ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Immunological Effects of Ceramic and Composite Subgingivally Extended Restoration

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Abstract

In this study, 80 samples of gingival crevicular fluid (GCF) from patients have their teeth filled with different restoration materials , collected 20 sample of composite resin restoration material ,20 sample of crown ceramic restoration material , 20 sample of metal ceramic restoration material and 20 sample of control non-filled teeth. The purpose of this study was determine the effects composite rein , ceramic crown and metal ceramic crown restoration material on (GCF) levels of interleukin 1(IL-1 ß) , interferon gamma (IFN- γ) , Matrix Metalloproteinase-8 (MMP8) and tumor necrosis factor (TNF- ß). Paper points (size 30) were inserted into the gingival crevice until mild resistance was felt, and kept in place for 30 seconds. Following collection of GCF the paper point placed in eppendroffs tubes contain (300 micro liter) phosphate buffer saline. GCF was eluted from paper point by centrifugation at 3000 rpm for 15 minutes after that the paper point was removed and GCF sample kept at -40 ˚C till analysis. Analysis of data for immunological parameter (IL-1ß, IFN- γ, TNF- ß and MMP8) that got from ELISA test. Finally, the results showed mean concentration of IL-1ß, IFN- γ, TNF- ß and MMP8 in patients with restoration material (composite resin , crown ceramic and metal ceramic) significant ($P < 0.05$) were higher than in control while MMP-8 in ceramic crown restoration material high but no significant ($P > 0.05$).

Keywords: *Composite resin, Ceramic crown, Metal ceramic crown, Periodntitis IL-1β, IFN- γ, TNF- β, MMP8.*

Introduction

Dental restorative materials are in direct or indirect contact with various tissues, such as enamel, dentin, pulp and gingiva. Therefore, dental materials should be risk-free for all oral tissues and should not cause toxic, mutagenic or cancerogenic effects [1]. The main purpose of restorative dentistry is to restore and maintain tooth health by an adequate restorative treatment. Although developing technology in the field of dentistry, there is a continuing need for biomaterials with high biocompatibility, mechanical competence and antimicrobial effects [2, 3].

The crown restoration material better to other composite resin material. Crown did not cause any inflammation, but the accumulated plaque on the crwon surface is the main cause of gingival inflammation.

Dental materials can cause gingival inflammation because of its placement near the gingival and other periodontal tissues [4, 5]. Biomarkers can be derived directly from inflamed periodontal tissue during biopsy, from oral fluids, such as gingival crevicular fluid (GCF), peri-implant sulcular fluid (PISF), mouth-rinse and saliva or blood circulatory system - serum or plasma. GCF and saliva are particularly suitable, as they can be easily and noninvasively collected and comprise both locally and systematically synthesized molecules [6].

The content of the gingival crevicular fluid (GCF) reflects inflammatory reactions taking place in the gingival tissue [7, 8]. GCF can be considered among the most nontraumatic investigational methods used to provide information about periodontal tissue

conditions, including the status of the connective tissue and the degree of hard tissue destruction [9, 10]. An increased volume of GCF is positively associated with the degree of gingival inflammation [11]. Cytokines are glycoproteins with a low molecular weight, which play a role in important biological events, such as cellular growth, inflammation, immunity, tissue repair and hematopoiesis [12, 13]. They also play an important role in the inflammatory response related to gingivitis, tissue destruction in periodontal diseases and the regulation of the adaptive immune response [14, 13]. The restoration material increased the progressive gingival inflammation [15].

Composition of the materials may cause inflammatory responses by monocyte activation and changes in the levels of cytokine released from monocytes. This condition causes variations in the levels of some cytokines, such as IL-1ß, TNF, IFN- γ, and MMP8 in the gingival crevicular fluid (GCF) of the infected region [16]. IL1beta levels can increase in GCF during the initial phase of gingival inflammation before the onset of clinical signs of gingivitis [17, 8]. Innate-immune-system triggered inflammation is activated after periodontal pathogens have invaded through the first line of defense in the periodontium; comprising intact junctional epithelium and a constant flow of GCF and antibacterial products. Proinflammatory cytokines (IL-1 -ßand TNF) link the innate and the adaptive immune systems together by recruiting and activating the adaptive immune system related cells [18, 20].

Materials and Methods

Samples Collection of GCF

To avoid contamination with saliva, they were isolated from saliva with cotton wool rolls and dried with short air blasts. Immediately following isolation and removal of supragingival plaque, paper point (size 30) were inserted into the gingival crevice until mild resistance was felt, and kept in place for 30 seconds. Blood contaminated paper point were excluded. Following collection of GCF the paper point placed in eppendroffs tubes contain (300 micro liter) phosphate buffer saline. GCF was eluted from paper point by centrifugation at 3000 rpm for 15 minutes after that the paper point was removed and GCF sample kept at -40 ˚C till analysis [21, 23].

Statistical Analysis

Statistical Package for Social Science (SPSS) program was used in this study. All values were expressed as mean ± standard deviation (S.D). A nova test (F.) used to compare between all groups and When P values were less than or equal to 0.05 considered as statistical significance while p value were larger than 0.05 as statistical non-significant [24].

Results

Measurement of Cytokines Concentration of Composite Resin Restoration Material and Healthy Control

All GCF samples measured by using ELISA test for detection the concentration of cytokines (IL-1ß, IFN-γ, MMP-8 and TNF-ß) all showed high significant ($p<0.0001$) in composite restoration material comparison to healthy control Table (1).

Table 1: Concentration of cytokines in patients of Composite Resin Restoration Material and Healthy control

Cytokines	Groups n =20	Concentration (pg / mL , ng/mL) Mean ±SD	*P.* Value
IL-1ß (pg/mL)	Composite resin	*27.041±10.069	0.0001
	Control	4.239±1.727	
IFN-γ (pg/mL)	Composite resin	*41.19±14.4	0.0001
	Control	12.791±3.641	
MMP-8 ng/mL)(	Composite resin	*0.396±0.193	0.0001
	Control	0.123±0.046	
TNF-ß (pg/mL)	Composite resin	*36.177±12.641	0.0001
	Control	9.581±1.855	

*significant differences $p<0.0001$

Measurement of Cytokines Concentration of Ceramic Crown Restoration Material and Healthy Control

All GCF samples measured by using ELISA test for detection the concentration of cytokines (IL-1ß, IFN-γ and TNF-ß) all showed high significant (p<0.0001) in ceramic crown restoration material comparison to healthy control .While concentration of cytokines MMP-8 high but no significant (p> 0.1324) in ceramic crwon restoration material comparison to healthy control .Table (2).

Table 2: Concentration of cytokines in patient of ceramic crown Restoration Material and Healthy control

Cytokines	Groups n =20	Concentration (pg / mL , ng/mL) Mean ±SD	*P.* Value
IL-1ß (pg/mL)	Crown ceramic	*21.21±8.689	0.0001
	Control	4.239±1.727	
IFN-γ (pg/mL)	Crown ceramic	*34.178±10.02	0.0001
	Control	12.791±3.641	
MMP-8 ng/mL)(	Crown ceramic	0.250±0.131	0.1324
	Control	0.123±0.046	
TNF-ß (pg/mL)	Crown ceramic	*31.645±15.086	0.0001
	Control	9.581±1.855	

Measurement of Cytokines Concentration of Metal Ceramic Crown Restoration Material and Healthy Control

All GCF samples measured by using ELISA test for detection the concentration of cytokines (IL-1ß, IFN-γ, MMP-8 and TNF-ß) all showed high significant (p<0.0001) in metal ceramic restoration material comparison to healthy control Table (3).

Table 3: Concentration of cytokines in patients of metal ceramic crown Restoration Material and Healthy control

Cytokines	Groups n =20	Concentration (pg / mL , ng/mL) Mean ±SD	*P.* Value
IL-1ß (pg/mL)	Metal ceramic	*26.509±7.333	0.0001
	Control	4.239±1.727	
IFN-γ (pg/mL)	Metal ceramic	*30.777±10.946	0.0001
	Control	12.791±3.641	
MMP-8 ng/mL)(	Metal ceramic	0.437±0.277*	0.0001
	Control	0.123±0.046	
TNF-ß (pg/mL)	Metal ceramic	29.848±15.738*	0.0001
	Control	9.581±1.855	

*significant differences p<0.0001

Discussion

Measurement of Cytokines Concentration of Composite Resin Restoration Material and Healthy Control

The results found the concentration of cytokine IL-1ß in patient for composite resin compared to healthy group were significant statically differences between two groups were ($p<0.0001$) table (1). The results indicated that composite resin restoration material that case toxic and inflammatory effect. This result is agreement with Sakallioglu *et al* [25]. IL-1ß is important in periodontal diseases due to its potency in inhibiting bone formation and enhancing bone resorption stimulating the production of prostaglandin E2, collagenase, and proteinase [26].

Gingival crevicular fluid (GCF) is often attracted as a marker of periodontal disease activity. It is an inflammatory exudate that can be collected at the gingival margin or gingival crevice. Host response in periodontal disease can be assessed non-invasively by the biochemical analysis of GCF [27]. The interleukin one beta (IL-1ß) is a potent inflammatory cytokine, since it recruits neutrophils to the inflamed site, being generally induced by bacterial antigens. Additionally, it has been suggested and used as one of the main markers of acute inflammation [28].While concentration of the cytokine (IFN-γ) in patient have composite resin and control.

The result found the concentration of IFN-γ for composite resin compared to healthy group which were significant statically a difference between two groups was ($p<0.0001$) table (1). The results indicated that composite resin restoration material that case toxic and inflammatory effect. This result is agreement with Tsai *et al* [29], Fu *et al* [30]. Gurban *et al* [31]. IFN-γ together may regulate the immunoinflammatory response [32, 29] .The IFN-γ bearing cells in human periodontitis gingival tissue [33].

The IFN-γ-bearing cells might be involved in the destruction of periodontal tissue. The advanced adult periodontitis patients showed higher GCF IFN-γ suggesting that longitudinal follow-up in the levels of IFN-γ in GCF might be a useful indicator of periodontal tissue destruction [34, 29].

Inflammatory cell infiltrate containing lymphocytes and macrophages is often observed in periodontal lesions. It is hypothesized that T-lymphocytes are predominant in stable lesions, whereas B-lymphocytes and plasma cells are increased in progressive lesions [35, 29].

This suggests that Th1 cytokine expression T-cells are the main regulators of inflammation in the early/ stable lesions. Production of interferon-gamma (IFN-γ) can increase the phagocytosis of polymorphonuclear neutrophils and macrophages, therefore inhibiting the progression of infection. Also concentration of the cytokine (MMP-8) in patient of composite resin and control group. The results found the concentration of MMP-8 for composite resin compared to healthy group were significant statically a difference between two groups was ($p<0.0001$) table (1).

The results indicated that composite resin restoration material that case toxic and inflammatory effect. This result is agreement with Leppilahti *et al* [36]. Ou *et al* [37].

MMPs play a minor role in periodontal tissue destruction [38]. MMP-8 possesses the unique capacity to disrupt collagen type I and III which is essential in periodontitis but not in normal gingival tissue remodelling and is considered to be one of the key mediators of tissue destruction during inflammation of periodontal tissues [39, 40]. It is the most frequently found MMP in inflamed periodontal tissue, gingival crevicular fluid and saliva [41].

MMP-8 levels can identify sites or patients who are at risk of periodontitis progression or have poor response to standard treatment [42].Also concentration of the cytokine (TNF-ß) in patient of composite resin and control group. The results found the concentration of TNF-ß for composite resin compared to healthy group were significant statically a difference between two groups was ($p<0.0001$) as shown in the Table (1).

The results indicated that composite resin restoration material that case toxic and inflammatory effect. Tumor necrosis factor-beta (TNF-ß), also known as lymphotoxin-alpha, is a member of the TNF family. It is a pro-inflammatory cytokine that activates NF-kB, MAPK, and PI3K/AKT pathways upon binding to TNF receptors 1 and 2. It is

produced mainly by T cells, though other cells can express TNF-ß at lower levels [43]. TNF-ß is involved in autoimmune disorders, lymph node development, and mediating the inflammatory demyelination process [44, 45].

Measurement of Cytokines Concentration of Ceramic Crown Restoration Material and Healthy Control

Concentration of the cytokine (IL-1ß) in patient of ceramic crown (zirconia) restoration and control group. The results found the concentration of IL-1ß for crown ceramic compared to healthy group were significant statically a difference between two groups was ($p<0.0001$) table (2). The results indicated that crown ceramic (zirconia) restoration material that case toxic and inflammatory effect. This result is agreement with Ozen *et al* [21]. Saravanakumar *et al* [46].

Interleukin-1 beta (IL-1b) is of particular interest since it is a proinflammatory, multifunctional cytokine, which promotes bone resorption and stimulates eicosanoid production. IL-1b also participates in many aspects of the immune response and has been shown to be present and elevated in the tissues and gingival crevicular fluid (GCF) of patients with periodontal disease. IL-1b is a key mediator of the host inflammatory and tissue regulatory pathways in a number of chronic inflammatory disorders, such as periodontitis [47, 48].

As well as Guzeldemir *et al* [49].Found that IL-1ß increased levels in GCF correlate with the severity of the periodontal disease. While concentration of the cytokine (IFN-γ) in patient of ceramic crown (zirconia) restoration and control group.

The result found the concentration of IFN-γ for ceramic crown (zirconia) compared to healthy groups were significant statically differences between two groups was ($p<0.0001$) table (2). The results indicated that crown ceramic (zirconia) restoration material that case toxic and inflammatory effect. This result is agreement with Tsai *et al* [29]. Fu *et al* [30]. Gurban *et al* [31]. IFN-γ is the sole type II IFN. It is structurally unrelated to type I IFNs, binds to a different receptor, and is encoded by a separate chromosomal locus. Initially, it was believed that CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes, and NK cells exclusively produced IFN -γ [50].

However, there is now evidence that other cells, such as B cells, NKT cells, and professional antigen-presenting cells (APCs) secrete IFN- γ. IFN- γ production by professional APCs [monocyte/macrophage, dendritic cells (DCs)] acting locally may be important in cell self-activation and activation of nearby cells [51]. IFN- γ secretion by NK cells and possibly professional APCs is likely to be important in early host defense against infection, whereas T lymphocytes become the major source of IFN- γ in the adaptive immune response [52]. The IFN- γ in GCF were increased in progressive periodontal lesions [53].Also concentration of the cytokine (MMP-8) in patient of ceramic crown (zirconia) restoration and control group.

The result found the concentration of (MMP-8) for ceramic crown (zirconia) compared to healthy groups were highly but no significant statically differences between two group was ($p>0.1324$) as table (2). The results indicated that crown ceramic (zirconia) restoration material that case toxic and inflammatory effect. This result is agreement with Indriani *et al* [54]. MMP-8 or collagenase-2 has been identified as the central biomarker in connective tissue injury that is caused by periodontitis and their relation to the gingival tissue inflammation and periodontal [55].

It is a protein that is produced by Th1 type T-cells and induces vascular endothelial cells to change their surface adhesion molecules to allow phagocytic cells to bind to them. It is also known to be required for normal development of Peyer's patches.

Lymphotoxin is homologous to Tumor Necrosis Factor beta, but secreted by T cells. It is paracrine due to the small amounts produced. The effects are similar to TNF-alpha, but TNF-beta is also important for the development of lymphoid organs [56]. Also concentration of the cytokine (TNF-ß) in patient of ceramic crown (zirconia) restoration and control group. The result found the concentration of TNF-ß for ceramic crown (zirconia) compared to healthy groups were significant statically differences between two groups was ($p<0.0001$) Table (2).

The results indicated crown ceramic (zirconia) restoration material that case toxic and inflammatory effect.

Measurement of Cytokines Concentration of Metal Ceramic Crown Restoration Material and Healthy Control

This healthy periodontium must exist prior to the fabrication of a crown and must be maintained after the crown has been placed. Despite the long-standing use of alloys and ceramics as fixed and removable restoration materials, there are still questions about their behavior in the oral environment [57]. These materials come into close and prolonged contact with gingival and oral mucosa and have been claimed to cause inflammation of these tissues. Concentration of the cytokine (IL-1ß) in patient of metal ceramic crwon restoration material and control group.

The result found the concentration of IL-1ß for metal ceramic crwon restoration compared to healthy group were significant statically differences between two groups was ($p<0.0001$) as table (3). The results indicated that metal ceramic crown restoration material that case toxic and inflammatory effect. This result is agreement with Ozen *et al* [58]. Saravanakumar *et al* [46]. Among the numerous cytokines involved in the induction and regulation of host responses in inflammation, IL-1ß seems to play a central role in the inflammatory reaction. Evaluated the importance of interleukin 1-ß and its association with inflammatory periodontal disease [59] and showed that an increased production of the gingival crevicular fluid and salivary IL-1ß predisposes the patient to chronic periodontitis due to an exaggerated inflammatory response by the immune system. It is well established that IL-1ß plays the central role in inflammatory reactions and also enhances various immune responses *In Vitro* [60].

However, there is at present no direct evidence that an increased IL-1ß release after exposure to dental alloys and ceramic specifically indicates inflammatory reactions in vivo. The results of this study support the hypothesis that alloys and ceramic affect the activity of cells and IL-1ß secretion from gingival fibroblasts. While concentration of the cytokine (IFN-ɣ) in patient of metal ceramic crwon restoration material and control group. The result found the concentration of IFN-ɣ for metal ceramic crwon restoration compared to healthy group were significant statically differences between two groups was ($p<0.0001$) as table (3). The results indicated that metal ceramic crown restoration material that case toxic and inflammatory effect. This result is agreement with Tsai *et al* [29]. Fu *et al* [30]. Gurban *et al* [31]. Proinflammatory interferon gamma (IFN-ɣ) markers have been identified in gingival crevicular fluid (CGF).Correlation between the effect of interferons in crevicular fluid and periodontitis. Interferon gamma (IFN-ɣ) is an immune regulatory cytokine that works through its receptor and plays an important role in the progression of inflammation [61]. Also concentration of the cytokine (MMP-8) in patient of metal ceramic crwon restoration material and control group.

The result found the concentration of MMP-8 for metal ceramic crwon restoration compared to healthy group were significant statically differences between two groups was ($p<0.0001$) as table (3). the results indicated that suffering metal ceramic crown restoration material that case toxic and inflammatory effect. This result is agreement with Thalib *et al* [5]. Indriani *et al* [54]. Zhang *et al* [62]. The inclusion of porcelain in the porcelain fused to metal crown affecting the periodontal health.

The study result showed that MMP-8 expression was elevated due to the porcelain materials. Paired t test analysis result showed that the MMP-8 gene expressions was significantly increased. This also means that the elevation of MMP-8 gene expressions was affected by the porcelain materials. Who showed that artificial crown play a role in the periodontal inflammations [63]. The MMP-8 is mainly secreted by polymorphonuclear leukocytes, and other cells, such as oral epithelium, plasma cells, and fibroblast. MMP-8 was expressed in inactive form during the latency periods and was activated by the so-called cysteine switch [64].

MMP-8 was expressed on the periodontal tissues and its elevation had been reported in the tissues of individuals with inflammation and periodontitis [65].The decreased of MMP-8 levels in the GCF showed that this enzyme can be used as a current status indicator and

as a predictor of the prognosis of the disease [66].

Neutrophil is a main cellular source of MMP-8 which also increased in periodontitis patients. Furthermore, bacterial proteinase in microbial plaque may be able to activate the MMP-8 production by neutrophil [67].Also concentration of the cytokine (TNF-ß) in patient of metal ceramic crown restoration material and control. The result found the concentration of TNF-ß for metal ceramic crown restoration compared to healthy group were significant statically differences between two groups was ($p<0.0001$) table (3). The results indicated that metal ceramic crown restoration material that case toxic and inflammatory effect.

Conclusion

They study has arrived at the following conclusion: Concentration of cytokines (IL-1ß, IFN-ɣ, MMP-8 and TNF-ß) all showed high significant in restoration material (composite resin, ceramic crown and metal ceramic crown) comparison to healthy control, while concentration of cytokines (MMP-8) high but no significant in crown ceramic restoration material comparison to healthy control.

References

1. Costa CA, Giro EM, do Nascimento AB, Teixeira HM, Hebling J (2003) Short-term evaluation of the pulpo-dentin complex response to resin- modified glass-ionomer cement and a bonding agent applied in deep cavities. Dental Materials, 19(8): 739-746.

2. Modena KC, Casas-Apayco LC, Atta MT, Costa CA, Hebling J, Sipert CR (2009) Cytotoxicity and biocompatibility of direct and indirect pulp capping materials. Journal of Applied Oral Sciences, 17(6): 544-554.

3. Eskandarizadeh A, Parizi MT, Goroohi H, Badrian H, Asadi A, Khalighinejad N (2015) Histological assessment of pulpal responses to resin modified glass ionomer cements in human teeth. Dent Res J. (Isfahan), 12(2): 144-149.

4. Vacaru R, Podariu AC, Jumanca D, Galuscan A, Timisoara RM and Romania. (2003). Periodontal-restorative interrelationships. OHDMBSC 3(5): 12-5

5. Thalib B, Damayanti I, Arafi A, Iqbal M (2015) Changes in gene expression of matrix metalloproteinase-8 after insertion porcelain fused to metal crown. Department Prostdontic, Faculty of dentistry Hasanuddin University, Makassar, South Sulawesi, Indonesia.

6. Buduneli N, Kinane DF (2011) Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. J. Clin Periodontol 38: 85-105.

7. Sakai A, Ohshima M, Sugano N, Otsuka K, Ito K (2006) Profiling the cytokines in gingival crevicular fluid using a cytokine antibody array. J. periodontal, 77(5):856-64.

8. Ramberg P, Albertsson KW, Derks J, Duken JV (2016) A randomized controlled cross-over study of the effect of alcohol-free chlorhexidine and essential oils on interleukin -1 levels in crevicular fluid .Swed dent J., 40:143-152.

9. Bostanci N, Ilgenli T, Emingil G (2007) Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio, *Journal of Clinical Periodontology*, 34(5): 370-376.

10. Majeed ZN, Philip K, Alabsi AM, Pushparajan S, Swaminathan D (2016) Identification of Gingival Crevicular Fluid Sampling, Analytical Methods, and Oral Biomarkers for the Diagnosis and Monitoring of Periodontal Diseases: A Systematic Review. Hindawi Publishing Corporation Volume, Article ID 1804727, 23. http://dx.doi.org/10.1155/1804727

11. Emingil G, Cinarcik S, Baylas H, Coker I, Huseyinov A (2001) Levels of leukotriene B4 in gingival crevicular fluid and gingival tissue in specific periodontal diseases. Journal of Periodontology, 72(8): 1025-1031.

12. Gornowicz A, Bielawska A, Bielawski K, Grabowska SZ, Wojcicka A, Zalewska M (2012) Pro-inflammatory cytokines in saliva of adolescents with dental caries disease. Annals of Agricultural and Environmental Medicine, 19(4): 711-716.

13. Celik N, Askın S, Gul MA, Seven N (2017) The effect of restorative materials on cytokines in gingival crevicular fluid. Journal homepage. Archives of Oral Biology, 84: 139-144.

14. Giannopoulou C, Kamma JJ, Mombelli A (2003) Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. Journal of Clinical Periodontology, 30(2): 145-153.

15. Knoernschild KL, Campbell SD (2000) Periodontal tissue responses after insertion of artificial crowns and fixed partial dentures. *J. Prosthet. Dent*., 84 5: 492-8.

16. Nelson SK, Wataha JC, Cibirka RM, Lockwood PE (2001) In vitro TNF-alpha release from THP-1 monocytes in response to dental casting alloys exposed to lipopolysaccharide. Journal of Prosthetic Dentistry, 85(5): 466-471.

17. Zhang J, Kashket S, Lingstrom P (2002) Evidence for early onset of gingival inflammation following short-term plaque accumulation. J. Clin. periodontal., 29(12):1082-5.

18. Ebersole JL (2003) Humoral immune responses in gingival crevice fluid: local and systemic implications. *Periodontology 2000* 31: 135-166.

19. Preshaw PM, Taylor JJ (2011) How has research into cytokine interaction and their role in driving immune responses impacted our understanding of periodontitis? J. Clin. Periodontol, 38: 60-84.

20. Kinane DF, Preshaw PM, Loos BG (2011) Working Group 2 of Seventh European Workshop on Periodontology. Host-response: understanding the cellular and molecular mechanisms of host- microbial interactions - consensus of the Seventh European Workshop on Periodontology. J. Clin. Periodontol, 38 (11): 44-48.

21. Ozen J, Beydemir B, Serdar M A, Dalkiz M, Saygun I, Ozdemir A (2001) The Effect of Fixed Restoration Materials on the IL-1b Content of Gingival Crevicular Fluid. Turk. J. Med. Sci., 31: 365-369.

22. Hussain AA, Ali BG (2014) Evaluation of Interleukin 1ß Levels in Gingival Crevicular Fluid and Serum of Patients with Gingivitis and Chronic Periodontitis. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) *e*-ISSN: 2279-0853, p-ISSN: 2279-0861.13 11 Ver. VI 70-75.

23. Escalona LA, Alberga PM, Correnti M (2016) Cytokine and metalloproteinases in gingival fluid from patients with chronic periodontitis. Invest Clin. 57(2):131-142.

24. Daniel WW (1999) Probability and t distribution biostatistics: A foundation for analysis in health science.7th ed.83-123.John willey and Sons, INC-USA.

25. Sakallioglu EE, Lutfioglu M, Sakallioglu U, Ceylan GK, Pamuk F, Dede FO, Dede D (2015) Gingival crevicular fluid levels of neuropeptides following dental restorations. J. Appl. Biomater Funct Mater 13(2): e186-e193. DOI: 10.5301/JABFM.5000197. EISSN 2280-8000.

26. Montserrat BC, Montserrat CP, Jose V (2014) Salivary and crevicular fluid interleukins in gingivitis. J. Clin. Exp. Dent., 6:175-9.

27. Lamster IB (1997) Evaluation of components of gingival crevicular fluid as diagnostic tests. Ann Periodontal. 2.1.123. https://doi.org/10.1902/annals.1997.2.1.123 .

28. Oliveria Silva AC, Rubio Faria M, Fontes A (2009). Interleukin-1 beta and Interleukin-8 in healthy and inflamed dental pulps. J. Appl. Oral Sci., 17: 527-32.

29. Tsai CC, Ku CH, Ho YP, Ho KY, Wu YM, Hung CC (2007) Changes in gingival crevicular fluid interleukin-4 and interferon-gamma in patients with chronic periodontitis before and periodontal initial therapy. Kaohsiung J. Med. Sci., 23:1-7.

30. Fu QY, Zhang L, Duan L, Qian SY, Pang HX (2013) Correlation of chronic periodontitis in tropical area and IFN - γ, IL-10, IL-17 levels. Asian Pacific Journal of Tropical Medicine 489-492.

31. Gurban CV, Suciu O, Vlaia L, Gotia LS, Cornianu M, Zambori C, Motoc M, Vlaia V, Velea I, Pricop M (2016) Inflammatory Markers from Crevicular Fluid in Periodontal Disease REV. CHIM. (Bucharest). 67: 10.

32. Kobayashi M, Okada N, Okamatsu Y (1999) Intracellular interleukin-1 alpha production in human gingival fibroblasts is differentially regulated by various cytokines. *J. Dent. Res.,* 78: 840-9.

33. Ukai T, Mori Y, Onoyama M (2001) Immunohistological study of interferon-gamma- and interleukin-4-bearing cells in human periodontitis gingiva. Arch. Oral. Biol., 46: 901-8.

34. Salvi GE, Brown CE Fujihashi K (1998) Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. *J. Periodontal. Res.,* 33: 212-25.

35. Seymour GJ, Gemmel E, Walsh LJ (1988) Immunological analysis of experimental gingivitis in humans. Clin. *Exp. Immunol.,* 71: 132-7.

36. Leppilahti JM, Ahonen MM, Hernandez M, Munjal S, Netuschil L, Uitto VJ, Sorsa T, Mantyla P (2011) Oral rinse MMP-8 point-of-care immuno test identifies patients with strong periodontal inflammatory burden. Oral Diseases, 17: 115-122.

37. Ou Q, Hu Y, Yao S, Wang Y, Lin X (2018) Effect of matrix metalloproteinase 8 inhibitor on resin-dentin bonds. Journals Dental Materials, 34 (5): 756-763.

38. Sapna G, Gokul S, Bagri-Manjrekar K (2013) Matrix metalloproteinases and periodontal diseases. *Oral Dis*. doi:10.1111/odi.12159.

39. Rai B, Kharb S, Jain R, Anand SC (2008) Biomarkers of periodontitis in oral fluids. *J. Oral. Sci.,* 50**:** 53-56.

40. Sorsa T, Tjaderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mantyla P (2006) Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med.,* 38: 306-321.

41. Dejonckheere E, Vandenbroucke RE, Libert C (2011) Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. Cytokine *Growth Factor Rev.,* 22**:** 73-81.

42. Leppilahti JM, Sorsa T, Kallio M A, Tervahartiala T, Emingil G, Han B, Mantyla P (2015) The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome. J. *Periodontol.,* 86**:** 777-787.

43. Chu W-M (2013) Tumor necrosis factor. Cancer Lett., 328(2): 222-5.

44. McCarthy DD et al (2006) The lymphotoxin pathway: beyond lymph node development. Immunol Res 35(1-2): 41-54.

45. Seleznik GM et al (2014) The role of lymphotoxin signaling in the development of autoimmune pancreatitis and associated secondary extra-pancreatic pathologies. Cytokine Growth Factor Rev., 25(2): 125-37.

46. Saravanakumar P, veeravalli PT, V AK, Mohamed K, Mani U, Grover M, Thangarajan TT (2017) Effect of Different Crown Materials on the Interleukin-One Beta Content of Gingival Crevicular Fluid in Endodontically Treated Molars: An Original Research. Cureus 9(6): e1361. DOI 10.7759/cureus.1361

47. Toker H, Poyraz O, Eren K (2008) Effect of periodontal treatment on IL-1beta, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis Journal of Clinical Periodontology, 35(6): 507-513.

48. Firat E, Ercan E, Gurgan S, Cakir FY and Berker E (2011) The Effect of Bleachıng Systems on the Gingiva and the Levels of IL-1b and IL-10 in Gingival Crevicular Fluid. Operative Dentistry, 36-6, 572-580.

49. Guzeldemir E, Gunhan M, Ozcelik O, Tastan H (2008) Interleukin-1 and tumor necrosis factor-alpha gene polymorphisms in Turkish patients with localized aggressive periodontitis. J. Oral. Sci., 50: 151-159.

50. Bach EA, Aguet M, Schreiber RD (1997) The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.,* 15: 563-591.

51. Frucht DM, Fukao T, Bogdan C, Schindler H, O'Shea JJ, Koyasu S (2001) IFN-gamma production by antigen-presenting

cells: mechanisms emerge. *Trends Immunol.,* 22**:** 556-560.

52. Sen GC (2001) Viruses and interferons. *Annu. Rev. Microbiol.* 55: 255-281.

53. Dutzan N, Vernal R, Hernandez M, Dezerega A, Rivera O, Silva N, Aguillon JC, Puente J, Pozo P, Gamonal J (2009) Levels of Interferon-Gamma and Transcription Factor T-Bet in Progressive Periodontal Lesions in Patients With Chronic Periodontitis. J Periodontol 80:290-296.

54. Indriani L, Dharmautama M, Machmud E, Djide MN, Hatta M (2016) Effect of Roselle Extract in Expression of Matrix Metallo Proteinase - 8 (MMP-8) in Gingival Crevicular Fluid (GCF). American Journal of Clinical and Experimental Medicine, 4(3): 56-62.

55. Sorsa T, Tjaderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mantyla P (2006) Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med.,* 38: 306-321.

56. Parham and Peter (2005) "Chapter 6: T-cell mediated immunity". The immune system (2nd Ed.). New York: Garland Science, 172. ISBN 0-8153-4093-1.

57. Joe BH, Borke JL, Keskintepe M (2001) Interleukin-1 beta regulation of adhesion molecules on human gingival and periodontal ligament fibroblasts. J. Periodontol., 72: 865-70. 10.1902/jop. 72.7.865.

58. Ozen J, Ural AU, Dalkiz M, Beydemir B (2005) Influence of Dental Alloys and an All-Ceramic Material on Cell Viability and Interleukin-1beta Release in a Three-Dimensional Cell Culture Model. Turk. J. Med. Sci., 35: 203-208.

59. Korrnman KS, Crane A, Wang HY (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. J. Clin. Periodontol., 24: 72-7.

60. Masada MP (1990) Measurement of interleukin 1a and 1b in gingival crevicular fluid. J Periodont Res 25: 156-63.

61. Niedzielska I, Cierpka S (2010) Interferon gamma in the etiology of atherosclerosis and periodontitis. Thromb. Res, 126: 324-327.

62. Zhang ZH, Zhang XH, Zhang Y, Fang M (2016) Detection of inflammatory factor and protease content as well as oxidative stress level in gingival crevicular fluid after fiber post and metal post repair. Journal of Hainan Medical University, 22(14): 148-151.

63. Campbell JY (2000) Asset pricing at the Millennium. Journal of Finance.

64. Passoja A, Ylipalosaari M, Tervonen T, Raunio T, Knuutitila M (2008) Matrix metalloproteinase-8 concentration in shallow crevices associated with the extent of periodontal disease. J. Clin. Periodontol., 35: 1027-1031

65. Liu KZ, Hynes A, Man A, Aksagheer A, Singer DL, Scoot DA (2006) Increased local matrix metalloproteinase-8 expression in the periodontol al connective tissues os smokers with periodontal disease. Biochimica et biophysica acta, 1762: 776-80.

66. Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L (2007) The role of matrix metalloproteinases in the oral environment. Acta Odont Scan, 65: 1-13

67. Holla I, Hrdlickova B, Vokurka J, Fassman A (2012) Matrix metalloproteinase 8 (MMP-8) gene polymorphisms in chronic periodontitis. Archieves of oral Biology, 5.

