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ORIGINAL ARTICLE

Evaluation of Salivary Interleukin-1ß Levels and Oral Health in Pakistani Male Smokers

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ABSTRACT

Aim: Smoking induces changes in salivary inflammatory biomarker levels associated with oral diseases. This study status and explored association among salivary Interleukin-1β, oral diseases and smoking.
Methods: Data of male smokers of a private institute recruited for a randomized clinical trial is analyzed for this paper. Demographic and systemic information were collected. Oral disease status was examined and saliva sample collected for IL-1β levels. IL-1β levels and other study variables were analyzed with respect to smoking status categorized into smoking years and cigarettes per day. The student's t-test and one-way ANOVA were used for statistical analysis using SPSS version 22) with significance level of p≤0.050.
Results: Analysis of baseline data of seventy-eight smokers showed elevated levels of IL-1β with increasing smoking, higher BMI. Smoking was higher among aged, married and low-income individuals. Pearson partial correlation analysis, after controlling age, marital status, education, income, and BMI, demonstrated a positive significant relationship of smoking per day with smoking years; dental caries with missing teeth and calculus; gingivitis with missing teeth and calculus; periodontitis with dental caries, calculus and gingivitis.
Conclusion: This analysis demonstrates that levels of IL-1β were raised in smokers, however; there was no association with oral disease parameters. Large studies may be conducted to observe status and association of smoking, oral disease and salivary biomarkers.
Keywords: Smoking, Oral Health, IL-1β, Males

INTRODUCTION

Smoking is a renowned global burden as it increases risk of contracting a wide range of diseases, many of which are fatal[1]. Use of smoking and tobacco products tends to disrupt the dynamic harmony between oral commensal and pathogenicmicrobiota as well as host immune inflammatory responses.[2–4] Smoke particles change the physiological pattern and nature of various oral microflorathat ultimately changes the innate and adaptive immune responses of an individual[4–6]. Smoking also alters and interplays with the physiological functioning of oral cytokines,[7]and thence, this imbalancedoral environment leads toinitiation of numerous oral diseases[8].

Various salivary microbial diagnostic studies have revealed that sub-gingival pathogenic microbial profile varies significantly in smokers from those who do not smoke.[9]Tobacco products impairs the body's adaptive immune responses against pathogenic oral flora[10] and enhances the host susceptibility for developing oral periodontal infection by downregulating the functions of pro-inflammatory cytokines and chemokines[5]. Studies have reported a positive association of smoking, nicotine levels, and pack years with enhanced inflammatory mediators like IL-6, IL-8, IL-1β and acute phase proteins[11,12]. Interleukin-1β is a pro-inflammatory cytokine that exerts a number of biological functions[13,14] and is expressed by monocytes, macrophages, fibroblasts and epithelial cells[15,16]. IL-1β also regulates antigen-presenting cells, enhance antigen mediation of T-cells, and plays a key role in adaptive immunity[17,18].

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Oral cavity and saliva are the first to be exposed and affected by smoking. Saliva contains thousands of multiple proteins and peptides that are identifiable[19] and the status of these proteins can be used to anticipate any pathological change associated with oral diseases[20]. The objective of this article was to explore and analyze status and association of IL-1β, with oral diseases and smoking.

MATERIALS AND METHODS

A randomized controlled trial[21] was conducted on smoker male employees of a private institution. Individuals aged 21-40 years with smoking habit and without any other tobacco addictionwere included for this RCT. Out of 400 smokers, 78 were recruitedin the study after screening as per inclusion / exclusion criteria and obtaining informed written consent.The inclusion criteria of the study subjects were that: (i) no one has consumed antibiotics for the last three months, (ii) all subjects smoked at least one cigarette per day. Study participants who had a history of any chronic oral or medical disease were excluded from study. Baseline information of the study sample, oral health parameters and oral biomarkers are summarized and analyzed in this paper.
Study Parameters: Demographic and medical data includedage, marital status, education, income, body mass index (BMI) and smoking habits.Oral health parameters of oral hygiene practices, missing teeth, carious teeth, calculus deposits, gingivitis, periodontitis, and soft tissue lesions were examined. Salivary specimens were collected for analysis of interleukin-1β.

Saliva collection and analysis: Overnight fasting whole saliva (unstimulated) sample was collected using the Pure-SAL^{TM} and RNA Pro-SAL^{TM} from Oasis Diagnostics Corporation (Vancouver WA, USA). Subjects were asked to refrain from drinking and eating 2 hours before sampling. Before the collection of saliva, subjects were invited to rinse with water for 15 seconds to remove any food debris, microorganisms, and desquamated epithelium cells. After rinsing subjects were asked to sit straight in a chair and wait for 1 minute. Manufacturer instructions were strictly observed for the use of saliva device for the passive drooling method. After the samples were obtained, they were transported in the icebox, and within two hours after sampling, each sample was centrifuged at 1000xg for 20 minutes in a benchtop centrifuge machine, later on the supernatant layer was separated into the new tube for storage at -80^{o}C freezer. IL-1β was determined for each subject using the conventional enzyme-linked immunosorbent assay (ELISA). The sandwich ELISA technique was used with interleukin-1βEIA Kit (Labscience, USA). ELISA well plates were prepared as instructed by the manufacturer. Seventy-one (n=70) samples were tested in duplicate. Assay sensitivity was 4.69 pg/mL Analysis was performed in the Department of Immunology, University of Health Sciences Lahore.

Data Analysis: Data was analyzed using SPSS version 22.0 and presented as a proportion (%) for categorical variables and Mean±SD for continuous variables. The Shapiro-Wilks test was used to determine the normality of the data distribution. Smokers were categorized on the basis of smoking years (≤5 years, 6-10 years and >10 years and number of cigarettes per day (≤10 or >10). All other variable was analyzed among these categories. The difference in number/proportions of subjects were analyzed using Chi-Squared (χ^2) test. Independent t-test and ANOVA test was applied to analyses status of continuous variables.For every analysis, the level of significance was considered as 5% (p≤0.05).

RESULTS

Four hundred (n=400) employees of a private organization in Lahore were screened. Two hundred fifty smokers agreed for general health assessment. One hundred and forty were excluded as they did not fulfill the inclusion and exclusion criteria. One hundred and ten qualified people were called for oral assessment. Twenty-five individuals were excluded on the basis of oral health measures and twelve individuals didn't sign written informed consent. Seven-eighthealthy volunteer smokers participated in the clinical trial and their baseline data is analyzed in this paper[21].

Table 1 presents distribution of demographic and oral health parameters with respect to smoking (number of cigarettes/day and number of smoking years). In the present analysis, the noteworthy finding of use of cigarettes/day was among the age group of 30-40 years while smoking years were pretty much same in both age groups. Smoking was less observed in overweight and obese group. Smoking habitin terms of smoking/years and number of cigarettes/day was high among married individuals (p≤0.036). Individuals with history of ≥10 smoking/years and ≥10 cigarettes/day was more common among the study participants with no education. Whereas, ≤10 smoking/years and ≤10 cigarettes/day were found in participants with intermediate education and least frequency was observe in participants with bachelors' education.

The smoking ratio was high among the individuals with low salary (≤16000 Pak rupees) both in term of cigarettes/day and smoking/years (category 6-10 smoking years)when compared with individuals who earn more than Rs.16000/- Pak rupees. A major proportion of individuals with more cigarette consumption/day or years exhibited no or mild calculus and gingivitis as compared to moderate and severe category, while for the periodontitis there was a noteworthy difference among mild and moderate category. Individuals with smoking frequency of ≤10 cigarettes/day and 6-10 smoking/years exhibit more missing teeth then the other categories of smoking frequency.Dental caries status was higher among individuals who smoke ≤10 cigarettes/day with ≤5 smoking/years history. Brushing habitofindividuals with once/day brushing frequencywas higher for smokers with ≥10 cigarettes/day among 6-10 years'group.

Table 2 explains status ofstudy variables of demographic, oral health, smoking categories and interleukin-1β. In age category, the level of interleukin-1β was somewhat high in 21-30 years when compared with 31-40 years. Among the BMI categories, IL-1β level was high in overweight and obese individuals. IL-1β levels show the slight difference between the single and married individuals. There was a significant difference in IL-1β levels between education groups with a p-value of <0.001. Slight difference of IL-1β levels was observed among income groups. There was a significant difference in IL-1β levels among calculus and gingivitis status categories. In periodontitis categories, the value of interleukin-1β is slightly high in mild category than moderate category. The value of interleukin-1β was significantly high among the individuals with no missing teeth and no dental caries. The value of interleukin-1β was showing a rise with the increasing number of smoking years. Value of IL-1β was marginally high among individuals who smoke >10 cigarettesper day.

Table 3 show correlation of smoking categories, oral health variables (calculus, periodontitis, gingivitis, missing teeth, dental caries and cleaning habits) and IL-1β; while the demographic variable (age, BMI, married status, education and salary) were controlled. Number of smoking years showed a significance association of (0.350; p=0.003) with cigarettes/day.The presence or absence of dental caries showed a mild significance relation with missing teeth (0.242; p=0.042). Calculus showed highly significant relationwith dental caries (0.317; p=0.007). Gingivitis have significant relation with missing teeth and calculus with p-values of (0.323; p=0.006) and (0.281; p=0.018) respectively. Calculus (0.540; p≤0.001) and gingivitis (0.335; p=0.004) showed a strong significant relation with periodontitis while dental caries (0.235; p=0.048) was least significant with periodontitis. Moderate to severe category of calculus also showed a significant relationship (0.342; p=0.004) (0.362; p=0.002) (0.408; p=0.000) (0.297; p=0.012) with missing teeth, gingivitis,

calculus and periodontitis respectively.Moderate to severe category of gingivitis showed a significant relationship with missing teeth gingivitis and mod-severe category of calculus with a P value of ≤0.005.Moderate to severe category ofperiodontitis are also significantly associated with all variables except dental caries and cigarettes per day.The association of IL-1β was significantly associated with missing teeth(-0.249; p=0.036), dental caries (-0.331; p=0.005) and moderate to severe category of calculus (-0.261; p=0.028).

Figure 1 show the distribution of IL-1β with respect to smoking years. The trend line on the graph represent that there is a significant positive relationship (p=0.024) between smoking years and IL-1β as the smoking years increase the value of IL-1β also increase.

Table 1: Distribution of Parameters {Mean ±SD / n(%)} with respect to smoking Status among Study Participants

Parameters	**≤5 smoking years n=29 (37%)**	**6-10 smoking years n=27(35%)**	**>10 smoking years n=22(54%)**	**≤10 cigarettes / day n=41(54%)**	**>10 cigarettes / day n=37(46%)**
Age					
Mean ±SD	26.17±6.87	30.78±5.87	33.86±5.42*	29.24±6.62	30.70±7.09
21-30 years	21(72.4)	14(51.9)	7(31.8)	25(61)	17(45.9)
31-40 year	8(27.6)	13(48.1)	15(68.2)**	16(39)	20(54.1)
Body Mass Index (BMI)					
Mean ±SD	19.45±3.19	19.82±3.86	21.32±6.87	19.44±3.54	20.84±5.71
Normal	27(93.1)	25(92.6)	17(77.3)	38(92)	31(83)
Overweight and above	2(6.9)	2(7.4)	5(22.7)	3(8)	6(17)
Marital status					
Married	12(41.4)	19(70.4)	19(86.4)**	24(58.5)	26(70.3)
Education					
No education	11(37.9)	12(44.4)	10(45.5)	15(36.6)	18(48.6)
Up to Intermediate	14(48.3)	12(44.4)	7(31.8)	19(46.3)	14(37.8)
Bachelor and above	4(13.8)	3(11.1)	5(22.7)	7(18)	5(14)
Income					
≤16000	11(37.9)	6(22.2)	11(50)	11(26.8)	17(46)
>16000	18(62.1)	21(77.8)	11(50)	30(73.2)	26(54)
Calculus					
Mild or no	19(65.5)	16(59.3)	15(68.2)	27(65.9)	23(62.2)
Moderate to Severe	10(34.5)	11(40.7)	7(31.8)	14(34.1)	14(37.8)**
Gingivitis					
Mild or no	24(82.8)	23(85.2)	19(86.4)	34(82.9)	32(86.5)
Moderate to Severe	5(17.2)	4(14.8)	3(13.6)	7(17.1)	5(13.5)

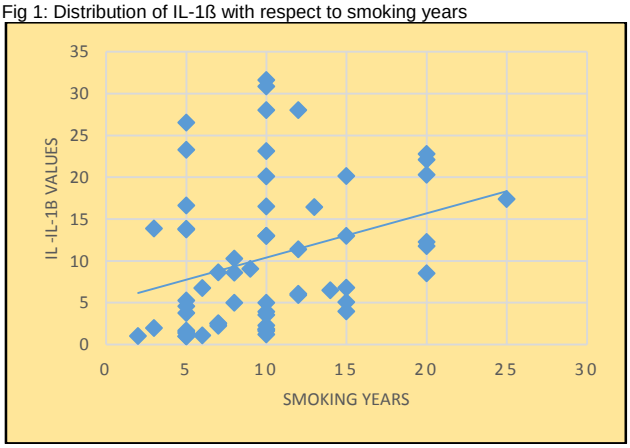
Table 2: Status of Interleukin-1β {Mean ±SD} with respect to Demographic, Oral Health Parameters and Smoking

Parameters		**Interleukin-1β** Mean ±SD
Age	21-30 years (n=42)	9.46±10.89
	31-40 year (n=36)	8.57±10.18
Body Mass Index(BMI)	Normal (n=69)	8.43±9.82*
	Overweight and above (n=9)	13.79±14.64
Marital status	Single (n=50)	8.53±9.93
	Married (n=28)	9.97±11.60
Education	No education (n=33)	7.54±8.40**
	Up to Intermediate (n=33)	12.50±12.58
	Bachelor and above (n=12)	3.72±5.83
Income	≤16000 (n=28)	8.45±8.56
	>16000 (n=50)	9.38±11.53
Calculus	Mild or no (n=50)	10.74±11.05*
	Moderate to Severe (n=28)	6.02±8.87
Gingivitis	Mild or no (n=66)	9.99±10.63*
	Moderate to Severe (n=12)	3.89±8.41
Periodontitis	Mild or no (n=47)	10.26±10.50
	Moderate to Severe (n=31)	7.21±10.43
Missing teeth	No (n=54)	10.64±10.80*
	Yes (n=24)	5.47±9.04
Dental caries	No (n=41)	11.83±10.99*
	Yes (n=37)	5.96±9.14
Smoking years	≤5 year	7.63±9.42
	6-10 years	9.00±10..50
	>10 years	10.98±11.98
Smoking per day	≤10 cigarettes	8.43±11.18
	>10 cigarettes	9.73±9.82

Table 3: Pearson Partial Correlations (2-tailed) between study variables

Control Variables	Variables		Cigarettesperday	MissingTeeth Yes/No	Dental Caries Yes/No	Calculus Yes/No	Gingivitis Yes/No	Periodontitis (Yes/No)	Calculus Mild/ mod-Severe	Gingivitis Mild/ mod-Severe
Age Marital status	Smoking Years	Correlation Significance	0.35 **.003**							
Education Income	Dental Caries (yes/No)	Correlation Significance	-0.02 .985	0.24 **.042**						
BMI Age (≤30/ >30 years) Income (Pak Rupees ≤15000/ >15000/-)	Calculus (Yes/No)	Correlation Significance	0.17 0.14	0.17 0.14	0.31 **.007**					
	Gingivitis (Yes/No)	Correlation Significance	.017 .891	.323 **.006**	.083 .491	.281 **.018**				
	Periodontitis (Yes/No)	Correlation Significance	0.03 .783	0.08 .497	0.23 **.048**	0.54 **.000**	0.33 **.004**			
	Calculus Mild/ mod-Severe	Correlation Significance	0.14 .223	0.34 **.004**	0.18 .127	0.36 **.002**	0.40 **.000**	0.29 **.012**		
	Gingivitis Mild/ mod-Severe	Correlation Significance	.034 .780	0.26 **.024**	0.14 .233	0.15 .185	0.44 **.000**	0.16 .174	0.51 **.000**	
	Periodontitis Mild/ mod-Severe	Correlation Significance	.015 .904	0.34 **.003**	.192 .110	0.29 **.013**	0.60 **.000**	0.37 **.001**	0.72 **.000**	0.45 **.000**
	IL1	Correlation Significance	.011 .924	-0.24 **.036**	-0.33 **.005**	-0.14 .218	-0.08 .484	0.05 .667	-0.26 **.028**	-0.22 .055

Fig 1: Distribution of IL-1ß with respect to smoking years



DISCUSSION

An ample evidence is available in literature showing the positive influence of smoking on oral diseases[9,10,12]. Oral diseases are broadly classifiedas gingivitis, periodontitis, taste disorder, tooth loss, impaired wound healing,oral lesionstoserious life threating conditions such as smoker's melanosis and potential malignant lesions causing morbidity and mortality worldwide[22]. Ryder et al.has reported that the mean IL-1beta levels were higher in smokers than in non-smokers[23]. Results of this study demonstratesa steady rise in levels of IL-1β with respect to smoking categories of participants.

To our best knowledge, this may be the first study from Pakistan to demonstrate the status of the IL-1β with reference to income, BMI, educational status and calculus and there was a dearth of previous studies in this respect. Higher percentage of smokers were found in age category 21-30 in comparison to the age category of 31-40. This finding attain support from a study by Nasim et al who reported the significant proportion of individual start smoking at the age of 14-16 years.[24] A Reports from National Health Interview Surveys (NIHS 2009) stated that majority of US male adults start smoking in early age 18-25 years of their life.[25]Lesser smoking frequency among obese and overweight individuals was a notable finding of this study which was consistent with a findings from a study by Tuovinen et al. who reported that smoking frequency was significantly low in obese and overweight[26].

Smoking prevalence with respect to education status was also observed in thepresent study, which exhibitsthat individuals with less education or no education had more smoking frequency than other more educated individuals. The findings of this study are in accordance with those of Kendal et al and Gilman et al who reported similar results[27,28]. This study also revealed that individuals with low salary were mild smokers. Although many other studies[29-31] are inconsistent with our results.

This study revealed that individuals with more smoking habit exhibit no or mild gingivitis and calculus but their periodontal condition was poor. Similar results were observed in different studies[30-32] while results reported by Bregstrome et al are inconsistent with this study, in that study calculus load increased with increasing smoking exposure[33]. Moreover, missing teeth and dental caries frequency in this study shows a positive relation with smoking and results are consistent with other studies[34-37].

Individuals having more missing teeth along with dental caries had elevated concentration of IL-1β. However, these findings contradict with another study,which reported that there was no significant difference of IL-1β in individuals with or without missing teeth or dental caries.Howsoever, this study shows that IL-1β value was significantly high in individuals with periodontitis and gingivitis and several studies support our results[38-41]. A cross-sectional study in adults (20–89 years) reported that IL-1β levels were significantly higher in participants with periodontitis (144pg/mL) compared to

healthy participants (61pg/mL)[42]. The value of IL-1β was foundrelatively high in young age individuals (21-30 years)when compared to individuals with age 30 years or above. This findingis consistent with a study by Gaphor et al who reported thatIL-1β value was higher in individuals with age above 30 years.[43] This study also revealed that the value of IL-1β was significantly high in chronic smokers and results are consistent with a study who also reported thatsmoking causes an increased expression of IL-1β tissues which induce tissue destruction, bone resorption[43].

Smoking play a vital role in dental caries[36] and this study demonstrates a significant relation of smoking with dental caries and a meta-analysis is consistent with this study which revealed that ten out of eleven studies indicated a positive association between tobacco smoking and dental caries[35]. The results of this study also concludedthat calculus status plays a significantcorrelation with dental caries but study by Keyes et al. reported that calculus is not responsible for dental caries[43].

A significant finding in this study was that gingivitis have a significant relation with calculus and missing teeth. The finding of this study is consistent with a study that reports higher numbers of missing teeth and calculus associated with higher chances of gingivitis[44]. This study also demonstrates the significantcorrelation of calculus (0.540; p=0.000) and gingivitis (0.335; p=0.004) with periodontitis. Albandar et al. reports consistent findingsthat individuals with early-onset periodontitis had significantly more sites with gingival bleeding and more sub gingival calculus than the controls[45].

Salivary cytokines are produced during periodontal inflammation and tissue destruction. Smoking also increases cytokine levels in the saliva and gingival crevicular fluid, accelerates inflammation, and destroys periodontal tissue[9,12]. Therefore, many studies have focused on the effect of smoking on cytokines in periodontitis, and adult participants including middle-aged and older people, have generally been targeted. In our current work, we also demonstrated that there was a significant positive relationship (p=0.024) between smoking years and IL-1β as the smoking years increase the value of IL-1β also increase. Suzuki et al investigated the relationship between salivary biomarkers and cigarette smoking. In his finding, he concluded that salivary IL-1β levels were significantly elevated in smokers than non-smokers. Furthermore, levels of other biomarkers, such as prostaglandin E2, matrix metalloproteinase-9, lactoferrin, albumin, and aspartate aminotransferase were significantly lower in active smokers. This reveals that IL-1β is a more important salivary biomarker.

This study has a few limitations. First, we only recruited male smoker individuals in this study so gender and non-smoking based comparison was not possible. Secondly, our sample size was small, due to which the precision of the study may be less.

CONCLUSION

This study supports the scientific notion that smoking has an effect onoral IL-1β levels andthat IL-1β is an important salivary cytokine, which shows a significant association withdifferent oral diseases. Further large-scale observational and experimental studies are required to gain evidence for the IL-1β with oral diseases.

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REFERENCES

1. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health. 2017/05/28. 2017;32(8):1018–36.
2. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis. 2012;18(2):109–20.
3. Kato I, Vasquez AA, Moyerbrailean G, Land S, Sun J, Lin H-S, et al. Oral microbiome and history of smoking and colorectal cancer. J Epidemiol Res. 2016;2(2):92–101.
4. Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, et al. Cigarette smoking and the oral microbiome in a large study of American adults. ISME J. 2016;10(10):2435–46.
5. Qiu F, Liang C-L, Liu H, Zeng Y-Q, Hou S, Huang S, et al. Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? Oncotarget. 2017;8(1):268–84.
6. Garmendia J, Morey P, Bengoechea JA. Impact of cigarette smoke exposure on host–bacterial pathogen interactions. Eur Respir J. 2012;39(2):467 – 477.
7. Al-Zyoud W, Hajjo R, Abu-Siniyeh A, Hajjaj S. Salivary Microbiome and Cigarette Smoking: A First of Its Kind Investigation in Jordan. Int J Environ Res Public Health. 2019;17(1):256.
8. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell. 2018;9(5):488–500.
9. Karasneh JA, Al Habashneh RA, Marzouka NAS, Thornhill MH. Effect of cigarette smoking on subgingival bacteria in healthy subjects and patients with chronic periodontitis. BMC Oral Health. 2017;17(1):64.
10. Zambon JJ, Grossi SG, Machtei EE, Ho AW, Dunford R, Genco RJ. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. J Periodontol. 1996;67(10 Suppl):1050–4.
11. Geng Y, Savage SM, Razani-Boroujerdi S, Sopori ML. Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell anergy. J Immunol. 1996;156(7):2384–90.
12. Ebersole JL, Steffen MJ, Thomas M V, Al-Sabbagh M. Smoking-related cotinine levels and host responses in chronic periodontitis. J Periodontal Res. 2014;49(5):642–51.
13. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. Brain Res Rev. 2008/12/31. 2009;60(1):57–64.
14. Essayan DM, Fox CC, Levi-Schaffer F, Alam R, Rosenwasser LJ. Biologic activities of IL-1 and its role in human disease. J Allergy Clin Immunol. 1998;102(3):344–50.
15. Eskan MA, Benakanakere MR, Rose BG, Zhang P, Zhao J, Stathopoulou P, et al. Interleukin-1β Modulates Proinflammatory Cytokine Production in Human Epithelial Cells. Infect Immun. 2008;76(5):2080–9.
16. Di Paolo NC, Shayakhmetov DM. Interleukin 1α and the inflammatory process. Nat Immunol. 2016;17(8):906–13.
17. Santarlasci V, Cosmi L, Maggi L, Liotta F, Annunziato F. IL-1 and T Helper Immune Responses. Front Immunol. 2013;4:182.
18. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010;10(2):89–102.
19. Castagnola M, Scarano E, Passali GC, Messana I, Cabras T, Iavarone F, et al. Salivary biomarkers and proteomics: future diagnostic and clinical utilities TT - Biomarkers e proteomica salivari: prospettive future cliniche e diagnostiche. Acta

Otorhinolaryngol Ital. 2017;37(2):94–101.
20. Roi A, Rusu LC, Roi CI, Luca RE, Boia S, Munteanu RI. A New Approach for the Diagnosis of Systemic and Oral Diseases Based on Salivary Biomolecules. Dis Markers. 2019 Feb;2019:8761860.
21. Hamza SA, Wahid A, Afzal N, Asif S, Imran MF, Khurshid Z, et al. Effect of Sodium Bicarbonate Mouth Wash on Salivary pH and Interleukin-1β Levels among Smokers. Eur J Dent. 2020;14(2):260–7.
22. Komar K, Glavina A, Boras VV, Verzak Ž, Brailo V. Impact of Smoking on Oral Health: Knowledge and Attitudes of Croatian Dentists and Dental Students. Acta Stomatol Croat. 2018;52(2):148–55.
23. Ryder MI, Saghizadeh M, Ding Y, Nguyen N, Soskolne A. Effects of tobacco smoke on the secretion of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta from peripheral blood mononuclear cells. Oral Microbiol Immunol. 2002;17(6):331–6.
24. Hossain M, Parveen R. Impacts of Smoking Habit by Young Generation in Our Society. AIUB J Bus Econ. 2011;10:45–64.
25. Freedman KS, Nelson NM, Feldman LL. Smoking initiation among young adults in the United States and Canada, 1998-2010: a systematic review. Prev Chronic Dis. 2012;9:E05.
26. Tuovinen E-L, Saarni SE, Männistö S, Borodulin K, Patja K, Kinnunen TH, et al. Smoking status and abdominal obesity among normal- and overweight/obese adults: Population-based FINRISK study. Prev Med reports. 2016;4:324–30.
27. Kandel DB, Griesler PC, Schaffran C. Educational attainment and smoking among women: risk factors and consequences for offspring. Drug Alcohol Depend. 2009;104(Suppl 1):S24-33.
28. Gilman SE, Martin LT, Abrams DB, Kawachi I, Kubzansky L, Loucks EB, et al. Educational attainment and cigarette smoking: a causal association? Int J Epidemiol. 2008;37(3):615–24.
29. Martire KA, Clare P, Courtney RJ, Bonevski B, Boland V, Borland R, et al. Smoking and finances: baseline characteristics of low income daily smokers in the FISCALS cohort. Int J Equity Health. 2017;16(1):157-59.
30. Jalayer Naderi N, Semyari H, Elahinia Z. The Impact of Smoking on Gingiva: a Histopathological Study. Iran J Pathol. 2015;10(3):214–20.
31. Gautam DK, Jindal V, Gupta SC, Tuli A, Kotwal B, Thakur R. Effect of cigarette smoking on the periodontal health status: A comparative, cross sectional study. J Indian Soc Periodontol. 2011;15(4):383–7.
32. Rosing CK, Gomes SC, Carvajal P, Gamez M, Costa R, Toledo A, et al. Impact of smoking on gingival inflammation in representative samples of three South American cities. Braz Oral Res. 2019;33. e094
33. Bergström J. Tobacco smoking and subgingival dental calculus. J Clin Periodontol.2005;32(1):81–8.
34. Souto MLS, Rovai ES, Villar CC, Braga MM, Pannuti CM. Effect of smoking cessation on tooth loss: a systematic review with meta-analysis. BMC Oral Health. 2019;19(1):245.
35. Jiang X, Jiang X, Wang Y, Huang R. Correlation between tobacco smoking and dental caries: A systematic review and meta-analysis. Tob Induc Dis. 2019;17:34.
36. Wu J, Li M, Huang R. The effect of smoking on caries-related microorganisms. Tob Induc Dis. 2019;17.
37. Mai X, Wactawski-Wende J, Hovey KM, LaMonte MJ, Chen C, Tezal M, et al. Associations between smoking and tooth loss according to the reason for tooth loss: the Buffalo OsteoPerio Study. J Am Dent Assoc. 2013;144(3):252–65.
38. Liukkonen J, Gürsoy UK, Pussinen PJ, Suominen AL, Könönen E. Salivary Concentrations of Interleukin (IL)-1β, IL-17A, and IL-23 Vary in Relation to Periodontal Status. J Periodontol. 2016;87(12):1484–91.
39. Abbasi ZA, Hadi NI, Zubairi AM, Hosein M. Salivary Interleukin 1-beta levels and clinical periodontal parameters in habitual naswar users and non-users. Pakistan J Med Sci. 2019;35(3):674–9.
40. Ülker AE, Tulunoglu Ö, Özmeric N, Can M, Demirtas S. The Evaluation of Cystatin C, IL-1β, and TNF-α Levels in Total Saliva and Gingival Crevicular Fluid From 11- to 16-Year-Old Children. J Periodontol. 2008;79(5):854–60.
41. Na HS, Song YR, Kim S, Heo J-Y, Chung H-Y, Chung J. Aloin Inhibits Interleukin (IL)-1β−Stimulated IL-8 Production in KB Cells. J Periodontol. 2016;87(6):e108–15.
42. Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, et al. Proinflammatory cytokines, aging, and age-related diseases. J Am Med Dir Assoc. 2013 Dec;14(12):877–82.
43. Abdullah SHA and MJ. Evaluation of Salivary Interleukin-1beta (IL-1ÃŽÂ²) Level in Relation to the Periodontal Status in Smoker and Non-smoker Individuals. Vol. 2, JBR Journal of Interdisciplinary Medicine and Dental Science. OMICS International; 2014. 1–5
44. Haas AN, Prado R, Rios FS, Costa R dos SA, Angst PDM, Moura M dos S, et al. Occurrence and predictors of gingivitis and supragingival calculus in a population of Brazilian adults. Braz Oral Res. 2019;33. e036
45. Albandar JM, Brown LJ, Brunelle JA, Löe H. Gingival State and Dental Calculus in Early-Onset Periodontitis. J Periodontol. 1996;67(10):953–9.