Scientific Research Journal of Clinical and Medical Sciences
ISSN Print : 2788-8843 | **ISSN Online :** 2788-8851
Frequency : Bi-Monthly
Language : English
Origin : Kenya
Website : https://www.iarconsortium.org/journal-info/srjcms

OPEN ACCESS JOURNALS

Review Article

Lyophilized Platelet-Rich Plasma: Advantages and Applications in COVID-19 Patients

Article History

Received: 20.10.2021
Revision: 30.10.2021
Accepted: 10.11.2021
Published: 20.11.2021

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How to Cite the Article:
Mahajan Pradeep, Kulkarni Ajit, Subramanian Swetha, & Mahajan Sanskruti. (2021); Lyophilized Platelet-Rich Plasma: Advantages and Applications in COVID-19 Patients. *SRJ Clin & Med Sci. 1*(3) 5-10.

DOI: 10.47310/srjcms.2021.v01i03.002

Abstract: The current coronavirus disease 19 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and its variants. Though various vaccines are now available in the market and become effective in reducing the severity of disease and hospitalization, still for those who are infected with SARS-CoV-2 there is no effective treatment to deal with this health emergency, and therefore, there is an urgent need for the new treatments. The design of new therapies must be focused not only on the elimination of the virus but also on the recovery of the affected lung tissue and the avoidance or reduction of the severe sequels. In that sense, treatments based on regenerative medicine and specifically platelet-rich plasma (PRP) could be found suitable and effective to tackle this disease. Lyophilized PRP in nebulized form can be effective as it directly enters the lungs and unique properties of PRP such as immunomodulatory, anti-inflammatory, angiogenic, anti-fibrotic and anti-apoptotic, anti-bacterial properties as well as stimulation of different cells, etc. exerts their effect to improve the health condition rapidly. However, further studies are warranted to prove its efficacy. In this review, we used words like COVID-19, drugs used in the nebulized form in COVID-19, platelets, the role of platelets in various disease conditions, platelet-rich plasma, nebulization, lyophilization for selecting, extracting, and synthesizing data using Google search engine, PubMed search. This review is focused on the advantages and applications of LPRP in nebulized form as a treatment in COVID-19 patients

Keywords: PRP, COVID-19, Nebulization, Platelets, Platelet rich plasma, Lyophilized platelet rich plasma.

Running title: Lyophilized platelet rich plasma in COVID-19 patients

Abbreviations:
COVID-19: Coronavirus disease-19
CTGF: Connective tissue growth factor
EGF: Epidermal growth factor
FGF-2: Fibroblast growth factor-2
HCV: Hepatitis C virus
HGF: Hepatocyte growth factor
HIV: Human immunodeficiency virus
IGF -1, 2: Insulin-like growth factor-1,2
IkB: Inhibitor of Nuclear factor kappa B cells
IL: Interleukin
LPRP: Lyophilized Platelet rich plasma
NF-kB: Nuclear factor kappa B cells
PDGF: Platelet-derived growth factor
PRF: platelet-rich fibrin
PRP: Platelet rich plasma
RBCs: Red blood cells
SARSCoV-2: severe acute respiratory syndrome coronavirus 2
TGF β: Transforming growth factor beta
TNF-α: Tumor necrosis factor alpha
VEGF: Vascular endothelial growth factor
WBCs: White blood cells

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) virus and its variants are responsible for current COVID-19 pandemic [Harvey WT *et al.*, 2021, Singh J *et al.*, 2021]. People infected by SARSCoV-2 virus can progress either asymptomatically or develop the coronavirus disease 2019 (COVID-19) that can cause acute respiratory distress syndrome (ARDS), cardiovascular complications which might progress to death in severe cases. COVID-19 infection causes sequels such as pulmonary fibrosis, which results in progressive reduction in the patients' respiratory capacity and thus severely affects their quality of life [Beitia M *et al.*, 2021, Zheng YY *et al.*, 2020, Rothan HA *et al.*, 2020, Guan WJ *et al.*, 2020, Wu C *et al.*, 2020, George PM *et al.*, 2020, Spagnolo P *et al.*, 2020]. Though the vaccines are now available and are effective in reducing severity of disease and hospitalization [Wang J *et al.*, 2020], still for those infected patients the effective treatment is not yet available to deal with this health emergency, so there is urgency for new treatments. The focus of new therapies should on virus elimination as well as on quick recovery of affected lung tissues and also prevention or minimize severe sequels. In that sense, PRP as a regenerative medicine therapy could be found suitable and effective to control this disease [Beitia M *et al.*, 2021]. Platelets are the important player in respiratory disorders caused by virus infections, act as first line of defence against viruses and also are essential in maintaining lung function [Qiu J *et al.*, 2020]. PRP is plasma having platelet count above that of peripheral blood. The PRP terminology is used by haematologists in 1970's. PRP is known by different names such as platelet- rich growth factors, platelet-rich fibrin (PRF), and platelet concentrate (PC) etc. PRP as a transfusion product was used initially to treat patients with thrombocytopenia. After a decade, PRP was started to be used in maxillofacial surgery because of the presence of fibrin which has adherence and homeostatic properties and also because of anti-inflammatory properties of PRP which stimulated cell proliferation. Subsequently, PRP has gained widespread attention and predominantly used in musculoskeletal field in sport injuries [Alves R *et al.*, 2018].

Platelet rich plasma (PRP) is a regulatory agencies approved biological therapy based on platelet concentration present in blood [Beitia M *et al.*, 2021]. PRP is a unique and advanced treatment which speedup the body's natural healing process. Platelet lysate obtained from platelet rich plasma of healthy donors consists of various growth factors, chemokines, cytokines and antibacterial molecules and have an anti-inflammatory, immunomodulatory, anti-fibrotic, and repairing effects which could help in the improvement of patient's health [Beitia M *et al.*, 2021, Alves R *et al.*, 2018, Pavlovic V *et al.*, 2016, Kieb M *et al.*, 2017]. Lyophilization of PRP causes concentration of growth factor can enable longer shelf life of product and makes it easier for transport and storage; enhance stability of product [Carpentier SC *et al.*, 2007].

PRP has now been used in other medical fields such as cardiac surgery, plastic surgery, paediatric surgery, ophthalmology, urology, gynaecology and dermatology etc. [Beitia M *et al.*, 2021].

The current review is focussed on advantages of lyophilized PRP, and its applications with special attention to COVID-19.

Methods Used for Locating, Selecting, Extracting, and Synthesizing Data

For searching the published article, we conducted an electronic search of the using Google search, Medline, PubMed databases. All literature was searched for and reviewed by two authors independently. Search results were then cross-examined, and any differences were settled by consensus. In this review, we used words like COVID-19, drugs used in nebulized form in COVID-19, platelets, role of platelets in various disease conditions, platelet rich plasma, nebulization, lyophilisation for selecting, extracting, and synthesizing data.

This review article reviews the advantages and applications of LPRP. This review article presents a baseline from where further prospective trials can be designed and help as a spur for further research in this commonly encountered clinical entity as well as current COVID-19 pandemic where not more studies are done.

Role of Platelet in Various Conditions

Platelets are originating from megakariocytes in bone marrow. Platelets or thrombocytes are small, discoid, anucleated, colourless cell fragments of 1-3 µm size and having 8 to 12 days life span. Platelets contain 3 types of granules namely α-granules, delta granules and lambda granules. The α-granules are about 200-500 nm in size and becomes the most abundant granule type in platelets (approx. 50 to 80 granules per thrombocyte and constitute approximate 10% of platelet volume).These granules mostly consist of various cytokines, chemokines, membrane proteins, and proteases, as well as pro-inflammatory and anti-inflammatory mediators. They are important for regulation of cellular processes such as chemotaxis, mitogenesis and differentiation; stimulation of local mesenchymal stem cells and epithelial cells to migrate, divide and increase the collagen and matrix synthesis which results in fibrous connective tissue and scar formation. Also, their combine action and interaction with each other activates different intracellular signalling pathways in damaged tissues that resulted in enhanced tissue repair [Pavlovic V *et al.,* 2016]. In spite of small size and relatively short life span, platelets are crucial for various significant biological processes such as haemostasis, thrombosis, angiogenesis, wound healing, immune response, and inflammatory responses. Platelets detect and respond to pathogens in the vasculature by exhibiting many pattern recognition receptors (PRR) including toll-like receptors, C-type lectin receptors, and NOD-like receptors etc. platelets play an important role in innate immunity in the lung. Activated platelets express Toll-like receptors (TLRs), FcγRIIA receptors to capture respiratory viruses (*Respiratory syncytial virus*, Influenza A H1N1 etc.) and endocytose them. After endocytosis α-granules containing microbicidal and antiviral components destroy the virions. Platelet-derived chemokine ligand 5 (CCL5) has been effective against various viruses such as HIV-1, hepatitis C virus (HCV), and influenza A viral infections [Qiu J *et al.,* 2020, Felix E *et al.,* 2018, Koupenova M *et al.,* 2019].

Platelet Rich Plasma (PRP)

PRP is a platelet concentrate obtained by the centrifugation process of an autologous blood. PRP was first introduced by Whitman *et al.*, to the oral surgery community and since then it has been widely used in dentistry and in other medicine branches [Trindade-Suedam IK *et al.*, 2007].

PRP is a rich source of effector molecules or growth factors as well as chemokines, cytokines, and other plasma proteins which includes platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), transforming growth factor beta (TGF-β), insulin-like growth factor-1,2 (IGF-1, IGF-2), matrix metalloproteinases 2,9, Sphingsosine-1, platelet microparticles, VEGF-A/TGF-β1, Connective tissue growth factor (CTGF), IL-8 and other growth factors [Kieb M *et al.*, 2017]. These growth factors like interleukin-1 receptor antagonist (IL-1RA), an anti-inflammatory cytokines can supress proinflammatory cytokines such as IL-6, TNF-α and IL-1β. VEGF in presence of present TNF*α* in PRP acts as a powerful angiogenic agent and in presence of TNF*α* it can reduce the activation of NF-kB by interfering with IkB degradation. NF-kB is a transcription factor which can lead to induce proinflammatory cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, and TNF*α.*) thus, has a crucial role in inflammation and immunity. Thus, PRP can suppress NF-kB and thus the inflammation which is beneficial in COVID-19 patients. VEGF can differentiate progenitor cells to angiogenic and osteogenic lineages and has an important role in vascularization and patterning of the bone growth plate [Gerber HP *et al.*, 1999] as well as differentiation of osteoblast in an autocrine fashion and osteoblast apoptosis inhibition [Street J *et al.*, 2002, Street J *et al.*, 2009, Kasten P *et al.*, 2012, Hosny N *et al.*, 2015]. VEGF also stimulates migration and mitosis of endothelial cells as well as chemotaxis of macrophages and neutrophils. Activated thrombocytes and macrophages secreted VEGF in damaged tissues that cause new blood vessel formation which increased blood flow and brings nutrients to the injured site. VEGF secretion can be increased by the presence of other growth factors such as TGF-β, PDGF and EGF. The role of FGF is also important in angiogenesis [Pavlovic V *et al.*, 2016].

PDGF name was given because it was first detected in platelets. However, it was also found in other cells such as endothelial cells, fibroblasts, monocytes, and macrophages. PDGF consists of two subunits A and B and exist in three isoforms AA, AB and BB. PDGF after released from platelets causes chemotaxis and mitosis of fibroblasts, collagen synthesis and extracellular matrix remodelling. PDGF also causes chemotaxis of macrophages and neutrophils and increased secretion of TGF-β from macrophages [Pavlovic V *et al.*, 2016].

TGF-β is a member of TGF-β superfamily and consists of three isoforms (TGF-β1, TGF-β2 and TGF-β3) and bone morphogenetic factors. After injury, the thrombocytes secrete active form of TGF-β1 which stimulates collagen production, prevent breakdown of collagen, and promote angiogenesis, regeneration of connective tissue and chemotaxis of immune cells to the injured site. TGF-β1, in bone injury, stimulates proliferation of osteoblasts and inhibition of osteoclast formation and thus favours bone formation over resorption. TGF-β can act as immunosuppressor and inhibit proinflammatory cytokines [Pavlovic V *et al.*, 2016].

EGF has a role in chemotaxis and angiogenesis stimulation of endothelial cells, epithelisation and mitosis of mesenchymal cells as well as cytokine secretion which resulted in speedup healing process [Pavlovic V *et al.*, 2016]. EGF also showed antioxidant effect which is cross-linked with inflammation.

FGF acts as most potent mitogen for osteoblasts, chondrocytes and mesenchymal cells. It is involved in angiogenesis process with VEGF and stimulates growth and differentiation of osteoblasts and chondrocytes [Pavlovic V *et al.*, 2016].

IGF-1 is a polypeptide hormone of 70 amino acids and is a normal plasma component. IGF binding protein transported it to the platelets. IGF has role in differentiation and mitogenesis of mesenchymal cells as well as in bone formation by proliferating and differentiating osteoblasts [Pavlovic V *et al.*, 2016]. IGF-1 also showed antioxidant and anti-fibrogenic effect which is cross-linked with inflammation in COVID-19 patients [Beitia M *et al.*, 2021].

HGF is also one of the main components of PRP which has an anti-inflammatory role to suppress NF-kB activity and thus reduce cytokine storm in COVID-19 patients. It also acts as an anti-apoptotic, angiogenic and anti-fibrogenic agent in COVID-19 patients [Beitia M *et al.*, 2021].

Moreover, interaction of PRP growth factors with endogenous stem cells and progenitor cells in lung tissue can initiate repairing process such as angiogenesis, fibroblast activation, and collagen remodelling. VEGF and FGF stimulate migration and proliferation of healthy endothelial cells in lungs of COVID-19 patients that leads to pulmonary capillary angiogenesis. EGF and TGF-α has a role in alveolar epithelium regeneration by stimulating proliferation of endogenous stem cells [Beitia M *et al.*, 2021, Alves R *et al.*, 2018, Pavlovic V *et al.*, 2016, Kieb M *et al.*, 2017].

Platelet-Rich Plasma (PRP) as a Treatment

PRP as a regenerative medicine contains the biomolecules present in both platelets and plasma which are capable of modulating the altered biological processes. PRP has immunomodulatory, anti-inflammatory, angiogenic, anti-fibrotic and anti-apoptotic properties. It can also stimulate different cells and has no general or adverse effects on other tissues [Beitia M *et al.*, 2021, Alves R *et al.*, 2018, Pavlovic V *et al.*, 2016, Kieb M *et al.*, 2017].

Application of PRP in Various Diseases

In 1970s, haematologists initiated the use of PRP in the form of transfusion product to treat patients with thrombocytopenia due to various causes of bone marrow suppression and viral infection. Subsequently, the use of PRP becomes popular in maxillofacial surgery, and musculoskeletal field in sports injuries. PRP now widely used in other medical fields such as paediatric surgery, cardiac surgery, plastic surgery, urology, gynaecology, ophthalmology and dermatology. In diabetic foot ulcers and joint degeneration of the knee or hip the PRP has already been proven its efficacy as a regenerative medicine. In dermatology, the use of PRP in tissue regeneration, wound healing, scar revision, skin rejuvenation and alopecia treatment has increased. In chronic ulcers, high protease activity and proinflammatory biochemical environment impairs the wound healing process by reducing the effective growth factor concentration. PRP can be used as an alternative treatment for recalcitrant wounds because it is a good source of growth factors and also possess chemotactic, mitogenic and angiogenic properties. In cosmetic dermatology, PRP played a role in induction of soft-tissue augmentation, dermal fibroblast stimulation, proliferation, increase collagen synthesis, and formation of new blood vessels and adipose tissue. PRP treatment improves the postsurgical scars, burn scars; acne scars increase collagen and elastic fibres and improve the skin quality. In Rheumatoid arthritis PRP treatment results in an improvement in joint inflammation with no adverse effect. The improvement was last for 1 year [Beitia M *et al.*, 2021, Alves R *et al.*, 2018, Pavlovic V *et al.*, 2016, Kieb M *et al.*, 2017, Kasten P *et al.*, 2012, Badsha H *et al.*, 2020].

PRP as a New Approach to Prevent Infection

PRP can also be used to prevent implant-associated infections because of its antibacterial properties. Thus, PRP can be used as an advanced alternative treatment to conventional antibiotic treatments as there is less chance of antibiotic resistance. Also, healing – promoting properties and antimicrobial properties of PRP synergistically act to prevent infection. PRP was found to have strong antimicrobial properties against MRSA, MSSA, Group A *Streptococcus*, and *Neisseria gonorrhoeae* [Li H *et al.*, 2013].

Because of healing –promoting properties of PRP, it has been widely used for various clinical applications such as periodontal and oral surgeries, orthopaedic surgeries, plastic surgeries, maxillofacial surgeries, sports medicine etc. PRP administration decreases the need of invasive mechanical ventilation and accelerates recovery of lung functioning from respiratory distress in COVID-19 patients [Li H *et al.*, 2013].

PRP in Nebulized form

Nebulization is a one of the method of drug delivery to the lungs. Nebulization can already be effectively used for Asthma, Chronic Obstructive Pulmonary Disease (COPD), Bronchitis, and Seasonal allergies etc. After nebulization, biomolecules present in Platelet lysate directly reached to the lung cells by the airway and can interact with cell receptors and triggers various cellular signals. These cell responses acts in autocrine as well as paracrine fashion which results in a global response throughout tissues and generate a biological environment favourable for healing lung injuries. It is clinically proved that PRP due to its anti-inflammatory and tissue repair mechanism can prevent fibrosis in other tissues. It has been observed that in COVID-19 patients PRP nebulization does not affect lung fibroblasts proliferation and thus repair process [Beitia M *et al.*, 2021].

Other drugs or biomolecules used in nebulized forms in COVID-19

There are several drugs or biomolecules in different clinical trial phases that are tried in nebulized forms in COVID-19 patients alone or along with other drugs. These are IFN-α-2b, IFN-β1a, novel gene recombinant super compound interferon, IFN-α-1b, Janus kinase inhibitor (TD-0903), COVID-19 Specific T Cell derived exosomes (CSTC-Exo), recombinant tissue-Plasminogen Activator (rt-PA), Nebulized amniotic fluid, and Alpha one antitrypsin etc. [Karina K *et al.*, 2021, Cazzola M *et al.*, 2021, Mary A *et al.*, 2020].

PRP in lyophilized form (LPRP)

Use of PRP in lyophilized form has several advantages such as consistency in growth factor concentrations that eliminate the variability among existing PRP formulations, availability in large quantities, no depletion of growth factors, anti-bacterial activity remains unchanged, denaturation of all allergenic proteins, very few or no adverse events as it is devoid of WBCs and RBCs, cost effective, increase in stability of product [Kieb M *et al.*, 2017].

LPRP in Nebulized form

The use of LPRP in nebulized form has several advantages such as biomolecules in PRP directly reach to the lungs (broncho-alveolar tree) by air. The other method to reach to the broncho-alveolar tree is instillation which would provoke coughing in COVID-19 patients with a high risk of contagion by aerosol formation. By nebulization the higher, more specific and effective pulmonary concentration of PRP biomolecules can be achieved. In the lungs, IGF-1 and HGF molecules present in LPRP act on intracellular pathways and favour the reparative macrophages M2 phenotype and anti-oxidative stress mechanism. The antiviral effect and stimulation of tissue repair mechanism re-establish the pulmonary structure as the alveolocapillary barrier. The biomolecules present in PRP stimulate the reparative action of Alveolar Type II Cells; modulate cellular activity, and promoting tissue repair [Beitia M *et al.*, 2021, Cazzola M *et al.*, 2021, Mary A *et al.*, 2020].

CONCLUSION

The role of platelets and its secretory biomolecules consisting various growth factors, chemokines, cytokines, antibacterial molecules etc. are well known and applied in various medical fields. The potential properties of PRP such as immunomodulatory, anti-inflammatory, angiogenic, anti-fibrotic and anti-apoptotic, stimulation of different cells and no adverse effect of other tissues etc. has advantages on various disease conditions including current COVID-19 pandemic. PRP has been found to be effective in reducing complications and improving health of COVID-19 patients. In this review we described the advantages of use of lyophilized PRP in nebulized form which might found to be effective in the treatment of COVID-19 patients. However, further studies are warranted to prove its efficacy.

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