Autologous Platelet-rich Plasma for Treatment of Ischemic Ulcers in Buerger’s Disease: A Pilot Study with Short-term Results

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Abstract

Background: Many treatment modalities are available for the treatment of ischemic ulcers in Buerger’s disease (thromboangiitis obliterans [TAO]). Objectives: The objective of the study was to evaluate the efficacy and clinical outcome of autologous platelet-rich plasma (PRP) for the treatment of ischemic ulcers in TAO patients. Methods: This prospective observational study was conducted on selected TAO patients who underwent autologous PRP treatment in surgery department of a teaching hospital in Central India. Diagnosis of TAO was made on clinical grounds and Color Doppler study. Autologous PRP was injected subcutaneously around the area of ulcer on day 0 and then on the 5th and 10th day. Results were noted on day 1, day 5, day 10, and on day 15. Outcome monitored was improvement in pain (using visual analog scale) and healing of ischemic ulcers. Results: All 14 patients were males, chronic smokers, and most of the patients were in the 4th decade of life. All had involvement of lower limbs; one had upper limb ischemia as well. All patients had ischemic ulcers. Pain relief, as measured with visual analog scale score, was good; most of the patients had 50% relief within 24 h of injecting PRP, which persisted/continued to improve on days 5, 10, and 15. Similarly, ulcer healing showed improvement on days 5, 10, and 15. Conclusions: PRP can provide efficient treatment for pain and healing of ischemic ulcers in TAO patients.

Keywords: Buerger’s disease, healing, ischemic ulcer, platelet-rich plasma

INTRODUCTION

Buerger’s disease aka thromboangiitis obliterans (TAO) is a nonatherosclerotic, segmental inflammatory disease that most commonly affects the small- and medium-sized arteries and veins in the lower extremities mainly affecting males smoking tobacco from the low socioeconomic strata. It is a common cause of peripheral vascular disease in India, Japan, Korea, and Bangladesh. In the past decade, platelet-rich plasma (PRP) has become known as a reservoir of various growth factors which lead to neoangiogenesis and enhance wound healing. Taking inspiration from these results, we studied the use of PRP in the treatment of ischemic ulcers in TAO patients.

METHODS

This is a prospective observational study conducted on TAO patients in surgery department of a teaching hospital in Central India. It received due clearance from Institutional Ethics Committee.

Diagnosis of TAO was made on clinical grounds, supplemented with two-dimensional (2D) Color Doppler study as per Shionoya’s criteria. Selected patients of TAO having ischemic ulcers (including those after excision of gangrenous patches and amputation of toes) were included in the present study. Patients were examined clinically, studied with 2D Color Doppler, and classified according to Rutherford and WIfI classifications. Symptom-free patients, those with deep ulcers and gangrene (Grade 6 of Rutherford classification and Grade 3 wound of WIfI classification, n = 20) were included in the present study. Patients were examined clinically, studied with 2D Color Doppler, and classified according to Rutherford and WIfI classifications [Tables 1 and 2].

Symptom-free patients, those with deep ulcers and gangrene (Grade 6 of Rutherford classification and Grade 3 wound of WIfI classification, n = 20) were included in the present study. Patients were examined clinically, studied with 2D Color Doppler, and classified according to Rutherford and WIfI classifications [Tables 1 and 2].

How to cite this article: Sharma D, Agarwal P, Jain S, Kothari R. Autologous platelet-rich plasma for treatment of ischemic ulcers in Buerger’s disease: A pilot study with short-term results. Indian J Vasc Endovasc Surg 2018;5:14-20.

Received: August, 2017. Accepted: September, 2017.
and patients with diabetes \((n = 1)\) and hypertension \((n = 1)\) were excluded from the study. Selected patients were admitted in surgical ward. They were explained about their disease, benefit of complete abstinence from tobacco smoking/chewing was vigorously counseled, its compliance/noncompliance noted, and their informed consent was obtained in vernacular language.

Autologous PRP was freshly prepared, each time, by standard technique of differential centrifugation.\(^{14}\) A volume of 40 ml whole blood was obtained by venipuncture in acid citrate dextrose tubes. Blood was centrifuged using a “soft” spin at constant acceleration to separate red blood cells (RBCs) from the remaining blood volume. After the first spin step, the whole blood separates into three layers: an upper layer that contains mostly platelets and white blood cells (WBC), an intermediate thin layer that is known as the buffy coat and that is rich in WBCs, and a bottom layer that consists mostly of RBCs. The supernatant plasma containing platelets was then transferred into another sterile tube (without anticoagulant). This tube was then centrifuged at a higher speed, a “hard” spin, to obtain a platelet concentrate. The lower one-third is PRP and upper two-third is platelet-poor plasma (PPP). At the bottom of the tube, platelet pellets are formed. Now, PPP was removed, and platelet pellets were homogenized in a minimum quantity of plasma \((4 \text{ mL})\) by gently shaking the tube to create the PRP.

A volume of 4 ml PRP was injected subcutaneously around the area of ulcer with all due aseptic precautions on day 0 and then on the 5th and 10th day. Standard wound and foot care was given to all patients.

Pain relief was noted on day 1, day 5, day 10, and on day 15. Clinical improvement was noted in pain relief by visual analog scale (VAS) score of \(0–10\) \((0\) representing no pain and 10 maximum pain; pain score on day 0 was taken as 10). Ulcer healing results were noted on day 5, day 10, and on day 15. We constructed a simple grading system to denote the healing of ulcer. As shown in Table 3, if healing was \(0–24\%\), it was graded as Grade 4, if healing was \(25–49\%\), it was graded as Grade 3, if healing was \(50–74\%\), it was graded as Grade 2, and if healing was \(75–100\%\), it was graded as Grade 1. Ulcer healing was noted by two observers (DS and PA). Kappa coefficient was used to check the degree of interobserver agreement.\(^{15}\)

| Grade | Clinical features |
|-------|------------------|
| 0     | Asymptomatic     |
| 1     | Mild claudication|
| 2     | Moderate claudication|
| 3     | Severe claudication|
| 4     | Ischemic rest pain|
| 5     | Minor tissue loss - nonhealing ulcer, focal gangrene with diffuse pedal ischemia|
| 6     | Major tissue loss, functional foot no longer salvageable|

| Grade | Clinical features |
|-------|------------------|
| 0     | No ulcer no gangrene |
| 1     | Small, shallow ulcer on distal leg or foot; no exposed bone, unless limited to distal phalanx no gangrene |
| 2     | Deeper ulcer with exposed bone, joint, or tendon; generally not involving the heel; shallow heel ulcer, without calcaneal involvement Gangrenous changes limited to digits |
| 3     | Extensive, deep ulcer involving forefoot and/or midfoot; deep, full-thickness heel ulcer, calcaneal involvement, extensive gangrene involving the forefoot/midfoot; full-thickness heel necrosis, calcaneal involvement |

| Grade | Clinical features |
|-------|------------------|
| 0     | No symptoms or signs of infection (infection present, as defined by the presence of at least two of the following items Local swelling or induration Erythema 0.5 cm-2 cm around the ulcer Local tenderness or pain Local warmth Purulent discharge (thick, opaque to white, or sanguineous secretion) |
| 1     | Local infection involving only the skin and the subcutaneous tissue exclude other causes of an inflammatory response of the skin (trauma, gout, acute Charcot, fracture, thrombosis, and venous stasis) |
| 2     | Local infection with erythema >2 cm, or involving structures deeper than skin and subcutaneous tissues, and no systemic inflammatory response signs |
| 3     | Local infection with the signs of SIRS, as manifested by two or more of the following: Temperature >38°C or <36°C Heart rate >90 beats/min Respiratory rate >20 breaths/min or PaCO\(_2\) <32 mmHg White blood cell count >12,000 or <4000 cu/mm or 10% immature bands |

SIRS: Systemic inflammatory response syndrome; ABI: Ankle-brachial index, ASP: Ankle systolic pressure

Patients were asked to come for follow-up after 3 months, those who could not come were followed up by a telephone call.

Patients who could not complete the protocol of three injections were excluded from the final observations.

**Results**

Seventeen selected TAO patients with ischemic ulcers were given PRP treatment in surgery department of a teaching hospital in Central India from February 2017 to April 2017.
All patients were chronic bidi smokers, males; most of the patients were in the 4th decade (mean age 43.2 years, range 30–51 years) of life. Sixteen out of seventeen patients had unilateral involvement of lower limbs; one patient had upper limb involvement in addition to lower limb ischemia. Demographic data and results are shown in Table 4.

Most of the patients were in Rutherford classification Grade 4 or 5 [Table 1]. All patients had ischemic ulcers (mean size 2.5 cm, range 2–5 cm), wound grade was I or II according to WIfI classification, ischemia grade was 3 (as per WIfI classification) in majority of the patients, while infection Grade varied from 0 to 2 [Tables 2 and 4].

Popliteal artery pulsations were present in all patients; however, ankle pressure index could not be measured in majority of the patients as their dorsalis pedis and posterior tibial arteries were not palpable [Table 4].

The actual process of injecting PRP subcutaneously around the ulcers was painful for patients. However, pain relief, as measured with VAS score, was good; most of the patients had 50% relief within 24 h of injecting PRP (mean VAS score 4.35), which persisted/continued to improve on days 5 (mean VAS score 4.35), 10 (mean VAS score 3.71), and 15 (mean VAS score 2.78) [Table 4]. Similarly, ulcer healing showed improvement on days 5, 10, and 15 [Tables 3 and 4]. There was good interobserver agreement as kappa coefficient was 1.00.

### Table 3: Healing of ulcer

| Ulcer healing | Grade | Observer 1 (%) | Observer 2 (%) |
|---------------|-------|----------------|----------------|
| Day 5         | 1     | 75-100         | 75-100         |
|               | 2     | 50-74          | 50-74          |
|               | 3     | 25-49          | 25-49          |
|               | 4     | 0-24           | 0-24           |
| Day 10        | 1     | 75-100         | 75-100         |
|               | 2     | 50-74          | 50-74          |
|               | 3     | 25-49          | 25-49          |
|               | 4     | 0-24           | 0-24           |
| Day 15        | 1     | 75-100         | 75-100         |
|               | 2     | 50-74          | 50-74          |
|               | 3     | 25-49          | 25-49          |
|               | 4     | 0-24           | 0-24           |

### Table 4: Demographic data and results

| Serial number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Age in years  | 35| 40| 45| 30| 37| 50| 40| 48| 50| 45 | 48 | 51 | 42 | 45 |
| Rutherford grade | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4/5 | 5 | 5 | 5 | 5 |
| Wound Grade I | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Wound Grade II | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Wound Grade III | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Ulcer size cm | 2 | 2 | 2 | 2 | 2 | 3 | 4 | 2 | 2 | 3 | 2 | 1 | 2 | 5 | 2/2 |
| Ischemia grade | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 1 | 0 | 0 |
| Ischemia ABI  | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 1 | 0 | 0 |
| Ischemia ASP  | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 1 | 0 | 0 |
| Infection Grade 0 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Infection Grade 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Infection Grade 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Infection Grade 3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Doppler femoral | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Doppler popliteal | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Doppler DP | - | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Doppler PT | - | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pain VAS day 1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 3 | 3 | 2 | 5 |
| Pain VAS day 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 3 | 3 | 2 | 5 |
| Pain VAS day 10 | 2 | 2 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 4 | 3 | 3 | 1 | 2 |
| Pain VAS day 15 | 2 | 2 | 4 | 5 | 5 | 3 | 4 | 5 | 3 | 0 | 0 | 3 | 1 | 2 |
| Ulcer healing day 5 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 3 | 4 | 3 | 3 | 3 | 2 | 3 |
| Ulcer healing day 10 | 2 | 2 | 2 | 2 | 1 | 2 | 4 | 2 | 2 | 2 | 1 | 2 | 2 |
| Ulcer healing day 15 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 4 | 2 | 1 | 1 | 2 | 2 |
| Remark | - | - | - | - | - | - | - | - | * | - | ** | - | *** | - |

*Presented with self-mediated herbal/chemical cauterization after gangrene of all toes; later underwent Syme’s amputation, **Presented after bilateral LS; history of amputations of multiple toes; ulcer over the right middle finger (weak radial artery on palpation but good flow on Doppler) and left great toe. Had 100% pain relief in the finger on day 1 after 1st injection of PRP; and good healing of ulcer of great toe after three injections of PRP. ***Presented after bilateral LS and omental transposition with 5 cm size nonhealing ulcer over dorsum of foot after failed split-thickness skin grafting. Ulcer showed good healing after three injections of PRP. ABI: Ankle-brachial index, ASP: Ankle systolic pressure, DP: Dorsalis pedis artery, PT: Posterior tibial artery, VAS: Visual analog scale for pain, PRP: Platelet-rich plasma, LS: Lumbar sympathectomy, +: Present.
Three out of seventeen patients left before completing protocol; one of them could not give up tobacco and dropped out of protocol. These patients were excluded from the study, and outcome was observed in 14 patients.

Three out of fourteen patients came back after 3 months for follow-up and had good healing and pain relief. Seven out of fourteen patients reported, through telephone, good healing and pain relief. Three out of fourteen patients admitted, through telephone, to restarting tobacco smoking resulting in recurrence of disease; two had progression to gangrene of foot and had to undergo below-knee amputation at their respective district hospitals. One out of fourteen patients could not be contacted by telephone.

**DISCUSSION**

PRP is defined as a small volume of plasma with a 4–7-fold increase in the concentration of platelets (at least 1,000,000 platelets/μL) above peripheral blood concentration. PRP, when exposed to endothelium within wounds or damaged tissues, essentially forms an in situ-generated fibrin matrix delivery system, releasing multiple growth factors, chemokines, and cytokines including platelet-derived growth factor, vascular endothelial growth factor (VEGF), connective tissue growth factor, fibroblast growth factor, epidermal growth factor, transforming growth factor-beta, and other bioactive molecules that play key roles in tissue regeneration by enhancing in vivo angiogenesis, improving reperfusion/microcirculation, tissue remodeling, and enhanced wound healing.

An additional advantage of PRP is that its preparation does not need an expansive commercial kit. Recent advent of hand-powered ultralow-cost hand-spun paper centrifuge can circumvent nonavailability of even a mechanical/electrical centrifuge.

A variety of medicines are tried for the treatment of TAO. Best known among them are prostaglandin analogs which facilitate relaxation of vascular smooth cells, inhibit platelet aggregation, and show promise in reducing peripheral vascular resistance with vasodilative effect. However, this effect is not seen with oral prostaglandin analogs. Recent Cochrane reviews have concluded that moderate-quality evidence suggests that intravenous iloprost (prostacyclin analog) is more effective than aspirin for eradicating rest pain and healing ischemic ulcers in Buerger’s disease (even this benefit is transient), but oral iloprost is not more effective than placebo. Very low and low-quality evidence suggests that there is no difference between prostacyclin (iloprost and clinprost) and the prostaglandin analog alprostadil for healing ulcers and relieving pain, respectively, in severe Buerger’s disease. However, no Randomized control trial (RCTs) have assessed other pharmacological agents such as cilostazol, clopidogrel, and pentoxifylline, and their usefulness remains unproven.

Publication of groundbreaking paper by Asahara et al. in 1997 showed that endothelial progenitor cells were capable of being isolated from human peripheral blood and that they also retain the capacity to differentiate into mature and functional endothelial cells. Before the discovery of this cell type, new vessel formation was believed to occur “only” due to proliferation of existing endothelial cells. These findings have overturned the previous dogma that vasculogenesis can only occur during embryogenesis. This prompted a revolutionary change in thinking and application of therapeutic neoangiogenesis in the management of critical limb ischemia (CLI). This discovery led to the treatment of CLI,
in both animal models and humans, by the use of peripheral blood/cord blood/adipose tissue/bone marrow mononuclear cells/stem cells.\textsuperscript{[13-45]}

Recent use of spinal cord stimulation has also led to a lot of interest among the research workers working in this field.\textsuperscript{[54-57]}

Similarly, many surgical procedures are used for TAO. Popularity of sympathectomy stems from the fact that it reduces peripheral resistance by opening of arteriovenous anastomoses (both dependent on sympathetic vasoactivity), thereby increasing blood flow in skin. This effect, even though transient, allows ischemic ulcers to heal.\textsuperscript{[48,49]} Segmental nature of disease affecting small and medium-sized arteries limits endovascular/vascular reconstruction therapy to small number of patients in highly specialized centers. Once the feeding vessels have undergone thrombosis and obliteration – basic problem in TAO – only alternative left is to achieve neovascularization. Omentum is a source of angiogenic growth factors and stem cells which promote neovascularization. This property has been utilized by transposing the omentum in revascularization of the extremities.\textsuperscript{[50-53]} Distraction osteogenesis, as shown by Ilizarov’s landmark study, can stimulate and maintain regeneration, neoangiogenesis, and active growth of tissues not only in the bones but also in muscle, fascia, nerve, vessels, skin, and its appendages.\textsuperscript{[34]} This is called the “law of tension stress” and its beneficial effect has been seen in many studies in TAO.\textsuperscript{[55-57]} Even insertion of intramedullary K-wire has shown relief of pain and clinical improvement in TAO patients.\textsuperscript{[58]} Hypothetically, this may be due to the release of stem cells from bone marrow.

In our study, most of the patients had Grade 3 ischemia [as per WIfI classification, Table 2 and 4], and their dorsalis pedis and posterior tibial arteries were not clinically palpable and showed weak pulsations by Doppler, respectively, in five out of fourteen and eight out of fourteen patients. Platelets have a half-life of 4.6 days; hence, we injected PRP after 5-day interval.\textsuperscript{[39]} We obtained good pain relief and ulcer healing in 14 selected TAO patients with ischemic ulcers [Table 4]. Pain relief, as measured with VAS score, was quite dramatic as most of the patients had 50% relief within 24 h of injecting PRP, which persisted/continued to improve on days 5, 10, and 15 [Table 4]. Similarly, ulcer healing grades showed improvement on days 5, 10, and 15 [Tables 3 and 4]. We used kappa coefficient to demonstrate good interobserver agreement.

One patient with a history of bilateral sympathectomy [patient no. 10] and another patient with a history of bilateral sympathectomy and omental transposition [patient no: 10] had good ulcer healing and pain relief after PRP. This suggests that PRP is a good addition to the armamentarium of physicians treating ischemic ulcers in TAO patients when other avenues have been exhausted.

After PRP, it is possible to show improved perfusion, increased blood flow, and greater angiogenesis (greater presence of endothelial cells, collagen and VEGF, improved angiographic score, and increased capillary density).\textsuperscript{[19,60]} However, in our study, these could not be shown more objectively as transcutaneous oxygen tension, toe brachial index, laser Doppler perfusion imaging, microangiography (showing improved angiographic score), and immune histology (showing increased capillary density) are not available in our setup.

The overall quality of evidence of autologous PRP for treating chronic wounds is low.\textsuperscript{[61]} There are many explanations for variability in the clinical benefit of PRP reported in the literature. the dose of platelets in PRP used may increase up to 25-fold increase depending on the amount injected, efficiency of preparation, activation of PRP, sustained release PRP, and leukocyte-rich PRP which releases more growth factors.\textsuperscript{[62-64]}

The fundamental principle of TAO management is “complete abstinence from tobacco.” We took pains to explain to the patients about etiology of TAO and vigorously counseled them about benefit of complete abstinence from tobacco. The management of TAO remains a multidisciplinary effort with emphasis on smoking cessation, pain control, and wound management, and moral support by family members is necessary.\textsuperscript{[65]} If patients start smoking again, the disease progression starts again, as seen in three out of fourteen of our patients. These patients confessed to a false sense of euphoria after their ulcers healed after PRP and mistakenly started smoking again.

Healing of ischemic ulcers in TAO needs total abstinence from tobacco, which is the cornerstone of preventing further progress of disease. Increased perfusion, even temporarily, can result in healing of these ulcers. All treatment modalities – prostaglandin analogs, stem cells, spinal cord stimulation, sympathectomy, omental transposition, and distraction osteogenesis led neoangiogenesis – aim at increasing perfusion. However, all these modalities have their inherent drawbacks; prostaglandin analogs are expansive, stem cells and spinal cord stimulation need upscale technology, and all surgical procedures are invasive and need indoor admission and anesthesia. In a global context, the Indian TAO patients are from the lowest socioeconomic strata which further limit their treatment options.

**Conclusions**

The use of autologous PRP fulfills the previously unmet need for treatment of TAO patients at grass-root level. It is very easy, reliable, and economical to prepare standard PRP and can be made available in the outpatient department of smallest of the hospitals. As the present observational study is based on a small number of patients, further validation in larger number of patients is desirable.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.
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