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Lipoxin A4 As Indicator for Anti-Inflammatory Role of Platelets Rich Plasma (PRP) in Treatment of Chronic Periodontitis

Maysaa Mahdi Nasser, B.D.S.[(1)]Basima Gh. Ali, B.D.S., M.Sc.[(2)]
[1]*Master Student, Department Of Periodontics, College Of Dentistry, University Of Baghdad.*
[2]*Professor, Department Of Periodontics, College Of Dentistry, University Of Baghdad.*

Abstract
Background: *chronic periodontitis is pathological multi causes inflammatory disorder and its represent the major cause for teeth losing in adults' patients' .Its affects the surrounding tissues of the teeth leading to pocket formation and alveolar bone loss. Platelets rich plasma (PRP) represents a new modality for treatment of periodontal diseases for its growth factors and anti-inflammatory effects. Lipoxin A4 play a role in inflammation resolution by encourages the apoptosis of neutrophils and act as a measurement for inflammatory response to the treatment. The purpose of this study is to evaluate the anti-inflammatory effect of PRP for treatment of chronic periodontitis.*
Materials and Methods: *two groups involved in this study; study group (20 systemically healthy patients with chronic periodontitis) their pockets sites treated with scaling and root planning (SRP) with PRP application. Control group (20 systemically healthy patients with chronic periodontitis) their pockets sites treated with scaling and root planing only. All patients with age ranged from (25-50) years. Patients followed up for one month divided on three visits. Clinical periodontal parameters (Plaque index (PI), Gingival index (GI), Bleeding on probing (BOP), Probing pocket depth(PPD), and Relative attachment level (RAL)) estimation and Blood samples collection were done in the first and third visits for both groups to estimate the platelets count, neutrophils count and Lipoxin A4 concentration.*
Results: *the clinical periodontal parameters for both groups showed reduction in median value between the first and third visits although it's not significant but the results showed more reduction in study group. The neutrophils and platelets count showed reduction in study group between the first and third visits whilein control group the neutrophils count showed reduction in median value but the platelets count showed increased in median value at third visit .there 'was significant difference in median value of Lipoxin A4 concentration level has been reveled in study group than in control group.*
Conclusion: *from the results of this study, it may be concluded that platelets rich plasma have a promising effects as adjunct treatment for SRP in term of reduction the clinical periodontal parameters, decrease the inflammatory response and exert anti-inflammatory effects.*
Keyword: *chronic periodontitistreatment, PRP,lipoxin A4.*

I. Introducton

Chronic periodontitis represent one of most dominant diseases in the elderly which may lead to teeth mobility and finally loss[(1)]. Periodontal diseases considered as multi bacterial disease that result in inflammatory reaction promotion in periodontal tissues [(2)].This inflammatory reaction end with damage of the periodontal attachment apparatus [(3).] Host response stimulated due to signal sent by the bacterial constituents which rested in biofilm on teeth surface [(4).]This lead to tissue breakdown after lysosome enzymes released by polymorph nuclear cells which reached to the inflammation site[(5).]The non-surgical treatment of periodontal diseases involved many modalities as scaling, root planing[(6)].New techniques involved laser,endoscopic therapy and the new method isplateletsrichplasma(PRP) injection. PRP which is a concentrated platelets on level ofplasma that produce from autologous blood; PRP work to improveperiodontal ligament(PDL) cell production, play a role in the (PDL) protein development and also involved in improvement of the extracellular matrix production, and this result in progresses postoperative periodontal wound healing [(7)].The activity of PRP is due to release many growth factors as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF)[(8)].Due to PRP anti-inflammatory effect as lipoxin A4 produced by platelets and neutrophils cell interaction and this lead to inflammation determination [(9)].Lipoxin A4 (LX4) defined as endogenous lipid particles resulting from cellular arachidonic acid [(10).]LX4 play arole in delaying the new neutrophils access to inflammation area and increase the apoptosis of neutrophil. LXs work as a factors to chemotactic non-phlogistic phenotype monocyte/macrophages for advance healing process [(11)]. Limited studies have made evident for the role of PRP in inflammation resolution due to its growth factors and anti-inflammatory effects [(12, 13)].the aims of this study weremeasurethe clinical periodontalparameters (PLI, GI, BOP, PPD, and RAL)and immunological

parameters (neutrophils counts, platelets counts and lipoxin A4 level). For both study group (treated with PRP as adjunct to scaling and root planing) and control group (treated with scaling and root planing alone) andstudy the anti-inflammatory effect of PRP as adjunct to scaling and root planning in treatment of chronic periodontitis compared to scaling and root planing alone

II. Materialsand Methods

1. Sample selection and study design

The participants in this study were patients who attending the Department of Periodontics in the Collage of Dentistry,University of Baghdad. The study population includes 40 patients male and females; all with chronic periodontitis and have periodontal pockets sites more than 4 mm and have no history of known systemic diseases and divided into two groups: control group (20 patients) and study group (20 patients). All patients age ranged from (25-50) years old**.** The exclusion criteriainclude**:** Alcohol drinker,Smoker,Pregnant and lactating womenand women that take contraceptive drug,Anti-inflammatory treatment and antibiotic treatment within the last three months, Patient made any periodontal treatment procedure with in last three months including local antimicrobial, Manifestation of local pathological condition in the area of working site.The control group periodontal pockets sites treated with scaling and root planing only while the study group periodontal pockets sites treated with scaling and root planing and PRP application

2.PRP Preparation : PRP that made by two centrifuge steps; the first cycle at 3000 rpm for 5 minutes and second cycle at 3500 rpm for 15 minutes . PRPapplication done locally in periodontal pockets and pressure Applied with wet gauze on working sites for 5 minutes.

3. Recording clinical periodontal parameters

All patientsfollowed up for one monthand recalled at three visits. **First visit** for control group; root planing treatment done to patients after clinical periodontal parameters (plaque index (PLI), gingival index (GI), bleeding on probing (BOP),probing pocket depth (PPD), and relative attachment level (RAL)) have been taken. **Secondvisit** after two weeks to estimate clinical periodontal parameters (PLI, GI) and keep in contact with patients.
Third visit ; clinical periodontal parameters (PLI, GI, BOP, PPD, and RAL) were estimated.

4. Immunological parameters record done in first and third visit

First visit: for **control** group 5ml blood samples drown to estimate the platelets count, neutrophils count and Lipoxin A4 concentration level. For **study** group the blood drowns about 10 ml. 6 ml for PRP preparation and 4 ml to estimate the platelets count, neutrophils count and Lipoxin A4 concentration level. **Third visit** 5ml of blood drowns for both groups to estimate the platelets count, neutrophils count and Lipoxin A4 concentration level. Blood samples for Lipoxin A4 concentration level reading were stored in deep freeze on -65 to -70 c until analyzed by ELISA human Lipoxin A4kit.

III. Results

1. Intra and Inter group comparison for clinical periodontal parameters:

The clinical periodontal parameters (PLI, GI, BOP, PPD, and RAL) showed reduction in the median value for both controland study groups as listed in table 1 and 2 ; but study group showed more reduction in median value of (PPD, and RAL)and significant differences in (GI) at third visits as listed in table 3.

2. Intra and Inter group comparison for immunological parameter neutrophils counts

Showed reduction in median value of Third visit when compared with first visit in both groups as listed in Table 1 and 2 but the platelets count in Control group showed increase while in study
group the platelets count showed reduction in third visit when compared with first visit. Study group whotreated with PRP revealed significant differences in Lipoxin A4 concentrationmedian value between first and third visitsthan control group as listed in table 3

Table 1: statistical difference of the median values of clinical periodontal parameters (PLI, GI, BOP, PPD and RAL) and immunological parameters (platelets count, neutrophils count and Lipoxin A4) for control group/ visits

Variables	**Visits**	**Median**	Friedman test	PLI
PLI	1st	0.87		Chi-Square8.04
	2nd	0.81		d.f. 2
	3rd	0.56		p-value 0.018
GI	1st	1.8	Friedman	GI

	2nd	1.6	test	Chi-Square 19
	3rd	1.5		d.f. 2
				p-value0.000
BOP	1st	87.5	Wilcoxon	3.352
	3rd	56.25	p-value	0.001
PPD	1st	4.78	Wilcoxon	-3.974
	3rd	4	p-value	0.000
RAL	1st	7.29	Wilcoxon	-3.830
	3rd	6.84	p-value	0.000
Neutrophil count(cell10e3/ul)	1st	5.41	Wilcoxon	-0.599
	3rd	4.95	p-value	0.549
Platelets count **(cell 10e3/ul)**	1st	277	Wilcoxon	-0.897
	3rd	281.5	p-value	0.370
Lipoxin (Pg/ml)	1st	0.26	Wilcoxon	-0.644
	3rd	0.26	p-value	0.519

Table 2: statistical analysis of the median values of clinical periodontal parameters (PLI, GI, BOP, PPD and RAL) and immunological parameters (platelets count, neutrophils count and Lipoxin A4) for study group/ visits

Variables	**Visits**	**Median**	Friedman test	PLI
PLI	1st	1		Chi Square 4.588
	2nd	0.78		d.f. 2
	3rd	0.78		p-value 0.101
GI	1st	1.8	Friedman test	GI
	2nd	1.5		Chi-Square21.233
	3rd	1.39		d.f. 2
				p-value 0.000
BOP	1st	83.3	Wilcoxon	-3.529
	3rd	37.5	p-value	0.000
PPD	1st	5	Wilcoxon	-3.734
	3rd	3.6	p-value	0.000
RAL	1st	8.19	Wilcoxon	-3.728
	3rd	7.3	p-value	0.000
Neutrophil count(cell10e3/ul)	1st	4.75	Wilcoxon	-2.315
	3rd	4.16	p-value	0.021
Platelets count**(cell 10e3/ul)**	1st	243.5	Wilcoxon	-2.091
	3rd	235	p-value	0.037
Lipoxin (Pg/ml)	1st	0.19	Wilcoxon	-0.322
	3rd	0.15	p-value	0.748

Table 3: Inter groups' statistical difference between two groups (control and study groups according to periodontal parameters (PLI, GI, BOP, PPD and RAL) and immunological parameters (platelets count, neutrophils count and Lipoxin A4) in each visits

Variables	Visits	Groups	Groups Difference		
			Median	MW	P-value
PLI	1st	control	0.87	177	0.499 (NS)
		study	1		
	2nd	control	0.81	182	0.621 (NS)
		study	0.78		
	3rd	control	0.56	150	0.168 (NS)
		study	0.78		
GI	1st	control	1.8	186.5	0.706 (NS)
		study	1.8		
	2nd	control	1.6	144	0.126 (NS)
		study	1.5		
	3rd	control	1.5	112	0.016 (S)
		study	1.39		
BOP	1st	control	87.5	181.5	0.605 (NS)
		study	83.3		
	3rd	control	56.25	107	0.011 (HS)
		study	37.5		
PPD	1st	control	4.78	122	0.030 (S)
		study	5		
	3rd	control	4	182.5	0.632 (NS)
		study	3.6		
RAL	1st	control	7.29	163.5	0.323

		study	8.19		(NS)
	3rd	control	6.84	176.5	0.525
		study	7.3		(NS)
Neutrophils	1st	control	5.41	194	0.871
		study	4.75		(NS)
Neutrophil count	3rd	control	4.95	143	0.123
		study	4.16		(NS)
Platelets count	1st	control	277	178	0.551
		study	243.5		(NS)
	3rd	control	281.5	141	0.110
		study	235		(NS)
Lipoxin	1st	control	0.26	143	0.122
		study	0.19		(NS)
	3rd	control	0.26	127.5	0.049
		study	0.15		(S)

IV. Discusson

Clinical periodontal parameters (PLI, GI, BOP, PPD, and RAL) showed reduction in third visit of study group and this could be explained that PRP has many growth factors that enhanced the treatment results and this in agreement with AshishAgarwal and NarinderDev Gupta 2014[12].The control group also revealed reduction in (PLI, GI, BOP, PPD, and RAL) due to effect of root planingwhich lead to remove rough dentin and cementum surface with calculus or contaminated with microorganisms or microorganisms toxin and this resulted in eradication of disease and the supporting structure of the teeth return to a healthystate; this in agreement withHammerleCHF 1991,Morrison EC1980,Taif M., MahaSh 2015[14-16]. There were significant differences in Lipoxin A4 concentration found between study and control group in third visit and this could be clarified thatPRP has anti-inflammatory effects which made inflammation determined in faster way and this in agreement withHesham El-Sharkawy 2007[13].Neutrophils count showed reduction in control group throughout visits and this in agreement withB Rai and S Kharb 2007[17]which showed that after treatmenttotal leukocytes counts significantly decrease. But platelets count showed increase in median value at third visit due to treatment response in control group is slow or the time that required to full inflammation resolution need to extend more than one month.This in disagreement with B Rai and S Kharb 2007[17].**Lipoxin A4** concentration in control group show no differences when compared the third visit with first visit and this in disagreement with BurakDoğan2015[18]. This may be due to small sample size and the resolution of inflammation is slow pattern. The study group also revealed reduction in (PLI, GI, BOP, PPD, and RAL)due to PRP growth factors effects and this in agreement with Piemontese M2008,Okuda K 2005[19, 20].

Study group showed **reduction** in **neutrophilscells count** when compare their number in the first visit with that in the third visit as LXs have ability to delay the entry ofnew neutrophils to inflammation sites and this in agreement withHesham El-Sharkawy 2007[13].**Platelets cells count** showed **reduction** in cells count at the third visit which agreewith B Rai and S Kharb 2007[17].**Lipoxin A4** concentration in study group showed reduction in median value between first and third visit and this in agreement withBurakDoğan2015[18].LXA4 is a marker for inflammation and that the persistent or resolved of inflammation will affect the level of LXA4.

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