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Periodontitis Borne Risk Factors & Oral Health: A Pilot Study

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Abstract

This study targets to earmark the risk factors that gets emerged in saliva and serum in the pathogenicity of periodontitis. ELISA based kits were used to estimate the risk components (TNF-α, IL-10 and IgA) in saliva and serum following company directed protocols. Colorimetry or spectrophotometry was used for the other estimations. Patients critically suffered from periodontitis, having pocket depth beyond 4mm, were found mildly affected with systemic hyperglycemia and dyslipidemia besides salivary hyperglycemia. The pro-inflammatory cytokine TNF-α was found exorbitantly high in the saliva of the periodontitis affected subjects. No significant change of the anti-inflammatory cytokine (IL-10) was observed in the saliva except, a marginal increase in serum as compared to the control group. A remarkably low IgA level was found in both serum and saliva of the patients suffered from periodontitis. Blood groups of the subjects in this study didn't show any correlation to the periodontitis infection. Females were found more vulnerable to periodontitis infection than the male counterparts. Lacking appropriate dental care could be the primary reason of making rooms for the settlement of microbes around teeth periphery causing periodontal pathogenicity.

Keywords: Periodontitis, TNF-α, IL-10, IgA, Oral infection

Introduction

This study was aimed to reach out the previously published reports on periodontitis showing multimodal relationships and factors between periodontal disease and systemic diseases (1,2) . Periodontitis is an inflammatory disease of the teeth and brought on by one particular microorganism or group of related microorganisms (3-5) . Established periodontal pathogens are *Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis.* Also, the emerging names of periodontal pathogens include *Filifactor alocis,* and *Peptoanaerobacter stomatis*. These microbes can destroy the periodontium, which is a complex structure of gingiva, periodontal ligament, cementum and alveolar bone.

The different types of periodontitis are simply classified to describe four categories: necrotizing periodontitis, chronic generalized periodontitis, aggressive periodontitis and periodontitis as a manifestation of systemic disease (6-7). Among all, commonly found type is chronic periodontitis and found to be linked with accumulation of plaque and calculus. Though, bacterial plaque is

the primary cause of periodontal disease; several factors affect the severity of the disease namely cigarette smoking, stress, and hyperglycemia. Diabetes is one of the major systemic factor (8), while smoking affects locally (9). The control of periodontal disease could be monitored by host-response (10-11) to it and that can be modulated by systemic health and genetic predisposition.

Recent research, however, has provided disease complexities by linking periodontal disease to systemic illnesses like cardiovascular disease (12), type 2 diabetes (8), poor pregnancy outcomes (13), osteoporosis (14,15) etc. Figure – 1 shows the inter-connections between periodontitis and various other comorbidities or systemic disorders.

In this study we have taken our target to find a comprehensive correlation, if present, to understand the severity of periodontal disease, in particular periodontitis, by aiming the risk factors in biological fluids viz. saliva, serum and whole blood.

MATERIALS AND METHODS

65 subjects, who attended the out-patient clinic of Dental Hospital of the SGT University, Gurugram, Haryana, India, with discomfort of swollen & bleeding gums, dental pain and / or ache, were screened to find 32 patients having periodontitis, who consented to volunteer in this study. The infected microorganism was detected by using PCR of the subgingival plaque samples. Among them, 20 were male and rest 12 were female subjects. On the other hand, 30 consented age matched healthy control subjects were randomly chosen from both male and female hospital workers after an initial clinical check-up and interrogation to rule out any existing physical and dental sickness. Thus, 62 subjects were included in this study considering both periodontal infection and healthy controls.

Inclusion criteria

1. ≥ 5mm or equivalent generalised pocket around the teeth periphery
2. Both the genders within age group 35 to 65 years
3. No periodontal therapy in last 6 months
4. Patients were otherwise healthy with no other clinical symptoms besides periodontitis
5. Non-smoker
6. Random samples were collected at any hour of the day as per patient's availability
7. Medically healthy control subjects having good oral health

Exclusion Criteria

- Edentulous subjects
- Pregnancy and lactation
- Genetic disorder
- Subjects diagnosed with oral cancer or any other oral disease besides periodontitis
- Critical illness
- Subjects, healthy or having periodontitis, not willing to participate in the study

Since the patients reported to the hospital clinic without any pre-appointment, only randomely collected samples, viz. blood and saliva, were used for this study. Venous blood (5ml) was taken in dry sterile vacutainers from all subjects included in the study after taking aseptic precautions. Each subject was also asked to rinse the mouth first thoroughly with water and then instructed not to swallow for 5 minutes. 3 ml of unstimulated intraorally retained saliva was expectorated in a dry sterile container kept in crushed ice in advance. Serum was separated by centrifuging 10 min at 3000 rpm. Serum and saliva were used for all the parameters included in this study.

The kit-based experimental procedures were used in this study for performing ELISA according to company directed protocols. Sandwich ELISA (Sunlong Biotech, Hanzghou,China) kit was used for estimation of TNF-α, IL-10 and IgA concentrations. Rest of the

assays were performed by colorimetric or spectrophotometric assays using the Erba-Transasia company manufactured kits under use in the clinical biochemistry laboratory of hospital facility. Glucose estimation was done in serum and saliva by GOD-POD method as was reported earlier (16,17), total cholesterol by CHOD-PAP method (18), VLDL and HDL by modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) protocol (19). TG was estimated by Glycerol phosphate oxidase (20) method. LDL was calculated by Friedewald equation [LDL = TC – HDL – TG/5]. Blood group was estimated by using ABO blood grouping kit (Tulip Diagnostics (P) Ltd, Goa, India).

Statistical analysis

Data and various parameters were analysed on SPSS software version 24. Variations in data were expressed by mean and standard deviation. The P values were calculated with Student's t-test and one-way variance analysis.

RESULTS

Table-1 shows the severity of periodontal infection in terms of pocket depths. If pocket depth goes beyond 4mm, the subject may be suspected of a victim of periodontitis. In this study a scale of infection severity, as per pocket depth in the affected area, has been decided in combination with clinical grading based on tooth pain/mobility, gingival inflammation & swelling, bleeding on probing, halitosis, stains and supra or sub-gingival calculus around the teeth. The net span of pocket depth was taken into consideration by watching the maximum pocket depth found among the participants in this study. Grading scale of infection severity as per pocket depth was decided as following, healthy teeth: 1 – 3mm; mild infection: 5.0 – 5.9mm; moderate infection: 6.0 – 6.9mm; critical infection: 7.0mm and above. At the same time the table-1 also shows the frequency of blood groups of both male and female subjects among the participants in this study. The result shows that there is no typical specificity of the blood group for being sensitive to periodontitis. The study shows that the periodontitis is independent of blood group class in the population. Rather, unhealthy mucky and soiled teeth might be the more vulnerable features for periodontitis infection.

Since glucose is the primary energy metabolite to the microbes; we checked the serum and salivary level of glucose in those periodontitis affected subjects participated in this study. The results are shown in table – 2A. In the case of mild and moderate types, serum glucose levels in both male and females were found within the normal range; though the males were remained towards upper normal limits. The critically affected males were only found hyperglycemic. Both male and female participants showed their salivary glucose level at far above of the normal limits of healthy saliva. This gives an indication that increased salivary glucose may help the bacteria to find their home at teeth periphery for their survival with abundance of energy resource.

Table-2B shows the serum lipid profile of subjects suffering from periodontitis. The overall serum lipid profile of the subjects participated in this study remained within desirable limits. Only males at the critically classified stage have shown mild dyslipidemia having a bit high values of TG, VLDL and LDL from the desirable limits. This dyslipidemia could be a characteristic reflection of infection related abnormality in critically ailing subjects having periodontitis.

Table-3A depicts the score of TNF-α in cases of periodontitis. TNF-α, a pro-inflammatory cytokine, gets increased in any inflammation related episode. The periodontium affected by periodontitis causing microbes (preferentially Treponema denticola and Porphyromonas gingivalis) develop inflammatory signs. The immunocompetent cells of the affected area may secrete TNF-α and related pro-inflammatory cytokines. The severity of the infection is expected to be proportionately related with the amount of secreted pro-inflammatory cytokines e.g. TNF-α. In this study, serum of infected males showed a modest rise of TNF-α as

compared to healthy control, whereas female subjects maintained the serum level in normal range only. The picture in saliva was completely different. Both male and female saliva of all staged infected subjects had shown exorbitantly high value of TNF-α and females showed even more than male counterparts. Since saliva remains in contact with the teeth and its supporting structures, the proportion of pro-inflammatory molecule like TNF-α surely reflects the degree of criticality of the lesion wound. Hence, this study shows that females are more vulnerable than men towards periodontal infection. This study also shows that females are more sensitive than males for developing comparatively severe infection even with similar pocket depth (table-1and-3A) i.e. having even similar teeth pocket depth (mild/moderate/critical) the severity of infection in females are more than male counterparts. In general term, this study shows that the females are more prone than the males to periodontitis. No correlation or specificity of blood group to inflammatory score was found either in male or female counterparts.

Table-3B is conveying the message on the anti-inflammatory cytokine IL-10. We measured IL-10 in order to compare and contrast its relative existence between local (saliva) and distal (blood) sites i.e. associated pro-inflammatory environment of periodontitis affected teeth (the saliva) with systemic body fluid i.e. the serum component of blood. No major difference was observed between serum and saliva. Serum of both affected male and female showed marginal to moderate increase of IL-10 as compared to healthy control; while the salivary level of IL-10 remained barely same as that of healthy control in both male and female subjects. The bit increased IL-10 in serum may be seen as a naturally preventive measure to keep blood circulation unaffected from the bacterial colony of periodontitis. No response of anti-inflammatory molecule (IL-10) in saliva to fight against its pro-inflammatory counterpart i.e. TNF-α, may be a reason for tight adherence of bacteria on teeth periphery in the ailment like periodontitis. Here also the blood group of infected subjects (male/female) had no specificity on the maintenance of anti-inflammatory score in serum or saliva. The blood groups were found very random among the periodontitis infected volunteers of this study.

Comparison of concentrations of immunoglobulin A (IgA) between serum and saliva is shown in table-3C. IgA is an antibody that exists as part of our immune system. IgA is present in blood as well as saliva as a molecule of immune surveillance. That is why IgA has been considered in this study to check if it has any role against periodontitis. Surprisingly, we found too low concentration of IgA in both serum and saliva as compared to the health controls. The result shows that periodontitis defeats the immune shield. So, system's own preventive measure cannot stop the growth of the microbe in the illness of periodontitis. It needs externally applicable means, mechanical or chemical, to heal the wound of infection i.e. surgical or non-surgical (scaling & root planning) dental therapy (mechanical) along with the drugs as appropriate (chemical). Since IgA values in serum and saliva were too low in study participants as compared to healthy control group, we have shown only the range of concentration found i.e. only lower and upper limits of concentrations obtained. As was found with other components in this study, the existence of blood group was very random in cases of IgA response too.

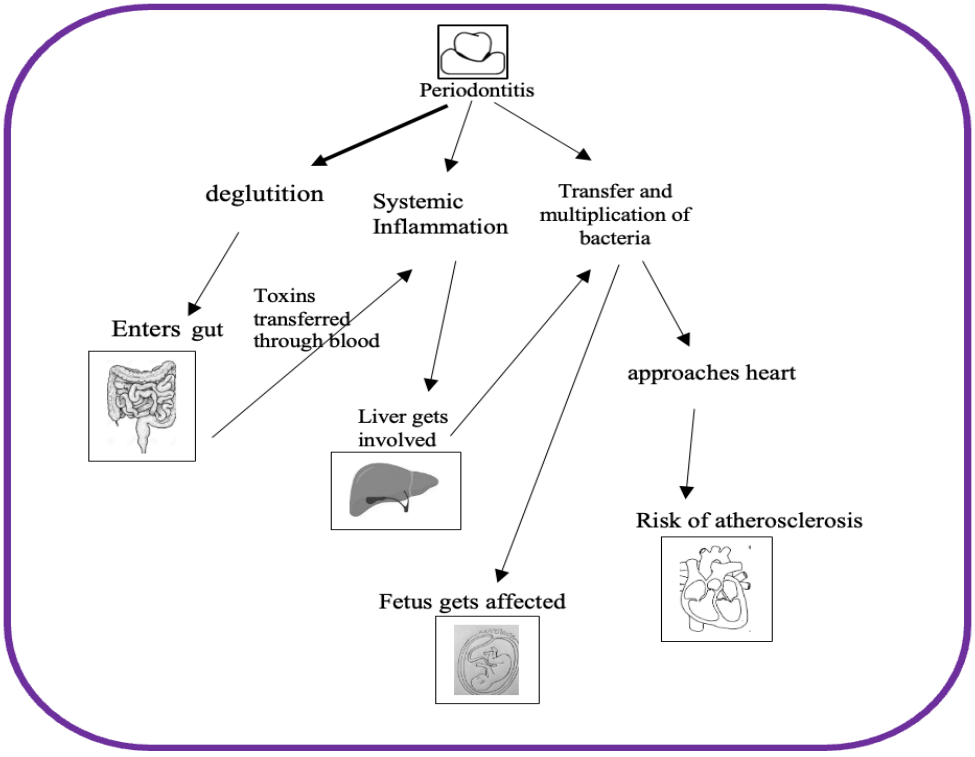

Figure 1: Correlation between periodontitis and medical disorders.

Table 1: Differential stages of periodontitis in terms Pocket Depth (n=32).

Stages of Infection	No. of Subjects	Pocket Depth (mm)	Male (n=20)				Female (n=12)			
			A	B	O	AB	A	B	O	AB
Mild	10	5.0-5.9		2	3			3	2	
Moderate	17	6.0-6.9	4	4		2	3	2	2	
Critical	5	≥ 7.0	2	3						

Table 2: Blood glucose and lipid profile of periodontitis affected subjects.

A- Profile of glucose concentration in serum and saliva of periodontitis affected subjects.

Site →		Serum		Saliva	
Normal range →		80 – 110 mg/dL		0.51 – 2.32mg/dL	
Gender [Male (M) / Female (F)] →		M	F	M	F
Stages	MILD n=10	105.66 ±6.17 n=5	98.80±13.52 n=5	36.20*±6.68 n=5	67.60±13.14 n=5
				$P_{M,F}$ <0.05	
	MODERATE n=17	103.39±9.6 n=10	98.67±18.93 n=7	67.30±23.60 n=10	31.57±3.20 n=7
				$P_{M,F}$ <0.05	
	CRITICAL n=5	127.68±24.10 n=5	—	52.6*±7.4 n=5 P* (mild, cri) < 0.05	—

M: Male; F: Female. P-values indicate the significant difference.

B-Serum lipid profile of periodontitis affected subjects

Parameters →		Total CHOL		HDL		TG		VLDL		LDL	
Desirable limit →		< 200 mg/dL		40-60 mg/dL		< 150 mg/dL		< 30 mg/dL		< 100 mg/dL	
Gender →		M	F	M	F	M	F	M	F	M	F
Stages	MILD n=10	151.10 ± 40.20 n=5	162.48 ± 19.92 n=5	40.18 ± 3.23 n=5	43.20 ± 8.83 n=5	146.068 ± 123.68 n=5	137.62 ± 54.95 n=5	32.84 ± 22.20 n=5	27.50 ± 11.0 n=5	76.88* ± 21.07 n=5	91.78 ± 10.61 n=5
	MODERATE n=17	174.29 ± 33.59 n=10	165.17 ± 20.04 n=7	41.52 ± 10.29 n=10	37.85 ± 7.08 n=7	147.12 ± 39.68 n=10	109.80 ± 48.80 n=7	29.44 ± 7.91 n=10	21.95 ± 9.75 n=7	103.3 ± 21.26 n=10	105.3 ± 14.09 n=7
	CRITICAL n=5	178.56 ± 42.45 n=5		31.32 ± 9.11 n=5		170.90 ± 30.80 n=5		34.16 ± 6.15 n=5		113.2* ± 30.31 n=5 $P^*_{mild,\ crt} = 0.05$	

M: Male; F: Female. P-values indicate the significant difference.

Table 3: Concentrations of TNF-α, IL-10, and IgA in periodontitis infected subjects.

A-Concentration of TNF-α (pg/ml) in periodontitis infected subjects

Healthy Control (n=30) Mean ± SD		Patients (n = 32) Mean ± SD				
Serum	Saliva	Stages with Blood gr.	Serum		Saliva	
			Male	Female	Male	Female
34.45 ± 1.05	55.19 ± 15.51	Mild (n=10) B, O	53.10±0.28 n=5	33.04±5.28 n=5	93.68±23.65 n=5	106.22±49.82 n=5
			$P_{M,F} < 0.05$			
		Moderate (n=17) A, B, O, AB	53.04± 8.44 n=10	36.24± 13.51 n=7	83.78± 9.95 n=10	132.33± 45.40 n=7
		Critical (n=5) A, B	56.64± 12.93 n=5	—	129.40± 39.14 n=5	—

B-Concentration of IL-10 (pg/ml) in periodontitis infected subjects

Healthy Control(n=30) Mean ± SD		Patients (n = 32) Mean ± SD				
Serum	Saliva	Stages with Blood gr.	Serum		Saliva	
			Male	Female	Male	Female
9.87 ± 4.23	24.53 ± 3.55	Mild (n=10) B, O	10.01± 4.80 n=5	17.28± 4.81 n=5	16.85*± 0.67 n=5	23.39± 6.80 n=5
		Moderate (n=17) A, B, O, AB	14.32± 2.98 n=10	11.68± 1.60 n=7	20.95± 4.0 n=10	23.04± 3.04 n=7
		Critical (n=5) A, B	13.61± 3.78 n=5	—	27.63*± 9.22 n=5 $P^*_{mild, crit} < 0.05$	—

C-Concentration of IgA (mg/dl) in periodontitis infected subjects

Healthy Control (n=30) Mean ± SD		Patients (n = 32) Mean ± SD				
Serum	Saliva	Stages with Blood gr.	Serum		Saliva	
			Male	Female	Male	Female
80 – 210	2.9 – 10.1	Mild (n=10) B, O	21 – 28 n=5	16 – 28 n=5	0.15 – 0.25 n=5	0.13 – 0.20 n=5
		Moderate (n=17) A, B, O, AB	11 – 22 n=10	14 – 27 n=7	0.02 – 0.06 n=10	0.10 – 0.18 n=7
		Critical (n=5) A, B	0.0 n=5	—	0.0 n=5	—

M: Male; F: Female. P-values indicate the significant difference

DISCUSSION

30 healthy subjects and 32 patients with periodontitis participated in this pilot study. Clinical evaluation based on pain, mobility, gingival inflammation & enlargement, bleeding on probing or with stiff stains, deposited calculus in and around the teeth etc., along with the size of the pocket depth in the infected area were used for rating the grade of infection e.g. mild, moderate and critical stage of pathogenesis. The rating of pocket depth was like, mild infection: 5.0 – 5.9mm; moderate infection: 6.0 – 6.9mm; critical infection: 7.0mm and above. We compared this rating of infection with various biochemical parameters used in this study including the blood group of those participants.

Carbohydrate is a fuel for any living bacterium (21). Therefore, aim was taken to check glucose level in serum and saliva of periodontitis affected subjects. In this study serum glucose level was found within the normal limits in both male and female subjects suffered with periodontitis, except critically affected male participants, who were found diabetic (serum glucose level > 126 mg/dL). A remarkable gap was noticed between serum and saliva of the periodontitis affected

subjects. Existing salivary hyperglycemia was noticed in periodontitis cases. On an average the salivary glucose concentration in periodontitis affected mouth was found to be 15 to 30 times higher than the concentration of a normal healthy person's saliva irrespective of male and female subjects. This makes it apparent that persistency of high glucose concentration in saliva around the teeth over a longer period of time might be the cause of inception of periodontitis. In our study no critically affected female participant was found.

Previous reports had shown incidence of dyslipidemia in subjects having periodontitis (22,23). In our study, we had found only critically affected males were marginally dyslipidemic; otherwise the serum lipid profile of infected subjects remained within desirable limits. This dyslipidemia of critically ailing subjects with periodontitis infection could be an infection related phenomenon as was reported previously (24).

Porphyromonas gingivalis, Treponema denticola and / or any other related bacterium may develop inflammatory lesion known as periodontitis. In response to antigens of infected bacterium the secreted pro-inflammatory cytokines from the lesion area of the immunocompetent cells can exaggerate the pathogenic outcome of bacterial infection. TNF-α, a pro-inflammatory cytokine, found to be increased in most bacterial infections including periodontitis (25). Kibune et al found a significant correlation between the levels of salivary TNF-α and the expansion of periodontal tissue inflammation (26) . Martinez-Aguilar et al observed variation of TNF-α levels in patients with periodontitis stage 2 grade B (POD2B) and having type-2 diabetes (T2D) (27). Varghese et al demonstrated considerably higher levels of TNF-α in cases of periodontitis (28). A modest increase of serum TNF-α was also noticed in our present study for infected male participants over healthy control group; while in case of female counterpart, serum values were remained only within the normal range. On the contrary, a pro-inflammatory environment was found within the oral cavity. Both male and female subjects, at all stages of infection severity, showed excessively high salivary TNF-α concentration. No correlation or specificity of blood group to the inflammatory score was found either in male or female counterparts.

In normal healthy individuals the effects of pro-inflammatory cytokines are neutralized by anti-inflammatory cytokines (29). Any imbalance in the activity of anti-inflammatory cytokines to counteract the effect of pro-inflammatory cytokines may develop inflammatory outburst. In this study an anti-inflammatory cytokine viz. IL-10 has been chosen to see its counter effect against pro-inflammatory flare of periodontitis. No significant change in the IL-10 concentration as compared to control values was noticed between different stages of infection, both in serum and saliva. Our study supports previous review by Varma et al., who also reported an insignificant change in salivary IL-10 concentration in patients having periodontitis (30). The marginally increased IL-10 in serum may be viewed as a naturally preventive measure to keep blood circulation unaffected from the bacterial contamination of periodontitis related pathogenesis. Here also the blood groups were found random among the volunteers of this study.

Besides anti-inflammatory cytokines, the inflammatory flare of an antigen can be shielded by respective antibodies against those foreign antigens of infected species. Since IgA is a known immune surveillance component of human saliva; IgA has been considered in this study to check its role against antigenic components of infected bacterium of periodontitis infection. Surprisingly, no stimulation by infected bacterium was observed to escalate the IgA concentration either in serum or saliva in reference to the concentration of healthy persons. Rather the concentrations, in both serum and saliva, were declined below the range normally found in healthy individuals. This showed a failure of body's immune machinery to

neutralize the bacterial antigenic power in periodontitis infection. Previous reports also showed low level of salivary IgA in patients having periodontitis (31,32). It has been recorded that bacterial species isolated from the oral cavity of patients with periodontitis were found to be the producers of IgA proteases (33). These enzymes cleave IgA molecules in serum and saliva equally well. The same IgA proteases can also cleave the A1 and A2 subclasses. Hence, infected bacterium of periodontitis may destroy IgA molecules in biological fluids.

CONCLUSION

This study shows salivary hyperglycemia is a pertinent factor for the inception of periodontitis over the teeth-gum juncture. Only critically affected male population is susceptible for dyslipidemic attack. Salivary inflammatory response by TNF-α (inflammatory cytokine) is immensely high over serum response. Anti-inflammatory safety shield responded by IL-10 (anti-inflammatory cytokine) and IgA (anti-immune antibody) are negligible in periodontitis infection. Blood groups of the population have shown no specificity or selectivity in this study to the incidence of periodontitis attack.

Ethical Clearance

The study was approved by the Institute Ethical Board. Reference no. SEC/FMHS/M.Sc/ 01/05/23-01, dated 01/05/2023, SGT University, Haryana, India.

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Informed consents: All authors have given their consents for publication of the result found in this study.

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REFERENCE

1. Kapila YL. Oral health's inextricable connection to systemic health: Special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. *Periodontology 2000* 2021; 87: 11–16.
2. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nature Reviews (Immunology)* 2021; 21: 426 – 440.
3. Saini R, Marawar PP, Shete S, et al. Periodontitis, a true infection. *J Glob Infect Dis* 2009; 1(2): 149-150.
4. American Academy of Periodontology. Informational paper. The pathogenesis of periodontal diseases. *J. Periodontol*.1999; *70:* 457–470.
5. Armitage GC. Development of classification system for periodontal diseases and conditions. *Ann. Periodontol*.1999; *4:*1–6.
6. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol, 89 (Suppl 1),* 2018*;* S159–S172.
7. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Periodontol* 2018; 89(Suppl 1),2018; S1–S8.
8. Păunică I, Giurgiu M, Dumitriu AS et al. The Bidirectional Relationship between Periodontal Disease and Diabetes Mellitus—*A* Review. *Diagnostics.2023;* 13(4): 681. doi: 10.3390/diagnostics13040681.

9. Sim KY, Jang YS, Jang YS, et al. Association between Smoking and Periodontal Disease in South Korean Adults. *Int J Environ Res Public Health,* 2023;20(5): 4423.
10. Bezerra B, Monajemzadeh S, Silva D et al. Modulating the immune response in periodontitis. *Front. Dent. Med.* 2022; *3:*879131.
11. Silva N, Abusleme L, Bravo D et al. Host response mechanisms in periodontal diseases. *J Appl Oral Sci.*; 2015: 23(3)*,* 329-355.
12. Zardawi F, Gul S, Abdulkareem A et al. Association between periodontal disease and atherosclerotic cardiovascular diseases: Revisited. *Front. Cardiovasc Med.*2021; 7: 625579.
13. Nannan M, Xiaoping L, Ying J. Periodontal disease in pregnancy and adverse pregnancy outcomes: Progress in related mechanisms and management strategies. *Front Med.*2022; *9:* 963956.
14. Yu B, Wang C-Y. Osteoporosis and periodontal diseases – An update on their association and mechanistic links. *Periodontology 2000.*2022; *89(1):*99 – 113.
15. Jayusman PA, Nasruddin NS, Baharin B et al. Overview on postmenopausal osteoporosis and periodontitis: The therapeutic potential of phytoestrogens against alveolar bone loss. *Front. Pharmacol, 14.*2023; 1120457.
16. Gupta S, Sandhu SV, Bansal H, et al. Comparison of Salivary and Serum Glucose Levels in Diabetic Patients. *Journal of Diabetes Science and Technology,* 2015;9(1): 91–96
17. Burrin JM,Price CP. Measurement of Blood Glucose. *Annals of Clinical Biochemistry.*1985; *22(4):* 327-342.
18. Allain CC, Poon LS, Chan CS et al. Enzymatic determination of total serum cholesterol. *Clin Chem,*1974*;20(4):* 470-475.
19. Shabana Shahid SU, Sarwar S. The abnormal lipid profile in obesity and coronary heart disease (CHD) in Pakistani subjects. *Lipids Health Dis,* 2020;*19(1):* 73.
20. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem,*1973; *19(5):* 476-482.
21. Chubukov V, Gerosa L, Kochanowski K et al. Coordination of microbial metabolism. *Nat Rev Microbiol,*2014; *12:* 327–340.
22. Bitencourt FV, Nascimento GG, Costa SA, et al.The role of dyslipidemia in periodontitis. Nutrients. 2023; 15(2): 300. doi: 10.3390/nu15020300.
23. Hagh LG, Zakavi F, Hajizadeh, F et al. The association between hyperlipidemia and periodontal infection. *Iran Red Crescent Med J.*2014; *16(12):* e6577.
24. Wang XE, Li W, Song W et al. Association of CYP 1A1 rs1048943 variant with aggressive periodontitis and its interaction with hyperlipidemia on the periodontal status. *Journal of periodontal research;* 2019: *54(5),* 546-554.
25. Rathinasamy K, Ulaganathan A, Ramamurthy S et al (2020). Estimation of TNF-α levels in saliva and serum of patients with periodontal health and chronic periodontitis: A case-control study. *J Contemp Dent Pract;* 2020: *21(2*), 148–151.
26. Kibune R, Muraoka K, Morishita M et al. Relationship between dynamics of TNF-α and its soluble receptors in saliva and periodontal health state. *Dent J (Basel),*2022; *10(2):* 25. doi: 10.3390/dj10020025.
27. Martinez-Aguilar VM, Carrillo-Avila BA, Sauri-Esquivel EA et al. Quantification of TNF-α in patients with periodontitis and type 2 diabetes. *BioMed research international.* 2019; 7984891: doi: 10.1155/2019/7984891.
28. Varghese SS, Thomas H, Jayakumar ND, Sankari M et al. Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients. *Contemporary clinical dentistry, 6(Suppl 1)*.2015; S152.

29. Cavaillon J-M. Pro-versus anti-inflammatory cytokines: Myth or reality. *Cell. Mol. Biol.2001; 47(4*), 695 – 702.
30. Varma SR, Thomas B, Ramesh A et al. Estimation of sialic acid and IL10 levels in stage 1 and 2 periodontitis patients. *International Journal of Dentistry, 2019.*2019*;* 2917124: doi: 10.1155/2019/2917124;
31. Giuca MR, Pasini M, Tecco S et al. Levels of salivary immunoglobulins and periodontal evaluation in smoking patients. *BMC Immunol.*2014; 15:5:doi: 10.1186/1471-2172-15-5.
32. Nikfarjam J, Pourpak Z, Shahrabi M et al. Oral manifestations in selective IgA deficiency. *Int J Dent Hyg.*2004; *2(1):* 19-25. doi: 10.1111/j.1601-5037.2004.00060.x.
33. Bokor-Bratić M. Clinical significance of analysis of immunoglobulin A levels in saliva. *Med Pregl.*2000; *53(3- 4):* 164 – 168.