Prostaglandin E2 (PGE2) Level in the Gingival Crevicular Fluid (GCF) Of Sudanese Patient with Chronic Periodontitis

[*1]Safinaz Hussien, [2]Ibrahim Ahmed Ghandour
[1]Tagelsir-Assistant Professor Of Periodontology[1],
[2]professor Of Periodontology[2] And Abdalla Aljabry-Assistant Professor Of Periodontology[2]*
Address For Correspondence: Dr. Abdalla Saleh Aljabry,

Abstract:
Background: *The purpose of the present study was to evaluate the level of prostaglandin E_2 in gingival Crevicular fluid of patients with chronic periodontitis.*
Material and Methods: *Thirty patients diagnosed with chronic periodontitis in the periodontal department in Khartoum Dental Teaching Hospital and sixty controls were enrolled in the study. GCF was collected using paper strips (periostrip) inserted into the gingival sulcus for 30 seconds then put in a cryoviale tube in frozen media and sent to the lab. Clinical examination is carried out using dental mirror and William's graduated periodontal probe. Plaque index (PI), gingival index (GI), periodontal pocket depth (PPD), Recession (R), clinical attachment loss (CAL), was recorded in the data sheets.*
Results: *Thirty case and sixty controls from both genders with age range 25-45 years were included in the study. The results showed that highest mean concentrations of PGE_2 in GCF were obtained from the study group; while the lowest concentrations were obtained from the control subjects with p value 0.001(statistically significant). There was a relationship between the mean PGE_2 concentration in GCF and mean of the clinical parameters (PI, GI, PPD, R, CAL) with p value 0.009(statistically significant).*
Conclusion: *There was strong association between the PGE_2 in GCF levels and severity of chronic periodontitis.*
Key words: *GCF, PGE2, Chronic periodontitis,Sudanese.*

Date of Submission: 28 -08-2017   Date of acceptance: 20-09-2017

I.   Introduction

Periodontal disease (PD) is an inflammatory process involving innate and adaptive immune responses characterized by irreversible loss of connective tissue attachment and supporting alveolar bone. This change may often lead to an esthetically and functionally compromised dentition (1). Periodontitis is a chronic inflammatory disease involving interactions between bacterial products, host cells, and inflammatory mediators. The inflammatory response results in destruction of the tissues and the alveolar bone supporting the teeth and can ultimately lead to tooth loss. The most common periodontal disease is chronic periodontitis. (2)Chronic periodontitis is a painless, slowly progressive disease characterized by inflammation of the gingiva and is caused by microbial colonization of the periodontal tissues and results in pocket formation, destruction of alveolar bone, mobility and exfoliation of teeth. (3) The disease is associated with variable microbial organisms; Porphyromonas gingivalis (P. gingivalis), provetella intermedia (P. intermedia), capnocytophaga, Aggregatobacteractinomycetemcomitans (A.a), Eikenella corrodens (E. corrodens), fusobacteriumnucleatum, campylobacter rectus (C.rectus), and Treponema denticola. (4). Chronic periodontitis is either localized or generalized. It is considered localized when less than 30% of the sites assessed in the mouth demonstrate attachment loss and bone loss while generalized periodontitis is considered when 30% or more of the sites assessed in the mouth demonstrate attachment loss and bone loss .(5) Chronic periodontitis may be classified according to the severity of the disease into mild (slight), moderate and severe.(6) Factors which are associated with increased risk of chronic periodontitis are either (local factors, systemic factors (diabetes) or environmental factors (smoking, stress factors). Local factors are the main etiological causes of chronic periodontitis. There is sufficient evidence that accumulation of plaque bioflim is necessary for the initiation and progression of periodontal disease, studies showed that bacterial species colonizing the gingival pocket play variable roles in the pathogenesis of this disease and may therefore poses different levels of risk for periodontal tissues loss (7). Smokers have a significant higher risk of developing chronic periodontal disease (8) there is a dose effect relationship between cigarette smoking and severity of the periodontal disease such that heavy smokers and those with a longer history of smoking show more sever tissue loss than light smokers (9). Diabetes is a widely

studied condition associated with periodontal disease; type 2 diabetes is the most prevalent type, which accounts for 85 %-95% of the diabetic population. (10)

Numerous studies have shown a correlation between chronic periodontitis and diabetes; it is concluded that these diseases may influence the progression and treatment response of each other. (11, 12). One of the etiological factors of chronic periodontitis is stress; the relationship is simply due to the fact that individuals under stress are less likely to maintain good oral hygiene. (13). Prostaglandins (PGs) are a group of lipid compounds derived from Arachidonic acid (AA); a polyunsaturated fatty acid found in plasma membrane of most cells. Arachidonic acid is metabolized by Cyclooxgenase 1 and 2 (COX -1 and COX-2) to generate a series of related compounds called prostanoids, which includes prostaglandins (PGs), thromboxanes and prostacyclins (14).Prostaglandins (PGs) are important mediators of inflammation particularly prostaglandin E_2 (PGE$_2$), which is a potent vasodilator and inducer of cytokines production by varies cells.PGE2 acts on fibroblast and osteoclast to induce production of MMPS, which are important for tissue turnover and tissue destruction in gingivitis and periodontitis (15). PGE$_2$ is produced via three different groups of enzymes; the first group of enzymes, phospholipase A2, converts membrane lipids to Arachidonic acid. (16, 17) while the second group of isoenzymes, Cyclooxgenase (COX1 and COX2) convert Arachidonic acid to prostaglandin H2 (49).The third group is prostaglandin E synthases (PGE synthases) which catalyze the conversion of COX-derived prostaglandin H2 to PGE2. (18, 19). PGE$_2$ is an important inflammatory mediator that is involved in all processes leading to the cardinal signs of inflammation; i.e. redness, swelling and pain (20). Redness and edema result from increased blood flow into the inflamed tissue through PGE$_2$ –mediated augmentation of arterial dilation and increased microvascular permeability (21). Pain results from the action of PGE$_2$ on peripheral sensory neurons and on central sites within the spinal cord and the brain (22). Fever is caused by PGE$_2$ released by inflammatory mediators from endothelial cells lining the blood vessels of the hypothalamus (23). Dysregulated PGE$_2$ synthesis or degradation has been associated with a wide range of pathological conditions. PGE$_2$ play role in certain systemic diseases for example, rheumatoid arthritis (RA) ,osteoporosis and preterm-low birth weight. (24).

PGE$_2$ play a key role in the progression of periodontal diseases; the endogenous PGE$_2$ production by host cells stimulated by plaque – associated bacterial endotoxins which is an important pathogenic factor in periodontal disease. (25). The levels of prostaglandin E_2 (PGE$_2$) in gingival crevicular fluid (GCF) have been reported to correlate positively with inflammation and impending tissue destruction. (26) This research is undertaken to study the relationship between prostaglandin E_2 (PGE$_2$) levels in the gingival crevicular fluid (GCF) in Sudanese patients with chronic periodontitis.

II.   Litreture Review

A case control study designed by Kumar et al, evaluated the gingival crevicular fluid (GCF) prostaglandin E_2 (PGE$_2$) levels. Samples were collected from 15 patients who were diagnosed with chronic periodontitis and 10 healthy. Age ranged between 20 and 55 years from both sexes. The samples were classified into groups; Group (1) healthy consist of 10 patients , Group (11) consist of 15 patients chronic periodontitis , Group (111) consist of 15 patients of Group (11) after treatment .PGE$_2$ levels were estimated in GCF samples by using the enzyme linked immunosorbent assay (ELISA) kits were obtained from R and D systems CO (USA) . Highest mean PGE$_2$ levels in GCF were obtained for group (11) while the lowest levels were seen in Group (1) and (111). (27). A cohort longitudinal study was designed by Preshow et al who monitored the gingival crevicular fluid prostaglandin E_2 (GCF-PGE$_2$) concentrations in patients with chronic periodontitis. Samples were collected from 41 patients who were diagnosed with (moderate to severe chronic periodontitis). GCF samples were collected from sites (with 5 – 8 mm pocket depth, and attachment loss); GCF-PGE$_2$ was estimated by using enzyme linked immunosorbent assay (ELISA). Every 30 days for 150 days and assayed for PGE$_2$. The results showed a significant increase in GCF-PGE$_2$ concentrations observed over the course of the study, from 40.3 ng/ml to 83.1 ng/ml (28). Another case control designed by Zhou et al measured the prostaglandin E_2 levels in gingival crevicular fluid and its relation to the depth of the pockets in patients with chronic periodontitis. Samples were taken from 46 normal controls and 90 patients suffering from chronic periodontitis with periodontal pockets with different depths measured by radioimmunoassay (RIA). The results demonstrated that PGE$_2$ levels in GCF of the periodontal pockets in chronic periodontitis with different depth were higher than controls. The PGE$_2$ levels increased with the increase in the depth of periodontal pockets, particularly when the depth exceeds 6 mm. The study concluded that the PGE$_2$ levels are significantly related to the severity of bone destruction in chronic periodontitis (29). Offenbacher et al to evaluated the level of gingival crevicular fluid (GCF) prostaglandin E_2 (PGE$_2$) in health, naturally occurring gingivitis and experimental gingivitis and concluded that PGE$_2$ levels are low in health and non-detectable at many sites, while in naturally occurring gingivitis there is modest rise in GCF-PGE$_2$ levels to about 32ng/ml, and a higher rise (about 53 ng/ml) is detected in experimental gingivitis (30).

Rationale / Justification:

1. Chronic periodontitis is the most common periodontal disease globally. PGE_2 is an important inflammatory mediator because it is involved in all processes leading to all signs of inflammation.
2. PGE_2 is a potent vasodilator and inducer of cytokines production by various cells.
3. PGE_2 plays a key role in the progression of periodontal diseases.
4. PGE_2 has many biological functions, such as regulation of blood pressure, gastrointestinal integrity.

Objectives:
General objectives: To determine prostaglandin E_2 (PGE_2) levels in the gingival crevicular fluid (GCF) of patients with chronic periodontitis at Khartoum Dental Teaching Hospital.

Specific objectives:
1- To study the relationship between oral hygiene (health) gingival crevicular fluid and prostaglandin E_2 levels.
2- To assess the relationship between gingival crevicular fluid, prostaglandin E_2 levels and chronic periodontitis.

Subjects and methods:
Study Design:
Case Control Study conducted at the department of periodontology, Khartoum Dental Teaching Hospital (KDTH), Khartoum, Sudan, during the period from 1/4 to 1/6/2016.

Study population:
Patients attending the department of periodontology from both sexes between 25-45 years age during the period of the study.
Study Sample:
30 Patients for study group and 60 controls from both sexes. Sample code (A for chronic periodontitis, B for controls).

Inclusion criteria:
All patients attending periodontal department in Khartoum Dental Teaching Hospital and agree to participate in this study, and fulfill the following criteria:-
1 – Patients age 25-45 years old of both sexes.
2 – Patients diagnosed with chronic periodontitis.
3 –Don't have chronic medical problems that affect the pathogens of periodontal diseases i.e. diabetes, kidney or liver problem etc.

Exclusion criteria:
1 – Pregnant females.
2 – Smokers.
3 – Patients who received periodontal treatment during the last three months.
4 – Patients using anti inflammatory or steroids drugs during the last three month (NSAIDs).

Methods:
Oral examination was performed using dental mirrors and William's graduated periodontal probes. All subjects were examined in day light, seated in dental chair. The teeth were dried with a piece of cotton before examination. The following parameters were recorded: personal data, medical history; oral hygiene , PI (plaque index) , GI (gingival index), PPD (periodontal pocket depth) , CAL (clinical attachment loss) , R (recession) on a special sheet (Appendix 5).

Procedures:
The standard of the oral hygiene of all subjects was evaluated by Sinless and Loe plaque index (1964), which is based on recording both soft debris and mineralized deposits. Each of the four surfaces of the tooth (buccal, lingual, mesial and distal) is given scores from 0-3. The scores from the four areas of the tooth are added and divided by four in order to calculate the plaque index for the tooth with the following scores and criteria:
0 = No plaque.
1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth.
2 = moderate accumulation of soft deposits within the gingival pocket or the tooth and gingival margin which can be seen within naked eye.

3 = Abundance of soft matter within the gingival pocket and / or on the tooth and gingival margin.
Gingival Index by Loe H and Sillness J (1963) was used to assess gingival inflammation. The Gingival Index System Scores Criteria is follows:-
0 = No inflammation
1 = Mild inflammation, no bleeding elicited on probing.
2 = Moderate inflammation, bleeding on probing.
3 = Sever inflammation, spontaneous bleeding.
Gingival Crevicular Fluid (GCF) was collected using paper strip (periostrip) inserted into the gingival sulcus for 30 second without touching the marginal gingiva; then the paper strip is removed from the sulcus and put in a cryoviale tube in frozen media then transferred to the lab. Before collection of GCF, area was isolated from saliva by cotton rolls and the supragingival plaque removed without touching the marginal gingiva to avoid bleeding.
PGE_2 level in GCF was measure by using the enzyme linked immunosorbent assay (ELISA).

INSTRUMENTS:
❖ William's graduated periodontal probe.
❖ Dental mirror.

Ethical Consideration :
The aims, the objectives and methodology of the study, were explained to the participants. Those who accepted to participate were asked to sign a consent form in Arabic (A ppendix 3).
Ethical approval was obtained from the ethical committees and concerned bodies at (KDTH), Sudan Medical Specialization Board (SMSB), and the Federal Ministry of Health (FMOH).

Statistical analysis :
Statistical analysis was performed with (SPSS) Statistical Package for the Social Sciences (version 16) by the help of a statistician. Subjects' characteristics were summarized using simple descriptive statistics. Many test used by statistician to analysis data ANOVA test, T- test and Cross ablation test.

PGE_2 Assay procedure:
The ELISA procedure was performed according to the instructions of the supplier (R&D System, USA). Samples were diluted 3-fold in Calibrator Diluents RD5-56. 200ul of Calibrator Diluents RD5-56 was added to the non-specific binding (NSB) wells .150 ul of Calibrator Diluents RD5-56 were added to the zero standard (B0) well. 150 ul of standard, controls, and samples were added to the remaining wells. Then 50 ul of the primary antibody solution were added to each well except NSB well. Plates were incubated for 1 hour at room temperature on a microplate shaker. 50 ul of PGE_2 Conjugate was added to each well except NBS well and the plates were covered with plate sealer.

Plates again incubated on the shaker for 2 hours, then washed 4 times before the addition of 200 ul of substrate solution. Reactions were terminated by adding 100 ul well of stop solution. Plates were then read immediately at 450 (VersaMaxmicroplate reader, Molecular Devices). Resulting Optical Density was obtained by calculating the average between each two readings. PGE_2 concentration of samples was extrapolated from a four parameter logistic standard curve constructed in Excel using the mean values obtained for the calibrators. Laboratory work was done by technician under supervision of candidate.
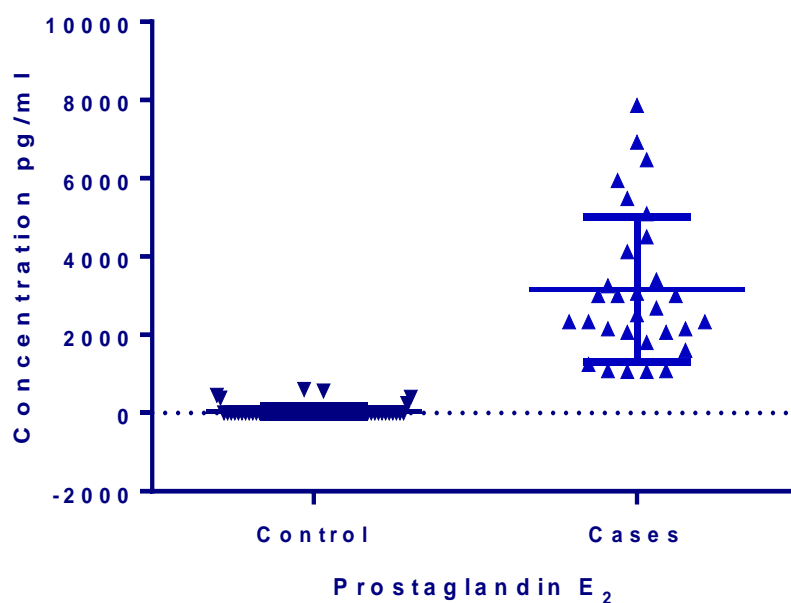
III.   Results

Figure (1) The Distribution and percentage of sample age Group in both study and control Groups.
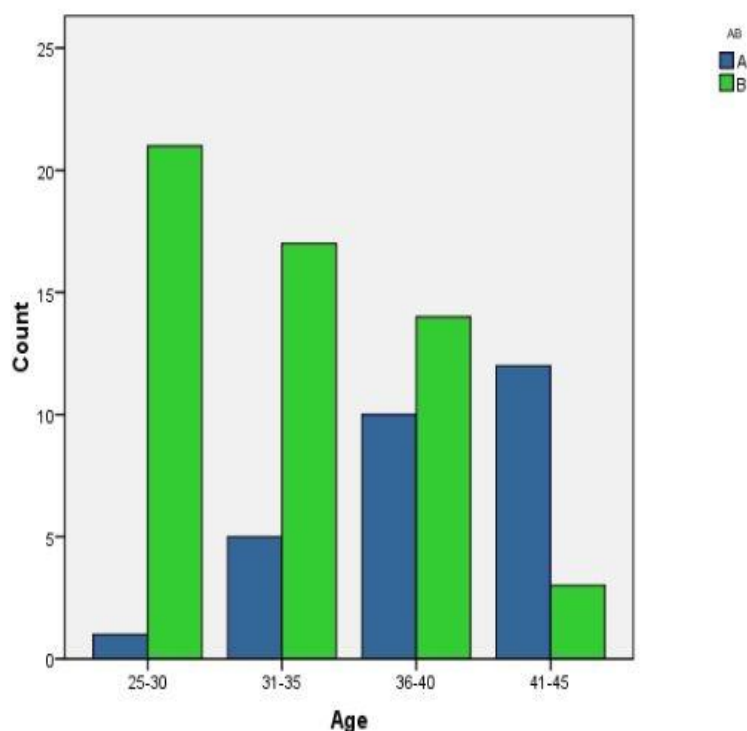
Table (1): The Mean PGE_2 Concentration in GCF and Standard Deviation for Both Study and Control Groups

Group	N	Mean ± SD	DF
Study	30	3158-6±1852.8	29
Control	57	46.5±142.2	56

P. value 0.001 (statically significant)

Figure (2) The distribution of PGE_2 concentration in GCF in study and control groups.

Table (2) The Distribution of sample clinical parameters (PI, GI, PPD, R, CAL) According to mean and standard deviation in study and control Group.

Clinical parameters	Mean ± SD	
	study	Control
PI	2.3 ± 1.2	.97 ± 1.2
GI	2.5 ± 1.4	.95 ± 1.2
PPD	1.7 ± 2.3	0
R	1.3 ± 4.6	0
CAL	3.1 ± 6.1	0

P.value 0.009 (statistically significant).

Table (3): Comparison between the Mean PGE_2 Concentration in GCF and Clinical Parameters in Both Study and Control Groups.

Group	PGE₂ Conc.	PI	GI	PPD	R	CAL
Study	3158.6	2.23	2.48	1.72	1.23	3.08
Control	46.5	.97	.95	0	0	0
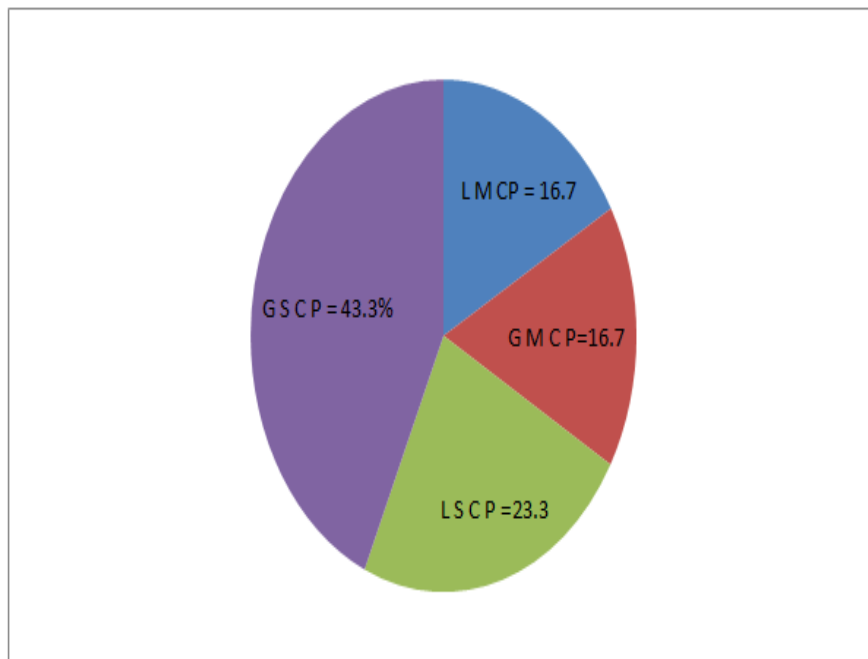
Figure (3) The Distribution of Study Group According to Severity of Chronic Periodontitis.

GSCP= Generalized severe chronic periodontitis.
LSCP= Localized severe chronic periodontitis.
GMCP= Generalized moderate chronic periodontitis.
LMCP= Localized moderate chronic periodontitis.

Table (4): The Relationship between the PGE_2 Concentration in GCF and Percentage of the Chronic Periodontitis.

PGE Conc pg/ml	Generalized moderate chronic Periodontitis No(%)	Localized moderate chronic Periodontitis No(%)	Generalized Severe chronic Periodontitis No(%)	Localized Severe chronic Periodontitis No(%)	Total No(%)
1000 – 2000	0(0)	2(3.3)	0(0)	0(0)	2(3.3)
2001 – 3000	3(10)	0(0)	2(6.7)	1(3.3)	6(20)
3001 – 4000	2(6.7)	2(10.1)	4(3.3)	2(6.7)	10(26.8)
4001 – 5000	0(0)	1(3.3)	1(3.3)	0(0)	2(6.6)
5001 – 6000	0(0)	0(0)	3(10)	3(10)	6(20)
6001 – 7000	0(0)	0(0)	2(6.7)	1(3.3)	3(10)
7001 – 8000	0(0)	0(0)	1(13.3)	0(0)	1(13.3)
Total No (%)	5(16.7)	5(16.7)	13(43.3)	7(23.3)	30(100)

IV. Discussion

In the present study the highest mean concentration of PGE_2 in GCF, obtained in chronic periodontitis (study group) is 3158.6 (table 1) compared to control subjects with p value 0.001which is statistically significant. This result agrees with the study done by Kumar et al (2013) in which the highest mean concentration of PGE_2 in GCF was found in group II (chronic periodontitis),while the lowest mean concentrations was found in group I(health subjects) and group III(group II patients after treatment) with p value 0.001. The results of the present study also agree with the longitudinal study done by Preshow et al (2002) in which they monitored the PGE_2 levels in GCF (observed over the course of the study 150 days). They concluded that there is a significant increase in the level of PGE_2 from 40.3 ng/ml to 83.1 ng/ml. the mean PGE_2 Concentrations in GCF increases progressively from healthy to diseased gingival tissues. The results are also similar to the results of the study done by Offenbacher et al (1993) who showed that the PGE_2 levels in GCF is low in health and may be un detectable in many sites, while a modest rise in PGE_2 in the GCF levels is found in naturally occurring gingivitis (32 ng/ml) and higher rise about (53ng/ml) in experimental gingivitis. (69). PGE_2 in GCF concentrations has strong positive correlation with clinical parameters and pathogenesis of periodontal disease. The present study showed that the highest mean concentration of PGE_2 in GCF is (3158.6) and the highest clinical parameters were found in the study group, while the lowest mean concentration of PGE_2 in GCF (46.5) and the lowest clinical parameters were found in the healthy subjects (Table 3). The mean of the clinical parameters of the study group was PI (2.23), GI (2.48), PPD (1.72), R (1.23), and CAL (3.08) with p value 0.009(statistically significant). This is also evident in the study done by Kumar et al (2013) in which they found that the highest mean concentration of PGE_2 in GCF was obtained in group II (chronic periodontitis) , while the lowest level in group I (health subject) and group III (patient after treatment) . The mean of the clinical parameters of Group II was PI (0.704), GI (0.816), PPD (0.826), and CAL (0.826).

The PGE_2 concentrations in GCF have strong correlations with clinical parameters and pathogenesis of periodontal disease. The results are comparable with those of the present study. Most previous studies did not compare the concentration of PGE_2 in GCF according to severity of chronic periodontitis (mild, moderate, severe) or the distribution of the disease according to site (localized and generalized). But in the present study (figure 3) shows the distribution of frequency and percentage of chronic periodontitis according to severity and site. The highest percentage 43.3% of patients were diagnosed with generalized severe chronic periodontitis, while the lowest percentages, 6.7% of patients were diagnosed with localized moderate chronic periodontitis. This indicates that the PGE_2 in GCF increases with the severity of the disease. (Table 4) shows the relationship between GCF PGE_2 concentrations (conc pg/ml) and the percentage of chronic periodontitis. The highest concentration of PGE_2 in GCF ranged between (7001 -8000 pg/ml) and was found in patients diagnosed with generalized severe chronic periodontitis with a percentage (13.3%),while 3.3% of patients diagnosed with localized moderate chronic periodontitis have the lowest concentrations of PGE_2 in GCF which ranged between (1000-2000 pg/ml) . the difference was statistically significant (P 0.001).

Strengths of the study:-

The present study is case control study .There is no long period of follow up for the patients. Most previous studies did not compare the concentration of PGE_2 in GCF a corroding to severity of periodontal disease (Mild, Moderate, Severe), or the distribution of the disease a corroding to site (Localized, Generalized).

Weakness of the study:-

All the participants in the study were collected from one hospital, which could lead to selection bias.

V. Conclusion

The highest Mean PGE_2 concentration in GCF were obtained in chronic periodontitis patients (study group), while the lowest concentrations were obtained in control subjects (health group) with P value o.oo1 which is statistically significant. The relationship between the mean PGE_2 concentrations in GCF and the clinical parameters (PI, GI, PPD, R, CAL) in patients with chronic periodontitis (Table 2) with p value 0.009 was statistically significant. There also a relationship between PGE_2 concentration in GCF and chronic periodontitis (Table 4). The results of the present study indicate that PGE_2 in GCF shows dynamic changes according to the severity of periodontal disease and concentration of PGE_2 and there is strong correlation with inflammation and clinical parameters.

VI. Recommendations

From the results of the present study the following may be recommended:

1- Further prospective investigations are necessary to study the relationship between the PGE_2 in GCF and other inflammatory mediators in patients with chronic periodontitis.

2- More research should be conducted in the mechanism by which PGE_2 causes destruction of periodontal tissue in chronic periodontitis.

3- The use of chair-side tests could help the dentist in determining the level of disease progression and guide the dentist in patient's treatment. It can be recommended as a routine investigation for patients with history to develop severe periodontal disease.

4- Studies are needed to find a substitute for NSAIDs in host modulation therapy, although it has a beneficial effect in reduction of PGE_2.

References

[1]. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical
[2]. periodontology. 10th ed. Philadelphia: Saunders publishers; 2007 .pp.222-3.
[3]. Pihlstrom B.L., Michalowicz B.S., Johnson N.W. periodontal diseases. Lancet. 2005; 366: 1809—1820.
[4]. Prichard, J.F, Kramer, Stahl, S.S., et al.; Glossary of terms. J periodontal (suppl.) 48: 1, 1977.
[5]. Loeshe WJ, Grossman NS, periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. Clin. Microbial. Rev. 2001; 14(14):7:27-55.
[6]. Armitage GC. Development of a classification system for periodontal Diseases and Conditions. Ann periodontal 1999:4:1-6.
[7]. Wolf L, Dahlen G, Aeppli D. Bacteria as risk markers for periodontitis. Journal of periodontology. 1994:65:498-510.
[8]. Grossi SG, Zambon JJ, & HO, AW. Assessment of risk for periodontal disease. Risk Indicators for attachment loss journal of periodontology .1994:65:260-267.
[9]. Tomar SL,& Asma S. Smoking attributable periodontitis in the United States: finding from NHANES 111. National health nutrition Examination survey. Journal of periodontology .2000:71:743-751.
[10]. Mokdad AH, Bowman BA, Ford ES, et al. the continuing epidemics of obesity and diabetes in united state. JAMA.2001:286:1195-1200.
[11]. Albrecht M, Banoczy J, Tamas GJ. Dental and oral symptoms of diabetes mellitus. Community Dental Oral Epidemiol. 1988:16:378-380.
[12]. Seppala B, Seppala M, Aminamo J, Longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. J Clin periodontal .1993:20:161-165.
[13]. Croucher R, Marcenes WS, Torres MC, Hughes F, Sheiham A. the relationship between life-events and periodontitis. A case control study. Journal of clinical periodontology .1997:24:39-43.
[14]. Carranza's, Clinical Periodontology, 11 Edition, Chapter 21, Page 201.
[15]. Lindhe J, Clinical periodontology and implant dentistry, fifth edition, chapter 11, page 296.
[16]. Bingham CO 3rd, Austen KF: phospholipase A2 enzyme eicosanoid generation proc Assoc Am physicians. 1999:111:516-524.
[17]. Needleman P: characterization of the reaction sequence involved in phospholipid labing and deacylation and prostaglandin synthesis and actions. J Allergy Clin immunol. 1978:62:96-102.
[18]. Smith WL, Song I: The enzamology of prostaglandin endoperoxide H synthases-1 and-2. Prostaglandin other lipid mediat .2002:68-69:115-128.
[19]. Jakobsson PJ, Thoren S, Morgenestern R, Samuelsson B: Identification of human prostaglandin E synthase: a microsomal, glutathione- dependent, inducible enzyme, constituting a potential novel drug target. Proc Natal Acad Sci: 1999:69:7220-7225.
[20]. Watanable K, Kurihara K, Tokunaga Y, Hayaishi O: Two types of microsomal prostaglandin E synthase: glutathione – dependent and independent prostaglandin E synthase. Biochem Biophys Res common .1997:235:148-152.
[21]. Funk CD. Prostaglandins and leukotrienes: Advances in eicosanoid biology science: 2001:294:1871-1875.
[22]. Cao C, matsumura K, Yamagata K, Watanable Y. cyclooxygenase-2 is induced in brain blood vessels during fever evoked by peripheral or central ministration of tumor necrosis factor. Mol Brain Res. 1998:56:45-56.
[23]. Legler DF, Bruckne M, Uetz-Von Allmen E, Krause P. prostaglandin E2 at new glance : Therapeutic chances . Int J Biochem cell Biol. 2010:42:198-201.
[24]. Miyauchi M, Ijuhin N, Nikai H, Takata T, Ito H, Ogawa I. effect of exogenously applied prostaglandin E2 on alveolar bone loss – histometric analysis . J periodontal: 1992:63:405-411.
[25]. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin , prostaglandin E2 and alkaline phosphate in gingival crevicular fluid : their relations to periodontal status . J Clin periodontal.1994:21:327-333.
[26]. Kumar A, Reddy N, Kumar P, Reddy, Chavan C. Estimation of PGE2 level in GCF in periodontal health, disease , and after treatment .2013:4(3):303-306.
[27]. Preshow PM, Heasman PA. Prostaglandin E2 concentrations in gingival crevicular fluid: observations in untreated chronic periodontitis .2002:29(1):15-20.
[28]. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE2 secretion as determinant of periodontal disease expression. J periodontal.1993:64:432-444.
[29]. Zhouj, Zou S, Zhao Y. Prostaglandin E2 levels in gingival crevicular fluid and its relation to the depth of the pocket in patients with periodontitis . 1994; 16(2):4-130.
[30]. Albander M, Thomas ER. Risk factors for periodontitis in children and young person .J periodontal 2000-2001:29:207-222.
[31]. Arora M, Weuve J, Schwartz J, et al. Association of environmental cadmium exposure with periodontal disease in U.S. adults. Environ Health Perspect. 2009 may; 117(5):44-739.

Appendix (1)

GCF prostaglandin E_2 level in patients with chronic periodontitis
Date Sheet :
Date…………………………… Case……………………
Name……………………….. Age……………………
Gender……………………….. Occupation…………………
Address…………………………. Telephone…………………
Marital Status…………………. …
Oral Hygiene :
Brushing……………………… Dental Floss………………

PI

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

GI

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

PPD

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

R

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

CAL

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

DIAGNOSIS……………………………………..
Sample collection:-
Site 1:
Presence of Blood: Yes / No
PPD:
PI:
GI:
R:
CAL:

Site 2:
Presence of Blood: Yes / NO
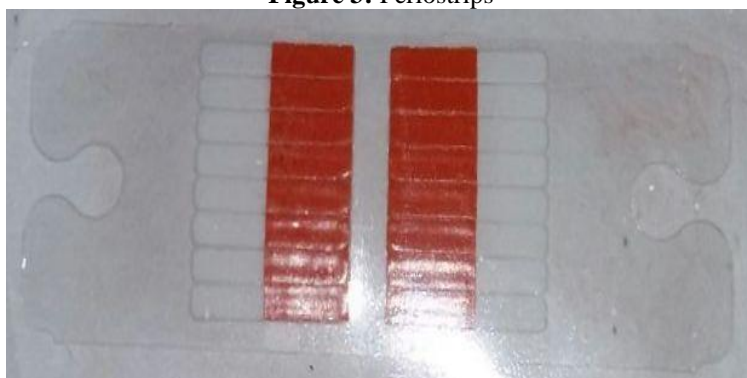PPD:
PI:
GI:
R:
CAL:

Figure 1: Collection of GCF from health subject
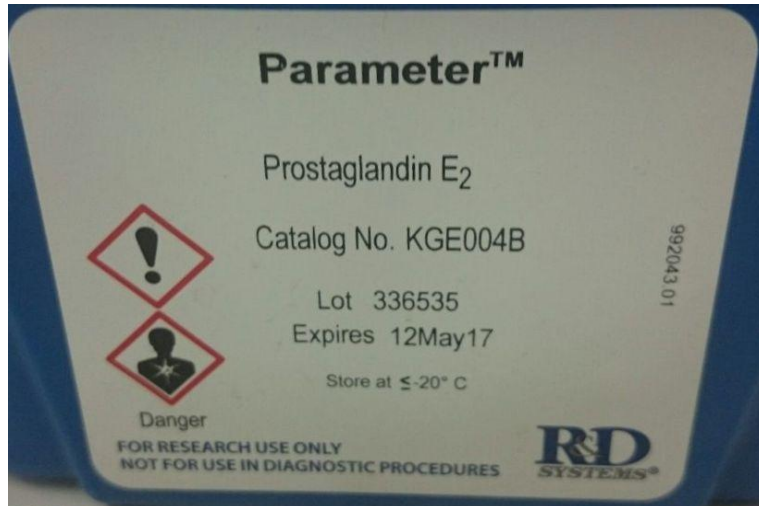

Figure 2: Collection of GCF in chronic periodontitis patient

Figure 3: Periostrips

Figure 4 & 5: ELISA KIT