar (OHI-S index level) and Bar error (Error with percentage).

Figure 1 shown that *T. denticola* bacteria reached 54% in 39 tested subjects. The rest were *T. forsythia* (25%) and *P. gingivalis* (21%). Based on the development of bacteria, it can be predicted that many subjects included in this study have good periodontal health. These three bacteria have their development intensity based on the phase of periodontal tissue infection. Based on the Wilcoxon test showed that *T. denticola* with *P. gingivalis* had a more significant influence on the periodontal bacterial population in 39 subjects and did not show a significant difference ($p>0.05$; 0.875), with positive rank 16 and sum of rank 240, negative rank 15. and the sum of rank 225. Then followed by the population ranking *T. forsynthia* with *P. gingivalis* and *T. forsynthia* with *T. denticola* these two population ranks did not show any significant difference.

Figure 2A shows Spearman's correlation PSQ has a shallow relationship with periodontal bacteria and periodontal index, as well as serum cortisol levels. But with salivary cortisol, there is no relationship. This correlation value can indicate stress activity with changes in oral biologic status and serum cortisol. The positive direction relationship indicates that the higher the stress experienced by the subject, the higher the changes in the periodontal index, the development of periodontal bacteria, and the quantity of the cortisol. The assessment criteria for Spearman's correlation are 1 (strong), 05-0.7 (moderate), 2-4.9 (low), 0-1.9 (very low). There are no significant differences in all tested relationships

Discussion

Ghosh (2013) reported that stress is related to the production of the hormone cortisol, which acts as an anti-inflammatory agent[24]. In addition, mental health also affects stress levels which tend to be influenced by demographic factors such as age, education, and marital status[25]. The phenomenon of cortisol involvement in periodontal disease (oral disease), when cortisol is produced in the periphery of the gums, directly provides a cellular response to induce mast cells to produce more allergen proteins, simultaneously increasing inflammation and the development of periodontal disease[26]. In addition, Kaur (2016) reported that stress correlates with oral disease, aphthous ulcers, oral lichen planus, xerostomia, burning mouth syndrome, and

bruxism[27]. Stress can also contribute to teeth grinding, gum disease, dry mouth, and canker sores and affect an oral health routine and diet.

Variables	Normal Score	N	Frequency (%)
A. PSQ-Stress Assessment			
- Minimal	0-50	18	46
- Mild	51-61	6	15
- Moderate	62-122	13	33
- Severe	123-201	2	5
B. Experience of Assignment to conflict areas			
- No experience		26	67
- 1 Time	-	8	21
- 2 Times	-	3	8
- 3 Times	-	1	3
- > 3 Times	-	1	3
C. Cortisol titers			
Serum cortisol:			
- Normal	3,95-27,23g/dL	39	100
- Abnormal	>27,23 g/dL	-	;-
Saliva cortisol:			
- Normal	0,5-2,16g/dL.	39	100
- Abnormal	> 2,16 g/dL	-	-
D. Periodontal Index			
Plaque Index:			
- No plaque	0		
- Mild plaque	1	33	85
- Moderate plaque	2	6	15
- Hight plaque	3		
Calculus Index:			
- Very good	0		
- Good	0,1-0,9	30	77
- Moderate	1-1,9	9	33
- Poor	2-3		
Bleeding Index :			
- No bleeding	0	39	100
- Bleeding (dot)	1	-	-
- Bleeding (lines)	2	-	-
- Bleeding (triangle)	3	-	-
- Bleeding (spreading)	4	-	-
Loss of Attachment:			
- No loss	0	39	100
- Litle loss	1-2	-	-
- Moderate loss	3-4	-	-
- Hight loss	5	-	-
E. Periodontal Bacteria			
- *Porphyromonas gingivalis*	-	39	100
- *Treponema denticola*	-	39	100
- *Tannerella forsythia*	-	39	100

Table 1. Subject demographic data based on the research variables.

Table 1 demonstrates that the subjects did not experience stress even though they had not been sent to the front lines of conflict. This information is consistent with the cortisol titer level, which is still considered normal on average. Stress hormones such as cortisol can identify specific body changes in response to stressors, individuals at risk for stress-related disorders, and the efficacy of stress-reduction interventions[28]. According to the periodontal index parameters, it was determined that only the plaque index and calculus index changed, while the bleeding index, attachment loss, and gingival recession remained unchanged. It means that changes in the periodontal index may be associated with the onset of stress, which changes the hormone cortisol's metabolism.

Similarly, the number of periodontal bacteria developed can indicate the severity of periodontal infection[29]. It is indicated that an increase in *T. denticola* bacteria was associated with the development of periodontal infection in all subjects, and that *T. denticola* and *T. forsythia* had a more significant number of early infection responses than *P. gingivalis*. This finding supports the theory that *P. gingivalis* infection tends to increase in chronic periodontitis cases[30]. Meanwhile, the findings of this study indicate that the periodontal index and PSQ belong to the same category.

% Titer	Saliva cortisol (g/dL)			Serum cortisol (g/dL)			p	R	Alpha
	N	Mean	SDV	N	Mean	SDV			
1-2	22	0,52	0,1093	29	2,27	0,295257			
3-4	14	0,88	0,1635	9	4,10	0,547915	0.000	0.69	0.81
≥5	3	1,69	0,3262	1	16,59	0			

Table 2. Statistical analysis of the percentage values of salivary and serum cortisol titer groups and the reliability.

Table 2 reported that the research subjects' salivary and blood serum cortisol levels were normal. It means that subjects dispatched to war zones have good mental and psychological well-being based on the cortisol profile. Assessment of cortisol as an indicator of stress is related to the body's metabolic activity when responding to stress[31]. When the body feels stressed, the adrenal glands make and release the hormone cortisol into the bloodstream. Stress triggers a combination of signals from hormones and nerves. This signal causes the adrenal glands to release hormones, including adrenaline and cortisol, as the effect of increasing heart rate and energy as part of the fight-or-flight response[32].

Cortisol causes an increase in heart rate and blood pressure. Indirectly, it can be ascertained that several stress factors have been eliminated through experience or decisive mental actions as soldiers[33]. According to Stults-Kolehmainen (2014), one of the efforts to prevent stress when facing war is to eat regularly, sleep and exercise, drink plenty of water, eat nutritious food, exercise and get enough sleep, practice relaxation techniques before, during, and after stressful events[34].

Another finding from the results of this study (Figure 3) is that the subjects had plaque index in good (63%) and moderate (37%). The calculus index was categorized as good (24%) and moderate (76%). The study results reported in Figure 2B show that the hormone cortisol derived from saliva and blood serum has a positive relationship with the level of pack index and calculus index. Meanwhile, bleeding index, pocket depth, gingival recession, and attachment loss did not show any change in status. All subjects from the four biological oral indicators have excellent and healthy hands. In general, although the plaque and calculus indexes experienced changes, they were still within normal limits. The subject has sufficient knowledge to maintain regular oral and dental health. At least, based on the data on the examination of oral biological parameters, it shows that the subject supports good oral health. Bauroth (2003) explained this indication by brushing teeth twice a day. Using dental floss daily and an antibacterial mouthwash twice a day can prevent oral disease, balanced with a regular exercise routine. So it can relieve stress, increase energy, and encourage healthier eating.[35] Military soldiers carry out this activity before being dispatched to war zones, including sports discipline, regular meals, and weekly medical check-ups. It prevents stress and oral disease.[36]

Cases of chronic stress usually cause an increase in periodontal disease. This process is influenced by a decrease in the immune system, causing the gums to become chronically inflamed, which leads to gum disease. Damage to the gums triggered by chronic stress can cause teeth to become loose due to loss of tooth attachment, damage to the supporting bone, and tooth loss[37]. In addition, the correlation of stress with periodontal disease occurs due to a temporary decrease in salivary flux, promoting plaque and calculus formation due to salivary composition changes resulting in periodontal disease[38].

Periodontal disease is a chronic inflammatory disease characterized by periodontal tissue damage caused by bacteria. Cortisol suppresses immunity by inhibiting the production of secretory immunoglobulins and neutrophil function. It can impair the defense against infection caused by pathogenic microorganisms that colonize the subgingival area and increase local and systemic proinflammatory cytokines, resulting in periodontal tissue destruction and periodontal disease[39].

The results of the study are presented in Figure 1. It was reported that the frequency of periodontal bacteria *T. denticola* was higher (54%) compared to T. forsythia (25%) and *P. gingivalis* (21%). Based on the reference, stress can disrupt the hormonal system, including blood glucose synthesis and carbohydrate fermentation in the dental pellicle[40]. These changes tend to increase the growth of bacteria. In cases of chronic periodontitis, *P. gingivalis* is the most dominant gingival commensal bacteria found, while other bacteria have very few populations. So, it can be assumed that the increase in the population of *T. denticola* is very identical to the early phase of stress and the early phase of periodontal disease[41].

The study results above are in line with research conducted by Duran-Pinedo (2018) that stress has been shown to cause an imbalance in the microbiome.[9] Another study conducted by Gunepin (2018) found that chronic stress harms periodontal treatment.[40] Through a cross-sectional study, Fukui showed that cortisol levels in saliva could provide information to evaluate the chronic stress condition of patients who complain of bad breath with their mouth[42]. In addition, the hormone cortisol influences the growth of *P. gingivalis*[7].

Criteria PSQ	N	Mean	SDV	Min	Max	References	p value	R	Alpha
Minimal	18	40,333	5,466	35	50	<51			
Mild	6	56,667	3,327	52	61	52-61	p<0.05 (0.000)	0.64	0.84
Moderate	13	86,692	17,909	67	114	62-123			
Severe	2	84,500	7,778	140	151	>123			

Table 3. The evaluation of stress level of subjects based on the police survey questionary.

The results of the study in Table 3 show that most of the subjects did not experience stress (18), moderate (13), mild (6), and severe (2). It means that the subject's activity influences the environment and activity as a parameter of the stress between subjects. Another finding in Figure 2A shows that PSQ has a positive relationship with periodontal bacteria and oral biology status, as well as serum cortisol levels. But with salivary cortisol, there is no relationship. The assessment data from the PSQ device shows the original information obtained from the research subject. They were positive linearity with the periodontal index, cortisol index, and

periodontal bacteria species' frequency. It has been assumed the psychological condition of the subject with stress intensity is related to the biological properties of body metabolism, including hormonal, immune system bacterial development[43]. This phenomenon aligns with the study results reported in Figure 3, where cortisol titers from saliva and serum have a shallow relationship with periodontal bacteria variables.

The scientific fact from the results of this study is that epidemiological data with in-vitro data information has a relationship to explain stress indicators for each subject examined in this study. Based on studies that reported that stress had been influenced to the metabolic activity of the cortisol hormone and changes in periodontal index dan development of periodontal bacteria.

Conclusion

Saliva and serum cortisol levels were normal in subjects with a good or moderate periodontal index. *T. denticola* was more prevalent than *T. forsythia* and *P. gingivalis*. PSQ values were significantly correlated with bacterial periodontal development, the periodontal index, and serum cortisol levels. Additionally, salivary cortisol levels were associated with *T. forsynthia*, the plaque index, the calculus index, and the PSQ, whereas serum cortisol levels were associated with *T. denticola* and the PSQ.

Ethical statement

The human subjects ethics board approved this study of the Faculty of Dentistry, University of Indonesia, Jakarta, Indonesia, No.6/Ethical-Approval/FKGUI/II/2019 and was conducted following the Helsinki Declaration of 1975, as revised in 2013.

Acknowledgment

Thank you to the Laboratory of Oral Biology, Faculty of Dentistry, Universitas Indonesia, which has assisted in reading cortisol results and readings of the periodontal bacterial population using qRT-PCR.

Declaration of Interest

The authors declare no conflicts of interest.

References

1. McKenzie SH. HM. Understanding the relationship between stress, distress and healthy lifestyle behaviour: a qualitative study of patients and general practitioners. BMC Fam Pract 2013;14:14-166.
2. A. TSY. Hans Selye (1907–1982): Founder of the stress theory. Singapore Med J 2018;59:170–1.
3. Fletcher D, Scott M. Psychological stress in sports coaches: A review of concepts, research, and practice. J Sports Sci 2010;28:127-37.
4. Hartley TA VJ, Mnatsakanova A, Andrew ME, Burchfiel CM. Military Experience and Levels of Stress and Coping in Police Officers. Int J Emerg Ment Health 2013;15:229–39.
5. Queirós C, Passos F, Bártolo A, Marques AJ, da Silva CF, Pereira A. Burnout and Stress Measurement in Police Officers: Literature Review and a Study With the Operational Police Stress Questionnaire. Front Psychol 2020;11:587.
6. Mendoza G, Clemente-Suárez VJ, Alvero-Cruz JR, Rivilla I, García-Romero J, Fernández-Navas M, et al. The Role of Experience, Perceived Match Importance, and Anxiety on Cortisol Response in an Official Esports Competition. Int J Environ Res Public Health. 2021;18:2893.
7. Akcalı A HO, Buduneli N, Davideau JL, Köse T, Tenenbaum H. Exposure of Porphyromonas gingivalis to cortisol increases bacterial growth. Arch Oral Biol 2014;59:30-4.
8. Payungporn S AP, Poomipak W, Praianantathavorn K, Charalampakis G, et al. . Identification of Bacteria Associated with a Periodontal Disease in Thai Patients Based on Next-Generation Sequencing. Jundishapur J Microbiol 2017;10:e13646.
9. Duran-Pinedo AE SJ, Frias-Lopez J. The effect of the stress hormone cortisol on the metatranscriptome of the oral microbiome. Npj Biofilms and Microbiomes 2018; 4.
10. Jo R, Nishimoto Y, Umezawa K, Yama K, Aita Y, Ichiba Y, et al. Comparison of oral microbiome profiles in stimulated and unstimulated saliva, tongue, and mouth-rinsed water. Sci Rep. 2019;9:1-7.
11. Nazaruddin HB, Gani BA, Jakfar S, Hasan M, Hanafiah M. Profile of allergy hyperplasma pathologic antibody and immunogenic characteristic. Jurnal Kedokteran Hewan-Indonesian Journal of Veterinary Sciences. 2017;11:35-8.
12. Al-Jasser RN. The effect of overbite and overjet on clinical parameters of periodontal disease: A case control study. Saudi Dent J 2021;33:201-6.
13. Al Shayeb K, Turner W, Gillam D. Accuracy and reproducibility of probe forces during simulated periodontal pocket depth measurements. Saudi Dent J. 2014;26:50-5.
14. Fageeh HN, Meshni AA, Jamal HA, Preethanath RS, Halboub E. The accuracy and reliability of digital measurements of gingival recession versus conventional methods. BMC oral health. 2019;19:1-8.
15. Delgado-Angulo EK, Bernabé E, Marcenes W. Ethnic inequalities in periodontal disease among British adults. J Clin Periodontol 2016;43:926-33.
16. Suharsini M, Budiarjo SB, Indiarti IS, Rudianto YE, Widyagarini A. Effect of tooth brushing, using song and dental model, on plaque index of children with visually impairment. J Int Dent Med Res 2017;10:608-11.
17. Newbrun E. Indices to measure gingival bleeding. J Periodontol 1996;67(6):555-61.
18. Soraya C, Mubarak Z, Gani BA. The growth and biofilm formation of Enterococcus faecalis in ethanol extract of Citrus aurantiifolia Indonesian species. J Pharm Pharmacogn Res 2020;8:558-68.
19. Khairani A, Budiardjo SB, Fauziah E. Correlation between Oral-Health-Related Quality of Life and Salivary Cortisol Level in Children Ages 8–10 Years. J Int Dent Med Res 2018;11(1):149-52.
20. Kato H, Yoshida A, Awano S, Ansai T, Takehara T. Quantitative detection of volatile sulfur compound-producing microorganisms in oral specimens using real-time PCR. Oral Dis 2005;11:67-

71.
21. Kawada M, Yoshida A, Suzuki N, Nakano Y, Saito T, Oho T, et al. Prevalence of Porphyromonas gingivalis in relation to periodontal status assessed by real-time PCR. Oral microbiol Immunol 2004;19:289-92.
22. Sedgley C, Nagel A, Shelburne C, Clewell D, Appelbe O, Molander A. Quantitative real-time PCR detection of oral Enterococcus faecalis in humans. Arch Oral Biol 2005;50:575-83.
23. Bachtiar BM, Gani BA, Deviana A, Utami NR, Andriyani AD, Bachtiar EW. The discrepancy between Clove and Non-Clove Cigarette Smoke-Promoted Candida albicans Biofilm Formation with precoating RNA-aptamer. F1000Research. 2021;10:372.
24. Ghosh SN, De A, Mondal S. Stress hormones and sports performance: A critical analysis. Stress.Int J Physiol Nut Physic Edu 2018; 3:1752-1757
25. Kim J, & Kim, H. Demographic and Environmental Factors Associated with Mental Health: A Cross-Sectional Study. Int J Environ Res Public Health 2017;14:431.
26. Masulili SL, Kemal Y, Soedarsono N, Widyastuti Y, Harsas NA, Maharani DA. The Relationship of Academic Stress to Periodontal Status and Level of Cortisol Hormone, Interleukin 1-[Beta] and Interleukin-6 in Gingival Crevicular Fluid (Study on Profession and Specialist Dental Students Faculty of Dentistry Universitas Indonesia. Jakarta). J Int Dent Med Res 2016;9(2):113-17.
27. Kaur D, Behl AB, Isher PP. Oral manifestations of stress-related disorders in the general population of Ludhiana. J Indian Acad Oral Med Radiol 2016;28:262.
28. King SL, Hegadoren KM. Stress hormones: how do they measure up? Biol Res Nurs 2002;4:92-103.
29. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim) 2017;11:72-80.
30. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernández M, Gamonal J. Host response mechanisms in periodontal diseases. J App Oral Sci :revista FOB 2015;23:329–355.
31. Hamer M, Steptoe A. Cortisol responses to mental stress and incident hypertension in healthy men and women. J Clin Endocrinol Metab 2012;97:E29-E34.
32. Yubiliana G, Raksanagara AS, Susilawati S. Dental Hypnosis Effectiveness to Cortisol Levels As Dental Anxiety Biomarker and Its Correlation with QoL. J Int Dent Med Res 2021;14(2):639-44.
33. Huang C-J, Webb HE, Zourdos MC, Acevedo EO. Cardiovascular reactivity, stress, and physical activity. Front Physiol. 2013;4:314.
34. Stults-Kolehmainen MA, Sinha R. The effects of stress on physical activity and exercise. Sports Med. 2014;44:81-121.
35. Bauroth K, Charles CH, Mankodi SM, Simmons K, Zhao Q, Kumar LD. The efficacy of an essential oil antiseptic mouthrinse vs. dental floss in controlling interproximal gingivitis: a comparative study. J Am Dent Assoc (1939) 2003;134:359-65.
36. Pols H, Oak S. War & military mental health: the US psychiatric response in the 20th century. Am J Public Health. 2007;97:2132-42.
37. Lamster IB. Geriatric periodontology: how the need to care for the aging population can influence the future of the dental profession. Periodontol 2000 2016;72:7-12.
38. Decker AM, Kapila YL, Wang HL. The psychobiological links between chronic stress-related diseases, periodontal/peri-implant diseases, and wound healing. Periodontol 2000 2021;87:94-106.
39. Jentsch HFR, März, D., & Krüger, M. . The effects of stress hormones on growth of selected periodontitis related bacteria. Anaerobe 2013;24 49–54.
40. Walkenhorst MS, Reyes L, Perez G, Progulske-Fox A, Brown MB, Phillips PL. A uniquely altered oral microbiome composition was observed in pregnant rats with Porphyromonas gingivalis induced periodontal disease. Front Cell Infect Microbiol 2020;10:92.
41. Cullinan MP, Seymour GJ. Periodontal disease and systemic illness: will the evidence ever be enough? Periodontol 2000. 2013;62:271-86.
42. Fukui M, Hinode, D., Yokoyama, M., Yoshioka, M., Kataoka, K., & Ito, H.-O. Levels of salivary stress markers in patients with anxiety about halitosis. Arch Oral Biol 2010;55:842-7.
43. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. EXCLI J 2017;16:1057.