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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL EFFECT OF AUTOLOGOUS PLATELET CONCENTRATES AGAINST PERIODONTAL DISEASE ASSOCIATED BACTERIA - AN INVITRO STUDY.

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Key words:-

Antimicrobial effect, blood platelets, platelet-rich fibrin, platelet-rich plasma.

Abstract

Aim: The aim of this study is to evaluate the antimicrobial effects of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) against periodontal disease associated bacteria at two different time intervals.

Subjects and Methods: Blood samples were obtained from ten patients. Procurement of PRP and PRF were done using centrifugation. The antibacterial activity of PRP and PRF was evaluated by microbial culturing using bacterial strains of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* at 2nd and 7th days.

Results: At 2nd and 7th day, both *P. gingivalis* and *A. actinomycetemcomitans* were inhibited by PRP but no zone of inhibition was seen by PRF.

Conclusions: PRP can be used as a potential autologous concentrate against periodontal pathogens and also it may serve as an adjunct in the enhancement of periodontal regeneration.

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Introduction:-

Periodontal disease is defined as a complex, multifactorial disease initiated by oral biofilm formation and if left untreated progress to gingivitis further leading to periodontal disease characterized by the loss of connective tissue attachment with destruction of periodontal tissues. The inter relation between periodontal disease and systemic diseases has been scientifically proven and the principle reason is due to dissemination of locally produced pro-inflammatory mediators such as C-reactive protein, interleukins-1 beta (IL-1 β) and IL-6, and tumor necrosis factor alpha.¹ Conventional periodontal therapy initially decreases the bacterial load at the diseased sites but it may show increased counts of periodontal pathogens weeks after treatment so prevention of such contaminations plays an important role in wound healing and regeneration procedures.

Autologous platelet concentrates (PCs) has gained great popularity in a variety of medical fields such as orthopedics, dermatology, ophthalmology, cosmetic and plastic surgery and dentistry since 2 to 3 decades. Platelet derived concentrates such as Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) have gained a lot of popularity as hard and soft tissues regenerative material and regeneration potential is thought to stem from the fact that platelets store growth factors such as Platelet Derived Growth Factors (PDGF), Transforming Growth Factor (TGF- β), Endothelial Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), Insulin like Growth Factor (IGF-1), Fibroblast Growth factor (FGF). Additionally, platelet-derived concentrates also impart anti-inflammatory properties as evident by reduction in postoperative pain and swelling.²

Along with these properties there are few studies on clinical and in vitro antibacterial effect of human PCs has against oral bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Klebsiella pneumoniae* while no activity has been found against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Bacillus cereus* and *Bacillus subtilis*.^{3,4} The mechanism of the antibacterial effect of PCs is not yet fully understood. Existing evidence suggests that platelets may play multiple roles in antimicrobial host defense.

Several centrifugation techniques are available for the production of PCs, leading to products with different biological characteristics. The various PCs can be classified into four main categories, depending on their leucocyte and fibrin content: pure

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platelet-rich plasma (P-PRP), pure platelet-rich fibrin (P-PRF), leukocyte- and platelet-rich plasma (L-PRP) and leukocyte- and platelet-rich fibrin (L-PRF).⁵

Although Platelet concentrates are used in variety of periodontal surgical procedures for its superior regenerative potential, there is no much evidence regarding their antibacterial potential. Hence, the aim of this study is to evaluate antibacterial effects of PRF and PRP against periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans* at 2nd and 7th days.

Methodology:-

In this study Blood samples were obtained from ten patients, age ranging from 25 to 40 years. Ethical clearance from the institutional review board was obtained prior to start of the study. All the patients provided written informed consent before beginning the study. Subjects who were systemically healthy, nonsmokers, with no symptoms of infection and took no antibiotics for at least 6 months before experiments the study were included in this study.

A volume of 10 ml of blood was collected from each patient and 5 ml was used for PRP and 5 ml for PRF procurement. The ten samples were randomly divided into two groups: 5 samples in *P. gingivalis* (ATCC no – 33277) group and 5 samples in *A. actinomycetemcomitans* group (ATCC no – 29523). Antimicrobial culturing was done on agar plates.

PRP Preparation:-

A volume of 5 ml of intravenous blood was collected into the blood collection tube coated with 3.2% sodium citrate solution used as an anticoagulant. The collected blood was firstly centrifuged in ROTEK Centrifuge machine at 1200 rpm for 20 minutes, at room temperature. Then, a red lower fraction (red cell component) and an upper straw-yellow turbid fraction (serum component) were observed. A point was marked at 1.4 mm below the line dividing the two fractions. All the content above this point was pipetted and transferred to other 5 ml vacuum tube, in which a line corresponding to 0.35 ml was drawn from the tube's bottom. The sample was then submitted to a new centrifugation at 3000, for 12 minutes, resulting in two components: one above the line drawn on the tube (platelet-poor plasma – PPP) and other below the line (platelet-rich plasma-PRP).

PRF Preparation:-

A volume of 5 ml of intravenous blood was collected in the plain bulb and centrifuged using centrifuge ROTEK Centrifuge machine at 3000 rpm for 10 min. After centrifugation, the PRF clot was removed from the tube using sterile tweezers, separated from the RBC base using scissors.

Antimicrobial Activity:-

Antimicrobial activity was done by Well Plate Method. Inoculum preparation was done using a loop or swab, transfer the colonies to the plates. Visually turbidity was adjusted with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum. Entire surface of agar plate was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. Hitting sides of petriplate and creating aerosols were avoided. Then the inoculated plate was allowed to stand for at least 3 minutes but no longer than 15 min before making wells.

Addition of PRF AND PRP into plate:-

Hollow tube of 5mm diameter was taken and heated followed by pressing it on above inoculated agar plate and removed it immediately by making a well in the plate. Likewise, two wells were made on each plate and L-PRF and PRP were added into the respective wells on each plate.

Incubation:-

For facultative anaerobes, *A. actinomycetemcomitans* (ATCC no – 29523) plates incubate in the CO₂ Jar and keep the jar in the incubator at 37 °C. For Anaerobic organisms, *P. gingivalis* (ATCC no – 33277) plates incubate in the anaerobic jar and keep the jar in the incubator at 37 °C.

Results:-

Analysis was performed using Independent samples t-test to compare PRP and PRF for *P. gingivalis* and *A. actinomycetemcomitans*. PRP has shown zone of inhibition on *P. gingivalis* in the mean range of 10.6±0.54 mm at 2nd day and 13.8±1.09 mm at 7th day and on *A. actinomycetemcomitans* in the mean range of 10.8±0.83mm at 2nd day and 14.6±1.51mm at 7th day. This shows that PRP dramatically inhibited the growth of both *P. gingivalis* and *A. actinomycetemcomitans*, which was statistically highly significant ($P < 0.0001$ HS) as compared to PRF where no activity was seen against these pathogens on both the bacteria at both the time intervals as shown in tables 1 and 2 and figures 1-8.

Discussion:-

The regenerative potential of autologous PCs has been explored considerably during the last two to three decades. The regenerative potential of platelet concentrates has been extensively reported but in the available literature only few studies are found about their antimicrobial properties and effects of those properties on pathogens.

The components which are responsible for the antimicrobial activity of PCs remain poorly understood till date because these materials are a complex mixture of platelets, white blood cells, and plasma. Platelets are anucleate cytoplasmic fragments derived from bone marrow megakaryocytes and measure 2–3 μm in diameter. They contain many granules, few mitochondria and 2 prominent membrane structures, the surface connected canalicular system and the dense tubular system. The alpha granules are spherical or oval structures with diameters ranging from 200 to 500 nm each enclosed by a unit membrane. They form an intracellular storage pool of proteins vital to wound healing, including platelet-derived growth factor (PDGF), transforming growth factor (TGF β), and insulin-like growth factor (IGF-I).⁶

The impact of the plasma and cellular components has not been studied in detail yet. The first studies hypothesizing the antimicrobial activity of platelets date back several decades.⁷ Platelets act as sentinels of the vascular system, express a wide range of potential bacterial receptors, may have the ability to internalize bacteria and are able to release a broad variety of molecules that provide an array of host defense functions.⁸ Current existing evidence suggests that platelets along with the regenerative potential of periodontal structures also may play multiple roles in antimicrobial host defense as they generate oxygen metabolites, including superoxide, hydrogen peroxide, and hydroxyl free radicals.^{9,10} Moreover, they are capable of binding, aggregating, and internalizing microorganisms, which enhances the clearance of pathogens from the bloodstream; they participate in antibody-dependent cell cytotoxicity functions to kill protozoal pathogens, and finally, platelets release an array of potent antimicrobial peptides.¹²

Several antimicrobial factors have been proposed, including platelet antimicrobial proteins and peptides of the innate immune defense, or platelet α -granules components, such as complement and complement-binding proteins.^{13, 14} Direct interaction of platelets with microorganisms and participation in antibody-dependent cell cytotoxicity and white blood cells in direct bacterial killing, release of myeloperoxidase, activation of the antioxidant responsive element and antigen-specific immune response have also been suggested.¹⁶

The antimicrobial activity of PCs could be due to direct interaction of platelets with microorganisms and participation in antibody-dependent cell cytotoxicity and white blood cells in direct bacterial killing. Release of myeloperoxidase, activation of the antioxidant responsive element, and antigen-specific immune response have also been suggested.¹⁰ Activated platelets could release various GFs that play an important role in improving the healing of ulcers and secreting platelet microbicidal proteins (PMPs).¹⁰ PMPs contain an array of materials which have antibacterial activity, including platelet factor 4, regulated upon activation of normal T-cell expressed and secreted protein, connective tissue-activating peptide 3, platelet basic protein, thymosin beta-4, fibrinopeptide A, and fibrinopeptide B. PMPs could play a role through the following mechanisms: Contacting the bacterial membrane, changing the membrane permeability, entering the cell, and inhibiting the synthesis of big molecules.¹²

The results of the present study clearly demonstrated that PRP has capability of inhibiting *P. gingivalis* and *A. actinomycetemcomitans* at 2nd day and 7th day of incubation where as PRF was unable to inhibit these bacteria.

PRF is a matrix of autologous fibrin, in which a large quantity of platelet and leukocyte cytokines are embedded intrinsically during centrifugation and it may be one of the reason for slow and progressive release over time of 7–11 days as the network of fibrin disintegrates.^{17, 18} But in this study at both 2nd and 7th day upon both the bacteria there was no zone of inhibition seen with PRF.

PRP contains a large number of platelets as well as a high concentration of leukocytes, which has been reported to be two to four times greater in PRP than in the whole blood. Among these leukocytes, neutrophils are known for their host defense actions against bacteria and fungi through the actions of myeloperoxidase, which presents in neutrophilic granulocytes; lymphocytes produce immunocompetent cells and one of their representative functions is found in immunologic defense; monocytes (precursors of macrophages) produce cytokines and chemotactic factors that participate in inflammation. Therefore, the concentrated leukocytes in PRP may enable PRP to play an important role in the immune defense against bacterial infection.⁴



Fig 1:- Zone of inhibition for platelet-rich plasma against *A.actinomycetemcomitans* at 2nd day.



Fig 2:- Zone of inhibition for platelet-rich plasma against *A.actinomycetemcomitans* at 7th day:



Fig 3:- Zone of inhibition for platelet-rich fibrin groups against *A.actinomyetemcomitans* at 2nd day.

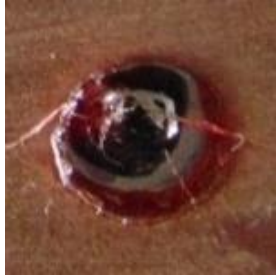


Fig 4:- Zone of inhibition for platelet-rich fibrin against *A.actinomyetemcomitans* at 7th day.



Fig 5:- Zone of inhibition for platelet-rich plasma in against *P. gingivalis* at 2nd day.



Fig 6:- Zone of inhibition for platelet-rich plasma in against *P. gingivalis* at 7th day.



Fig 7:- Zone of inhibition for platelet-rich fibrin in against *P. gingivalis* at 2nd day.



Fig 8:- Zone of inhibition for platelet-rich fibrin in against *P. gingivalis* at 7th day.

Table 1:- zone of inhibition for platelet-rich plasma and platelet-rich fibrin groups in millimeters (mm) against *P. Gingivalis* at 2nd and 7th day.

<i>P. gingivalis</i>	2 nd day		7 th day	
	PRP	PRF	PRP	PRF
Mean ± sd	10.6±0.54 mm	0	13.8±1.09 mm	0
p value	<0.0001 HS		<0.0001 HS*	

*Highly significant.

Table 2:- zone of inhibition for platelet-rich plasma and platelet-rich fibrin groups in millimeters (mm) against *a.actinomyetemcomitans* at 2nd and 7th day.

<i>A.actinomyetemcomitans</i>	2 nd DAY		7 th DAY	
	PRP	PRF	PRP	PRF
Mean ± sd	10.8±0.83mm	0	14.6±1.51mm	0
p value	<0.0001 HS		<0.0001 HS	

*Highly significant.

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