Gingival crevicular fluid levels of prolactin hormone in periodontitis patients before and after treatment and in healthy controls

Naglaa M. El-Wakeel[1], Olfat Shaker[2] and Eman M. Amr[3]

[1]Faculty of Dentistry, October University for modern sciences and arts (MSA), 6th October, Egypt, and Faculty of Dentistry, Al-Azhar University(Girls Branch), Cairo ,Egypt; [2]Faculty of Medicine, Cairo University, Cairo, Egypt; [3]Faculty of Dentistry, Cairo University, Cairo, Egypt

Abstract

Objective: Prolactin (PRL) acts as a hormone and as a cytokine and is linked to the pathogenesis of a variety of chronic inflammatory diseases. This study aimed to investigate a potential role for prolactin in the pathogenesis of periodontitis by assessing its local gingival crevicular levels before and after periodontal treatment, compared to controls. **Materials and Methods:** 40 participants were included and divided into 2 groups. Group 1; included 20 patients suffering from moderate to severe periodontitis and group 2; included 20 healthy controls. GCF samples were collected from both groups after initial clinical examination and 3 months after scaling and root planning for the periodontitis group only. Levels of prolactin were tested by enzyme linked immunosorbent assay. **Results:** At baseline, a statistically significant elevated PRL levels were shown in the periodontitis group compared to controls ($p<0.001$), with a non significant difference between males and females ($P >0.05$) . Periodontal debridement caused a significant reduction in PRL levels but these levels remained significantly higher compared to controls levels. A100% diagnostic accuracy was shown for PRL levels in the tested groups.

Conclusions: Our results suggest a role for PRL in the pathogenesis of periodontitis, further, it could represent a reliable biomarker for disease activity and prognosis.

Keywords: Prolactin, gingival crevicular fluid, periodontitis

Introduction

Periodontitis is a chronic inflammatory multifactorial disease affecting the tooth supporting structures and is associated with dysbiotic plaque biofilms resulting in progressive clinical attachment loss and alveolar bone destruction (Papapanou *et al*., 2018). Periodontal inflammatory processes are, by and large, immunologic, as bacterial factors induce a local inflammatory reaction and activate the innate immune system through activation of Toll-like receptors (TLR), located on the surface of resident cells and leukocytes. Activation of these cells results in the production of pro-inflammatory cytokines resulting in recruitment of phagocytes and lymphocytes into the inflammation zone (Gankovskaya *et al*., 2016). These bacteria-induced inflammatory mechanisms are the suspected links between periodontitis and inflammatory systemic conditions (Goldie, 2010; Berthelot and Le Goff, 2010).

Hormones are specific regulatory molecules that modulate reproduction, growth, development as well as maintenance of internal environment such as energy production, utilization and storage (Bhardwaj and Bhardwaj, 2012). It has been demonstrated that hormones can affect periodontal pathogenic mechanisms. Puberty, menstrual cycle, menopause and pregnancy are all biological phases that affect periodontal health in females (Khosravisamani *et al*., 2014). An association between periodontal inflammation and changes in certain hormone levels such as leptin and estrogen have been demonstrated (Gundala *et al*,. 2014; Shapiro and Freeman, 2014). Further, the link between some systemic diseases associated with hormonal disturbance and periodontitis has been defined in the latest classification of Periodontal Diseases and Conditions (Jepsen *et al*., 2018).

Correspondence to: Naglaa M, El-Wakeel El-sheikh Zayed, Giza, Egypt. Email nelwakeel@hotmail.com

Prolactin (PRL) is a 23-kD peptide hormone secreted in the pituitary gland, in extra-pituitary locations, such as adipose tissue, and produced by immune cells (Borba *et al.*, 2018). Although PRL's main function is to regulate differentiation and lactation of the mammary epithelium, it also plays an important regulatory role in inflammation, cell survival, and proliferation by binding to the PRL receptor (PRLR) - a member of the type 1 cytokine /hematopoietic receptor superfamily- which is widely expressed through the immune system cells, including monocytes, lymphocytes, macrophages, natural killer cells and granulocytes (Devi and Halperin, 2014; Krasselt and Baerwald, 2017).

For the many immune-stimulatory effects of prolactin, hyper-prolactinemia (HPRL) has been linked to the pathogenesis and activity of several inflammatory, immune-mediated disorders. A significant association between high PRL levels and disease activity has been found in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as well as during pregnancy and lactation periods, suggesting an active biologic role for PRL (Orbach and Shoenfeld, 2007; Tang *et al.*, 2017a). Further, many inflammatory diseases may have periodontal manifestations (Kira *et al.*, 1991; Fuggle *et al.*, 2016). The increased local production of PRL at sites of active inflammation and subsequent tissue destruction has been reported. For example, PRL levels are increased in the synovial fluid levels in patients with active RA compared to controls, suggesting that these elevated levels are due to increased local secretion by immune cells in relation to disease activity (Tang *et al.*, 2017a).

Assembling together the following facts; 1) periodontitis is, in part, an immunologically mediated disease; 2) the established relation between PRL and inflammation; 3) the proven association of some immunologically mediated diseases (e.g. RA) and periodontitis, and; 4) to date, data regarding the local levels of PRL in periodontal tissues in health and disease are not yet presented, we hypothesized that an up-regulation of local PRL levels would be present in periodontitis patients. The present study aimed to address whether there an increase in the local prolactin levels in periodontitis patients compared to controls and whether periodontal treatment can result in a decrease in these levels.

Material and Methods

This clinical trial has been registered at Clinical Trials.gov. (Identifier NCT03726411).

Study Population

Forty participants (20 males and 20 females; age range from 18-52 years) were randomly recruited from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry, MSA University, Egypt, into the present study from September 2017 to January 2018). Two groups were created as follows: systemically and periodontal healthy control group (n = 20), systemically healthy patients with periodontitis (n = 20).

A detailed medical history of each individual was obtained according to the modified Cornell Medical Index questionnaire (Abramson, 1966). After the study nature and aims were explained, each participant signed an informed consent form. This study was independently reviewed and approved by the Research Ethics Committee, Faculty of Dentistry, Cairo University.

Inclusion criteria

For the control group, subjects with clinically healthy gingiva (plaque index [PI] <1 and gingival index [GI] <1), zero clinical attachment loss (CAL) and with no previous history of periodontal disease were included. For the periodontitis group, subjects with moderate to severe periodontitis were included and diagnosed according to the American Academy of Periodontology, 2000 (AAP 2000). Periodontitis patients had 14 or more natural teeth, of which at least five had a site with probing depth (PD) $\geq$ 5 mm and CAL $\geq$ 4 mm. To confirm diagnosis, alveolar bone loss was examined using peri-apical radiographs.

Exclusion criteria

The following were use as exclusion criteria: 1) pregnancy or lactation; 2) any known systemic disease; 3) any medication and/or antibiotic 3 months before the study; 5) any type of previous periodontal treatment (surgical or non-surgical) in the preceding 6 months; 6) smoking (former or current); 7) abnormal body mass index BMI.

Periodontal Examination and Treatment:

At baseline, all participants received a full-mouth clinical examination, and the following periodontal parameters were recorded: Plaque Index (PI) (Silness and Loe, 1964), Gingival Index (GI) (Loe and Silness, 1963), Pocket Depth (PD), and Clinical Attachment Loss (CAL). All of these measurements were recorded by a single calibrated examiner (E.A.) at six sites (mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual, and mid-lingual) for all teeth. Calibration exercises for probing measurements were performed in five patients before the study. The intra-examiner reliability was acceptable, with a 0.82 k value. PD was measured from the gingival margin to the base of the periodontal pocket, and CAL was measured from the cemento-enamel junction to the base of the periodontal pocket. Measurements were rounded to the highest whole millimeter using the Michigan 0 probe with Williams markings (*Hu Friedy, Chicago, IL*).

Closed periodontal debridement was done using hand scalers and curettes with ultrasonic instrumentation, performed in two sessions with oral hygiene instructions.

Initial periodontal examination, full mouth scaling and root planning (SRP), clinical measurements before and after treatment, gingival crevicular fluid sampling as well as reinforcement of oral hygiene at baseline and during follow-up evaluations were performed by the same periodontist (E.A.). Reinforcement of oral hygiene was done every month for 3 months.

Gingival crevicular fluid (GCF) sampling:

Gingival crevicular fluid (GCF) collection was done the second day after clinical examination to prevent the contamination of sample with blood associated with the probing of inflamed sites. Samples were collected from the buccal aspects of two interproximal sites in teeth that had the highest signs of inflammation and attachment loss for periodontitis groups, this was done before, and 3 months after SRP. For the control group, samples were collected from the upper first molar.

Before GCF sampling, plaque was removed carefully by sterile curettes and the surfaces were dried and isolated by cotton rolls. Filter paper strip (Periopaper; ProFlow Inc., Amityville, NY, USA) was placed in the selected sites for 30 seconds. Care was taken to avoid mechanical trauma and strips visually contaminated with blood were discarded. The samples were assayed for PRL by using an enzyme linked immunosorbent assay (ELISA) kits (Prechek Bio, Inc. CA, USA). This assay is a sandwich technique ELISA and was performed according to manufacturer's instructions using human recombinant standards. The results were expressed as concentrations in nanograms per milliliter (ng/ml), sensitivity of the kit was 5 ng/ml.

Power Calculations

The Power analysis for this study used Prolactin level after 3 months as the primary outcome. The effect size (d = 1.0155) was calculated based upon a pilot study conducted on 5 periodontitis patients and 5 control subjects due to the absence of previous studies investigating the GCF levels of PRL. The mean (SD) of Prolactin levels were 17.4 (2.1) and 15 (2.6) ng/ml for the two groups, respectively. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%; the minimum estimated sample size was a total of 34 subjects. The sample size was increased to a total of 40 subjects (20 subjects per group) to compensate for a dropout rate of 15%. Sample size calculation was performed using G*Power Version 3.1.9.2.

Statistical Analysis

Numerical data were assessed for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Non-parametric data were presented as median and Inter-Quartile Range (IQR) values, for parametric data; Student's t-test was used. Repeated measures ANOVA test was used to compare between prolactin levels in the two groups as well as to study the changes in Prolactin levels within Periodontitis group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. For non-parametric data; Wilcoxon signed-rank test was used to study the changes after treatment within Periodontitis group. ROC (Receiver Operating Characteristic) curve was constructed to determine the cut-off values of Prolactin level for differentiation between the different groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM and SPSS Statistics Version 30 for Windows *(IBM Corporation, NY, USA.)*. ROC curve analysis was performed with MedCalc Version 11.3 for Windows (*MedCalc Software bvba).*

Results

Gender distributions showed no significant difference between the two groups was reported ($p > 0.05$). Similarly for age, patients in periodontitis group had a mean age values of 34.3 years (10.9) with a non-significant difference compared to the control group that had a mean of 31.4 years (4.8) ($p > 0.05$) (Table 1). Body mass index mean values were 23.52(0.83) and 23.38(0.9) kg/m2 for groups periodontitis and controls respectively, with a non-significant difference reported ($p > 0.05$).

At base line, PRL was increased in all GCF from periodontitis sites. The periodontitis group showed statistically significantly higher mean PRL levels (37.8(7.4) ng/ml) compared to the controls (12.6(1.5) ng/ml) ($p < 0.001$). For mean PRL levels between males and females at base line, a no significant difference was found, 27.7 (11.4) and 30.9 (10) ng/ml respectively) ($p > 0.05$). All tested clinical parameters at 3 months after SRP showed a statistically significant ($p<0.001$) reduction in PRL levels compared to the pretreatment levels (22.5(5) and 37.8 (7.4) respectively (Tables 2, 3; Figures 1, 2).

Table 1. Descriptive statistics and results of comparisons between base line characteristics in the two groups

	Periodontitis (n = 20)	**Control (n = 20)**	**P-value**
Age (Years)			
Mean (SD)	34.3 (10.9)	31.4 (4.8)	0.430
Gender [n (%)			0.206
Male	8/20 (40%)	12/20 (60%)	
Female	12/20 (60%)	8/20 (40%)	

*: Significant at $P \leq 0.05$

Table 2. Median, Inter-Quartile Range (IQR) values and results of Wilcoxon signed-rank test for comparisons between clinical parameters before and after treatment

Clinical parameters	Before treatment (n = 20)	After treatment (n = 20)	% Change	P-value
Plaque Index (PI)	2 (1.3 - 3)	0 (0 – 0)	-100 (-100 – -100)	<0.001*
Gingival Index (GI)	2 (2 - 2)	0 (0 – 0.8)	-100 (-100 – - 62.5)	<0.001*
Probing Depth (PD)	6 (6 - 7)	3 (2 – 4)	-50 (-66.7 – - 44.6)	<0.001*
Clinical Attachment Level (CAL)	7 (6 - 8)	4 (3 – 5)	-43.7 (-55.4 – - 34.1)	<0.001*

*: Significant at $P \leq 0.05$

Table 3. Mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between Prolactin levels in the two groups and the changes within Periodontitis group

Prolactin (ng/ml)	Periodontitis (n = 20)	Control (n = 10)	P-value
Before treatment	37.8 (7.4)	12.6 (1.5)	<0.001*
After treatment	22.5 (5)	12.6 (1.5)	<0.001*
% Change	-39.9 (10.1)	-	-
P-value (Within group)	<0.001*		

*: Significant at $P \leq 0.05$

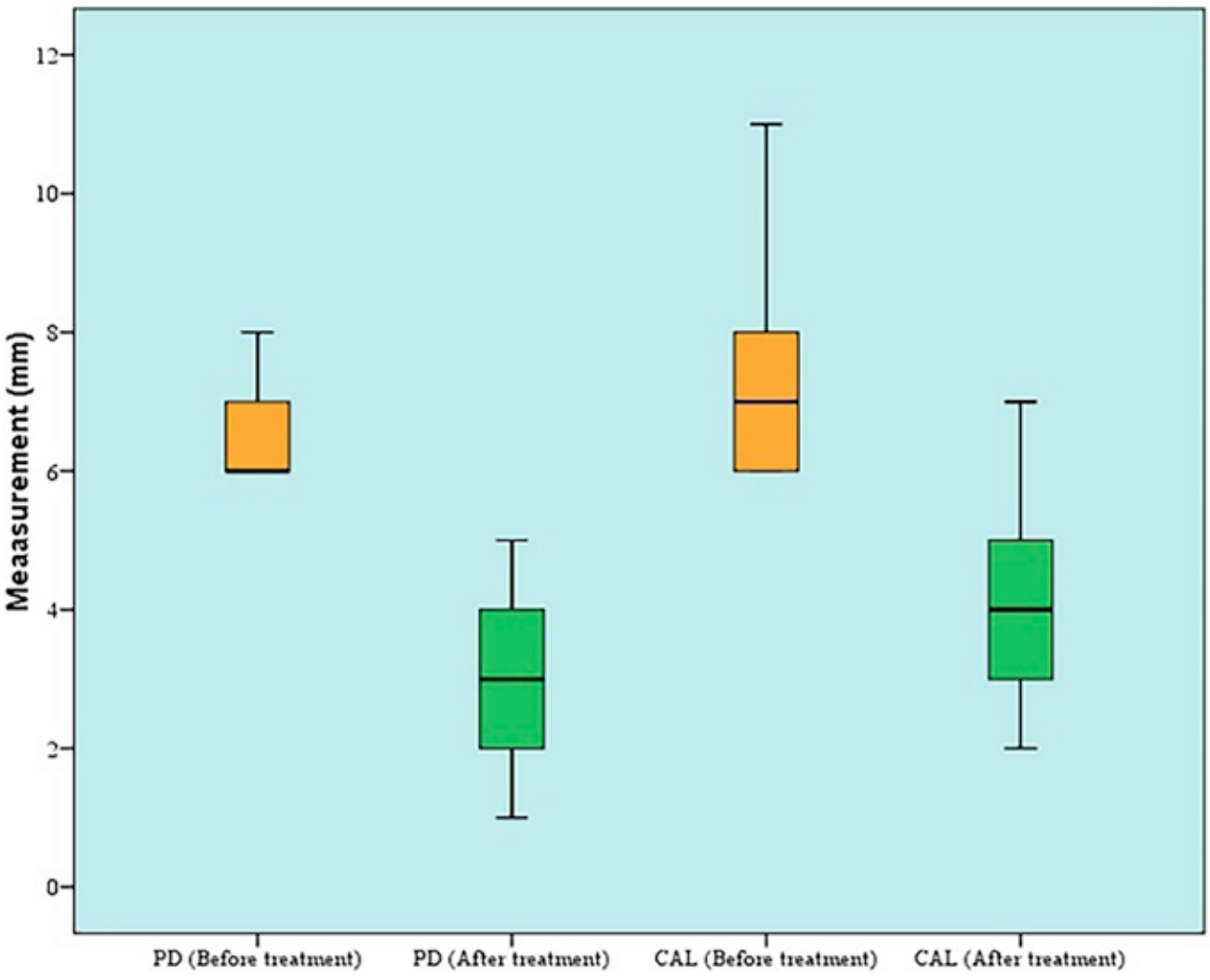

Figure 1

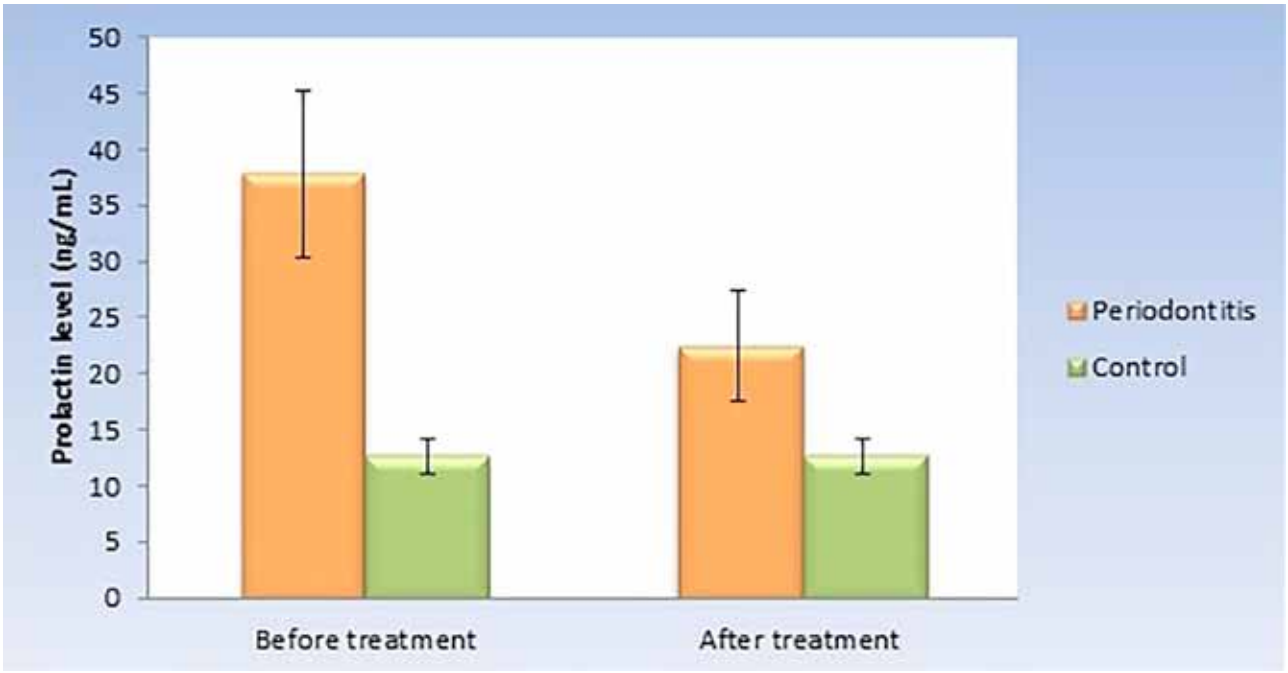

Figure 2

ROC curve analysis was used in this study to determine the diagnostic accuracy of PRL for the differentiation between periodontitis and control groups which is presented in Table 4. Analysis of the ROC curve revealed the highest area under the ROC curve (AUC) of 1.000 with a 100% sensitivity and specificity. The cut-off value was determined at14.6 ng/ml with a 100% diagnostic accuracy at this value. This means that subjects with GCF PRL level exceeding this cut-off value are considered to have periodontitis while those showing a reading below this level are considered to have healthy periodontium (Figure 3).

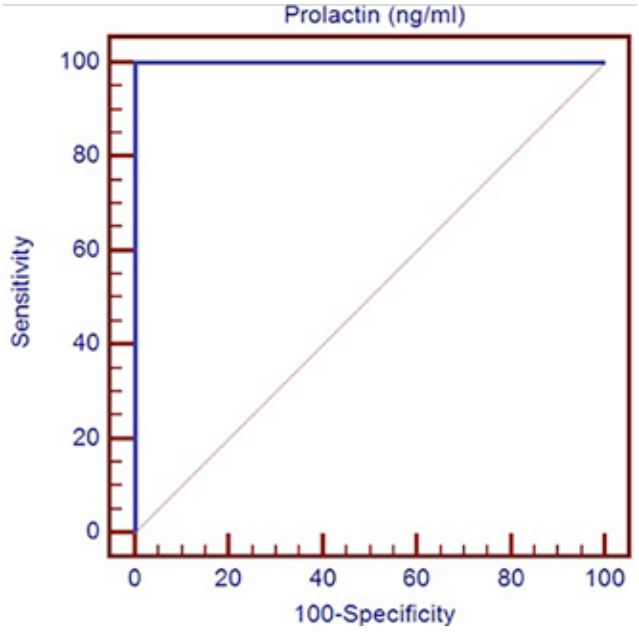

Figure 3

Discussion

Tissue destruction in periodontitis is due to complex interactions between pathogenic bacteria and the host immune- inflammatory response in which, a networks of cytokines are involved (Hajishengallis, 2014). The prolactin hormone has been linked to functional interactions within the immune system, having multiple immune modulatory effects. PRL significantly increases the ability of the immune cells to proliferate and to produce a wide variety of cytokines and chemokines such as TNF-alpha, IFN-gamma, IL-12, IL-1 beta (Kanda *et al.*, 2013). PRL enhances the differentiation of CD4+ and CD8+ cells, promotes dysfunction of regulatory T cells and increases T cells adhesion to endothelial cells (Tomio *et al.*, 2008). Furthermore, PRL influences B-cell maturation process, promoting the survival of self-reactive clones and increases the viability of immature B cells by rescuing them from apoptosis (Peeva and Zouali, 2005; Flores-Fernandez *et al.*, 2016). It also increases TNF-alpha expression by monocytes. Furthermore, PRL acts with other pro-inflammatory stimuli to activate macrophages via the cell surface PRLR (Carvalho-Freitas *et al.*, 2013; Tang *et al.*, 2017a).

The fact that PRL is associated with osteoporosis both in males and females has long been established, although the exact mechanism is not yet determined (Sperling and Bhatt, 2016). PRL has a direct effect on osteoblasts mediated through the expression of PRL receptors and results in decreased proliferation with an overall increased rate of apoptosis and decreased calcium content in these cells (Seriwatanachai *et al.*, 2009). In addition, high serum PRL levels are negatively associated with bone mineral content and bone density in patients with SLE (Elera-Fitzcarrald *et al.*, 2017).

Table 4. Cut-off values for Prolactin and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for differentiation between the groups

Cut-off value	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
14.6	100	100	100	100	100	1.000	0.884 – 1.000

+PV: Positive Predictive Value, -PV: Negative Predictive Value

This is especially relevant when considering a possible relationship between periodontal disease and PRL levels.

To the best of our knowledge, the present study is the first to assess local PRL protein levels in GCF of subjects suffering from periodontitis as well as in healthy controls, before and after periodontal treatment. In order to exclude the possibility of PRL derived from circulation, systemically healthy patients were included. Furthermore, pregnant or lactating females and patients suffering from any known condition that causes HPRL were excluded from the study.

Tang *et al.*, 2017a, suggested that systemic levels of PRL (originating from the anterior pituitary gland) may have a minor role in the pathology of inflammatory arthritis because of the inconsistent data targeting systemic levels of PRL in RA patients. They reported that an autocrine /paracrine loop of PRL acting locally in the synovial tissues may be more important because it is correlated with the clinical parameters of disease activity and systemic inflammatory markers like erythrocyte sedimentation rate. Accordingly, our main focus was to measure GCF levels of PRL in periodontitis patients. The results of the current study confirmed our research hypothesis, as a significant difference between mean GCF levels of PRL concentration in the healthy group(12.6 ng/ml) and in the periodontitis group(37.8 ng/ml) was demonstrated.

Our results could only be compared to a recently conducted cross sectional baseline study that estimated serum and GCF levels of PRL in subjects with acromegaly, periodontitis and healthy controls (Ozdemir *et al.*, 2019). This study has some interesting findings, first; there was no significant difference between serum levels of PRL in periodontitis subjects versus healthy controls, which supports our point of view regarding not measuring the serum PRL levels in our study. Second, in agreement with our results, this study reported elevated gingival crevicular PRL levels in periodontitis patients compared to controls, although a non significant difference was reported. However, in the Ozdemir study, the inclusion and exclusion criteria were not clearly described, for instance, it was not reported if periodontitis patients received any type of periodontal treatment in the past 3 months prior to the GCF sampling, or if they were taking anti-inflammatory treatment or antibiotics, moreover, and in accordance with our explanation, this study reported a non-significant difference between crevicular IL-6 levels in periodontitis patients versus controls, despite of the well known fact that IL-6 is strongly associated with periodontal disease destruction.

Our results could be interpreted in the context of previous data that reported elevated local PRL levels in association with other inflammatory conditions with immunologic background. Manconi *et al.*, 2018 reported elevated salivary PRL levels in patients with multiple sclerosis and highlighted its great potential to be a reliable biomarker for disease activity. Further, studies reported the high expression of synovial PRL in RA (Tang *et al.*, 2017a; Tang *et al.*, 2016). These studies showed that, PRL was synthesized by synovial T cells, macrophages and fibroblasts. Moreover, they proved that in active RA, synovial PRLR expression is enhanced. Tang *et al.*, 2017b, have suggested that PRL cooperates with other pro-inflammatory stimuli to activate monocytes via engagement with the PRL receptors. We propose that the elevated expression of pro-inflammatory cytokines in periodontitis may play a role in the overt expression of PRL in GCF. Mediators for periodontal tissue destruction like IL-1 and TNF- alpha are well known stimulators of PRL secretion (Gerlo *et al.*, 2006; Savino, 2017).

Another mechanism that can explain the association between local PRL and periodontitis is that PRL interferes specifically with B cell tolerance induction, enhances proliferative response to antigens and increases the production of immune globulins, cytokines and autoantibodies (Borba *et al.*, 2018; Peeva and Zouali, 2005; Tang *et al.*, 2017a). Several studies have recently highlighted the role of B cells in physio-pathogenesis of periodontal disease supported by the high predominance of B cell and plasma cell in advanced periodontal lesions (Kim *et al.*, 2010; Demoersman *et al.*, 2018). Coat *et al.*, 2015 showed that B cells deficiency lead to improved periodontal parameters in patients with RA. Whereas, Demoersman *et al.*, 2018 reported the increased prevalence of auto-reactive B cells with increased RANKL expression in subjects with severe periodontitis.

In our study, PRL in GCF was found to be significantly increased in both male and female patients with a non significant differences noted between them. This agrees with previous studies that have reported a no significant difference between males and females regarding elevated local and systemic PRL levels in RA patients (Tang *et al.*, 2016). Such findings strengthen the role of PRL as a cytokine and indicates that there is a local release of RRL in response to periodontal inflammation. On the other hand, it is worth mentioning that HPRL in males is a cause of erectile dysfunction (ED) (Zeitlin and Rajfer, 2000), and recently, Martín *et al.*, 2018 reported, based on clinical and biochemical findings, that; periodontitis is a risk factor for ED.

In our study a statistically significant decrease in PRL levels as well as all tested clinical parameters was noted 3 months after closed periodontal debridement. Further, a statistically significant increase in PRL levels was reported between the post-treatment levels compared to controls, this is specially relevant to one of the important remarks in the late consensus report of the 2017 World Workshop. Thus once a patient experiences periodontitis they will always be considered a periodontitis patient (Papapanou *et al.*, 2018). Finally, the ROC curve interpretation in the present work yielded 100% sensitivity and specificity of PRL level in GCF. This finding suggests that PRL could serve as a diagnostic indicator of periodontal disease activity with the highest diagnostic accuracy.

Study limitations and future directions in research

Study limitations that need to be considered are: 1. Relatively small sample size; 2. Lack of data among gingivitis patients to evaluate the levels of PRL as the inflammation progresses from health to severe periodontitis; 3. Effect of pregnancy and breast feeding on GCF levels of periodontitis patients. Ongoing investigations are running to answer these questions.

Conclusions

Within the limitations of the present study, our findings suggest a possible role for prolactin in periodontal disease pathogenesis and as a biomarker for disease activity. Further studies are indeed required to clarify the underlying mechanisms, especially regarding the relationship between periodontitis and some systemic diseases like Rheumatoid arthritis.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

References

Abramson JH. The Cornell Medical Index as an epidemiological tool. *American Journal of Public Health* 1966; **56**:287-298.

Albandar JM, Susin C and Hughes FJ. Manifestations of systemic diseases and conditions that affect the periodontal attachment apparatus: case definitions and diagnostic considerations. *Journal of Periodontology* 2018; **89**:S183–S203.

American Academy of Periodontology. Parameter on chronic periodontitis with slight to moderate loss of periodontal support. *Journal of Periodontology* 2000; **71**: 853–855.

Berthelot JM and Le Goff B. Rheumatoid arthritis and periodontal disease. *Joint Bone Spine* 2010; **77**:537-541.

Bhardwaj A and Bhardwaj SV. Effect of Androgens, Estrogens and Progesterone on Periodontal Tissues. *Journal of Orofacial Research* 2012; **2**:165-170.

Borba VV, Zandman-Goddard G and Shoenfeld Y. Prolactin and Autoimmunity. *Frontiers in Immunology* 2018; **9:**73.

Carvalho-Freitas MI, Anselmo-Franci JA, Palermo-Neto J and Felicio LF. Prior reproductive experience alters prolactin-induced macrophage responses in pregnant rats. *Journal of Reproductive Immunology* 2013; **99**:54–61.

Coat J, Demoersman J, Beuzit S, *et al.* Anti-B lymphocyte immunotherapy is associated with improvement of periodontal status in subjects with rheumatoid arthritis. *Journal of Clinical Periodontol*ogy 2015; **42**:817-823.

Demoersman J, Pochard P, Framery C, *et al.* B cell subset distribution is altered in patients with severe periodontitis. *PLoS ONE* 2018; **13**:e0192986.

Devi YS and Halperin J. Reproductive actions of prolactin mediated through short and long receptor isoforms. *Molecular Cell Endocrinology* 2014; **382**:400–410.

Elera-Fitzcarrald C, Ugarte-Gil MF, Gamboa-Cárdenas RV, *et al.* Prolactin levels are associated with a pro-inflammatory body mass distribution among women with systemic lupus erythematosus. *Lupus* 2017; **26**:808-814.

Flores-Fernandez R, Blanco-Favela F, Fuentes-Panana EM, *et al.* Prolactin rescues immature B-cells from apoptosis induced by B-cell receptor cross-linking. *Journal of Immunology Research* 2016; 2016:3219017.

Fuggle NR, Smith TO, Kaul A and Sofat N. Hand to mouth: a systematic review and meta-analysis of the association between rheumatoid arthritis and periodontitis. *Frontiers in Immunology* 2016; **7:**80.

Gankovskaya LV, Khelminskaya NM, Molchanova EA and Svitich OA. Role of innate immunity factors in periodontitis pathogenesis. *Zhurnal Mikrobiologii Epidemiologii Immunoogii* 2016; **2**:100-107.

Gerlo S, Verdood P and Kooijman R. Tumor necrosis factor-alpha activates the extrapituitary PRL promoter in myeloid leukemic cells. *Journal of Neuroimmunology* 2006; **172**:206-210.

Goldie MP. The relationship of inflammation to systemic diseases and chronic periodontitis. *International Journal of Dental Hygiene* 2010; **8**:253-255.

Gundala R, Chava VK and Ramalingam K. Association of leptin in periodontitis and acute myocardial infarction. *Journal of Periodontology* 2014; **85**:917-924.

Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends in Immunology* 2014; **35**:3-11.

Jepsen S, Caton JG, Albandar JM, *et al.* Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Periodontology* 2018; **89**:S237–S248.

Kanda N, Hau CS, Tada Y and Watanabe S. Prolactin May Promote the Development of Psoriasis: Reawakened Issue. *Journal of Clinical and Experimental Dermatology Research* 2013; **4**:198.

Khosravisamani M, Maliji G, Seyfi S, *et al.* Effect of the menstrual cycle on inflammatory cytokines in the periodontium. *Journal of Periodontal Research* 2014; **49**:770-776.

Kim YC, Ko Y, Hong SD, *et al.* Presence of Porphyromonas gingivalis and plasma cell dominance in gingival tissues with periodontitis. *Journal of Oral Diseases* 2010; **16**:375-381.

Kira J, Harada M, Yamaguchi Y, Shida N and Goto I. Hyperprolactinemia in multiple sclerosis. *Journal of Neurological Sciences* 1991; **102**:61-66.

Krasselt M and Baerwald C. Sex, symptom severity, and quality of life in rheumatology. *Clinical Review of Allergy and Immunity*. 2017; **11**:1–16.

Loe H and Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontologica Scandinavica* 1963; **21**:533–540.

Manconi B, Liori B, Cabras T, *et al.* Top-down proteomic profiling of human saliva in multiple sclerosis patients. *Journal of Proteomics*. 2018; **187**:212-222.

Martín A, Bravo M, Arrabal M, Magán-Fernández A and Mesa F. Chronic periodontitis is associated with erectile dysfunction. A case–control study in European population. *Journal of ClinicalPeriodontology*. 2018; **45**:791-798.

Orbach H and Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. *Autoimmunity Review*. 2007; **6**:537-542.

Ozdemir Y, Keceli G, Helvaci N, Erbas T and Nohutcu R. The tendency of reduced periodontal destruction in acromegalic patients showing similar inflammatory status with periodontitis patients. *Endocrine* 2019 Sep 2. doi: 10.1007/s12020-019-02060-2.

Papapanou P, Sanz M, Buduneli N, *et al.* Periodontitis: Consensus report of workgroup 2 of the 2017World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Periodontology*. 2018; **89**:S173–S182.

Peeva E and Zouali M. Spotlight on the role of hormonal factors in the emergence of autoreactive B-lymphocytes. *Immunology Letters* 2005; **101**:123–143.

Savino W. Prolactin: an immunomodulator in health and disease. *Frontiers in Hormone Research* 2017; **48**:69–75.

Seriwatanachai D, Krishnamra N and Van Leeuwen J P. Evidence for direct effects of prolactin on human osteoblasts: inhibition of cell growth and mineralization. *Journal of Cellular Biochemistry* 2009; **107**:677–685.

Shapiro LF and Freeman K. The relationship between estrogen, estrogen receptors and periodontal disease in adult women: a review of the literature. *The New York State Dental Journal*. 2014; **80**:30-34.

Silness J and Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica*. 1964; **22**:112–120.

Sperling S and Bhatt H. Prolactinoma: A Massive Effect on Bone Mineral Density in a Young Patient. *Case Reports in Endocrinology* 2016; 6312621.

Tang MW, Garcia S, Gerlag DM, Tak PP and Reedquist KA. Insight into the endocrine system and the immune system: a review of the inflammatory role of prolactin in rheumatoid arthritis and psoriatic arthritis. *Frontiers in Immunology* 2017;**8:**720.

Tang MW, Garcia S, Malvar Fernandez B, Gerlag DM, Tak PP and Reedquist KA. Rheumatoid arthritis and psoriatic arthritis synovial fluids stimulate prolactin production by macrophages. *Journal of Leukocyte Biology* 2017; **102:**897–904.

Tang MW, Reedquist KA, Garcia S, *et al.* The prolactin receptor is expressed in rheumatoid arthritis and psoriatic arthritis synovial tissue and contributes to macrophage activation. *Rheumatology (Oxford)* 2016; **55:**2248–2259.

Tomio A, Schust DJ, Kawana K, *et al.* Prolactin can modulate CD4+ T-cell response through receptor-mediated alterations in the expression of T-bet. *Journal of Immunology and Cell Biology* 2008; **86**:616–621.

Zeitlin S and Rajfer J. Hyperprolactinemia and Erectile Dysfunction. *Reviews in Urology* 2000; **2:**39–42.