European Review for Medical and Pharmacological Sciences 2022; 26: 6169-6175

Association of HMGBP1 expression with clinical periimplant parameters among smokers and never-smokers with and without peri-implantitis

A.R. AHMED[1], M.A. KAMRAN[2], G. SULEMAN[1], R.A. SHARIF[1], H.L. ABOUZEID[1], S.E. UDEABOR[3]

[1]Department of Prosthodontics, [2]Department of Pedodontics and Orthodontic Sciences, [3]Department of Oral and Maxillofacial Surgery, College of Dentistry, King Khalid University, Abha, Saudi Arabia

Abstract. – OBJECTIVE: With our study we aimed at investigating the levels of high mobility group box chromosomal protein-1 (HMGB-1), tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β in periimplant crevicular fluid (PICF) of smokers and never-smokers, with and without periimplantitis, and correlate these levels with the clinical and radiographic periimplant parameters.

SUBJECTS AND METHODS: Sixty participants (n=15/group) were recruited and divided into 4 groups: cigarette smokers with periimplantitis (CSPI); cigarette smokers without periimplantitis (CSNPI); never-smokers with periimplantitis (NSPI); and never-smokers without periimplantitis (NSNPI). Clinical and radiographic periimplant parameters, including plaque scores (PS), bleeding on probing (BOP), probing depth (PD) and crestal bone level (CBL), were assessed. Crevicular levels of HMGB-1, TNF-α, and IL-1β were quantified using human enzyme linked immunosorbent assay. *p*-values were generated using Kruskal-Wallis' test for comparison between the study groups, while correlations between HMGB-1, TNF-α, IL-1β levels and clinical variables were analyzed using Spearman rank correlation coefficient analysis.

RESULTS: Bleeding on probing was least in NSNPI and CSNPI followed by CSPI and NSPI ($p<0.05$). The highest PD and CBL was recorded for CSPI and NSPI groups, while the least PD and CBL were recorded among non-periimplantitis groups. HMGB-1 and IL-1β were found to be significantly highest in CSPI groups followed by NSPI and CSNPI groups with no statistically significant difference between CSPI and NSPI groups ($p<0.05$). CSPI groups reported the highest TNF-α levels in the PICF in comparison to other groups ($p<0.05$). A significant negative correlation was observed between plaque scores ($p=0.0187$) and CBL ($p=0.0049$) in NSNPI and CSPI groups with HMGB-1, respectively. A significant positive correlation was seen for HMGB-1 in groups CSPI ($p=0.0023$) and NSPI ($p=0.0018$) for BOP. In CSPI group, a significant positive correlation was observed between TNF-α and PD ($p=0.0443$). On correlating IL-1β, a significant positive correlation was observed for CBL in CSPI ($p=0.0006$) and NSPI ($p=0.0275$) groups, respectively.

CONCLUSIONS: HMGB-1 could play a significant role in periimplant inflammatory response and inflammation. Higher crevicular fluid HMGB-1 levels are indicative of a possible surrogate biomarker for peri-implantitis.

Key Words:
Periimplantitis, Smokers, High mobility group box chromosomal protein 1, Crevicular fluid, Biomarkers.

Introduction

High mobility group box chromosomal protein 1 (HMGB-1) is an intracellular DNA binding protein that possesses nuclear functions essential for survival[1]. HMGB1 orchestrates inflammatory and immune response *via* its cytokine, chemokine, and growth factor activity. HMGB1 is an intracellular protein that when present in the extracellular milieu acts as a "necrotic marker" for the immune system[2]. Recent studies[2] indicate that damaged or necrotic cells can release HMGB1 into the extracellular milieu, where it triggers inflammatory responses. Extracellular HMGB1 acts as an immune-stimulatory signal that indicates the extent of tissue injury[3]. Exposure to inflammatory stimuli such as lipopolysaccharides, tumor necrosis factor-α (TNF-α) and/or interleukins stimulates the release of HMBG-1 by macrophages, monocytes, endothelial cells and necrotic cells[4].

Corresponding Author: Abdul Razzaq Ahmed, MD; e-mail: aamoshtag@kku.edu.sa

Periimplantitis is a microbial infection of the supporting structures around dental implants that results in the destruction of periimplant connective attachment and progressive loss of alveolar bone[5]. It is a result of an interplay between key molecular pathways and bacterial challenge that enable periodontal ligaments attachment, apical migration of the epithelial attachment and apical spread of the bacterial biofilm along the implant surface[5]. Among various predisposing and modifying factors associated with peri-implantitis, such as type 2 diabetes mellitus, and obesity[6,7], smoking has been demonstrated as the well-recognized risk factor for periimplantitis[8]. Smoking increases the expression of inflammatory cytokines, such as TNF-α, interleukin (IL)-6 and IL-1β in the peri-implant crevicular fluid (PICF), which contribute to progression of peri-implant deterioration and alveolar bone loss[9,10]. HMGB1 has been found to be associated with the initiation, progression, and prolongation of periodontal inflammation *via* a positive feedback mechanism whereby the initial release of chemokines and cytokines by the epithelial cells cause HMGB1 secretion, which in turn causes inflammation and immune reaction in the periodontium[11]. Continuous release of HMGB1 enhances the release of IL-1β and TNF-α promoting bone resorption and osteoclastogenesis[11].

Although HMGB1 has been extensively studied and has a role in the progression of chronic systemic diseases including periodontitis[12,13], HMGB1 has never been compared among smokers and never smokers with and without periimplantitis. Therefore, the aim of this study was to investigate the levels of HMGB1, TNF-α and IL-1β in PICF of smokers and never-smokers, both, with and without periimplantitis, and correlate these levels with the clinical and radiographic periimplant parameters.

Subjects and Methods

Ethical Guidelines

This study followed the guidelines recommended by the Declaration of Helsinki and was approved by King Khalid University. The participating volunteers were requested to read the information sheet written in simple English, stating the aims and procedure of the current study, and clearly informing the participants that there would have been no consequences in case they wished to withdraw from the study at any time. Individuals who gave consent were invited to ask questions before signing a written informed consent form. Oral hygiene instructions and data regarding the hazardous effects of smoking was conveyed to all the volunteers.

Sample Size and Study Groups

Sixty participants (n=15/group) were recruited for the study considering a 50% difference in mean PICF HMGB-1 with a total of 90% power, and α=0.05. The study included self-reporting 'Cigarette-smokers (CS)' fulfilling the criteria of smoking at least 5 cigarettes daily, for at least 12 months[14]. 'Never-smokers (NS)' were defined individuals who reported to have never smoked tobacco neither consumed smokeless tobacco products. The exclusion criteria were as follows: a) smoking other than cigarette smoking; b) non-steroidal anti-inflammatory drug use, antibiotic, and/or steroid therapy in the last 3 months; c) self-reported systemic disorders such as acquired immunodeficiency syndrome/HIV, diabetes mellitus and/or cardiac, renal, hepatic disease; d) patients having undergone periodontal therapy in the past 6 months; e) third molars; f) pregnant and lactating females.

The selected individuals were then stratified into the following four groups:

1. Cigarette smokers with periimplantitis (CSPI);
2. Cigarette smokers without periimplantitis (CSNPI);
3. Never-smokers with periimplantitis (NSPI);
4. Never-smokers without periimplantitis (NSNPI).

Questionnaire

A pretested questionnaire in English and Arabic language was distributed among the participating subjects to gather information related to demographics, duration, and daily frequency of smoking (pack years), and family history of smoking. Implant related characteristics, such as number of dental implants, position of dental implants and duration of implants in service, were recorded.

Clinical and Radiographic Examination

A trained examiner blinded to the study groups estimated all the clinical and radiographic variables. Intra-assessor standardized calibration was carried out on peri-implant probing depth (PD) (kappa statistical value=0.92). Four sites on each implant (mesiobuccal, midbuccal, distobuccal, palatal/lingual) were assessed for plaque score (PS), bleeding on probing (BOP), and PD. Digital radiographs were taken using a dental radiogra-

phy machine and viewed on a calibrated computer screen (Samsung SyncMaster digital TV monitor, Seoul, Korea) using a software program (Image Tool 3.0 Program, Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). Crestal bone level (CBL) was calculated as the vertical distance from implant-abutment junction to the highest point of alveolar bone.

Collection of Periimplant Crevicular Fluid (PICF) and Biomarker Quantification

Periimplant crevicular fluid samples were collected from the deepest PD of the buccal side of the dental implant. The selected implant site was initially dried and isolated using sterile cotton rolls. Supragingival oral biofilm was gently removed from the crown surface and a sterile paper-strip (Periopaper, Amityville, NY, USA) was pushed inside the pocket until resistance was sensed. The paper was held in place for 30 s and volume of GCF collected was quantified based on the measurements on a calibrated digital machine (Periotron 8000, Oraflow Inc., Plainview, NY, USA). Visibly contaminated strips were discarded, and an alternate site was chosen for evaluation. All the strips were kept in labelled tubes containing 300 µL of a 0.01 M sodium phosphate buffer, pH 7.2, and protease inhibitor (Complete Mini; protease inhibitor cocktail tablets; Roche Applied Science, Indianapolis, IN, USA). To obtain the supernatant, centrifugation was carried out at 8,000 rpm for 5 min at 4°C and the final samples were stored at -80°C till further analysis. The levels of HMGB-1 (Biocompare, CA, USA), TNF-α (Abcam, UK) and IL-1β (Abcam, UK) were estimated using human enzyme linked immunosorbent assay (ELISA).

Statistical Analysis

A specific software (SPSS v.15, IBM Corp., Chicago, IL, USA) was used to analyze all statistical tests. Variables were processed for normality testing using Shapiro-Wilk test and homogeneity of variances using inferential statistic Levene's test. *p*-values were generated using Kruskal-Wallis' test for comparison between the study groups. Post-hoc two-group comparisons using Bonferroni-corrected Mann-Whitney U tests were used. The correlations between PICF HMGB-1, TNF-α, and IL-1β levels and clinical variables were analyzed using Spearman rank correlation coefficient analysis. *p*-values <0.05 were deemed significant.

Results

Baseline demographics are reported in Table I. Each group consisted of 15 male participants with mean age ranging between 45.9-48.8 years. The mean duration of pack years among cigarette smoking participants in CSPI was 18.7 years while in CSNPI was 10.6 years. The daily frequency of smoking ranged from 13.4 to 8.9 cigarettes daily among CSPI and CSNPI groups, respectively. A total of 125 dental implants were studied within the study groups out of which 76 dental implants were maxillary, while 49 dental implants were mandibular. The duration of dental implants ranged from 43.8 to 51.6 months. A higher number of participants reported once daily toothbrushing.

Clinical, radiographic and laboratory parameters are shown in Table II. Plaque scores were significantly higher in CSPI, CSNPI and NSPI groups ($p<0.05$). Bleeding on probing was least

Table I. Baseline demographic of the study groups.

Parameters	CSPI	CSNPI	NSPI	NSNPI
Number of study participants (n)	15	15	15	15
Gender (Male/Female)	15/0	15/0	15/0	15/0
Mean age in years (± SD)	48.8 ± 7.8	46.2 ± 6.6	45.9 ± 10.2	46.4 ± 7.2
History of cigarette smoking (pack years)	18.7 ± 0.7	10.6 ± 1.5	-	-
Daily frequency of smoking (mean ± SD)	13.4 ± 1.2	8.9 ± 0.6	-	-
Total number of implants included	39	28	26	32
Implant position (maxilla/mandible)	20/19	21/7	10/16	25/7
Duration of implants in months (mean ± SD)	43.8 ± 10.3	51.6 ± 16.8	48.1 ± 9.8	45.6 ± 3.4
Brushing frequency (%)				
Once daily	85	92	88	78
Twice daily	15	8	12	22

Table II. Clinical, radiographic and laboratory parameters among study groups,

Variables	CSPI	CSNPI	NSPI	NSNPI
Clinical parameters				
Mean plaque score (%)	38.6 (14.2)^{A}	33.8 (10.6)^{A}	31.4 (15.8)^{A}	15.3 (6.1)^{B}
Mean bleeding on probing (%)	28.5 (7.8)^{B}	11.4 (6.4)^{A}	35.7 (14.6)^{B}	9.8 (4.5)^{A}
Mean probing depth (mm)	5.8 (2.3)^{A}	1.4 (0.7)^{B}	5.2 (2.0)^{A}	1.1 (0.3)^{B}
Mean crestal bone level (mm)	2.8 (0.6)^{A}	1.5 (0.3)^{C}	2.6 (0.4)^{A}	0.6 (0.2)^{B}
Laboratory parameters				
PICF flow rate (µl/min)	1.98 (0.88)^{B}	1.56 (0.59)^{B}	1.24 (0.45)^{B}	0.71 (0.39)^{A}
HMGB-1 (pg/ml)	169 (85)^{A}	34 (18)^{B}	143 (68)^{A}	0.0 (0.0)^{C}
TNF-α (pg/ml)	342 (129)^{A}	119 (65)^{C}	289 (112)^{B}	33 (18)^{D}
IL-1β (pg/ml)	188 (32)^{A}	93 (37)^{B}	149 (76)^{A}	28 (13)^{C}

Data are expressed in median and interquartile range.

in NSNPI and CSNPI followed by CSPI and NSPI (p<0.05). The highest PD and CBL was recorded for CSPI and NSPI groups while the least PD and CBL were recorded among non-periimplantitis groups. The PICF flowrate was highest for CSPI, CSNPI and NSPI groups (p<0.05). Crevicular fluid HMGB-1 and IL-1β was found to be significantly highest in CSPI groups followed by NSPI and CSNPI groups with no statistically significant difference between CSPI and NSPI groups (p<0.05). The lowest levels were recorded for NSNPI groups. CSPI groups reported the highest TNF-α levels in the PICF in comparison to other groups (p<0.05).

Table III describes the Spearman rank correlation coefficient. On correlating HMGB-1, a significant negative correlation was observed between plaque scores (p=0.0187) and CBL (p=0.0049) in NSNPI and CSPI groups, respectively. A significant positive correlation was seen for HMGB-1 in groups CSPI (p=0.0023) and NSPI (p=0.0018) for BOP. The same trend was seen for TNF-α, in which a significant positive correlation was seen in groups CSPI (p=0.0291) and NSPI (p=0.0013) for BOP. In CSPI group, a significant positive correlation was observed between TNF-α and PD (p=0.0443). On correlating IL-1β, a significant positive correlation was observed for CBL in CSPI (p=0.0006) and NSPI (p=0.0275) groups, respectively.

Discussion

This study aimed at investigating the levels of HMGB-1, TNF-α and IL-1β in periimplant crevicular fluid of smokers and never-smokers, with and without periimplantitis, and correlate these levels with the clinical and radiographic periimplant parameters. The outcome of the present study suggests that HMGB-1 could play a significant role in peri-implant inflammatory response and inflammation, and higher crevicular fluid HMGB-1 levels are indicative of a possible surrogate biomarker for periimplantitis.

The role of HMGB-1 in inflammatory pathways is well-known. For instance, HMGB-1 plays a significant proinflammatory role in lung function and intratracheal injection of HMGB-1 could lead to necrosis of the alveolar cells and thereby cause lung injury[15]. In addition, anti-HMGB-1 antibody, when injected within mice, could protect the mice against lipopolysaccharide-induced lethality and could decrease the levels of other proinflammatory cytokines and incidence of acute lung inflammation[16,17]. HMGB-1 is also believed to play a significant role in various other dental infections including periodontitis and periimplantitis[18,19]. In periodontal infections, HMGB-1 is believed to stimulate the release of other proinflammatory biomarkers, such as IL-6 and IL-22 from the periodontal ligament fibroblast cells[20]. Furthermore, higher levels of salivary HMGB-1 were observed in the patients with Sjogren's syndrome and together with other proinflammatory biomarkers could form an inflammatory loop in the salivary glandular function[21]. Therefore, the function of HMGB-1 in periodontal and periimplant function enticed more consideration in research. Similarly, the role of HMGB-1 is widely studied in cigarette smoking. Evidence suggests that cigarette smoking induces HMGB-1 translocation and release in lung alveolar tissue[22,23]. Consistent with the previous evidence, it could be inferred that our study, that consisted in cigarette smokers having periimplantitis, justified the upregulated levels of HMGB-1 in their PICF.

Table III. Spearman rank correlation analysis between clinical and PICF biomarkers among study groups.

Parameters	CSPI	CSNPI	NSPI	NSNPII
HMGB-1				
Plaque score				
Correlation coefficient	-0.6397	-0.4577	0.3049	-0.6102
p-value	0.2555	0.9571	0.2317	0.0187*
Bleeding on probing				
Correlation coefficient	0.0531	0.1941	0.8236	-0.3143
p-value	0.0023*	0.0863	0.0018*	0.1860
Probing depth				
Correlation coefficient	0.8165	0.0037	0.0167	-0.1711
p-value	0.9815	0.4615	0.9958	0.2764
Crestal bone level				
Correlation coefficient	-0.700 4	0.2139	-0.2250	-0.7334
p-value	0.0049*	0.1376	0.3560	0.8879
TNF-α				
Plaque score				
Correlation coefficient	0.4474	-0.4300	0.1953	-0.3483
p-value	0.2215	0.3414	0.4985	0.1680
Bleeding on probing				
Correlation coefficient	0.9612	0.9321	0.0013*	0.0013*
p-value	0.0291*	0.8093	0.4055	0.9332
Probing depth				
Correlation coefficient	0.6853	0.0945	0.4283	0.2756
p-value	0.0443*	0.1217	0.3874	0.8743
Crestal bone level				
Correlation coefficient	-0.3498	0.3264	0.1265	-0.2384
p-value	0.0593	0.5486	0.4532	0.9237
IL-1β				
Plaque score				
Correlation coefficient	0.2821	-0.2364	0.3454	-0.8964
p-value	0.4229	0.8522	0.9734	0.3045
Bleeding on probing				
Correlation coefficient	-0.1296	0.5634	-0.2342	0.4503
p-value	0.0991	0.7856	0.7856	1.9234
Probing depth				
Correlation coefficient	0.9823	0.2348	-0.5498	0.8475
p-value	0.0834	0.5432	0.9274	0.8473
Crestal bone level				
Correlation coefficient	0.7644	0.3442	0.7433	-0.8510
p-value	0.0006*	0.8347	0.0275*	0.9322

*Significant at $p < 0.05$.

Age and cigarette smoking are two of the common risk factors in the pathogenesis of periimplantitis[24,25]. It is noteworthy that the included participants age ranged from 46 to 49 years and that the history of smoking in pack years was >10 years. These factors may prove to increase the extent of periimplant infections. Moreover, the majority of patients daily brushed only once, and this reflected in the scoring of plaque index, which was generally higher among cigarette smokers and patients having periimplantitis. With regards to bleeding on probing, some interesting findings were noted. The BOP was significantly lower among cigarette smokers, as compared to never-smokers. This could be attributed to the extent of bleeding, which is comparably lower among tobacco users as compared to never-smokers[26,27]. Research indicates that nicotine exerts a powerful vasoconstrictive effect on the gingival vasculature, which significantly reduces the level of bleeding in tobacco smokers[28,29].

It has been documented[30] that the concentration of IL-1β, IL-6 and TNF-α in PICF is significantly elevated in periimplantitis and can potentially be considered a prognostic marker of implant failure. Our results show that there are statistically significant differences in the concentration of IL-1β and TNF-α in the PICF

of cigarette smokers, with and without periimplantitis. These data suggest that these cytokines could stimulate HMGB-1 production in later stage and then HMGB-1 further promotes the release of these cytokines in periimplant inflammation.

Limitations

Some limitations exist in the present study. For instance, this study was a case-control study and present outcomes from a single time point investigation. The follow-up of participants could determine the evolution of HMGB-1 and other biomarkers and how they impact peri-implant health. In addition, this study did not investigate the levels of various pathogenic biofilms associated with periimplantitis. The determination of various bacteria could provide a link of HMGB-1 with periimplant inflammation along with other systemic diseases[31,32]. Future studies are warranted to investigate this association.

Conclusions

Within the limitations of the present study, HMGB-1 could play a significant role in periimplant inflammatory response and inflammation. Higher crevicular fluid HMGB-1 levels are indicative of a possible surrogate biomarker for periimplantitis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

The authors extend their appreciation to the Deanship Scientific Research at King Khalid University for funding this work through the Small Groups Project under grant No.: RGP-1/184/43.

References

1) Mandke P, Vasquez KM. Interactions of high mobility group box protein 1 (HMGB1) with nucleic acids: Implications in DNA repair and immune responses. DNA Repair 2019; 83: 102701.
2) Ulloa L, Messmer D. High-mobility group box 1 (HMGB1) protein: friend and foe. Cytokine Growth Factor Rev 2006; 17: 189-201.
3) Yang H, Wang H, Czura CJ, Tracey KJ. The cytokine activity of HMGB1. J Leukoc Biol 2005; 78: 1-8.
4) Morimoto Y, Kawahara KI, Tancharoen S, Kikuchi K, Matsuyama T, Hashiguchi T, Izumi Y, Maruyama I. Tumor necrosis factor-α stimulates gingival epithelial cells to release high mobility-group box 1. J Periodontal Res 2008; 43: 76-83.
5) Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Clin Periodontol 2018; 45: S246-S266.
6) Al-Sowygh ZH, Ghani SM, Sergis K, Vohra F, Akram Z. Peri-implant conditions and levels of advanced glycation end products among patients with different glycemic control. Clin Implant Dent Relat Res 2018; 20: 345-351.
7) Alkhudhairy F, Vohra F, Al-Kheraif AA, Akram Z. Comparison of clinical and radiographic peri-implant parameters among obese and non-obese patients: a 5-year study. Clin Implant Dent Relat Res 2018; 20: 756-762.
8) Al-Sowygh ZH, Al-Kheraif AA, Akram Z, Vohra F, Javed F. Peri-implant soft tissue inflammatory parameters and crestal bone loss among waterpipe (narghile) smokers and never-smokers with and without type 2 diabetes mellitus. J Periodontol 2018; 89: 645-652.
9) Al-Sowygh ZH, Aldamkh MK, Binmahfooz AM, Al-Aali KA, Akram Z, Qutub OA, Javed F, Abduljabbar T. Assessment of matrix metalloproteinase-8 and-9 levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis. Inhal Toxicol 2018; 30: 72-77.
10) Akram Z, Vohra F, Bukhari IA, Sheikh SA, Javed F. Clinical and radiographic peri-implant parameters and proinflammatory cytokine levels among cigarette smokers, smokeless tobacco users, and nontobacco users. Clin Implant Dent Relat Res 2018; 20: 76-81.
11) Sha Y, Zmijewski J, Xu Z, Abraham E. HMGB1 develops enhanced proinflammatory activity by binding to cytokines. J Immunol 2008; 180: 2531-2537.
12) Ebe N, Hara-Yokoyama M, Iwasaki K, Iseki S, Okuhara S, Podyma-Inoue KA, Terasawa K, Watanabe A, Akizuki T, Watanabe H, Yanagishita M. Pocket epithelium in the pathological setting for HMGB1 release. J Dent Res 2011; 90: 235-240.
13) Yoshihara-Hirata C, Yamashiro K, Yamamoto T, Aoyagi H, Ideguchi H, Kawamura M, Suzuki R, Ono M, Wake H, Nishibori M, Takashiba S. Anti-HMGB1 neutralizing antibody attenuates periodontal inflammation and bone resorption in a murine periodontitis model. Infect Immun 2018; 86: e00111-e00118.
14) BinShabaib M, ALHarthi SS, Akram Z, Khan J, Rahman I, Romanos GE, Javed F. Clinical periodontal status and gingival crevicular fluid cytokine profile among cigarette-smokers, electronic-cigarette users and never-smokers. Arch Oral Biol 2019; 102: 212-217.
15) Watanabe T, Kubota S, Nagaya M, Ozaki S, Nagafuchi H, Akashi K, Taira Y, Tsukikawa S,

Oowada S, Nakano S. The role of HMGB-1 on the development of necrosis during hepatic ischemia and hepatic ischemia/reperfusion injury in mice. J Surg Res 2005; 124: 59-66.

16) Lutz W, Stetkiewicz J. High mobility group box 1 protein as a late-acting mediator of acute lung inflammation. Int J Occup Med Environ Health 2004; 17: 245-254.

17) Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. Am J Respir Crit Care Med 2001; 164: 1768-1773.

18) Luo L, Xie P, Gong P, Tang XH, Ding Y, Deng LX. Expression of HMGB1 and HMGN2 in gingival tissues, GCF and PICF of periodontitis patients and peri-implantitis. Arch Oral Biol 2011; 56: 1106-1111.

19) Nogueira AV, Chaves de Souza JA, de Molon RS, da Silva Mariano Pereira E, de Aquino SG, Giannobile WV, Cirelli JA. HMGB1 localization during experimental periodontitis. Mediators Inflamm 2014; 2014.

20) Hasegawa N. Effect of high mobility group box 1 (HMGB1) in cultured human periodontal ligament cells. Kokubyo Gakkai zasshi. J Stomatol Soc Jap 2008; 75: 155-161.

21) Barbieri M, Mencio F, Papi P, Rosella D, Di Carlo S, Valente T, Pompa G. Corrosion behavior of dental implants immersed into human saliva: preliminary results of an in vitro study. Eur Rev Med Pharmacol Sci 2017; 21: 3543-3548.

22) Meo SA, Al Asiri SA. Effects of electronic cigarette smoking on human health. Eur Rev Med Pharmacol Sci 2014; 18: 3315-3319.

23) Chen J, Zhang W, Wu YQ, Chen H, Zhao JF. Correlations of acute myocardial infarction complicated by cerebral infarction with insulin resistance, adiponectin and HMGB1. Eur Rev Med Pharmacol Sci 2019; 23: 4425-4431.

24) De Angelis F, Papi P, Mencio F, Rosella D, Di Carlo S, Pompa G. Implant survival and success rates in patients with risk factors: results from a long-term retrospective study with a 10 to 18 years follow-up. Eur Rev Med Pharmacol Sci 2017; 21: 433-437.

25) Akram Z, Javed F, Vohra F. Effect of waterpipe smoking on peri-implant health: A systematic review and meta-analysis. J Investig Clin Dent 2019; 10: e12403.

26) Akram Z, Aati S, Alrahlah A, Vohra F, Fawzy A. Longitudinal evaluation of clinical, spectral and tissue degradation biomarkers in progression of periodontitis among cigarette and electronic cigarette smokers. J Dent 2021; 109: 103678.

27) Akram Z, Abduljabbar T, Hosain M, Al-Sowygh ZH, Al-Hamoudi N, Vohra F, Javed F. Comparison of periodontal inflammatory parameters among habitual gutka-chewers and naswar-dippers: a split-mouth retrospective clinical study. Acta Odontol Scand 2018; 76: 141-147.

28) Dietrich T, Bernimoulin JP, Glynn RJ. The effect of cigareté smoking on gingival bleeding. J Periodontol 2004; 75: 16-22.

29) Daood U, Abduljabbar T, Al-Hamoudi N, Akram Z. Clinical and radiographic periodontal parameters and release of collagen degradation biomarkers in naswar dippers. J Periodontal Res 2018; 53: 123-130.

30) Abduljabbar T, Akram Z, Vohra F, Warnakulasuriya S, Javed F. Assessment of interleukin-1β, interleukin-6, and tumor necrosis factor-A levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis. Clin Implant Dent Relat Res 2018; 20: 144-150.

31) Mencio F, De Angelis F, Papi P, Rosella D, Pompa G, Di Carlo S. A randomized clinical trial about presence of pathogenic microflora and risk of peri-implantitis: comparison of two different types of implant-abutment connections. Eur Rev Med Pharmacol Sci 2017; 21: 1443-1451.

32) Papi P, Letizia C, Pilloni A, Petramala L, Saracino V, Rosella D, Pompa G. Peri-implant diseases and metabolic syndrome components: a systematic review. Eur Rev Med Pharmacol Sci 2018; 22: 866-875.