ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 04 | April - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CLINICOPATHOLOGICAL STUDY, MANAGEMENT AND OUTCOMES OF AUTOLOGOUS BONE MARROW DERIVED MONONUCLEAR CELL CONCENTRATE IN CHRONIC LIMB ISCHEMIA.

General Surgery

Srijan Raj* Department of General Surgery, GSVM Medical College, Kanpur, Uttar Pradesh, India *Corresponding Author

Sanjay Kala Department of General Surgery, GSVM Medical College, Kanpur, Uttar Pradesh, India

Siddharth Singh Department of General Surgery, GSVM Medical College, Kanpur, Uttar Pradesh, India

B.S. Rajput Consultant stem cell transplant surgeon, Criticare Asia Hospital & Research centre, JVPD, Andheri West, Mumbai,India

Neeraj Kumar 3Department Of Cardiothoracic and Vascular Surgery, LPS Institute of Cardiology, Kanpur, Uttar Pradesh, India

ABSTRACT

Background: Chronic limb ischemia (CLI) is condition of gradual narrowing of the arterial lumen causing reduction of blood flow to the peripheral limbs. There are multiple factors causing chronic limb ischemia like Diabetes, Smoking, tobacco intake (Buerger's Disease), hypertriglyceridemia etc, which cause gradual reduction of blood flow to the limb. **Methods:** In this prospective study conducted on the patients admitted in the Surgery department of LLR Hospital, GSVM Medical College Kanpur with Chronic limb ischemia between October 2021 to October 2022, we've compiled the results of 25 patients who underwent Autologous Bone Marrow Derived Mononuclear cell concentrate (BM-MNC) for chronic limb ischemia. **Results:** The study showed significant improvement in Rest Pain (40% to 10% in BM-MNC) , accelerated wound healing (84% healed in BM-MNC) and Ankle Brachial Index (ABI) (0.50 ± 0.19 to 0.83 ± 0.14) in the Mononuclear cell concentrate. **Conclusions:** The patients treated with BM-MNC had significant improvement in rest pain, ABI, Pain VAS score wound healing status, less recurrences.

KEYWORDS

Chronic Limb Ischemia, Buerger's Disease, Autologous Bone Marrow derived Mononuclear Cell Concentrate, ABI

INTRODUCTION

Peripheral vascular disease commonly affects the arteries supplying the leg and is mostly caused by atherosclerosis. Restriction of blood flow, due to arterial stenosis or occlusion, often leads patients to complain of muscle pain on walking (intermittent claudication). Any further reduction in blood flow causes ischaemic pain at rest, which affects the foot. Ulceration and gangrene may then supervene and can result in loss of the limb if not treated.[1]

Globally around 236.62 million people over 25 had the peripheral arterial occlusive disease (PAOD) in 2015, out of which 72.91 were in lower and middle-income countries. It will be a significant health problem in our country as the Indian population is aging.[2]

Bone marrow (BM) primarily produces the red blood cells during haematopoiesis. It mainly consists of hematopoietic cells, adipose tissue (adipocyte), and supportive stromal cells[3]. The organized stroma of the BM promotes the proliferation and differentiation of the hematopoietic cells, and contains supporting cells such as fibroblasts, macrophages, adipocytes, osteoblasts, osteoclasts, and endothelial cells.[4]Particularly, the BM also contains marrow stromal cells (MSCs). Numerous studies have documented that MSC in BM itself is insufficient not to have a therapeutic property.[5] Bone marrow derived concentrate can produce higher concentration of chondrogenic cells, MSCs, growth factors and other alternative stromal cells, in comparison with BM itself.[6]

Surgical modalities such as revascularization offer limited efficacy for the treatment of TAO patients with clinical symptoms such as rest pain, claudication, ulceration, and gangrene. Unfortunately, revascularization is rarely possible since there is poor outflow, because of the absence of distal vascular targets, and thus results of surgical intervention are poor. In addition, according to one study, there was a 53%graft failure rate, which was mainly due to complications of anastomosis to a diseased artery, disease progression as patients continued to smoke, and vein graft stenosis. Furthermore, failure of secondary revascularization procedure increased the risk of both persistent disabling claudication and amputation.[7]

METHODS

The prospective study was conducted on the patients admitted in the Surgery department of LLR Hospital, GSVM Medical College, Kanpur with Chronic limb ischemia from October 2021 to October 2022. 25 patients were selected for BM-MNC transplantation. The inclusion criteria for BM-MNC transplantation included patients with chronic Limb Ischemia who had failed revascularization treatment, had diagnosed Thromboangiitis Obliterans (Buerger's Disease), had rest Pain (Fontaine stage III), had small ulcers in the affected limb (2-20cm^2), ABI <0.6 to >0.25, age>18 years - <85 years.

The exclusion criteria included patients having history of DVT (Deep Vein Thrombosis), Trauma/Burn/Poisoning, Impending gangrene involving large surface area. (Fontaine stage IV with area >40cm^2, malignant ulcers, patient having history of immunosuppressive therapy.

All patients gave full consent for research study participation. After informed consent was obtained from the patient, Bone marrow withdrawal of 35 ml was performed using a single aspiration method, from the posterior iliac crest into a syringe containing preservative-free heparin. The amount of bone marrow aspirated was based on the size and frailty of the patient. The bone marrow was then concentrated by the TriPReP® Tubex® PRP/Cell Concentration system, (Figure 1) which consists of a centrifuge (Figure 2) that rotates at 2000 rpm for 6 minutes. Morphological analysis of the bone marrow aspirates was performed, pre, and post concentration. Mononuclear Cells (MNC) were used to evaluate bone marrow cell concentration. MNC in Bone marrow concentrate post centrifugation was found to be 132.3 x 10^6 ± 32.87 in 7ml of bone marrow concentrate. It was re centrifuged for 5 minutes on 2000 rpm. (Figure 3) Plasma was then discarded, and the cellular component was collected and injected intramuscularly in 1-mL increments into the ischemic leg in at least 40 locations based on a standardized grid. Injections were placed below the knee and between the web spaces in the foot. Patients were discharged the same day. Patients who had clinical improvement after the initial set of injections but who either subsequently deteriorated or failed to continue to improve were offered a second treatment.

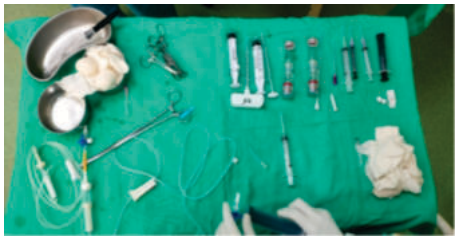

Figure 1: The Total BMC Kit Used For Bone Marrow Derived Cell Transplantation.

Figure 2: The Centrifuge Machine.

Figure 3: Centrifuged TUBEX Kit With Filtered Plasma And Bone Marrow Cell Extract

RESULTS

In our study the mean age group of the patients getting affected with Chronic Limb Ischemia was 51.64 ± 12.99. Also the mean Triglyceride levels of the patients getting affected with Chronic Limb Ischemia was 200 ± 80.97. In the study around 92% of the population undergoing BM-MNC therapy were consuming tobacco in either oral form or smoking. Only 8% weren't consuming tobacco which makes tobacco consumption an important risk factor for chronic limb ischemia. While on the other hand only 28% of the study population (n=50) had a history of Diabetes Mellitus. Rest 72% weren't having any diabetes. Also what we saw that amongst the affected population 88% of the affected patients were belonging from male sex and only 12% were female patients.

Rest Pain , 40 % had it in Pre-Operative stage. After therapy 2 months later only 12% had rest pain and during follow up 12 months later only 10% had rest pain. (Figure 4) .

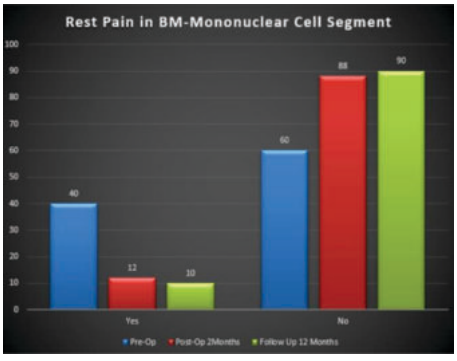

Figure 4: Rest Pain in BM-MNC Group

In the BM-MNC group, 76% of patients had Small Active Ulcer in Pre-Operative stage. (Figure 5) After therapy 2 months later 24% had Small Active ulcer and during follow up 12 months later only 16% had active healing ulcer. 24% of patients had Healing Ulcer in Pre-Operative stage. After therapy 2 months later 60 % had healing ulcers. And 16% of the patients had healed ulcers after 2 months of therapy while 84% of all the ulcers were healed completely (Figure 6,7).

Figure 5: Small Active Ulcer In Pre-op Stage In The Patient Of BM-MNC Group.

Figure 6 : Healed Ulcer After 12 Months Of Follow Up In The Patient Of BM-MNC Group.

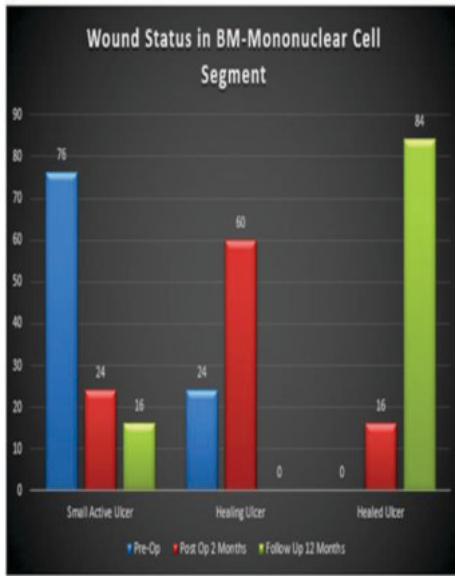

Figure 7: Wound Status Of The Bm-mnc Group With Complete Follow Up.

Only 16% patients suffered recurrence while the rest 84% didn't. 50% of the patient population complained of fever, 20% for pain at site of injections and only 8 % complained of Nausea and vomiting.

The Pain VAS score mean was 2.64 ± 2.71 in pre-op setting which later reduced to 1.72 ± 2.15 two months later & further reducing to 0.28 ± 0.61, 12 months later during follow up. (Figure 8)

Figure 8 : PAIN VAS Score In the BM-MNC group.

Pain VAS SCORE	MEAN	SD	LOWER LIMIT	UPPER LIMIT
			95% CONFIDENCE INTERVAL	
INITIAL	2.64	2.71	1.5213	3.7586
2 MONTHS	1.72	2.15	0.8325	2.6074
12 MONTHS	0.28	0.61	0.0282	0.5317

(F=8.608978, P=0.000444 P<0.001 Highly Significant, F Critical Value – 3.123907)

The mean ABI score of patients of BM-MNC group was 0.50 ± 0.19 in pre-op setting. Which increased to 0.55 ± 0.18 after two months. In the follow up stage 12 months later the ABPI score hit 0.83 ± 0.14. (Figure 9).

Figure 9 : ABI Score in the BM-MNC Group.

ABI Score	MEAN	SD	LOWER LIMIT	UPPER LIMIT
			95% CONFIDENCE INTERVAL	
INITIAL	0.50	0.19	0.4215	0.5784
2 MONTHS	0.55	0.18	0.4756	0.6243
12 MONTHS	0.83	0.14	0.6680	0.7919

(F=12.12011, P=2.9E-05, P<0.0001 Highly Significant, F Critical Value – 3.123907)

(F=7.02189, P=0.00163708, P<0.0001 Highly Significant, F Critical Value – 3.123907)

The data predicts that the t value of student unpaired t test for Pain VAS score was 0.51 (p>0.05 i.e., Non Significant) in pre op setting, further reducing to 0.06 (p>0.05 i.e., Non-Significant) but 12 months later it increased to 2.35 (p<0.05 i.e., Significant) Which implies that the reduction of Pain VAS score is significant for BM-MNC.

DISCUSSION

Mark D. Iafrati & et al [8] demonstrated 44% improvement in the Pain VAS score in the Bm-MNC group while in our study, the patients had significant improvements in the mean VAS score (2.64 ± 2.71 to 0.28 ± 0.61). Mark D. Iafrati & et al [8] also demonstrated the mean ABI improvement of 32.4% (n=34) in BM-MNC vs Controls (7.1%) (n=14) during a 12 week period. In our study the pain vas score in BM-MNC group was 2.64 ± 2.71 in pre-op setting which later reduced to 0.28 ± 0.61 twelve months later.

Kristina A. Giles & et al [9] demonstrated the mean ankle-brachial index increase of 0.23 ±0.25 after BM-MNC vs 0.25 ± 0.4 after high-risk bypass in 2 years & a 75% rate of complete wound healing at 1.5 years & mostly complete resolution from the rest pain while in our study the mean ABI increase was to 0.83 ± 0.14 twelve months later from 0.50 ± 0.19 in the pre-op stage & also 84% of all the ulcers were healed in the BM-MNC group after 1 year and the rest pain fell to 10% after 1 year of follow up from 40% in the beginning of the study in the BM-MNC group.

In the BM-MNC group who had recurrence were offered for repeated treatments.

CONCLUSION

Chronic Limb Ischemia is a condition where the most preferred modalities of treatment are generally medical therapy or, bypass surgeries or sympathectomies etc. But due to emerging modalities like that of Bone marrow derived mononuclear cell concentrate where the patients who have had failed revascularization procedures can be a promising treatment in these patients with great results. Since the cells have been derived from the patient's own body (autologous) it reduces the risk of any anaphylaxis drastically in the patients. In the whole procedure and follow up of the BM-MNC group we also noticed very less n of complications like fever, pain at the site of injection etc etc. which were probably due to anaesthesia side effects. But in some patients with recurrence of ulcers in the BM-MNC group we will have to plan for another session of therapy to ensure complete advantage of the treatment. It is still not sure how many of repeated applications might be required in certain cases and might be totally evaluated based on clinical skills and experience. Though the treatment is a bit costly as compared to revascularization procedures but the effects which it brings in the disease compensated for its price.

Acknowledgements

I sincerely thank Dr. Sanjay Kala & Dr. B.S. Rajput Sir for providing me immense support and guidance for this research & knowledge in the field of regenerative medicine which could pave a way for incurable diseases.

Declarations

Funding: No Funding source

Conflict Of Interest: None Declared

Ethical Approval: The study was approved by the GSVM Institutional Ethics Committee.

REFERENCES

1. J.D Beard. Chronic lower limb ischaemia. BMJ 2000; 320 doi: https://doi.org/10.1136/bmj.320.7238.854 (Published 25 March 2000).
2. Sawan Sharma Venkata Vineeth Vaddavalli Ajay Savlania Ashwitha Ravi Lileswar Kaman Arunanshu Behera. Demographics and Prevalence of Risk Factors in Patients With Peripheral Arterial Disease Presenting to a Tertiary Care Center in Northern India. DOI: 10.7759/cureus.32397
3. Rubin, R.; Strayer, D.S.; Rubin, E. Rubin's pathology: Clinicopathologic foundations of medicine; Lippincott
4. Lucas, D. The Bone Marrow Microenvironment for Hematopoietic Stem Cells. Adv. Exp. Med. Biol. 2017, 1041, 5–18. [PubMed]
5. Buda, R.; Vannini, F.; Cavallo, M.; Grigolo, B.; Cenacchi, A.; Giannini, S. Osteochondral lesions of the knee:A new one-step repair technique with bone-marrow-derived cells. J. Bone Jt. Surg Am. 2010, 92 (Suppl. 2), 2–11. [CrossRef]
6. Huh, S.W.; Shetty, A.A.; Ahmed, S.; Lee, D.H.; Kim, S.J. Autologous bone-marrow mesenchymal cell inducedchondrogenesis (MCIC). J. Clin. Orthop. Trauma 2016, 7, 153–156. [CrossRef]
7. Sasajima, T.; Kubo, Y.; Inaba, M.; Goh, K.; Azuma, N. Role of infrainguinal bypass in Buerger's disease: An eighteen-year experience. Eur. J. Vasc. Endovasc. Surg. 1997, 13, 186–192. [CrossRef]
8. Mark D. Iafrati, MD,a John W. Hallett, MD,b George Geils, MD,b Gregory Pearl, MD,cAlan Lumsden, MD,d Eric Peden, MD,d Dennis Bandyk, MD,e K. S.Vijayaraghava, MD,fR. Radhakrishnan, MD,f Enrico Ascher, MD,g Anil Hingorani, MD,g and Sean Roddy, MD: Early results and lessons learned from a multicenter, randomized, double-blind trial of bone marrowaspirate concentrate in critical limb ischemia: DOI: https://doi.org/10.1016/j.jvs.2011.06.118
9. Kristina A. Giles, MD, Eva M. Rzucidlo, MD, Philip P. Goodney, MD, MS, Daniel B. Walsh, MD, and Richard J. Powell, MD, Lebanon, NH : Bone marrow aspirate injection for treatment of critical limb ischemia with comparison to patients undergoing high-risk bypass grafts : DOI: https://doi.org/10.1016/j.jvs.2014.06.089