QUANTITATIVE ANALYSIS AND COMPARISON OF GROWTH FACTORS (PDGF-AB AND TGF-β1) IN ADVANCED AND INJECTABLE – PLATELET RICH FIBRIN: AN IN-VITRO STUDY

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By

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LIST OFABBREVIATIONS

aFGF	Acidic Fibrobast Growth Factor
A-PRF	Advanced-Platelet Rich Fibrin
ABC	Avidin-Biotin-Peroxidase Complex
ADM	Acellular Dermal Matrix
AGA	Adrogenetic Alopecia
ALP	Alkaline Phosphatase
AM	Amniotic Membrane
bFGF	Basic Fibroblast Growth Factor
BMPs	Bone Morphogenic Proteins
CAF	Coronally Advanced Flap
CCL5	C-C Motif Chemokine Ligand 5
cPRP	Concentrated Platelet Rich Plasma
CTG	Connective Tissue Graft
DMEM	Dulbeco'sMoodified Eagle Medium
EGF	Epidermal Growth Factor

ELISA	Enzyme Linked Immuno Sorbent Assay
EMD	Enamel Matrix Derivative
FDBA	Freeze Dried Bone Allograft
GF	Growth Factor
GR	Gingival Recession
GTR	Guided Tissue Regeneration
HDI	Hair Density Index
IIEF5	International Index of Erectile Function-5
IGF	Insulin like growth factor
IL	Inter Leukin
Il-PRF	Injectable Platelet Leuckocyte-Fibrin Rich Plasma
i-PRF	Injectable-Platelet Rich Fibrin
L-PRF	Leukocyte-Platelet Rich Fibrin
LSCC	Low Speed Centrifugation Concept
mRNA	Messenger RNA
MSCs	Messenchymal Stem Cells

NLFs	Nasolabial Folds
O.D	Optical Density
OECs	Outgrowth Endothelial Cells
PDGF	Platelet Derived Growth Factor
PDGF-AB	Platelet Derived Growth Factor-AB
PDL	Periodontal Ligament
PRF	Platelet Rich Fibrin
PRFr	Platelet Rich Fibrin Releasate
PRFM	Platelet Rich Fibrin Matrix
PRGF	Platelet Rich in Growth Factors
PRP	Platelet Rich Plasma
RCF	Relative Centrifugal Force
rhPDGF	Recombinant Human Platelet Derived Growth Factor
RCF	Relative Centrifugal Force
rpm	Revolutions Per Minute
Runx-2	Runt-related transcription factor 2

TGF-β	Transforming Growth Factor-β
TGF-β1	Transforming Growth Factor-β1
VEGF	Vascular Endothelial Growth Factor
WAS	Wrinkle Assessment Scale
XCM	Xenogenic Collagen Matrix

ABSTRACT

ABSTRACT

The use of platelet concentrates has gained increasing awareness in recent years for regenerative procedures in modern dentistry. The aim of the present study was to compare growth factor release over time from Advanced Platelet-Rich Fibrin (A-PRF) and Injectable Platelet-Rich Fibrin (i-PRF). Thirty blood samples were collected from fifteen donors (2 samples each for A-PRF and i-PRF). Following preparation, samples were incubated in a plate shaker and assessed for growth factor release at 1 day, 3 days, and 10 days. Thereafter, growth factor release of Platelet Derived Growth Factor-AB (PDGF-AB) and Transforming Growth Factor-β1 (TGF β1) was quantified using Enzyme Linked Immunosorbent Assay (ELISA). The highest reported growth factor released from platelet concentrates was PDGF-AB followed by TGF β1.

It was found that i-PRF released the highest total growth factors. Furthermore, i-PRF released significantly higher total protein accumulated over a 10-day period when compared to A-PRF.

The results from the present study indicate that the various platelet concentrates have quite different release kinetics. It was observed that the new formulation of PRF (i-PRF) released significantly higher total quantities of growth factors when compared to A-PRF.

Keywords: PDGF-AB, TGF β1, A-PRF, i-PRF, Growth factors.

INTRODUCTION

INTRODUCTION

Regenerative potential of platelets was introduced in 1974, and Ross et al.[1] were amongst the pioneers who first discovered growth factor from platelets. Growth factors (GF) release upon activation of platelets trapped within fibrin matrix. GF have been shown to stimulate mitogenic response in periosteum for bone repair during normal wound healing[2]. Last two decades have witnessed better understanding of physiologic properties of platelets in wound healing that led to increased therapeutic applications in various forms with varying results. However, due to controversies regarding production protocols of autologous fibrin adhesives, risk of cross infection and legal restrictions on blood handling with concentrated platelet rich plasma (cPRP), a new family of platelet concentrate, an autologous cicatricle matrix, platelet rich fibrin (PRF) appeared in France[3].

Choukroun's PRF is a fibrin matrix in which platelet cytokines and cells are trapped which is released after a certain time, and PRF can serve as a resorbable membrane[4]. More recently, Gassling et al.[2] have shown that PRF is a suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering applications[5].

Autologous PRF, considered to be a healing biomaterial, was initially used in oral implantology by its promotors, and presently, studies have shown its application in various disciplines of dentistry[4].

PRF is also known as leukocyte- platelet rich fibrin (L-PRF), or Choukroun's PRF after its inventor, to avoid any confusion with other techniques using similar names such as Vivostat PRF (Vivolution, Alleroed, Denmark), a pure platelet rich plasma (PRP) or Fibrinet PRF (Cascade Medical, Wayne, NJ) matrix (without leukocyte). PRF belongs to

the second generation of platelet concentrates, collecting on a single fibrin membrane containing constituents of blood sample favorable for healing and immunity [6, 7].

Although PRF belongs to a new generation of platelet concentrate, it is in the first place a fibrin technology. Biologic activity of the fibrin molecule is enough in itself to account for significant cicatricial capacity. Perfect understanding of its components and their significance will comprehend the clinical results obtained and subsequently extend the fields of therapeutic applications of this etiquette [4].

Developed in France by Choukroun et al.[8] the PRF production protocol attempts to accumulate platelets and released cytokines in a fibrin clot. Granules present in platelets contain many proteins, which may be platelet specific (eg. betathromboglobulins) or non-platelet specific (fibronectin, thrombospondin, fibrinogen, and other coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins etc) calcium and serotonin etc. Also phospholipids double layer of platelet membrane constitute many receptors for other molecules. Growth factors released by alpha granules encompass a group of cytokine polypeptides with relatively low molecular weight ranging from 6 to 45 kDa[9]. Activation and degranulation is important to initiate and support aggregation at the healing site and the release of the cytokines [Inter Leukin-1 beta (IL), IL-6, Tumor Necrosis Factor-alpha (TNF)][10] and growth factors [Transforming Growth Factor-β1 (TGF-β1), Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF)] that stimulates cell migration and proliferation within the fibrin matrix and thus begins the first stage of healing.[11]

Over the past 5 years, further modifications to centrifugation speed and time have additionally improved PRF into a concept now known as the "low-speed centrifugation concept." Recent investigators have formulated new generation of platelet concentrations i.e. A-PRF and i-PRF. Despite these significant alterations, little is known about the release pattern of growth factors from various platelet concentrates over time. To best of our knowledge, no data is available till date on release profile of growth factors from A-PRF and i-PRF.

AIM AND OBJECTIVES

REVIEW OF LITERATURE

ADVANCED-PLATELET RICH FIBRIN

The new protocol Advanced Platelet Rich Fibrin (A-PRF),based on the low speed centrifugation concept, is powerful as it combines the fibrin network, the growth factors from the platelets, and the white cell activity for only one goal: getting rapid and thorough vascularization. A-PRF is a "blood concentrate" and not a "platelet concentrate".

Mendonça-Caridad J, Lopez PJ, Fayos FV, Miery G (2013)[12] evaluated the feasibility of a new approach for the treatment of frontal sinus disease and other related cranial osseous derangements, pathologies ranging from devastating infection to invasive tumours or trauma were treated with this regenerative procedure in a single-stage surgery, based autogenous platelet-rich/fibrin-rich composite matrix as an alternative to fat or other obliterating or grafting materials and found an uneventful recovery with bone formation and no complications or recurrences over the years.

Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, et al. (2014)[13] Compared standard platelet-rich fibrin and advanced platelet-rich fibrin to establish histological cell detection and histomorphometrical measurement of cell distribution and the effects of the centrifugal force (speed and time) on the distribution of cells relevant for wound healing and tissue regeneration and found decreasing the revolutions per minute (rpm) while increasing the centrifugation time in the A-PRF gave an enhanced presence of neutrophilic granulocytes in the distal part of the clot and concludes A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors.

Kawase T, Kamiya M, Kobayashi M, Tanaka T, Okuda K, Wolff LF, Yoshie H. (2014)[14] developed and optimized a heat compression technique for PRF and tested the feasibility of the resulting PRF membrane. The heat-compressed PRF appeared plasmin resistant and remained stable for longer than 10 days in vitro. Additionally, in animal implantation studies, the heat compressed PRF was observed at least for 3 weeks post

implantation in vivo whereas the control PRF was completely resorbed within 2 weeks and suggests that the heat-compression technique reduces the rate of bio-degradation of the PRF membrane without sacrificing its bio-compatibility and that the heat-compressed PRF membrane easily could be prepared at chair-side and applied as a barrier membrane in the guided tissue regeneration (GTR) treatment.

Jain S, Rai R, Mohan R, Mehrotra S (2014)[15] PRF and β-Tricalcium phosphate with coronally advanced flap (CAF) have been shown to be a promising and successful approach for the management of grade II furcation defect. Its gaining clinical attachment significantly manages both the gingival recession and furcation involvement simultaneously.

Femminella B, Iaconi MC, Di Tullio M, Romano L, Sinjari B, D'Arcangelo C, et al. (2015)[16] compared the effects of PRF and gelatin sponge on the healing of palatal donor sites and the patient's morbidity on Miller's class I or II gingival recession treated by a coronally advanced flap with connective tissue graft (CTG) resulting from the de-epithelialization of a free gingival graft and suggested that PRF-enriched palatal bandage significantly accelerates palatal wound healing and reduces the patient's morbidity.

Mishra D, Kalapurakkal VB, Misra SR (2015)[17] treated gingival recession treatment in maxillary anterior region with synthetic collagen membrane and platelet rich fibrin and suggested that platelet-rich fibrin can be used as a membrane for periodontal tissue regeneration as it has the ability to promote platelet aggregation, be chemotactic for fibroblast and enhance wound stability and proper healing.

Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, et al. (2016)[18] compared PDGF-AA, PDGF-AB, PDGF-BB, TGFβ1, VEGF, EGF, and IGF release over time quantified using enzyme linked immuno sorbent assay (ELISA) from platelet-rich plasma (PRP), PRF, and a modernized protocol for PRF, A-PRF and reported with highest growth factor released from platelet concentrates was PDGF-AA followed by PDGF-BB, TGFB1, VEGF, and PDGF-AB. PRP released

significantly higher growth factors when compared to PRF and A-PRF within 15– 60 min incubation and at later time points up to 10 days, it was routinely found that A-PRF released the highest total growth factors. Furthermore, A-PRF released significantly higher total protein accumulated over a 10-day period when compared to PRP or PRF.

Kotsakis GA, Boufidou F, Hinrichs JE, Prasad HS, Rohrer M, Tosios KI (2016)[19] introduced a protocol for ''accelerated-early'' implant placement, PRF is employed to accelerate soft and hard tissue healing and provide a better-healed recipient site for accelerated, early implant placement. Six weeks post-extraction histological analysis reveals the application of autologous growth factors resulted in delicate newly formed bone showing intense osteoblastic activity surrounded by connective tissue as well as areas of mineralized tissue.

Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. (2017)[20] demonstrated that modifications to centrifugation speed and time with the low-speed concept was shown to favor an increase in growth factor release from PRF clots which in turn may directly influence tissue regeneration by increasing fibroblast migration, proliferation and collagen messenger RNA (mRNA) levels.

Culhaoglu R, Taner L, Guler B (2017)[21] evaluated the effects of different multiple layers of PRF membranes for the treatment of gingival recession compared with the CTG procedure. Miller class I gingival recession (GR) were treated with two layers of PRF membranes+CAF, four layers of PRF membranes+CAF and CTG+CAF and concluded that the PRF membrane+CAF technique may be an alternative to the CTG+CAF technique for postoperative patient comfort. However, PRF membranes should use as many layers as possible.

Kuka S, Ipci SD, Cakar G, Yılmaz S (2017)[22] assessed the clinical effect of PRF in combination with CAF on root coverage, esthetics, and patient satisfaction when compared to CAF alone for the treatment of multiple Miller class I recessions and concluded that the additional use of PRF membrane did not provide additional benefits

in terms of root coverage outcomes compared with CAF alone. The use of PRF membranes increases tissue thickness significantly.

Clark D, Rajendran Y, Paydar S, Ho S, Cox D, Ryder M, Dollard J, Kao RT. (2017)[23] evaluated the efficacy of A-PRF alone or with freeze-dried bone allograft (FDBA) in improving vital bone formation and alveolar dimensional stability during ridge preservation and draws a result that A-PRF alone or augmented with FDBA is a suitable biomaterial for ridge preservation.

K. Amine a, Y. El Amrani b, S. Chemlali a, J. Kissa (2017)[24] systemically reviewed the clinical efficacy of alternatives procedures; Acellular Dermal Matrix (ADM), Xenogeneic Collagen Matrix (XCM), Enamel Matrix Derivative (EMD) and PRF, compared to conventional procedures in the treatment of localized gingival recessions. The comparison between PRF and others technique was affected by large uncertainties. They concluded that ADM, XCM and EMD assisted to CAF might be considered alternatives to CTG in the treatment of Miller class I and II gingival recession.

Maluf G, Caldas RJ, Silva Santos PS (2017)[25] Lesions associated with medication-related osteonecrosis of the jaws (MRONJ) are refractory to different treatment approaches. The LPRF membrane contributes to a successful outcome, and acts as a physical barrier against microorganisms, thus preventing secondary infections.It shows anti-infectious activity through immune regulation and accelerates the angiogenesis and multiplication of fibroblasts and osteoblasts; consequently, it stimulates soft-tissue healing and prevents the exposure of alveolar bonsse in the oral cavity.

Chenchev IL, Ivanova VV, Neychev DZ, Cholakova RB (2017)[26] assessed the possibility for augmentation of the alveolar ridge in the frontal region of the upper jaw, utilizing a combination of bone graft material, i-PRF and A-PRF and successful clinical and radiographic results of the case concludes that using A-PRF and i-PRF can be beneficial for bone augmentation of the alveolar ridge before implant placement.

Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, Guillemette V, et al. (2017)[27] systematically reviewed the use of platelet-rich fibrin in regenerative dentistry and draws a conclusion that PRF has been most investigated in periodontology for the treatment of periodontal intrabony defects and gingival recessions where the majority of studies have demonstrated favorable results in soft tissue management and repair and it also significantly decrease sockets by tenfold dry of third molars.

Kumar A, Bains VK, Jhingran R, Srivastava R, Madan R, Rizvi I (2017)[28] evaluated autologous PRF and autogenous CTG in gingival recession defects in conjunction with CAF using a microsurgical technique and at the end of 6 months follow up draws a significant increase in gingival thickness measurements using transgingival probing in CAF with CTG , whereas nonsignificant changes were observed in CAF with PRF and CTG alone.

Rehan M, Khatri M, Bansal M, Puri K, Kumar A (2018)[29] evaluated the effectiveness of CAF with either PRF membrane or bioresorbable amniotic membrane (AM) in treatment of localized gingival recession defects and concluded that both CAF + PRF and CAF + AM are equally effective in providing clinically significant outcomes with respect to root coverage with AM showing the better percentage of root coverage as compared to PRF.

Consuelo C. Zumarán, Marcelo V. Parra, Sergio A. Olate, Eduardo G. Fernández, Francisco T. Muñoz, Ziyad S. Haidar (2018)[30] reviewed PRF, a three-dimensional (3-D) autogenous biomaterial obtained via simple and rapid centrifugation from the patient's whole blood samples, without including anti-coagulants, bovine thrombin, additives, or any gelifying agents. They stated that PRF promotes early bone formation and maturation; accelerated soft-tissue healing; and reduced post-surgical edema, pain, and discomfort. An advanced and original tool in regenerative dentistry, PRFs present a strong alternative and presumably cost-effective biomaterial for oro-maxillo-facial tissue (soft and hard) repair and regeneration. Yet, preparation protocols continue to be a source of confusion, thereby requiring revision and standardization.

Lei L, Yu Y, Ke T, Sun W, Chen L (2018)[31] treated a patient of severe chronic periodontitis. A novel surgical technique was used for treating maxillary right lateral incisor. A 3D printer was used to produce a photosensitive resin bony anatomy replica, A-PRF and i-PRF were mixed with Bio-Oss, and packed onto the 3D replica. A-PRF membrane was applied over the replica along with a Bio-Gide collagen membrane. Clinical and radiographic follow-up 15 months after the surgery revealed greatly reduced pocket depths and significant 3D alveolar bone fill at the treatment site.

Cabaro S, D'Esposito V, Gasparro R, Borriello F, Granata F, Mosca G, et al. (2017)[32] compared the release of chemokines and growth factors in standard leukocyte-PRF (L-PRF) with the experimental low-force modified procedure defined as A-PRF entrapped the same content of viable leukocytes, released a similar amount of inflammatory cytokines, but secreted 3-, 1.6-, 3-, and 1.2- fold higher levels of Eotaxin, C-C Motif Chemokine Ligand 5 (CCL5), PDGF and VEGF, respectively. A leukocyte-free scaffold, such as plasma rich in growth factors (PRGF), released only platelet-specific factors and, in particular, the F3 fraction, the richest in growth factors, secreted higher amount of CCL5 and PDGF compared to F1 and F2 fractions. In conclusion, different procedures and leukocyte content affect cytokine, chemokines, and growth factor release from platelet derivatives, which may be helpful in different clinical settings.

Miron RJ, Zhang Y. (2018)[33] reviewed the use of liquid PRF as an advanced local delivery system for small and large biomolecules. Potential target molecules including large (growth factors/cytokines and morphogenetic/angiogenic factors), as well as small (antibiotics, peptides, gene therapy and anti-osteoporotic) molecules are considered potential candidates for enhanced bone/cartilage tissue regeneration. Furthermore, liquid-PRF is cocluded as a potential carrier system for various cell types and nano-sized particles that are capable of limiting/by-passing the immune system and minimizing potential foreign body reactions within host tissues following injection.

INJECTABLE PLATELET RICH FIBRIN

Further reduction of relative centrifugal force (RCF) and fine tuning of the centrifugation setting, that is, revolution per minute (rpm) and centrifugation time, enables the production of fluid PRF matrix i.e. injectable platelet rich fibrin which coagulates few minutes after the end of spin.

Sclafani AP (2009)[34] used platelet-rich fibrin matrix to enhance healing after facial procedures as well as to rejuvenate the face without tissue manipulation. Platelet rich fibrin matrix (PRFM) provides autologous, natural, but concentrated platelet growth factor release and stimulation of surrounding tissue with the potential to stimulate natural biologic processes to achieve aesthetic improvements essentially by replicating the wound-healing process, but the lack of erythrocytes may assist in bypassing the inflammatory stage.

Sclafani AP (2010)[35] evaluated the efficacy of a single injection of autologous platelet-rich fibrin matrix (PRFM) for the correction of deep nasolabial folds (NLFs). NLFs were rated before and after treatment using the Wrinkle Assessment Scale (WAS) and patients rated their appearance at each post-treatment visit using the Global Aesthetic Improvement Scale and concluded that PRFM can provide significant long-term diminution of deep NLFs without the use of foreign materials. PRFM holds significant potential for stimulated dermal augmentation.

Sclafani AP (2014)[36] studied the effect of PRFM treatment on androgenetic alopecia (AGA) and concluded that a series of intradermal injections of autologous PRFM increased the Hair density index (HDI) in patients with AGA at 2 and 3 months after initial treatment; this improvement approached statistical significance at 6 months after initiating treatment and autologous PRFM injections are valuable treatment for AGA, particularly in cases with mild hair loss.

Fabi S, Sundaram H (2014)[37] reviewed the potential of topical and injectable growth factors and cytokines for skin rejuvenation, mechanism of action of growth factors (GFs) can potentially advance our general understanding of dermal signaling pathways, and hence of hyaluronic acid and other alloplastic fillers; and allow the development of protocols for synergistic combination of GFs with other skin rejuvenation modalities and suggested the clinical applications of topical and injectable GFs are promising, and remain to be fully defined

Mourão CF, Valiense H, Melo ER, Mourão NB, Maia MD (2015)[38] obtained injectable platelets rich-fibrin and used with particulated bone graft materials in the polymerized form and achieved the possibility of bonding of i-PRF with biomaterials for bone grafting creates an alternative to PRP as a platelet aggregate for bone regeneration and represented a promising and innovator tools in the medicine and dentistry today.

Wu CC, Sheu SY, Hsu LH, Yang KC, Tseng CC, Kuo TF (2016)[39] Studied the therapeutic effects of Platelet Rich Fibrin Releasate (PRFr) in combination with bone marrow-derived messenchymal stem cells (MSCs) for articular cartilage regeneration were evaluated in a rabbit model and concluded that intra-articular injections of MSCs+PRFr into the knee can reduce cartilage defects by regenerating hyaline-like cartilage without adverse events and this approach may provide an alternative method of autologous chondrocyte implantation to repair cartilage defects with an unlimited source of cells and releasate.

Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ (2017)[40] compared i-PRF to standard PRP with gingival fibroblasts cultured on smooth and roughened titanium implant surfaces and demonstrated that i-PRF had much translational potential as it induced significantly higher cell migration, as well as higher messenger RNA (mRNA) levels of PDGF, TGF-β, collagen1 and fibronectin. Furthermore, collagen1 synthesis was highest in the i-PRF.

Karde PA, Sethi KS, Mahale SA, Khedkar SU, Patil AG, Joshi CP (2017)[41] evaluated the antimicrobial property, and platelet count of i-PRF in comparison to other platelet concentrates, i.e., PRF, PRP and concluded that i-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to other platelet concentrates, thereby indicating to have a better regenerative potential then others.

Wend S, Kubesch A, Orlowska A, Al-Maawi S, Zender N, Dias A, et al. (2017)[42] evaluated the influence of RCF on cell types and growth factor release within injectable PRF- in the range of 60–966 g using consistent centrifugation time and suggested that LSCC (low speed centrifugation concept), confirms that improved PRF based matrices are generated through RCF reduction. The enhanced regenerative potential of PRF-based matrices makes them a potential source to serve as a natural drug delivery system.

Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S, Choukroun J. (2017) [43]investigated a liquid formulation of PRF termed i-PRF without the use of anti-coagulants and suggested that general PRP had higher early release of growth factors whereas i-PRF showed significantly higher levels of total long-term release of PDGF-AA, PDGF-AB, EGF, and insulin like growth factor-1 (IGF-1) after 10 days. PRP showed higher levels of TGF-β1 and VEGF at 10 days. While both formulations exhibited high biocompatibility and higher fibroblast migration and proliferation when compared, to control tissue-culture plastic, i-PRF induced significantly highest migration whereas PRP demonstrated significantly highest cellular proliferation. Furthermore, i-PRF showed significantly highest mRNA levels of TGF-β at 7 days, PDGF at 3 days, and collagen1 expression at both 3 and 7 days when compared to PRP.

Abd El Raouf M, Wang X, Miusi S, Chai J, Mohamed AbdEl-Aal AB, Nefissa Helmy MM, Ghanaati S, et al. (2017)[44] evaluated the effect of i-PRF on cultivated chondrocytes and osteochondral regeneration in critical-sized osteochondral defect of the rabbit's knee in comparison to autologous PRP and concluded that the use of i-PRF using the low speed centrifugation concept significantly promoted chondrocyte activity and further improved cartilage regeneration when compared to PRP. The histological

results also revealed early and better cartilage regeneration within 4 weeks postoperatively when i-PRF was utilized and the results were maintained at 12 weeks.

Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ (2017)[45] investigated effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma and demonstrated a significant increase in mRNA levels of alkaline phosphatase (ALP), Runt-related transcription factor-2 (Runx-2) and osteocalcin, as well as immune fluorescent staining of osteocalcin was also observed in the i-PRF as compared to PRP and concluded to favored the use of the naturally-formulated i-PRF compared to traditional PRP with anti-coagulants.

Matz EL, Pearlman AM, Terlecki RP (2017)[46] reviewed an initial series of patients receiving PRFM for urologic pathology to assess safety and feasibility and observed no patients compliance at follow-up and no decline in men completing pre/post International Index of Erectile Function (IIEF-5) evaluations. Concluded PRFM to be a safe and feasible treatment modality in patients with urologic disease.

Choukroun J, Ghanaati S (2017)[47] systematically analyzed the influence of the RCF on leukocytes, platelets and growth factor release within fluid PRFM and demonstrated that to enrich PRF-based fluid matrices with leukocytes, platelets and growth factors by means of a single alteration of the centrifugation settings within the clinical routine and postulated the so-called low speed centrifugation concept (LSCC) selectively enriches leukocytes, platelets and growth factors within fluid PRF-based matrices.

Dohle E, El Bagdadi K, Sader R, Choukroun J, James Kirkpatrick C, Ghanaati S (2018)[48] analyzed the effect of i-PRF on wound healing processes and angiogenic activation of outgrowth endothelial cells (OECs) in the co-culture with regard to proinflammatory factors, adhesion molecules and proangiogenic growth factor expression and suggested the i-PRF to positively influence wound healing processes, in particular angiogenesis, in the *in vitro* co-culture, making autologous PRF-based

matrices a beneficial therapeutic tool for tissue engineering purposes by simply profiting from the PRF, which contains blood plasma, platelets and leukocytes.

Varela HA, Souza JCM, Nascimento RM, Araújo RF Jr, Vasconcelos RC, Cavalcante RS, et al. (2018)[49] evaluated the blood cell content, morphological aspects, gene expression of type I collagen, and release of growth factors on an i-PRF and concluded Injectable platelet rich fibrin to be a good approach for soft and mineralized tissue healing considering the formation of a three-dimensional fibrin network embedding platelets, leukocytes, type I collagen, osteocalcin, and growth factors. Indeed, the injectable platelet rich fibrin can be indicated in several medical applications regarding bioactivity, simplied technique, and flowable mixing with other biomaterials.

Schiavone G, Paradisi A, Ricci F, Abeni D. (2018)[50] Studied the role of Injectable Platelet-, Leukocyte-, and Fibrin-Rich Plasma (iL-PRF) in the Management of AGA and observed a larger improvement after treatment in iL-PRF and concluded that the biological composition of the iL-PRF is of crucial importance in ensuring a good degree of clinical efficacy in patients with AGA

MATERIAL AND METHODS

Methodology

A prospective experimental in-vitro study evaluating the Quantitative and comparative analysis of PDGF-AB and TGF β1 levels in A-PRF and i-PRF was carried out in the department of Periodontology, Babu Banarasi Das College of Dental Sciences (BBDCODS), BBD University Lucknow in collaboration with Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow.

Ethical clearance was obtained from institutional ethical committee. **(Appendix-I)**

STUDY DESIGN

Inclusion criteria

Systemically healthy patients with the age range of 20-60 years.

Co-operative patients.

Exclusion criteria

Pregnant/lactating women

Patients taking any drug known to affect the number and function of platelets in the past 3 months.

Patients with abnormal platelet count.

Patients with immunologic diseases.

Patients having antibodies, antiepileptic, or immunosuppressive drugs.

Patients having any systemic disease.

Armamentarium

Mouth mirror

Tweezer

Explorer

UNC-15 probe

Gloves

Kidney tray

Goldman fox scissor

Syringe

Tourniquet

Spirit

Cotton

Vacutainers

Needle destroyer

Petri dish

Incubator

Refrigerator

Eppendorf tube

Micropipette

Sterilization hood

DMEM F-12 culture media

Table top centrifuge (Eppendorf 5702)

Enzyme-linked immunosorbent assay (ELISA) KIT

Microplate reader (Robonik)

MATERIALS

15 systemically healthy individuals were selected from the out-patient department of periodontology Babu Banarasi Das College of Dental Sciences. A-PRF and i-PRF was prepared from venous blood of subjects in department of biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. Quantitative and comparative analysis of PDGF-AB and TGF β1 levels in A-PRF and i-PRF was quantified using ELISA assay over a period of 1st, 3rd, and 10th day as explained underneath. All the reagents, sterile plastic ware, flasks culture plates, ELISA Kits etc. were mainly procured from Reagen info bio.

COLLECTION OF BLOOD SAMPLES

Blood samples collection was done at Emergency pathology, Department of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow (Plate - I).

PREPARATION OF A-PRF AND i-PRF

A-PRF and i-PRF was prepared from 10 ml of venous blood collected from subjects at Emergency pathology, Department of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. The i-PRF was prepared through centrifugation of blood for a period of 3 minutes at 700 rpm in uncoated vacutainer and A-PRF was centrifuged at higher speed 1500 rpm for 14 min in uncoated vacutainer (Plate – I and II).

.CULTURE OF A-PRF AND i-PRF

Experiment was conducted with A-PRF and i-PRF at Emergency pathology, Department of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. For experiment the A-PRF and i-PRF was cultured in Dulbecco's Modified Eagle Medium F-12 , PH 7.4 (DMEM F-12 1: 1) containing amino acids, vitamins, inorganic salts, phenol red in humidified incubator at 37°C for release of growth factor (Plate – III, IV AND V).

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ASSESSMENT OF GROWTH FACTORS RELEASE

At each point of time i.e. 1st day, 3rd day and 10th day 2ml of culture media was collected (Plate - VI) and replaced with 2ml additional culture media and remnant culture media was frozen at -80°c (Plate -VII). The collected sample was quantified for both PDGF-AB and TGF β1 release at desired time points by ELISA (Plate –VIII, IX and X).. Absorbance was measured at 450 nm on a microplate reader. Concentrations of the samples was interpolated from the standard curve, the standard curve was plotted as the relative Optical Density (O.D.) 450 [(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well)] of each standard solution (Y-axis) vs. the respective concentration of the standard solution (X-axis)

ELISA ASSAY

Avidin-Biotin-Peroxidase Complex (ABC) working solution and TMB substrates were equilibrated for 30 min at room temperature (37°C). Samples were added to wells. Standard curve for each test was plotted. Standard, test sample and control (zero) wells

were set on the pre-coated plate respectively, and then their positions were recorded. Each standard was measured in duplicate and test samples were measured in singles. Aliquoting of standard solutions into the standard wells was done as following: 0.1 ml of 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml. for PDGF-AB and 0.1 ml of 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, 15.6 pg/ml for 0.1 ml of Sample / Standard diluent buffer was added into the control (zero) well. 0.1 ml of properly diluted samples of A-PRF and i-PRF were added into test sample wells. Plate was sealed with a cover and incubated at 37°C for 90 min. Cover was removed, plate content was discarded and plate was clapped on the absorbent filter papers. 0.1 ml of Biotin conjugated anti-Human PDGF-AB and TGF-β1 antibody work solution was added into the above wells (standard, test sample & zero wells). Solution was added at the bottom of each well without touching the side walls. Plate was sealed with a cover and incubated at 37°C for 60 min. Cover was removed, followed by washing of plate 3 times with Wash buffer. Solution in the plate was discarded without touching the side walls. Plate was clapped on absorbent filter papers. Each well was filled completely with Wash buffer and vortexed mildly on ELISA shaker for 2 min. Contents were aspirated from the plate, and plate was clapped on absorbent filter papers. This procedure was repeated two more times for a total of three washes. 0.1 ml of ABC working solution was added into each well. Plate was covered and incubated at 37°C for 30 min. Cover was removed and plate was washed 5 times with Wash buffer, each time the wash buffer was let to stay in the wells for 1-2 min. 0.1 ml of TMB substrate was added into each well and plate was immediately covered and incubated at 37°C in dark. Shades of blue were seen in the first 3 wells, because these were most concentrated

Human PDGF-AB and TGF β1 standard solutions. The other wells showed no obvious color. 0.1 ml of Stop solution was added into each well and mixed thoroughly. Color changed into yellow immediately. Within 30 minutes after adding the stop solution, O.D. absorbance at 450 nm was read on a microplate reader.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

DESCRIPTIVE STATISTICS

Descriptive analysis includes the expression of study variables in terms of mean and standard deviation.

INFERENTIAL STATISTICS

Wilcoxon Signed Rank Test was used to compare the mean PDGF-AB & TGF-β1 concentration levels between A-PRF & i-PRF groups at different time intervals. Similar comparisons were done to estimate the difference in mean PDGF AB & TGF-B1 total Concentration from Day 1 to day 10 between A-PRF & i-PRF using the same test. Friedman's Test followed by Wilcoxon Signed Rank Test was used to compare the Mean PDGF-AB & TGF-β1concentration levels between different time intervals in A-PRF & i-PRF groups.

PREPARATION OF A-PRF AND i-PRF

Collection of venous blood

Centrifuged at 1500 rpm for 14 minutes and 700 rpm for 3 minutes

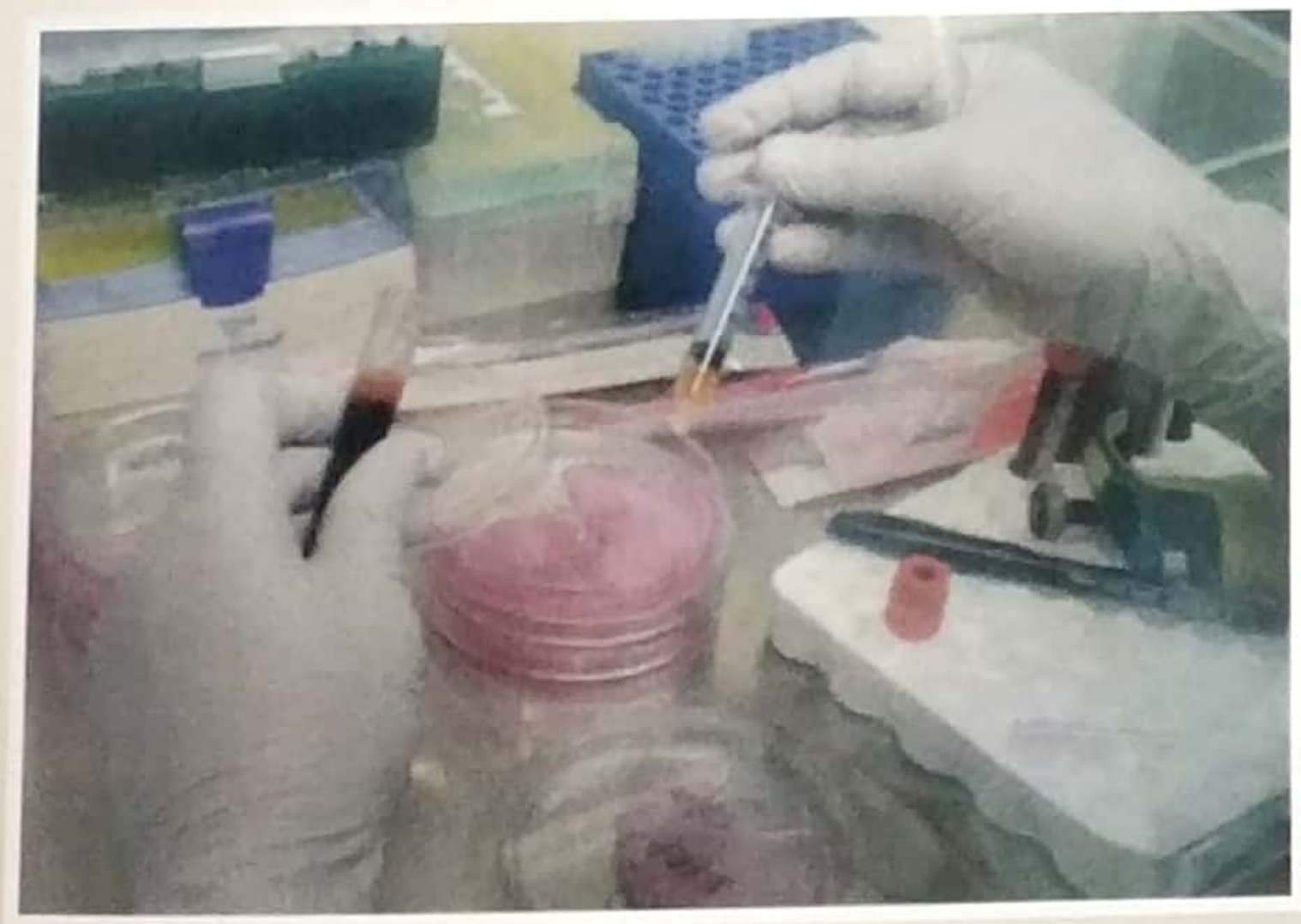
Plate -I

Prepared A-PRF and i-PRF

Plate -II

Transferring A-PRF and i-PRF to culture media

Transferring A-PRF



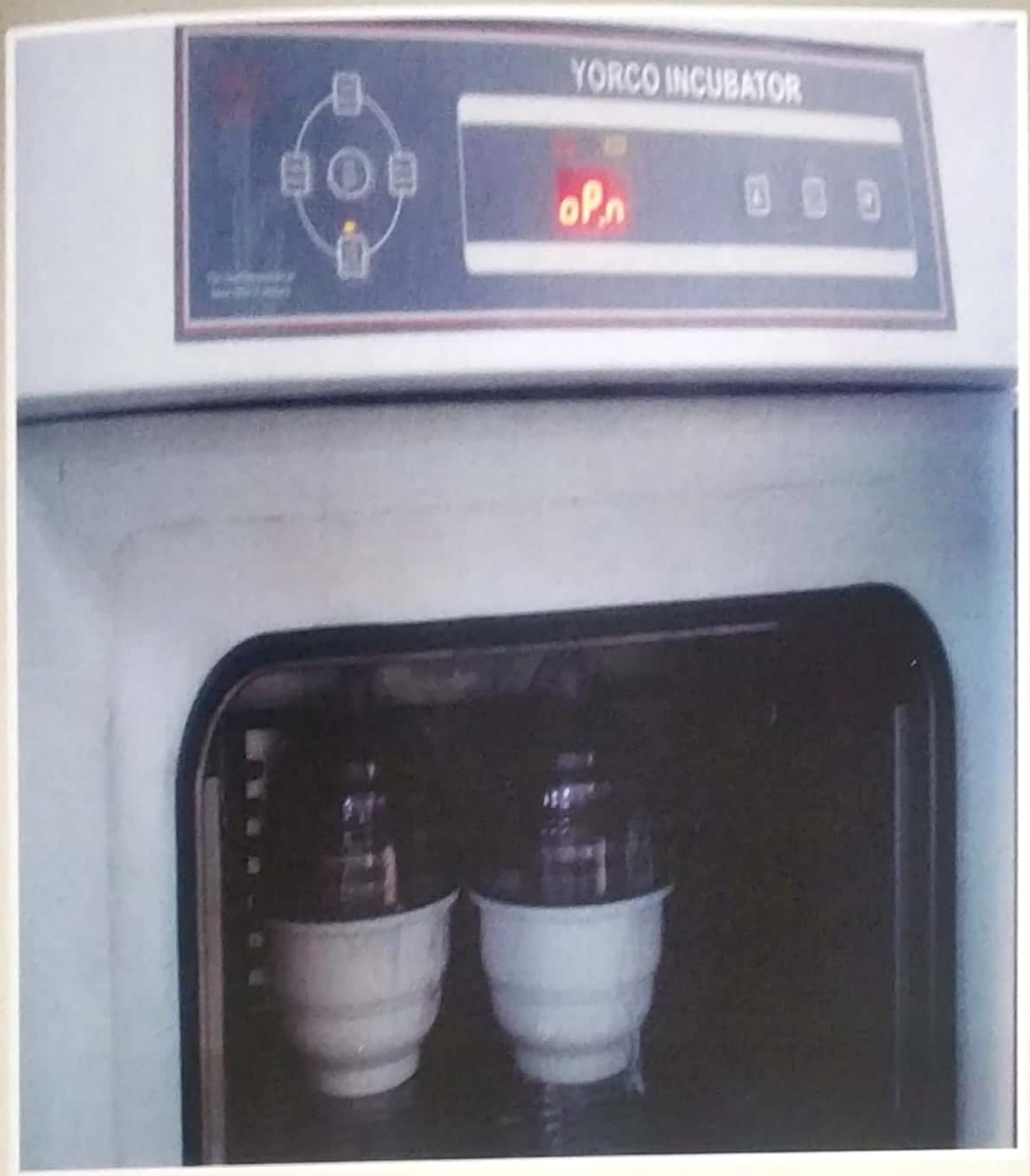
Transferring i-PRF

Plate - III

Incubating samples

Incubator

Plate -IV



Samples cultured at 37°c in incubator

Plate -V

Collection of culture media

Plate -VI

Stored culture media samples

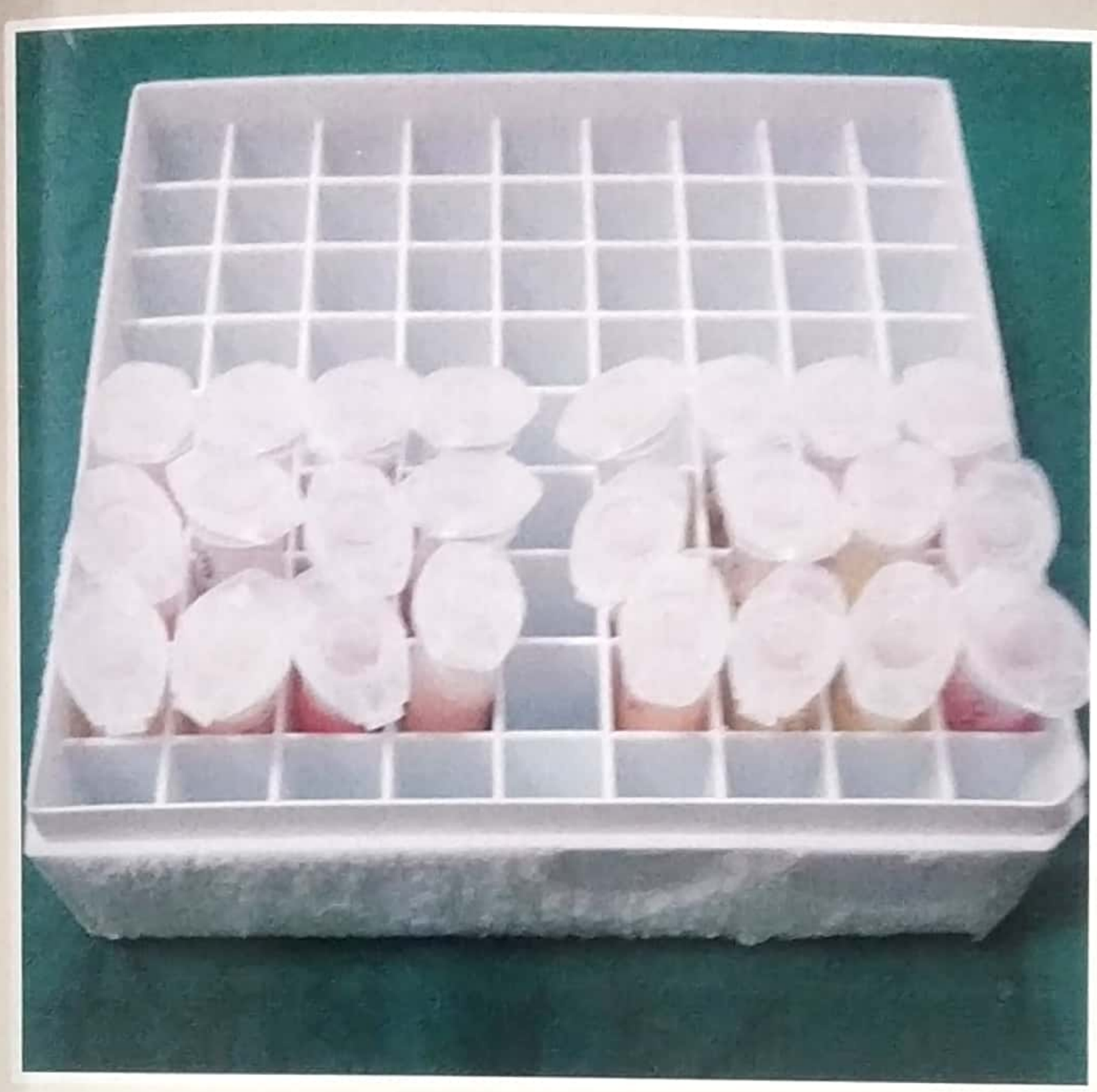


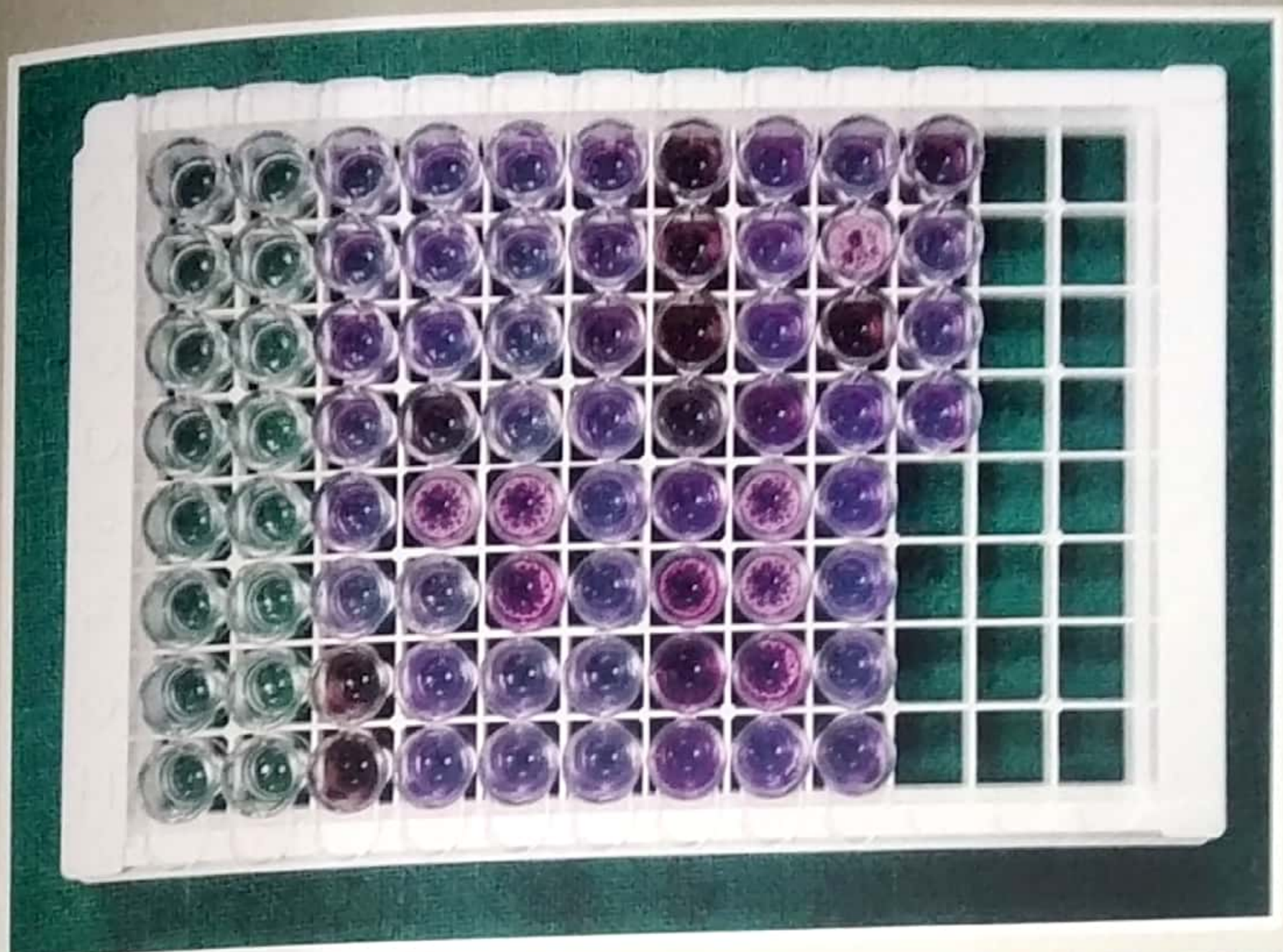
Plate -VII

ELISA Assay

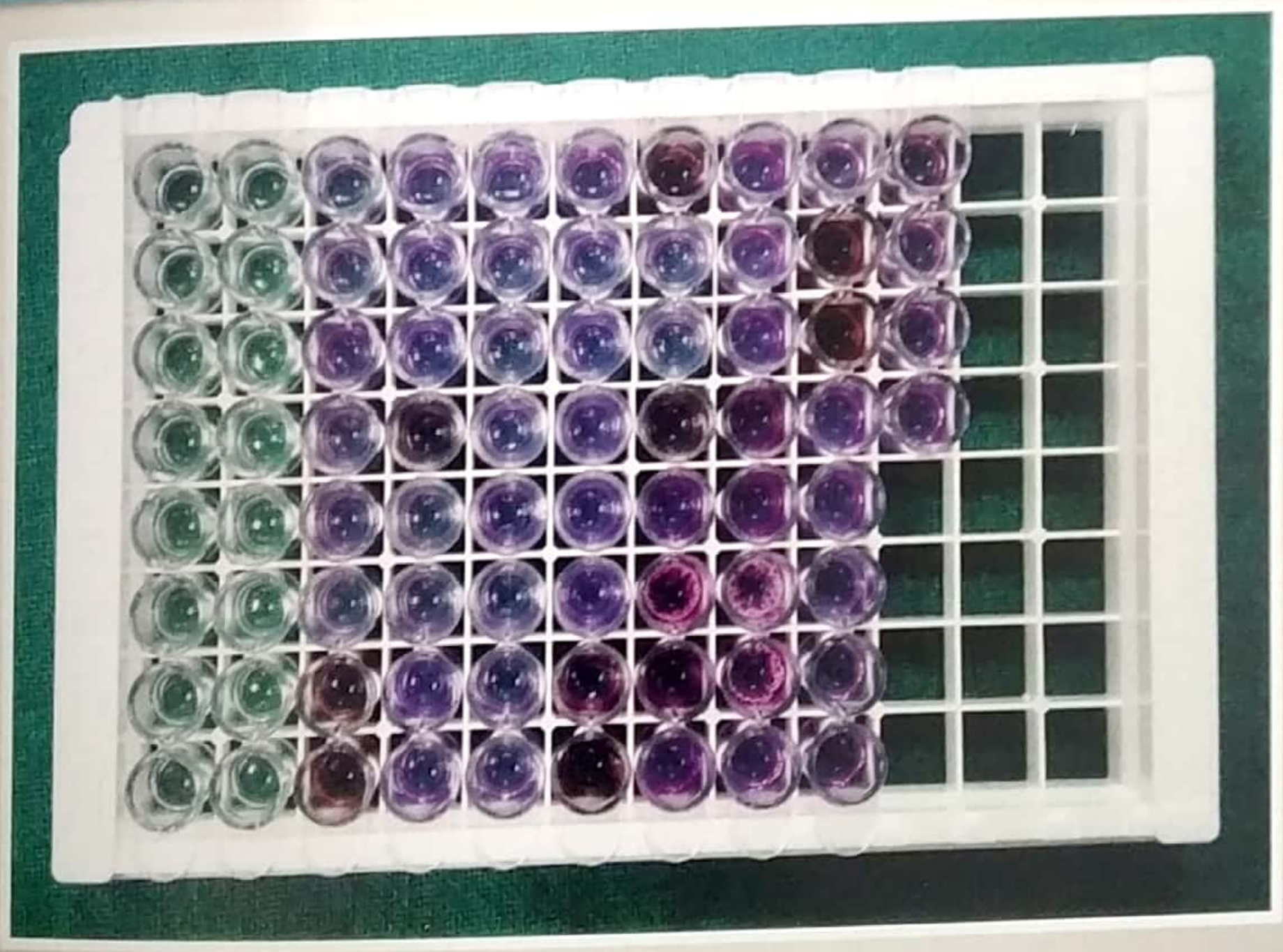
TGF-β1 ELISA Kit

PDGF-AB ELISA Kit

Plate -VIII



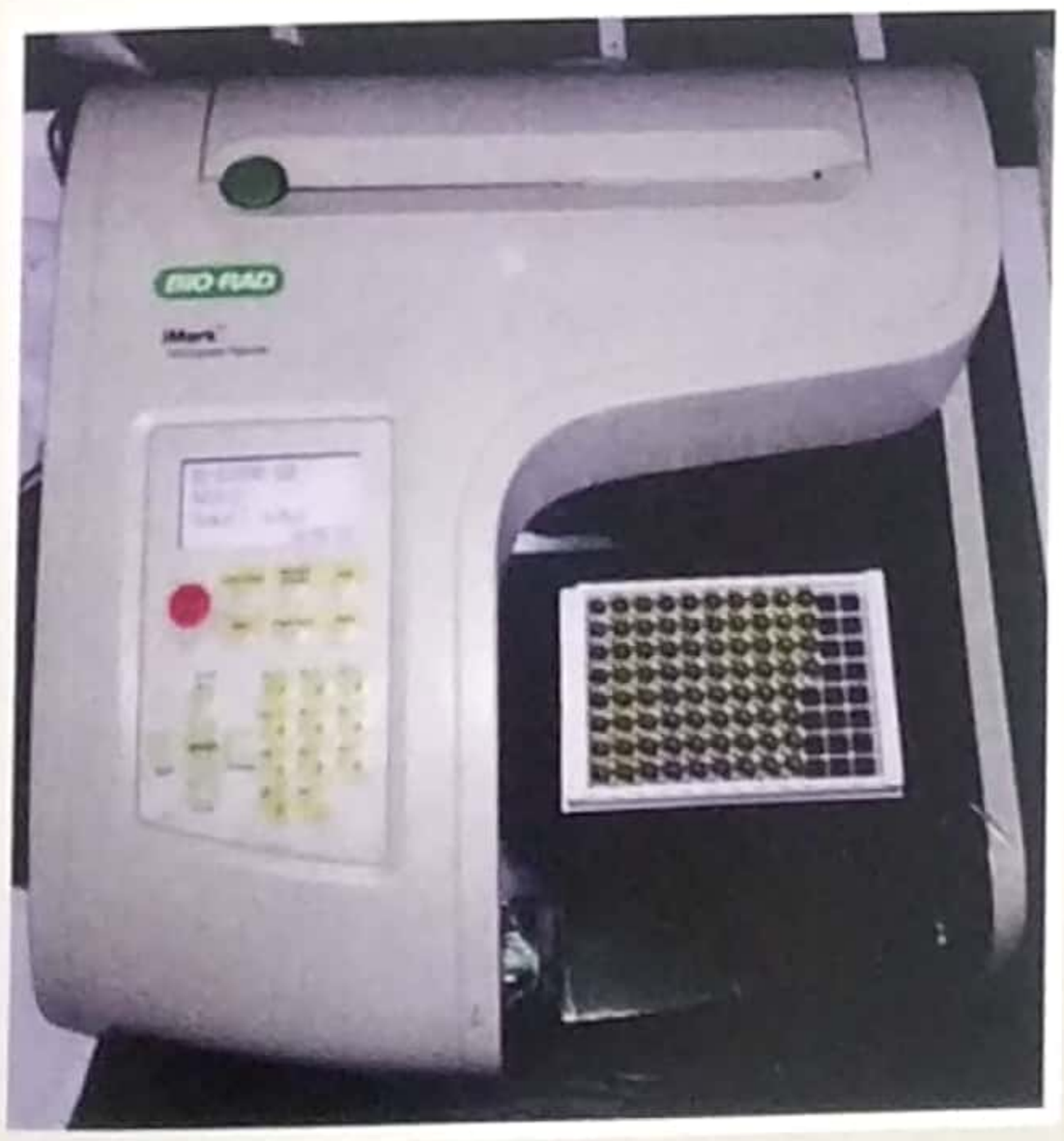
PDGF-AB ELISA assay



TGF-β1 ELISA assay

Plate -IX

Microplate Reader



ELISA reading

Plate -X

OBSERVATIONS AND RESULTS

RESULTS AND OBSERVATIONS

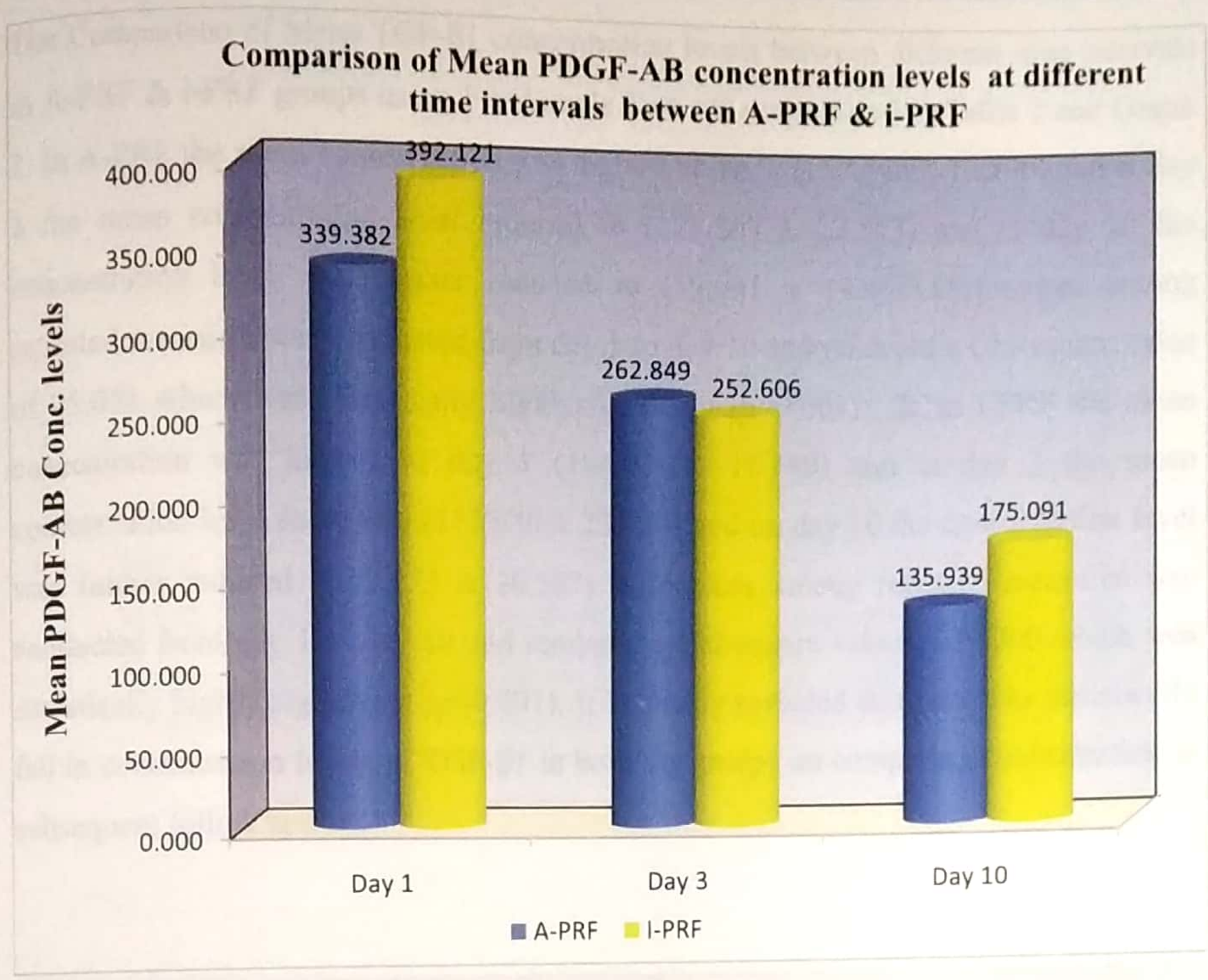
The present study deals with comparison of PDGF-AB and TGF-β1 growth factor release from A-PRF and i-PRF. Total 15 samples for each group were evaluated. The outcome measure of the study was growth factors concentration release. The outcome measure was assessed on 1st day, 3rd day and 10th day. The objective of the study was to compare outcome measures over the test periods.

RESULTS AND OUTCOME MEASURES

The Comparison of Mean PDGF-AB concentration levels between different time intervals in A-PRF & i-PRF groups using Friedman's Test are summarized in Table 1 and Graph 1. In A-PRF at day 1 the mean concentration was highest (339.382 ± 44.410), at day 3 the mean concentration levelsreduced to (262.849 ± 82.901) and at day 10 the concentration level was further reduces to (135.939 ± 66.001)differences among repeated measures was conducted from day 1 to day 10 andrendered a Chi-square value of 26.533 which was statistically highly significant ($p<0.001$).In i-PRF the mean concentration was highest atday 1 (392.121 ± 67.781) and at day 3 the mean concentration level reduced to (252.606 ± 70.681) and at day 10 the concentration level was further reduces to(175.091 ± 149.419).Differences among repeated measures was conducted from day 1 to day 10 and rendered a Chi-square value of 24.400 which was statistically highly significant ($p<0.001$). It is clearly revealed that here was statistically fall in concentration levels of PDGF-AB in both the groups on comparing concentration at subsequent follow ups.

Comparison of Mean PDGF-ABconcentration levels between different time intervals in A-PRF & i-PRF groups using Friedman's Test								
Groups	Time	N	Mean (pg/ml)	SD	Min	Max	χ^2 Value	P-Value
A-PRF	Day 1	15	339.382	44.410	244.55	486.36	26.533	<0.001*
	Day 3	15	262.849	82.901	109.09	457.27		
	Day 10	15	135.939	66.001	72.73	314.55		
i-PRF	Day 1	15	392.121	61.781	289.09	456.36	24.400	<0.001*
	Day 3	15	252.606	70.683	149.09	410.00		
	Day 10	15	175.091	149.419	17.27	629.09		

Table 1: Comparison of PDGF-AB concentration levels between different time intervals in A-PRF & i-PRF

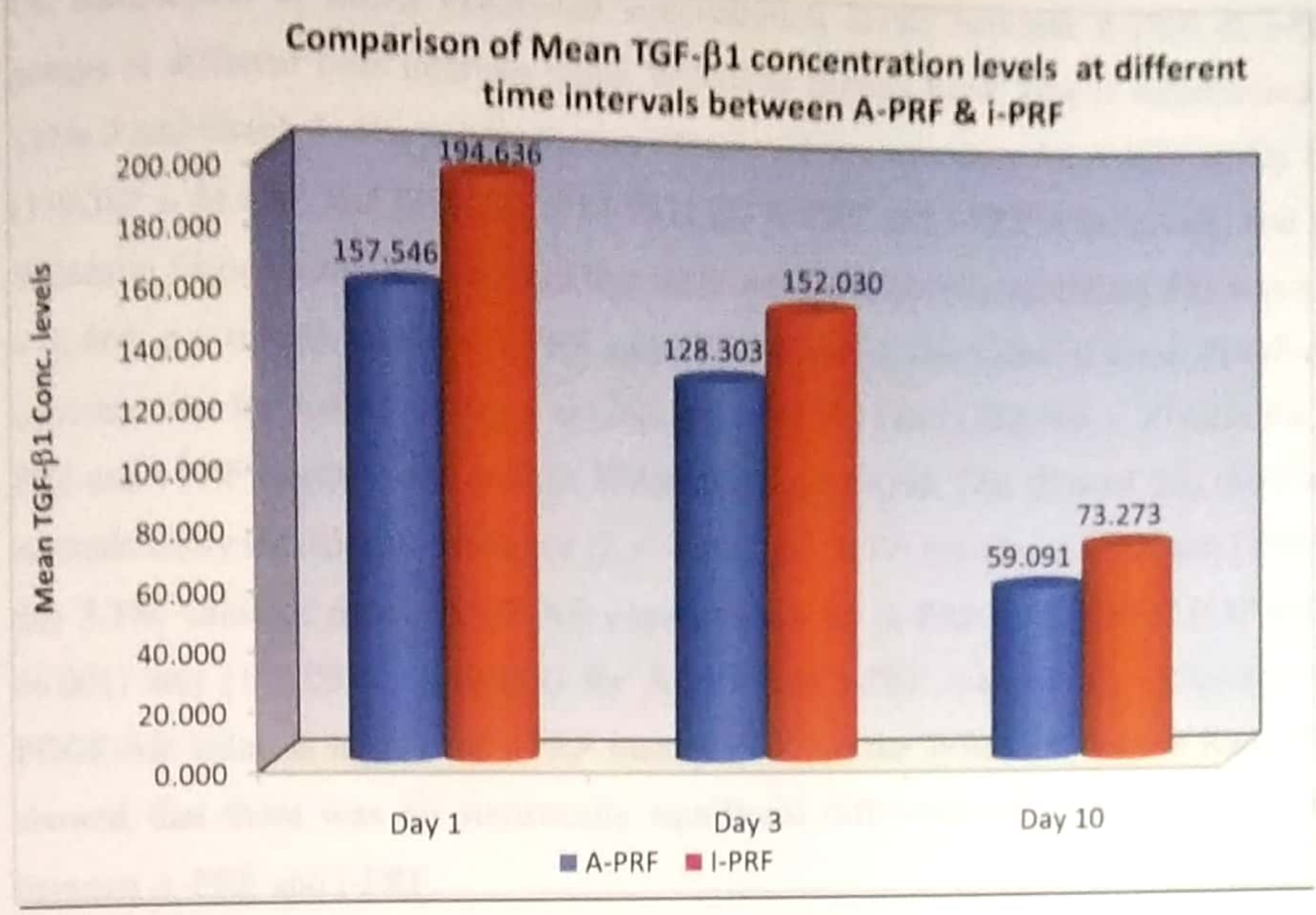


Graph 1: Comparison of Mean PDGF-AB concentration levels at different time intervals in A-PRF &i-PRF

The Comparison of Mean TGF-β1 concentration levels between different time intervals in A-PRF & i-PRF groups using Friedman's Test are summarized in Table 2 and Graph 2. In A-PRF the mean concentration was highest at day 1 (157.546 ± 19.890) and at day 3 the mean concentration level reduced to (128.303 ± 22.563) and at day 10 the concentration level was further reduced to (59.091 ± 14.483).Differences among repeated measures was conducted from day 1 to day 10 and rendered a Chi-square value of 25.051 which was statistically highlysignificant ($p<0.001$). In in i-PRF the mean concentration was highest at day 1 (194.636 ± 18.189) and at day 3 the mean concentration level reducedto (152.030 ± 23.257) and on day 10 the concentration level was further reduced to(73.273 ± 26.587) differences among repeated measures was conducted from day 1 to day 10 and rendered a Chi-square value of 30.000 which was statistically highly significant ($p<0.001$). It is clearly revealed that here was statistically fall in concentration levels of TGF-β1 in both the groups on comparing concentration at subsequent follow ups.

Comparison of Mean TGF-β1 concentration levels between different time intervals in A-PRF & i-PRF groups using Friedman's Test								
Groups	Time	N	Mean (pg/ml)	SD	Min	Max	χ^2 Value	P-Value
A-PRF	Day 1	15	157.546	19.890	161.82	207.82	25.051	<0.001*
	Day 3	15	128.303	22.563	81.36	167.27		
	Day 10	15	59.091	14.483	34.09	99.55		
i-PRF	Day 1	15	194.636	18.189	168.18	235.91	30.000	<0.001*
	Day 3	15	152.030	23.257	116.36	198.64		
	Day 10	15	73.273	26.587	46.82	123.64		

Table 2: Comparison of TGF-β1concentration levels between different time intervals in A-PRF & i-PRF

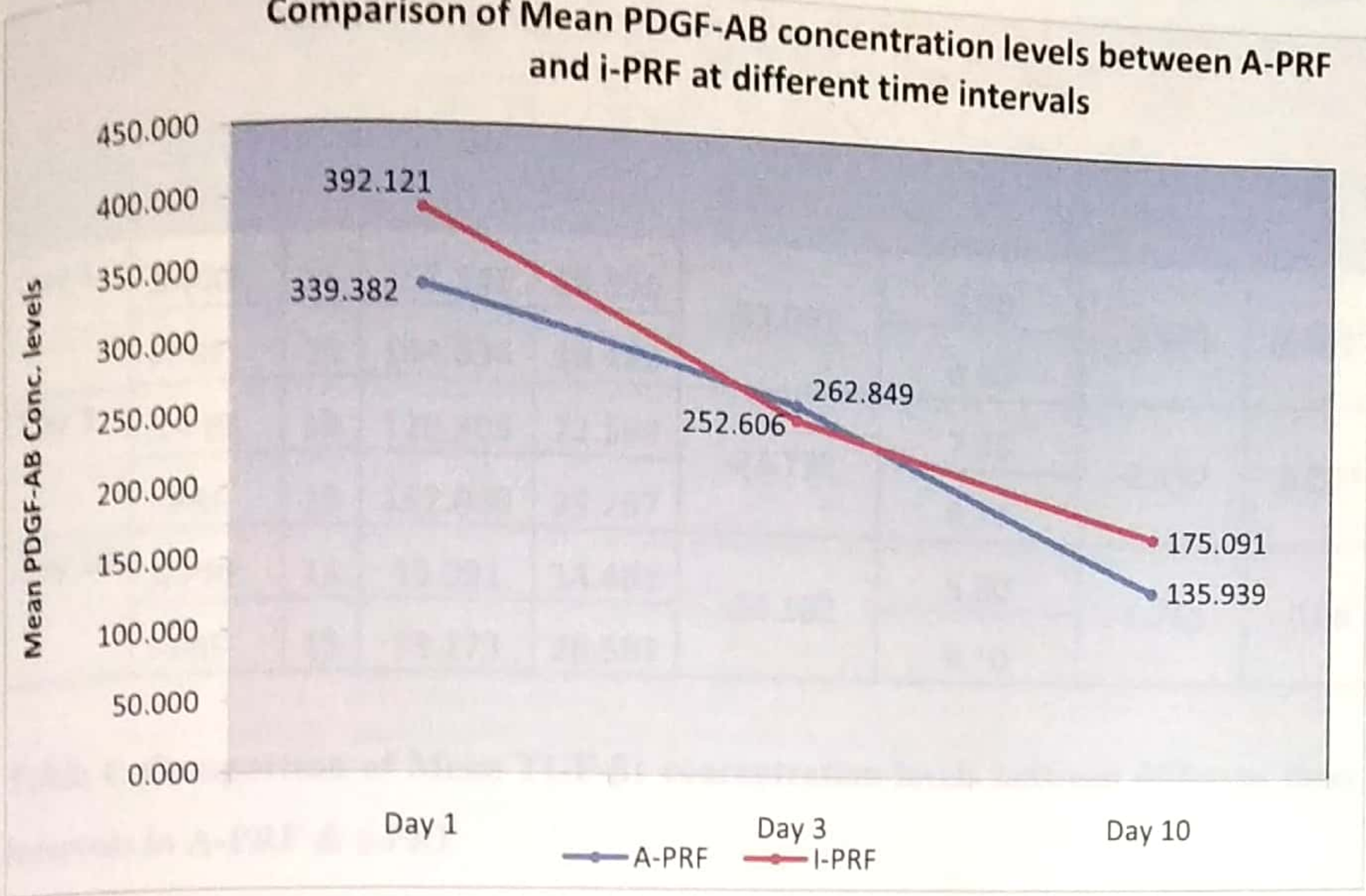


Graph 2: Comparison of Mean TGF-β1concentration levels between different time intervals in A-PRF & i-PRF

On comparison of Mean PDGF-AB concentration levels between A-PRF & i-PRF groups at different time intervals using Wilcoxon Signed Rank Test is summarized in Table 3 and Graph 3. The value of mean PDGF-AB concentration for A-PRF at day 1 is (339.382 ± 44.410) and (392.121 ± 61.781) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statisticallysignificant difference (Z = -2.669, p < 0.008) between A-PRF and i-PRF at day 1.The value of mean PDGF-AB concentration for A-PRF at day 3 is (262.849 ± 82.901) and (252.606 ± 70.683) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was no statistically significant difference (Z = -0.852, p < 0.39) between A-PRF and i-PRF at day 3.The value of mean PDGF-AB concentration for A-PRF at day 10 is (135.939 ± 66.001) and (175.091 ± 149.419) for A-PRF and i-PRF respectively. Although the PDGF-AB value is higher for i-PRF than A-PRF but the Wilcoxon Signed Rank Test showed that there was no statistically significant difference (Z = -0.795, p < 0.43) between A-PRF and i-PRF.

Comparison of Mean PDGF-AB concentration levels between A-PRF & i-PRF groups at different time intervals using Wilcoxon Signed Rank Test								
Time	Groups	N	Mean (pg/ml)	SD	Mean Diff	Mean Ranks	Z	P-Value
Day 1	A-PRF	15	339.382	44.410	-52.739	3.25	-2.669	0.008*
	i-PRF	15	392.121	61.781		9.73		
Day 3	A-PRF	15	262.849	82.901	10.242	8.33	-0.852	0.39
	i-PRF	15	252.606	70.683		7.50		
Day 10	A-PRF	15	135.939	66.001	-39.152	9.20	-0.795	0.43
	i-PRF	15	175.091	149.419		7.40		

Table 3: Comparison of Mean PDGF-AB concentration levels between different time intervals in A-PRF & i-PRF

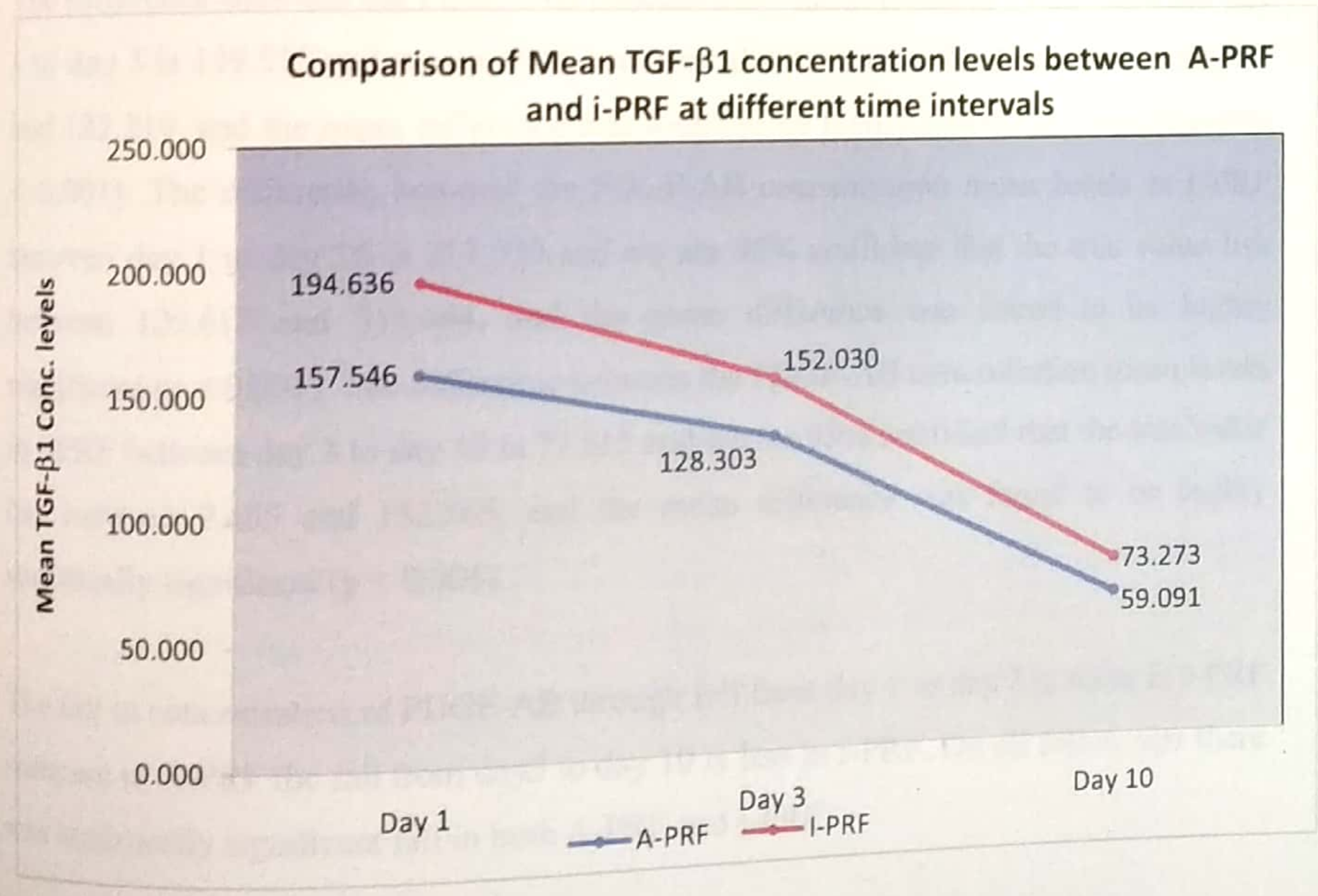


Graph 3: Comparison of Mean PDGF-AB concentration levels betweenA-PRF & i-PRFat different time intervals

On comparison of Mean TGF-β1concentration levels between A-PRF &i-PRF groups at different time intervals using Wilcoxon Signed Rank Test is summarized in Table 4 and Graph 4. The value of mean TGF-β1concentration for A-PRF at day 1 is (157.546 ± 19.890) and (194.636 ± 18.189) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statistically significant difference ($Z = -3.408$, $p < 0.001$) between A-PRF and i-PRF on day 1. The value of mean TGF-β1concentration for A-PRF at day 3 is (128.303 ± 22.563) and (152.030 ± 23.257) for A-PRF and i-PRF respectively, the Wilcoxon Signed Rank Test showed that there was a statistically significant difference ($Z = -2.158$, $p < 0.03$) between A-PRF and i-PRF at day 3. The value of mean TGF-β1concentration for A-PRF at day 10 is (59,091 ± 14.483) and (73.273 ± 26.587) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was no statistically significant difference ($Z = -1.761$, $p < 0.08$) between A-PRF and i-PRF.

Comparison of Mean TGF-β1 concentration levels between A-PRF & i-PRF groups at different time intervals using Wilcoxon Signed Rank Test								
Time	Groups	N	Mean (pg/ml)	SD	Mean Diff	Mean Ranks	Z	P-Value
Day 1	A-PRF	15	157.546	19.890	-37.091	0.00	-3.408	0.001*
	i-PRF	15	194.636	18.189		8.00		
Day 3	A-PRF	15	128.303	22.563	-23.727	7.33	-2.158	0.03*
	i-PRF	15	152.030	23.257		8.17		
Day 10	A-PRF	15	59.091	14.483	-14.182	5.80	-1.761	0.08
	i-PRF	15	73.273	26.587		9.10		

Table 4: Comparison of Mean TGF-β1 concentration levels between different time intervals in A-PRF & i-PRF



Graph 4: Comparison of TGF-β1 concentration levels between A-PRF & i-PRF at different time intervals

Multiple comparison of mean fall in PDGF-ABconcentrations between time intervals in A-PRF & i-PRF groups using Wilcoxon Signed Rank Post Hoc Test is summarized in Table 5.The difference between the PDGF-AB concentration mean levels in A-PRF betweenday 1 to day 3 is 76.533 and we are 95% confident that the true value lies between 19.364 and 133.672, and the mean difference was found to be statistically significant ($p < 0.003$). The difference between the PDGF-AB concentration mean levels in A-PRF between day 1 to day 10 is 203.442 and we are 95% confident that the true value lies between 159.312 and 247.573, and the mean difference was found to be highly statistically significant ($p < 0.001$). The difference between the PDGF-AB concentration mean levels in A-PRF between day 3 to day 10 is 126.909 and we are 95% confident that the true value lies between 76.569 and 177.249, and the mean difference was found to be highly statistically significant ($p < 0.001$).

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The difference between the PDGF-AB concentration mean levels in i-PRF between day 1 to day 3 is 139.515 and we are 95% confident that the true value lies between 92.811 and 182.219, and the mean difference was found to be highly statistically significant ($p < 0.001$). The difference between the PDGF-AB concentration mean levels in i-PRF between day 1 to day 10 is 217.030 and we are 95% confident that the true value lies between 120.617 and 313.444, and the mean difference was found to be highly significant ($p < 0.001$). The difference between the PDGF-AB concentration mean levels in i-PRF between day 3 to day 10 is 77.515 and we are 95% confident that the true value lies between 2.465 and 152.565, and the mean difference was found to be highly statistically significant ($p < 0.001$).

The fall in concentration of PDGF-AB through fall from day 1 to day 3 is more in i-PRF compare to A-PRF the fall from day3 to day 10 is less in i-PRF. On all follow ups there was statistically significant fall in both A-PRF and i-PRF

Multiple comparison of mean fall in PDGF-AB concentrations between time intervals in A-PRF & i-PRF groups using Wilcoxon Signed Rank Post Hoc Test						
Groups	(I) Time	(J) Time	Mean Diff. (I-J)	95% CI for Diff.		P-Value
				Lower	Upper	
A-PRF	Day 1	Day 3	76.533	19.364	133.672	0.003*
		Day 10	203.442	159.312	247.573	0.001*
	Day 3	Day 10	126.909	76.569	177.249	0.001*
i-PRF	Day 1	Day 3	139.515	92.811	186.219	0.001*
		Day 10	217.030	120.617	313.444	0.001*
	Day 3	Day 10	77.515	2.465	152.565	0.01*

Table 5: Multiple comparison of mean fall in PDGF-AB concentrations between time intervals in A-PRF & i-PRF groups

Multiple comparison of mean fall in TGF-β1concentrations between time intervals in A-PRF & i-PRF groups using Wilcoxon Signed Rank Post Hoc Test is summarized in Table 5. The difference between the TGF-β1concentrationsmean levels in A-PRF between day 1 to day 3 is 29.242 and we are 95% confident that the true value lies between 6.602 and 51.883, and the mean difference was found to be statistically significant ($p< 0.005$). The difference between the TGF-β1concentration mean levels in A-PRF between day 1 to day 10 is 98.455 and we are 95% confident that the true value lies between 78.134 and 118.775, and the mean difference was found to be highly statistically significant ($p< 0.001$). The difference between the TGF-β1concentration mean levels in A-PRF between day 3 to day 10 is 69.212 and we are 95% confident that the true value lies between 49.881 and 88.543, and the mean difference was found to be highly statistically significant ($p< 0.001$).

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The difference between the TGF-β1concentration mean levels in i-PRF between day 1 to day 3 is 42.606 and we are 95% confident that the true value lies between 27.473 and 57.739, and the mean difference was found to be highly statistically significant ($p< 0.001$). The difference between the TGF-β1concentration mean levels in i-PRF between day 1 to day 10 is 121.364 and we are 95% confident that the true value lies between 104.894 and 137.834, and the mean difference was found to be highly statistically significant ($p < 0.001$). The difference between the TGF-β1concentration mean levels in i-PRF between day 3 to day 10 is 78.758 and we are 95% confident that the true value lies between 57.783 and 99.732, and the mean difference was found to be highly statistically significant ($p < 0.001$). The fall in concentration of TGF-β1was found to be statistically significant in both A-PRF and i-PRF at all follow ups.

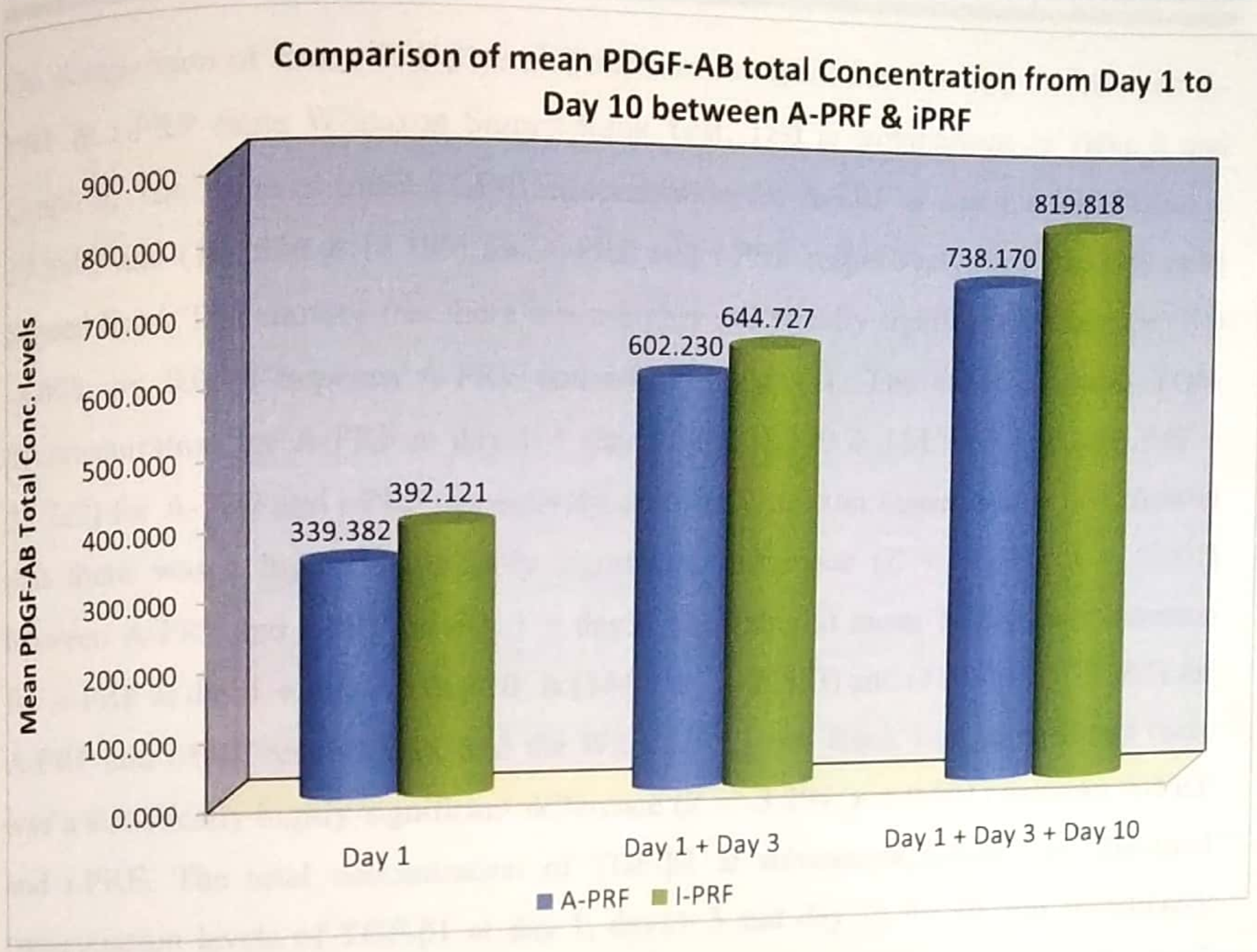
Multiple comparison of mean difference in TGF-β1 concentrations between time intervals in A-PRF & i-PRF groups using Wilcoxon Signed Rank Post Hoc Test						
Groups	(I) Time	(J) Time	Mean Diff. (I-J)	95% CI for Diff.		P-Value
				Lower	Upper	
A-PRF	Day 1	Day 3	29.242	6.602	51.883	0.005*
		Day 10	98.455	78.134	118.775	0.001*
	Day 3	Day 10	69.212	49.881	88.543	0.001*
i-PRF	Day 1	Day 3	42.606	27.473	57.739	0.001*
		Day 10	121.364	104.894	137.834	0.001*
	Day 3	Day 10	78.758	57.783	99.732	0.001*

Table 6: Multiple comparison of mean fall in TGF-β1concentrationsbetween time intervals in A-PRF & i-PRF groups

On comparison of mean PDGF-AB total Concentration from day 1 to day 10 between A-PRF & i-PRF using Wilcoxon Signed Rank Test is summarized in Table 7 and Graph 5. The value of mean PDGF-AB concentration for A-PRF at day 1 is (339.382 ± 44.410) and (392.121 ± 61.781) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statistically significant difference ($Z = -2.669$, $p < 0.001$) between A-PRF and i-PRF at day 1. The value of mean PDGF-AB concentration for A-PRF at day 1 + day 3 is (602.230 ± 105.163) and (644.727 ± 114.874) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statistically significant difference ($Z = -0.738$, $p < 0.001$) between A-PRF and i-PRF on day 1 + day 3. The value of mean PDGF-AB concentration for A-PRF at day 1 +day 3 + day 10 is (738.170 ± 154.503) and (819.818 ± 242.221) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statistically insignificant difference ($Z = -0.625$, $p < 0.53$.) The total concentration of PDGF-AB on day 1 was statistically significant and higher in i-PRF as compared to A-PRF but on day1+3 and day1+3+ 10 the differences were statistically insignificant although the total concentration was always more in i-PRF than A-PRF.

Comparison of mean PDGF-AB total Concentration from Day 1 to day 10 between A-PRF & I-PRF using Wilcoxon Signed Rank Test								
Time	Groups	N	Mean (pg/ml)	SD	Mean Diff	Mean Ranks	Z	P-Value
Day 1	A-PRF	15	339.382	44.410	-52.739	3.25	-2.669	0.008*
	i-PRF	15	392.121	61.781		9.73		
Day 1 + Day 3	A-PRF	15	602.230	105.163	-42.497	7.83	-0.738	0.46
	i-PRF	15	644.727	114.874		8.11		
Day 1 + Day 3 + Day 10	A-PRF	15	738.170	154.503	-81.649	9.80	-0.625	0.53
	i-PRF	15	819.818	242.221		7.10		

Table 7: Comparison of mean PDGF-AB total Concentration from Day 1 to day 10 between A-PRF & i-PRF

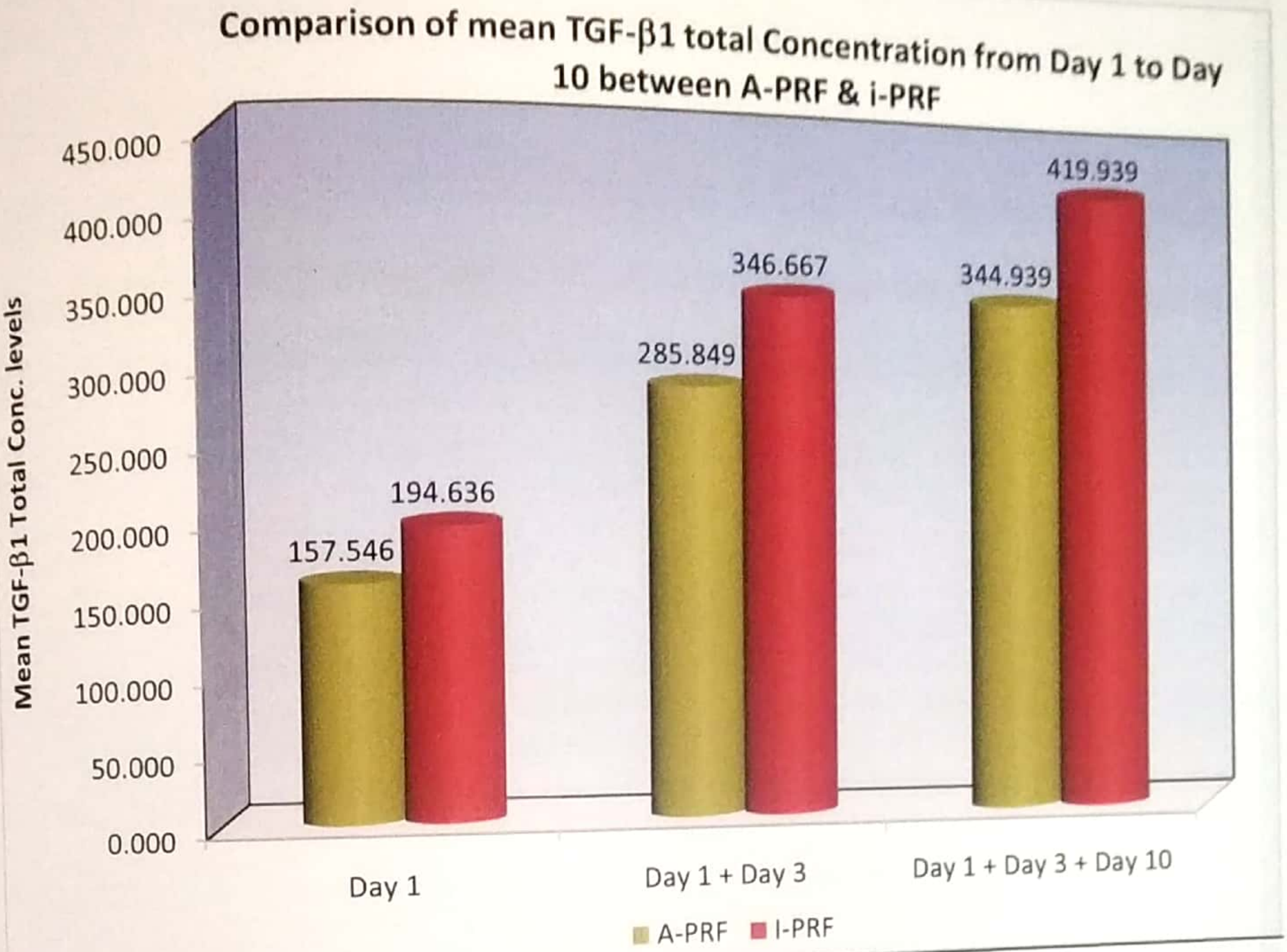


Graph 5: Comparison of mean PDGF-AB total Concentration from Day 1 to Day 10 between A-PRF & i-PRF

On comparison of mean TGF-β1total Concentration from day 1 to day 10 between A-PRF & i-PRF using Wilcoxon Signed Rank Test, Test is summarized in Table 8 and Graph 6. The value of mean TGF-β1concentration for A-PRF at day 1 is (157.546 ± 19.890) and (194.636 ± 18.189) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a highly statistically significant difference (Z = -3.408, p< 0.001) between A-PRF and i-PRF on day 1. The value of mean TGF-β1concentration for A-PRF at day 1 + day 3 is (738.170 ± 154.503) and (285.849 ± 27.722) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a highly statistically significant difference (Z = -3.351, p < 0.001) between A-PRF and i-PRF on day 1 + day3. The value of mean TGF-β1concentration for A-PRF at day 1 + day 3 + day 10 is (344.939 ± 26.533) and (419.939 ± 53.165) for A-PRF and i-PRF respectively, and the Wilcoxon Signed Rank Test showed that there was a statistically highly significant difference (Z = -3.294, p < 0.001) between A-PRF and i-PRF. The total concentration of TGF-β1 at subsequent follow ups, the total concentration levels of TGF-β1 at day 1, day1+ 3 and day 1+ 3+ 10 was statistically highly significant and higher in i-PRF as compared to A-PRF although the total concentration was always more in i-PRF than A-PRF.

Comparison of mean TGF-β1 total Concentration from Day 1 to day 10 between A-PRF & i-PRF using Wilcoxon Signed Rank Test								
Time	Groups	N	Mean (pg/ml)	SD	Mean Diff	Mean Ranks	Z	P-Value
Day 1	A-PRF	15	157.546	19.890	-37.091	0.00	-3.408	0.001*
	i-PRF	15	194.636	18.189		8.00		
Day 1 + Day 3	A-PRF	15	285.849	27.722	-60.818	1.00	-3.351	0.001*
	i-PRF	15	346.667	35.755		8.50		
Day 1 + Day 3 + Day 10	A-PRF	15	344.939	26.553	-75.000	2.00	-3.294	0.001*
	i-PRF	15	419.939	53.165		8.43		

Table 8: Comparison of mean TGF-β1 total Concentration from Day 1 to day 10 between A-PRF & i-PRF



Graph 6: Comparison of mean TGF-β1 total Concentration from Day 1 to Day 10 between A-PRF & i-PRF

DISCUSSION

When the concept of platelet rich fibrin was established almost 20 years ago, it was simply a means to provide a more natural way to bring blood-derived growth factors and vascularization to human tissues. By developing new protocols utilizing 100% natural methods (anti-coagulant removal) while simultaneously providing a three dimensional scaffold made of autologous fibrin, an array of possibilities was created in regenerative medicine. This new field, now known as Platelet Rich Fibrin or PRF

Over the past 5 years, further modifications to centrifugation speed and time have additionally improved PRF into a concept now known as the "low-speed centrifugation concept." Wound healing is a complex biological process where many cellular events taking place simultaneously leading to the repair or regeneration of damaged tissues[51-54]. Many attempts have been made in the field of tissue regeneration with the aim of predictably repairing, regenerating, or restoring damaged and diseased tissues[51-54]. These include strategies with foreign materials often derived from allografts, xenografts, or synthetically produced alloplasts to regenerate host tissues[51-54]. While many of these materials have shown promise in various aspects of regenerative medicine, it is important to note that all create a "foreign body reaction," whereby a foreign material is introduced into human host tissues. Platelet concentrates collected from whole blood was first introduced over 20 years ago.

The concept was developed with the aim of utilizing human blood proteins as a source of growth factors capable of supporting angiogenesis and tissue ingrowth based on the notion that blood supply is a prerequisite for tissue regeneration[55]. Four aspects of

wound healing have since been described as key components for the successful regeneration of human tissues (Figure 1). These include 1) hemostasis, 2) inflammation, 3) proliferation, and 4) maturation.

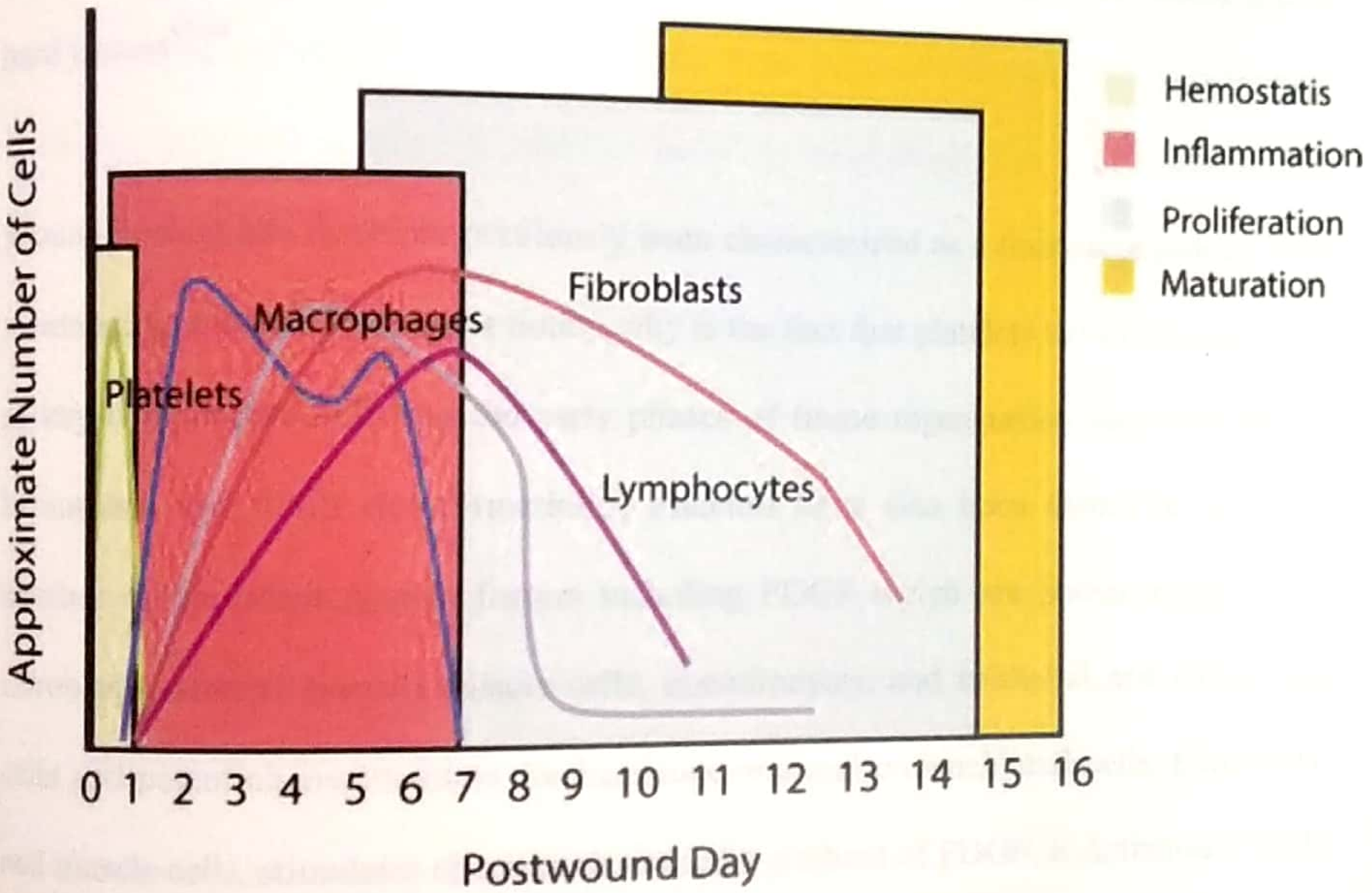


Figure 1. Four phases of wound healing including 1) hemostasis, 2) inflammation, 3) proliferation, and 4) maturation.

Noteworthy are the overlaps between each of the phases and the population of cells found in each category. Whereas lymphocytes typically arise at 7 days, the ability for

PRF to introduce a high number at day 0 acts to speed the regenerative phase during this process.

It must further be noted that in general, wound healing demands the complex interaction of various cell types with a three dimensional extracellular matrix as well as soluble growth factors capable of facilitating regeneration[56]. Certainly, one area of research in dentistry that has gained tremendous momentum in recent years is that of recombinant growth factors where a number have been used to successfully regenerate either soft or hard tissues[57-59].

Wound healing has therefore previously been characterized as a four-stage process with overlapping phases[57-59]. What is noteworthy is the fact that platelets have been described as key components affecting the early phases of tissue regeneration important during hemostasis and fibrin clot formation[56]. Platelets have also been shown to secrete a number of important growth factors including PDGF which are potent mitogens for fibroblasts, arterial smooth muscle cells, chondrocytes, and epithelial and endothelial cells and potent chemoattractant for hematopoietic and mesenchymal cells, fibroblasts, and muscle cells, stimulates chemotaxis toward a gradient of PDGF, it Activates TGF-β, stimulates neutrophils and macrophages, mitogenesis of fibroblasts and smooth muscle cells, collagen synthesis, collagenase activity, and angiogenesis. TGF-β1 stimulates fibroblast chemotaxis and proliferation and stimulates collagen synthesis and decreases dermal scarring. It is Growth inhibitor for epithelial and endothelial cells, fibroblasts, neuronal cells, hematopoietic cell types, and keratinocytes and antagonizes the

biological activities of EGF, PDGF, acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF).

It is interesting to point out that the use of platelet concentrates have dramatically increased in popularity over the past decade since the discovery of PRF. These first attempts to use concentrated platelet growth factors was derived from the fact that supra-physiological doses could be obtained from platelets to promote wound healing during and following surgery[60-61]. These concepts were later established into what is now known as "platelet rich plasma" (PRP). One of the other drawbacks of PRP was the fact that it was liquid by nature, and therefore required its combination with other biomaterials including bone grafts derived from human cadavers (allografts) or animal products (xenografts), thereby further combining its use with other "unnatural" products.

From this perspective, a second generation platelet concentrate, without the use of anti-coagulants, was therefore developed with shorter preparation times termed platelet rich fibrin (PRF) [8]. During this harvesting procedure, many of the cells were trapped within the fibrin matrix along with growth factors[3]. PRF contains a variety of cells, which have individually been studied for their role in the regeneration process The protocol was developed using a simpler centrifugation protocol requiring only 1 cycle of 12 minutes at 2700 rpm (750 g). The original objective was to spin at high centrifugation speeds in order to phase separate the layers between the red corpuscle base and the overlaying clear liquid containing leukocytes and plasma. As no anti-coagulants were utilized, the resultant formulation came with a three-dimensional fibrin scaffold termed PRF[11, 62, 63].

PRF macrophages and neutrophils contained within PRF are naturally one of the first cells found within infected wounds.

Three main components of PRF have been noted as being key components assisting in tissue regeneration. PRF not only contains host cells, but also contains a three-dimensional fibrin matrix containing various growth factors. These include (TGF-β), PDGF and VEGF, IGF, and EGF. Recent research has more specifically shown how leukocytes (as opposed to platelets) are the main implicators in the tissue wound healing process capable of further enhancing new blood vessel formation (angiogenesis) and tissue formation[10, 11, 62-64].

PRF serves all three important criteria for tissue regeneration including 1) serving as a three-dimensional fibrin scaffold, 2) includes autologous cells such as leukocytes, macrophages, neutrophils, and platelets, and 3) serves as a reservoir of natural growth factors that may be released over a 10- to 14-day period.

To our great interest, wounds that were initially covered with PRF and plastic "Saran" wrap began to heal in as early as 10 days,

TGF-β1: Transforming growth factor β (TGF-β) is a superfamily of more than 30 members described in the literature as fibrosis agents[65-66]. Platelets are known to be a major source of TGF-β production. The role of TGF-β mediates tissue repair, immune modulation, and extracellular matrix synthesis. Bone morphogenetic proteins (BMPs)

are also part of the TGF subfamily. TGF-β1, the predominant isoform, is important in wound healing, with roles in inflammation, angiogenesis, re-epithelialization, and connective tissue regeneration[67]. This growth factor is crucial during bone formation contributing to osteoblast precursors in chemotaxis and mitogenesis, and stimulates osteoblast deposition of mineralized tissue on the bone collagen matrix. It is also reported that TGF-β1 can upregulate VEGF, thereby favoring angiogenesis and recruitment of inflammatory cells. Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among all cytokines and the growth factor commonly released from autogenous bone during tissue repair and remodeling[67].

PDGF: Platelet-derived growth factors (PDGFs) are essential regulators for the migration, proliferation, and survival of mesenchymal cell lineages and promotes collagen production for remodeling of ECM during wound healing[68-73]. Platelets are the major source of PDGF with various groups divided into homo- (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and hetero-dimeric (PDGF-AB) polypeptide dimers linked by disulfide bonds. They are present in large amounts in platelet α-granules. Interestingly, PDGF is accumulated in high quantities in the PRF matrix and are considered one of the important released molecules over time from PRF. It is important to note that since PDGF has an extremely short half-life, the PRF matrix acts to support its slow and gradual release over time. PDGF is also a major mitogen for osteoblasts and undifferentiated osteoprogenitor cells, fibroblasts, smooth muscle cells, and glial cells. Since it plays such a critical role in the mechanisms of physiologic healing, a

commercially available recombinant source (rhPDGF-BB) was made available having received FDA approval for the regeneration of various defects in medicine and dentistry.

The release profile of growth factors has been an important and highly debated research topic over the past years. These differ significantly between PRP and PRF. Development of PRF enabled scientists to control and enrich growth factors from platelet concentrates. This won achieved by allowing a slower and gradual release of growth factors over time. This second generation of platelet concentrate contains leukocytes within the fibrin matrix also allowed for an enhanced secretion of growth from these cells involved in tissue regeneration[74]. PDGF-AB and TGF-β1 released from two different platelet concentrates including A-PRF and i-PRF. The result of our study shows that i-PRF released a higher total amount of growth factors when compared to A-PRFS over a 10-day period.

The aim of the present study was to compare PDGF AB and TGF β1 release from two different platelet concentrates i.e. A-PRF and i-PRF, while the advancements made in terms of platelet concentrates have been hypothesized to improve tissue regeneration[75], no information is available to date regarding the comparative growth factors released from these two platelet concentrates over time.

Therefore, the aim of the present study was to investigate in detail two different growth factors including PDGF-AB, and TGF-β1 on protein release over time from A-PRF and i-PRF. Three things stand out from the results in the current investigation. First, it was found that i-PRF released the highest amount of growth factors at early time points when

compared to A-PRF. The fast action of released proteins found in i-PRF concentrates may be hypothesized to speed the recruitment of incoming progenitor cells in defect locations and could prove a valuable means in medical and dental procedures requiring rapid incoming recruitment of regenerative cells.

Secondly, while i-PRF had rapid release of growth factors, it was interesting to note that over time, i-PRF not only had more growth factor released at later time points but also contained more growth factors as a whole from within their fibrin matrix. One of the hypothesized reasons for this is the fact that i-PRF have been shown to contain more living cells as the low-speed centrifugation concept (LSCC) indicates that reducing the relevant centrifugation force (RCF) advances PRF matrices with an enhanced number of inflammatory cells and platelets. Therefore, these cells are likely the contributing difference between the results observed between A-PRF and i-PRF.

Lastly, one of the surprising findings from the present study was the significant increase in total protein released between A-PRF and i-PRF. The subsequent significant increase in total protein release may therefore present additional advantages for clinical use.

Although this is the first report to investigate release of growth factors from A-PRF and i-PRF, previous authors have investigated protein release from either PRP or PRF and further investigated its subsequent effect on cell activity. In a first study, El- Sharkawy et al. demonstrated that PRP was able to promote increases in PDGF-AB, PDGF-BB, TGF-B1, VEGF, and EGF when compared to whole blood[76].

Furthermore, in another previous study it has been shown that PRF could release various growth factors including PDGF-AB, TGFβ1, VEGF, EGF, and IGF-1[77]. The results from that study demonstrate an increase in growth factor over time from 5 to 300 min but did not look at later time points. Furthermore, the release of growth factors was not compared to a second platelet concentrate making it difficult to investigate the potency of PRF in comparison to either PRP or A-PRF[77]. It has also previously been shown that PRP has high levels of secreted PDGF and TGFβ[78]. This subsequently stimulates collagen synthesis in periodontal ligament (PDL) cells and gingival fibroblasts[79, 80,] induces cell proliferation[81], and mineralization potential in osteoblasts[82], and increases endothelial cell activity[83] in vitro. Gassling et al. showed that osteoblasts and fibroblasts that were cultured with PRP or PRF demonstrated varying expression of various growth factors with those cultured with PRP favoring significantly higher levels of PDGF-AB and TGFβ1 expression[84]. Furthermore, a second report by this group compared PRF (as a membrane) to bovine-derived collagen membranes (BioGide) and tested osteoblast response to the two biomaterials[85]. It was found that cell growth was significantly higher on PRF when compared to the bovine collagen membrane[85].

Another aspect that needs to be considered when comparing in vivo work with in vitro studies is the variability in growth factor concentrations between donors. In the present study, the patient age range was between 20-60 years, and limited to a particular epidemiological area. We found reported mean differences between PDGF-AB and TGF-β1 growth factor accumulation between donors as reported in Table 7 and 8. Furthermore, with an increasingly aging population continuously requiring regenerative

procedures, one can only expect that with advanced age (and the likelihood of increased systemic diseases and medications), a much larger variability may also be expected. Therefore, ongoing research investigating the optimal concentrations may be required to further optimize this avenue of research. It was also found in the present study that the slower spinning protocols of i-PRF released more growth factor than the prototype A-PRF.

As one previous report demonstrated that i-PRF contains more platelets and neutrophilic granulocytes[43], it may be hypothesized that these cells contributed to the slight increase in total growth factor accumulation after a 10-day period. This hypothesis however requires further investigation. There remain several aspects of research necessary to further compare the various platelet formulations investigated in this study. First, it is unclear how the release of the various platelet concentrates including PRP, PRF, and A-PRF will affect cell behavior over time.

Therefore, a comparative in vitro cell study further investigating the use of A-PRF and i-PRF on cell behavior of various cell types including osteoblasts, gingival fibroblasts, and periodontal ligament cells could further provide rationale for which treatment modalities stimulates a higher cell response. Furthermore, it is known that platelet concentrates are often combined with various biomaterials such as collagen membranes and bone grafting materials. Therefore, it would also be worthwhile to compare growth factor release from a variety of biomaterials following coating with either A-PRF or i-PRF. Future research comparing the various platelet formulations in a clinical setting

would also be valuable to compare which indications may serve better for various clinical scenarios.

CONCLUSION

CONCLUSION

Within limitations, the present study concluded that:

- Protein quantification done by ELISA reveals that i-PRF has greater concentration release of PDGF-AB and TGF-β1 over a period of 10 days.
- i-PRF may serves as a better alternative to A-PRF for future regenerative procedures in periodontal fields.

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APPENDICES

APPENDIX-I

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL FORM

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **Quantitative Analysis and Comparison of Growth Factors (PDGF-AB and TGF β_1) in Advanced and Injectable –Platelet Rich Fibrin: An In-Vitro Study** submitted by **Dr. Anshul** Post graduate student from the Department of **Periodontics** as part of MDS Curriculum for the academic year 2016-2019 with the Accompanying proforma was reviewed by the institutional research committee present on **7th and 8th December 2016** at BBDCODS. The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the institutional ethics committee for granting ethical approval.

Prof. (Dr). Vivek Govila
Principal

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
Faizabad Road, Lucknow-226028

Chairperson Institutional Research Committee

APPENDIX-II

ETHICAL COMMITTEE APPROVAL FORM

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the Vth Institutional Ethics Sub-Committee

IEC Code: 01

BBDCODS/03/2017

Title of the Project: Quantitative Analysis and Comparison of growth factors (PDGF-AB and TGF β1) in advanced and Injectable–Platelet Rich Fibrin: An in vitro study.

Principal Investigator: Dr. Anshul

Department: Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Anshul,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 02nd March, 2017.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Neerja Singh Member	Prof. & Head, Department of Pedodontics, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Manu Narayan Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The proposal was reviewed, comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 01/05/17

(Dr. Lakshmi Bala)
Member-Secretary
IEC

(Dr. Vivek Govila)
Principal
BBDCODS

APPENDIX- III

FORMULA USED FOR THE ANALYSIS

Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Standard deviation and standard error

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

and SE (standard error of the mean) is calculated as

$$SE = \frac{SD}{\sqrt{n}}$$

Where, n= no. of observations

Minimum and Maximum

Minimum and maximum are the minimum and maximum values respectively in the measure data and range may be dented as below

$$\text{Range} = \text{Min to Max}$$

and also evaluated by subtracting minimum value from maximum value as below

$$\text{Range} = \text{Maximum value-Minimum value}$$

Median

The median is generally defined as the middle measurement in an ordered set of data. That is, there are just as many observations larger than the median as there are smaller. The median (M) of a sample of data may be found by first arranging the measurements in order of magnitude (preferably ascending). For even and odd number of measurements, the median is evaluated as

$$M = [(n+1)/2]^{th} \text{ observation- odd number}$$

$$M = [n(n+1)/2]^{th} \text{ observation – even number}$$

Wilcoxon Signed Rank Test

The Wilcoxon signed-rank test is a non-parametric statistical hypothesis test used to compare two related samples, matched samples, or repeated measurements on a single sample to assess whether their population means ranks differ. And also for ordered

categorical data where a numerical scale is inappropriate but where it is possible to rank the observations.

The formula for calculating the Z statistic for Wilcoxon Signed Rank Test is given below:

$$z = \frac{T - \frac{n(n+1)}{4}}{\sqrt{\frac{n(n+1)(2n+1)}{24}}}$$

Friedman's Test

The Friedman test is a **non**-parametric alternative to ANOVA with repeated measures. It is used to test for differences between groups when the dependent variable being measured is non-parametric in nature and a continuous data. The Friedman test tests the Null hypothesis of identical populations for dependent data.

The formula for calculating the Q statistic for Friedman's Test is given below:

$$\chi r^2 = \left[\left(\frac{12}{N*C*(C+1)}\right)*\Sigma Tc^2\right] - 3*N*(C+1)$$

Post Hoc Test

Post-hoc (Latin, meaning "after this") means to analyze the results of experimental data. In a scientific study, **post hoc analysis** (from Latin **post hoc**, "**after** this") consists of **analyses** that were not specified before seeing the data. This typically creates a multiple testing problem because each potential **analysis is** effectively a statistical test.

$$\alpha = \frac{\alpha}{\text{Number of tests}}$$

Statistical significance

Level of significance "p" is the probability signifies level of significance. The mentioned p in the text indicates the following:

$p > 0.05$ Not significant

$p < 0.05$ Just significant

$p < 0.01$ Moderate significant

$p > 0.05$ Highly significant

APPENDIX- IV

CASE RECORD PROFORMA

Name of the patient:

Age / Sex: **O.P. No. :**

Address & Ph. No.: **Occupation:**

BLOOD PREPARATIONS REQUIRED FOR HAEMATOLOGICAL INVESTIGATIONS

1) Previous hemorrhagic episodes after trauma or surgery, or even spontaneous bleeding:

2) Family history regarding hereditary bleeding disorders:

3) Current illness i.e. Hepatic/renal failure:

4) List of medications interfering with haemostatis:

a) Nonsteroidal anti-inflammatory drugs

b) Antibiotics

5) Anticoagulation medication:

- **Coumarin**
- **Heparin**

APPENDIX- V

Consent Form (English)

Title of the Study .....

Study Number.....
Subject's Full Name.....
Date of Birth/Age .....
Address of the Subject.....
Phone no. and e-mail address.....
Qualification .....
Occupation: Student /Self Employed / Service / Housewife/
Other (Please tick as appropriate)
Annual income of the Subject.....
Name and of the nominees(s) and his relation to the subject..... (For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated .....for the above study and have had the opportunity to ask questions. OR I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. Yes [ ] No [ ] Not Applicable [ ]
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:..... Date .....
Signatory's Name..... Date.....
Signature of the Investigator..... Date.....
Study Investigator's Name..... Date.....
Signature of the witness.....
Name of the witness.....
Received a signed copy of the PID and duly filled consent form
Signature/thumb impression of the subject or legally Acceptable representative Date.....

सहमति पत्र

अध्ययन शीर्षक.....

अध्ययन संख्या.....

प्रतिभागी के पूर्ण नाम.....

जन्म तिथि / आयु.....

पता .....

1. मेरी पुष्टि है कि मैनें अध्ययन हेतु सुचना पत्र दिनांक ..... को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैं यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और मै किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हुँ ।

3. मै यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हुँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।

5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हुँ। हॉ [ ] नही [ ] अनउपयुक्त [ ]

6. मै परीक्षण की अनुमति देता हुँ। मुझे इसके द्वारा यदि कोई परेशानी होती है, के बारे में जानकारी दे दी गई है। मैने रोगी जानकारी सूचना पत्र को पढ तथा समझ लिया है।

प्रतिभागी / कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान.....

हस्ताक्षरकर्ता का नाम..... दिनांक .....अन्वेषक के

हस्ताक्षर ..... दिनांक .....

अध्ययन अन्वेषक का नाम .....

गवाह के हस्ताक्षर ..... दिनांक .....गवाह के

नाम .....मैनें हस्ताक्षर युक्त सुचना तथा सहमति पत्र प्राप्त किया।

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर/ अंगूठे का निशान ..... दिनांक.....

Babu Banarasi Das College of Dental Sciences

(A constituent institution of Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Participant Information Document (PID)

1. Study title

QUANTITATIVE ANALYSIS AND COMPARISON OF PDGF-AB AND TGF β1 IN A-PRF AND i-PRF: AN IN VITRO STUDY

2. Invitation paragraph

You are being invited to take part in a research study, therefore it is important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

3. What is the purpose of the study?

The purpose of this study is to quantitative analysis and comparison of PDGF-AB and TGF β1 levels in A-PRF and i-PRF.

4. Why have I been chosen?

You have been chosen for this study as you are fulfilling the required criteria for this study.

5. Do I have to take part?

Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

Twenty milliliter venous blood sample will be collected.

7. What do I have to do?

You do not have to change your regular lifestyles for the investigation of the study.

8. What is the procedure that is being tested?

Twenty milliliter venous blood will be collected in a vacutainer, PDGF-AB and TGF β levels will be quantified using ELISA assays on 1st, 3rd and 10th day, according to manufacturer's protocol. The obtained data will be subjected to statistical analysis.

9. What are the interventions for the study?

Collection of twenty milliliter venous blood.

10. What are the side effects of taking part?

There are no side effects on patients of this study.

11. What are the possible disadvantages and risks of taking part?

There is no risk or disadvantages of taking part in this study.

12. What are the possible benefits of taking part?

This study will analyze and compare PDGF-AB and TGF β1 levels in A-PRF and i-PRF at specific time intervals. This will help in formulating treatment plan for affected population.

13. What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

14. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

15. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and Institutional ethical community, and treatment cost will be beard by principal investigator.

16. Will my taking part in this study be kept confidential?

Yes it will be kept confidential.

17. What will happen to the results of the research study?

The results of the study will be used to compare the levels of PDGF-AB and TGF β1 in A-PRF and i-PRF. This will help in determining their use in surgical procedure.

18. Who is organizing the research?

This research study is organized by the academic institution (BBDCODS).

19. Will the results of the study be made available after study is over?

Yes.

20. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Department, and the IEC/IRC of the institution.

21. Contact for further information

Dr. Anshul

Department of periodontics

Babu Banarasi Das College of Dental Sciences.

Lucknow-227105

Mob- 9198726666

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Dr. Laxmi Bala,

Member Secretary,

Babu Banarasi College of Dental Sciences.

Lucknow

bbdcods.iec@gmail.com

Signature of PI ..... eAnshul .....

Name ..... ANSHUL .....

Date ..... 20-2-17 .....

Babu Banarasi Das College of Dental Sciences

(A constituent institution of Babu Banarasi Das University)

BBD City, Faizabad road, Lucknow – 227105 (INDIA)

प्रतिभागी जानकारी दस्तावेज (पीआईडी)

1. **अध्ययन का शीर्षक**

मात्रात्मक विश्लेषण एवं तुलना करना PDGF-AB एवं TGF β1 की A-PRF एवं i-PRF में: एक पात्रे अध्ययन

2. **निमंत्रण अनुच्छेद**

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किये जा रहा है, इसलिए आप का जानना महत्वपूर्ण हैं कि ये अध्ययन क्यों किया जा रहा है और इसकी क्या प्रक्रिया हैं कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने के लिए समय लें। किसी भी स्पष्टीकरण या अधिक जानकारी के लिए पूछें। चाहे आप भाग लें या न लें यह आपका निर्णय है।

3. **अध्ययन का उद्देश्य क्या है?**

अध्ययन का उद्देष्य A-PRF एवं i-PRF में PDGF-AB एवं TGFβ1 की मात्रात्मक विश्लेषण एवं तुलना करना है।

4. **मैं क्यों इस अध्ययन के लिए चुना गया ?**

आप इस अध्ययन के लिए चुने गये है क्योंकि अवश्यक मानदण्डों को पूरा कर रहे है।

5. **क्या मुझे भाग लेना है।**

अनुसंधान के क्षेत्र में आपकी भागीदारी पूरी तरह स्वैच्छिक है। यदि आप भाग लेते है तो आपको इस जानकारी को रखने के लिए पत्र दिया जाएगा और एक सहमति पत्र पर हस्ताक्षर करने के लिए कहा जाएगा। अध्ययन के दौरान आप किसी भी समय बिना कारण बतायें निस्कासित होने के लिए स्वतंत्र है।

6. **अगर मैं इस अध्ययन में भाग लेता हूं तो मुझे का क्या होगा?**

मेरे अध्ययन के लिए आपके 20 मि0ली0 शिरा रक्त का नमूना लिया जायेगा।

7. **मुझे क्या करने की जरूरत है?**

आपको अध्ययन की जॉच के लिए अपने नियमित जीवन शैली बदलने की जरूरत नहीं है। इस शोध अध्ययन आत्म–प्रायोजित उम्मीदवार के द्वारा होता है। आपको किसी भी शामिल प्रक्रियाओं के लिए भुगतान करने की जरूरत नहीं हैं।

8. **किस प्रक्रिया का परीक्षण किया जा रहा है?**

20मि0ली0 शिरा रक्त को वैक्यूटेनर में एकत्रित किया जाना है तदापरान्त PDGF-AB एवं TGFβ1 के स्तर का मात्रात्मक विश्लेषण ELISA विधि द्वारा प्रथम, तृतीय और दसवां दिन उत्पादक संलेख के अनुसार किया जाना है। एकत्रित संगृह का संख्यिकिक विश्लेषण किया जायेगा।

इस अध्ययन के लिए कौन हस्तक्षेप कर रहे है?

9. 20 मि0ली शिरा रक्त ।

भाग लेने के दुष्प्रभाव क्या है?

10. इस अध्ययन का मरीजों पर कोई दुष्प्रभाव नहीं है।

संभावित नुकसान और भाग लेने का जोखिम क्या है?

11. इस अध्ययन में मरीजों पर कोई सम्भव नुकसान/जोखिम नहीं हैं।

भाग लेने के संभावित लाभ क्या है?

12. इस अध्ययन के द्वारा PDGF-AB एवं TGF β1 के स्तर का विश्लेषण एवं तुलना A-PRF एवं i-PRF में एक निर्धारित समय पर किया जाना है जिसके माध्यम से इलाज की प्रक्रिया को निर्धारित कर सकते है।

13. अगर कोई नई जानकारी उपलब्ध हो जाती है?

यदि शोध के दौरान कोई नई जानकारी उपलब्ध हो जाती है तो आपको सूचित किया जायेगा और आप इस पर चर्चा करने के लिए स्वतंत्र हैं। यदि आप शोध से निष्कासित होने का निर्णय लेते हैं तो शोधकर्ता आपकी निष्कासिता की व्यवस्था कर देगें। यदि आप भाग लेते हैं तो आपको एक अद्यतन सहमति पत्र पर हस्ताक्षर करने के लिए कहा जायेगा।

14. जब शोध अध्ययन बंद हो जाता है क्या होता है?

जब अध्ययन निर्धारित समय से पहले बंद हो जाता है/खत्म हो जाता है। इसका कारण मरीज/स्वयंसेवक को समझाया जायेगा।

15. जब कुछ गलत हो जाता है?

किसी भी गम्भीर प्रतिकूल घटना होती है, या कुछ और अंध्ययन के दौरान गलत हो जाता है, शिकायतों को संस्था (एस), और आईईसी में रिपोर्टिंग करके नियंत्रित किया जाएगा और इलाज का सारा खर्च शोधार्थी द्वारा व्यय किया जायेगा।

16. क्या मेरी सहभागिता को गोपनीय रखा जाएगा?

हाँ, आपकी सहभागिता गोपनीय रखी जायेगी

17. शोध अध्ययन के परिणामों का क्या होगा?

अध्ययन के परिणाम A-PRF एवं i-PRF में PDGF-AB एवं TGF β1 के स्तर की तुलना की जायेगी। जिसकी मदद से हम शल्य चिकित्सा में इनकी उपयोगिता देख सकते है।

18. इस शोध का आयोजन कौन कर रहा है?

इस शोध का आयोजन अकादम्कि संस्थान (बी0बी0डी0को0ड0स0) द्वारा किया जा रहा है।

19. अध्ययन खत्म होने के बाद क्या परिणामों को उपलब्ध कराया जायेगा?

हाँ,

20. किसने अध्ययन की समीक्षा की है?

अध्ययन की समीक्षा विभाग के प्रमुख और संस्था के आईईसी0 द्वारा अनुमोदित किया गया है।

अधिक जानकारी के सम्पर्क करें:–

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APPENDIX- VI

Master Chart

Sl	Groups	pdgfab_D1	pdgfab_D3	pdgfab_D10	tgfB1_D1	tgfB1_D3	tgfB1_D10	pdgf ab	tgf b1
1	A-PRF	357.27	268.18	129.09	184.18	130.45	61.82	251.52	125.48
2	A-PRF	244.55	207.27	81.82	161.82	81.36	57.27	177.88	100.15
3	A-PRF	264.55	226.36	72.73	171.36	122.73	66.82	187.88	120.30
4	A-PRF	387.27	109.09	78.18	207.82	126.82	62.27	191.52	132.30
5	A-PRF	390.91	320.00	314.55	185.64	165.45	67.27	341.82	139.45
6	A-PRF	325.45	160.00	110.91	206.82	128.64	43.18	198.79	126.21
7	A-PRF	335.45	303.64	120.91	187.27	167.27	65.00	253.33	139.85
8	A-PRF	366.36	291.82	110.91	165.45	105.91	99.55	256.36	123.64
9	A-PRF	486.36	457.27	193.64	170.91	136.36	34.09	379.09	113.79
10	A-PRF	330.00	311.82	244.55	195.09	147.27	59.55	295.45	133.97
11	A-PRF	350.91	259.09	125.45	187.27	131.36	56.82	245.15	125.15
12	A-PRF	323.64	164.55	115.45	191.36	105.91	58.64	201.21	118.64
13	A-PRF	360.91	286.36	110.00	167.27	107.73	45.45	252.42	106.82
14	A-PRF	290.91	291.82	123.64	189.55	139.55	55.45	235.45	128.18
15	A-PRF	378.18	285.45	107.27	196.36	127.73	53.18	256.97	125.76
16	I-PRF	370.00	310.91	131.82	209.09	158.64	107.73	270.91	158.48
17	I-PRF	289.09	149.09	113.64	168.18	130.00	112.27	183.94	136.82
18	I-PRF	452.73	182.73	99.09	175.00	153.64	55.00	244.85	127.88
19	I-PRF	456.36	356.36	629.09	235.91	181.36	123.64	480.61	180.30
20	I-PRF	451.82	267.27	140.91	183.64	116.36	62.27	286.67	120.76
21	I-PRF	449.09	410.00	356.36	207.27	198.64	104.09	405.15	170.00
22	I-PRF	295.45	185.45	103.64	215.00	130.00	93.64	194.85	146.21
23	I-PRF	341.82	190.00	59.09	185.91	123.18	46.82	196.97	118.64
24	I-PRF	444.55	208.18	17.27	191.82	164.09	50.45	223.33	135.45
25	I-PRF	328.18	215.45	60.91	182.73	174.09	58.64	201.52	138.48
26	I-PRF	425.45	274.55	174.55	195.45	154.09	62.27	291.52	137.27
27	I-PRF	388.18	240.00	157.27	206.82	164.09	56.82	261.82	142.58
28	I-PRF	324.55	277.27	201.82	204.09	153.18	52.27	267.88	136.52
29	I-PRF	439.09	229.09	151.82	180.91	126.82	58.64	273.33	122.12
30	I-PRF	425.45	292.73	229.09	177.73	152.27	54.55	315.76	128.18