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TITLE: “Relationship between Psychological Stress, Serum Cortisol, Expression of MMP-1 and Chronic Periodontitis in Male Police Personnel”

Little Mahendra, Ravi David Austin, S. Senthil Kumar, Jaideep Mahendra, A. John William Felix

Abstract
Aim: The aim of the study was to assess the relationship between Stress, Serum Cortisol, expression of mmp-1 and Chronic Periodontitis in male police personnel. Methodology: Fifty male police personnel were assigned for the study and grouped into Test (Group 1 and Group 2) and Control depending on their probing pocket depth. Control group (Probing pocket depth ≤ 3 mm, n = 10), Test group 1 (at least four sites with probing pocket depth ≥4mm and ≤6 mm, n = 20) and Test group 2 (at least four sites with probing pocket depth > 6 mm, n = 20). The Clinical parameters such as plaque index, Sulcus Bleeding Index, Probing Pocket Depth and Clinical attachment level were recorded. Stress was measured using a stress questionnaire. Blood sample was collected and serum cortisol level was evaluated using ELISA. Expression of MMP-1 was evaluated by RT-PCR. Results: The clinical, psychological and biochemical data were statistically significant with p value<0.001 Conclusion: The present study showed a positive correlation between stress, serum cortisol level, expression of MMP-1 and chronic periodontitis.

Index terms: Chronic Periodontitis, Clinical attachment level, MMP, Probing pocket depth, RT-PCR, Serum Cortisol, Stress.
INTRODUCTION

Periodontitis is an inflammatory disease of bacterial origin leading to progressive destruction of tooth supporting tissues, specifically periodontal attachment loss and alveolar bone loss. Periodontal disease is multifactorial with a complex interaction between bacteria and host responses which is often modified by behavioural factors. Several risk factors and some systemic diseases can contribute to periodontal destruction. Stress is also a contributing factor in periodontal disease. Moreover, the term stress has a precise physiological definition. It is a state of physiological or psychological strain caused by an adverse stimuli, physical, mental or emotional, internal or external that tends to disturb the functioning of an organism and which an organism naturally desires to avoid. Thus “stress” can be viewed as, which process both psychological and physiological components (15).

The role of stress in human periodontal disease has a plausible pathophysiological basis. This is because stress can cause behaviour modification like smoking, alcohol abuse, etc. as well as some immunosuppressive effects (2).

Periodontal disease has been found to be associated with psychosocial stress (3), undesired life events (4), and job stress (5). Among job stress “Police personnel” has an increased risk for emotional disorder due to inherently adversarial nature of police work (6). They are also prone to infectious diseases, physical injury and death.

For many years, the police profession has been ranked among the top five of the most stressful occupations. Police personnel are exposed to chronic non-traumatic stress arising from the demands of their work environment. Police stress, however, refers to the negative pressures related to police work (4).

Almost any type of stress causes an immediate and marked increase in adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary gland followed by an increased secretion of Cortisol from the adrenal cortex. Cortisol is a stress hormone which decreases the permeability of the capillaries, migration of the white blood cells into the inflamed areas as well as suppresses the immune system, thereby causing decrease in the lymphocyte production (7,8) and phagocytosis of the damaged cells.

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Peripathogenic bacteria and their products are the primary etiological agents for the initiation of periodontitis. Among host factors implicated in the destruction of periodontal supporting structures, MMPs are key enzymes for the degradation and remodeling of the extracellular matrix.

MMPs are a family of zinc dependent endopeptidases, which are collectively capable of degrading extracellular matrix, composed of collagenous and non-collagenous proteins (9). They have an important role in various biological processes, including tissue remodeling, wound healing and pathological conditions such as periodontitis, arthritis, tumor cell invasion and metastasis (10). Human MMPs are classified into different groups in relation to their structure and function: collagenases, gelatinases, stromelysins, membrane-type (MT)-MMPs and other MMPs (11). The activity of MMPs is regulated by endogenous tissue inhibitors, especially TIMPs. TIMP-1, 30 kDa glycoprotein, is the main inhibitor of MMPs synthesized by most cells. It forms high-affinity complexes with the active forms of MMPs and might be specially bound to proMMP-9 (12, 13).

Metalloproteinases have mainly been studied in gingival tissues by immunohistochemistry at protein level and by RT-PCR at mRNA level (12). There are two distinct types of MMP-1, 52 kDa glycoprotein and 57 kDa glycoprotein. MMP-1 is expressed by fibroblasts, by keratinocytes during healing and by defence cells during inflammation (10).

The aim of the present study was to find out the levels and the association between, psychological stress, serum Cortisol and expression of MMP-1 in male police personnel with and without Chronic Periodontitis.

MATERIALS AND METHOD

The present study was conducted by the Department of Periodontology, Rajah Muthiah Dental College & Hospital, Annamalai University and Department of Animal Biotechnology Tamil Nadu Veterinary and Animal Science University. The experiment protocol was approved by Institutional Human Ethical Committee, Annamalai University. For the present investigation 50 male police personnel were recruited on the basis of
socio-demographic data. These subjects were divided into three groups on the basis of periodontal examination.

All the subjects selected were based according to the following criteria.

Inclusion criteria:

1. The subjects within the age group of 30 to 50 years.
2. Without any systemic ailments as mentioned in the exclusion criteria.
3. All the subjects had Silness and Löe Plaque score more than or equal to 1.

Exclusion criteria:

1. Participants who went through periodontal treatment in past 6 months.
2. Subjects taking corticosteroid/immunosuppressant drugs for medical conditions like asthma, arthritis, collagen disease etc or in the recent past for ailments like allergies, hay fever, acute adrenal insufficiency, status asthmaticus, arthritis, ocular inflammation etc.

Prior written consent from all the participants was obtained, mentioning the study in detail.

Study Design:

The selected patients were assigned to three groups in accordance with their levels of probing pocket depth (PPD) (13):

Control Group - Probing Pocket Depth ≤ 3 mm.

Test Group 1 - At least four sites with Probing Pocket Depth ≥ 4 mm & ≤ 6 mm.

Test Group 2 - At least four sites with Probing Pocket Depth > 6 mm.

Clinical examination

The clinical examination included

1. Silness-Löe plaque index (PlI)
2. Sulcus Bleeding Index ( SBI )
3. Assessment of Probing Pocket Depth.
4. Clinical attachment level.

Evaluation of stress

All the 50 subjects were evaluated for stress, using Occupational stress questionnaire

The questionnaire consisted of 46 questions. Each question is rated on the five-point scale.

Estimation of Serum Cortisol:

Collection of blood Sample

About 1 ml of blood was collected by venipuncture, from the median cubital vein, between 8:00 - 10:00 a.m. for both test and control groups.

Analysis of Serum Cortisol

Blood was centrifuged, and was used to estimate serum Cortisol level using Cortisol ELISA-kit.

Collection of gingival tissue samples:

Experimental group -

Gingival tissue samples were harvested during extraction of the periodontally hopeless tooth and during closed curettage with deepest periodontal pocket sites for the Periodontitis patients.

Control group -

For the healthy controls, the samples were collected during the surgical crown lengthening or third molar extraction (14)

Table: Details of primer used in for amplifying various MMP genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>F 5'ATGCTGAAACCTGAAGGTG 3'</td>
</tr>
<tr>
<td></td>
<td>R 5'CCTGCTTGACCCTCAGACG3'</td>
</tr>
</tbody>
</table>

Real time PCR results analysis

The Ct values were recorded for each gene expression assayed in real time PCR using the SYBR Green chemistry. All the Ct values were mean of duplicate samples tested as diseased condition. The ∆Ct values indicate the difference in the Ct values between the target gene and the endogenous control gene β-actin.

RESULTS

Human gingival tissues expressed MMP-1, Cortisol and stress affected the regulation of MMP-1.

Fig -1

Pearson's correlation co-efficient values between occupational stress, serum cortisol and MMP-1

<table>
<thead>
<tr>
<th></th>
<th>OCCUPATIONAL STRESS</th>
<th>SERUM CORTISOL</th>
<th>MMP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>0.881***</td>
<td>0.716***</td>
<td>0.360**</td>
</tr>
<tr>
<td>SBI</td>
<td>0.903***</td>
<td>0.619***</td>
<td>0.446**</td>
</tr>
<tr>
<td>PPD</td>
<td>0.842***</td>
<td>0.614***</td>
<td>0.405***</td>
</tr>
<tr>
<td>CAL</td>
<td>0.762***</td>
<td>0.500***</td>
<td>0.327***</td>
</tr>
</tbody>
</table>

***P<0.001,  **P<0.01,  *P<0.05
Discussion

The known correlation between periodontal breakdown and psychosocial stress status may derive from the effect of increased cortisol levels. Periodontitis is characterized by connective tissue breakdown, with proliferation and apical migration of the junctional epithelium. Cortisol seems to play an important role in the pathogenesis of periodontal disease, probably exerting a strong inhibitory effect on the inflammatory process and immune responses.(15) Individuals with psychologic stress may have decreased cell-mediated immune activity, natural killer cell function and lymphocyte responses to mitogenic stimulation.(16). Chronic activation of the HPA axis may also influence the initiation and progression of periodontitis through dysregulation of circulating cortisol and other hormones that affect immune function.(17). In contrast, the development of periodontal disease might also be related to conditions that alter the host’s resistance to periodontopathic bacteria. A positive relationship exists among stress scores, serum cortisol and severity of periodontitis. This is likely because of factors such as altered immune responses that facilitate increased colonization by pathogenic bacteria and the breakdown of the periodontal attachment.(16) The immune response plays an important protective and destructive role. If the immune system cannot protect the body against infection, the body has to rid itself of the bacteria by exfoliating the teeth to which the bacteria are attached.(17). When the inflammatory action is sufficiently long and profound, systemic manifestations of the disease might become evident, as could happen with periodontitis.

In the present study, the subjects were grouped into two test groups and one control group. This method of grouping the sample with 2 study groups and 1 control also called as 2:1 Case-Control Match (18), was similar to the study done by Vetore et al(19). A 2:1 Case-Control ratio was chosen to increase the power of the Study.

The results of the the study suggests an immunologic explanation for the cortisol and periodontitis relationship, which is contrary to some previous studies.(20) The inconsistency might be due to difference in sampling techniques. The present study also suggests that stress and cortisol are positively associated with periodontal destruction and its level of severity. The higher cortisol concentrations might have significantly upregulated the expression of MMP-1, in human gingival samples, which could constitute a mechanism underlying the increased periodontal breakdown associated with psychosocial stress status.

CONCLUSION

Cortisol produces regulation of MMP-1 expression. Higher Cortisol levels significantly up regulate the expression of MMP-1 in human gingival tissue samples, which may constitute a mechanism likely to be involved in the increased periodontal breakdown, associated with psychosocial stress status.

ACKNOWLEDGMENTS

The Author is grateful to Mr. Abhay kumar, I.P.S, Chennai. For permitting to conduct this study on police personnel.

<table>
<thead>
<tr>
<th></th>
<th>MEAN VALUE</th>
<th>STANDARD ERROR</th>
<th>ANOVA F</th>
<th>P value</th>
<th>Bonferroni multiple comparison test result</th>
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<tbody>
<tr>
<td>Control</td>
<td>25.37</td>
<td>1.253</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Test group 1</td>
<td>25.03</td>
<td>0.640</td>
<td>10.671</td>
<td>&lt;0.001</td>
<td>Control,mild periodontitis Vs severe periodontitis</td>
</tr>
<tr>
<td>Test group 2</td>
<td>29.34</td>
<td>0.734</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reference:

6. Police literature 2005 - An Australian focused compendium
12. Police literature 2005 - An Australian focused compendium