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ROLE OF STEM CELLS IN TREATMENT OF UNCORRECTABLE CRITICAL LOWER LIMB ISCHAEMIA IN DIABETIC PATIENTS

By

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ABSTRACT

Background: Stem cells have the remarkable potential to renew themselves. They can develop into many different cell types in the body during early life and growth. Researchers study many different types of stem cells. There are several main categories: the "pluripotent" stem cells (embryonic stem cells and induced pluripotent stem cells) and nonembryonic or somatic stem cells (commonly called "adult" stem cells). Pluripotent stem cells have the ability to differentiate into all of the cells of the adult body. Adult stem cells are found in a tissue or organ and can differentiate to yield the specialized cell types of that tissue or organ.

Objective: This study aims at evaluating the efficacy of autologous injection of bone marrow stem cells concentrates in achieving tissue regeneration, relieving symptoms, improving clinical signs, saving limbs and reducing the level of amputation in cases of critical limb ischemia.

Patients and methods: In the present study, 30 patients with diabetic non correctable peripheral arterial diseases were included in study. The study was conducted at Al Hussein University Hospital in the period from January 2019 to June 2020.

Results: In this study, 30 patients with diabetic non correctable peripheral arterial diseases had bone marrow stem cells concentrates. Age ranged from 55-76 years with mean value 66.10 ± 5.967 years. 24 patients (80%) were over 60 years. 17 patients (56.7%) were male while 13 patients (43.3%) were female. 18 patients (60%) had urban residency while 12 patients (40%) came from rural areas. The duration of DM ranged from 7 to 31 years. 5 patients (16.6%) had Type I while 25 patients (83.3%) had Type II DM.

In this study, 17 patients (56.7%) had ischemic ulcer while 13 patients (43.3%) had neuroischemic ulcer. Ulcer presented in forefoot in 25 patients (83.3%), midfoot in 3 patients (10.0%) and hind foot in 2 patients (6.7%). Mean size of ulcer 2.31 ± 0.649 cm2 with a range of 1.5-3.5 cm2.

Tissue regeneration was achieved in 24 patients (80%), relieved symptoms in 25 patients (83.3%), improved clinical signs in 26 patients (86.7%), saved limbs in 28 patients (93.3%) and reduced the level of amputation in 28 patients (93.3%). In this study, 27 patients (90%) were satisfied with the results while 3 patients (10%) were not.

Conclusion: Injection of bone marrow mesenchymal stem cells (BMMSC) may provide safety and feasibility for the enhancement of limb salvage in patients with critical ischemic limb.

Keywords: Stem cell therapy, Diabetic, Peripheral arterial diseases.

INTRODUCTION

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person is still alive *(Garg et al., 2012).*

When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell *(Biehl and Russell, 2010).*

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions *(Li and Ikehara, 2013).*

In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions *(Biehl and Russell, 2010).*

Bone marrow is a rich reservoir of tissue-specific stem and progenitor cells. Experimental and clinical studies have shown that endothelial progenitor cells (EPCs) are mobilized from bone marrow (BM), migrate to ischaemic tissues, and contribute to the neo-vascularization process in response to tissue ischaemia *(Du et al., 2012).*

The autologous transplantation of bone marrow mononuclear cells (ATBMMC) has shown great therapeutic potential in vitro as well as in vivo Angiogenesis induced by the treatment with stem cells, coupled with the practice of exercises and pharmaceutical therapy can be a therapeutic alternative for patients with peripheral arterial occlusive disease (PAOD) *(Wei et al., 2020).*

Critical limb ischaemia (CLI) related to atherosclerosis is associated with a high risk of amputation and death. Despite improvements in the medical and surgical treatment of CLI, 10–40% of patients still require amputation (*Kinlay, 2016).*

Cell-based therapy is a novel and attractive potential treatment strategy for patients with CLI, based on the fact that endothelial cells and haematopoietic stem cells derived from a common precursor, the haemangioblast. Endothelial progenitor cells derived from bone marrow circulate in peripheral blood and are involved in regenerating injured endothelium and in neo-angiogenesis after tissue ischaemia *(Jaluvka et al., 2020).*

A number of trials are now ongoing to evaluate the safety and efficacy of autologus bone marrow mono nuclear cells (BM-MNCs) transplantation in CLI. The aim in most trials is to reduce the number of bone marrow mono nuclear cells (BM-MNCs) transplantation, reduce rest pain, induce wound healing and reduce the number of necessary leg amputations in cases of CLI. Successful injection of bone marrow mono nuclear cells (BM-MNCs) in CLI may be the only hope in patients who seem to have no

other alternative therapy to save their limbs *(Tateishi- Yuyama et al., 2002).*

This study aimed at evaluating the efficacy of autologous injection of bone marrow stem cells concentrates in achieving tissue regeneration, relieving symptoms, improving clinical signs, saving limbs and reducing the level of amputation in cases of critical limb ischemia.

PATIENTS AND METHODS

In the present study, 30 patients with diabetic non correctable peripheral arterial diseases were included in study. The study was conducted at Al Hussein University Hospital in the period from January 2019 to June 2020.

Inclusion criteria: Diabetic patients with peripheral arterial diseases, patients not fit for surgery, patients with longstanding diabetic foot ulcer and patients with microvascular ischemia.

Exclusion criteria: Patients fit for angioplasty, non-diabetic peripheral arterial disease, correctable peripheral arterial disease and patients fit for plastic procedure

All patients were subjected to the following:

Full history taking: Personal history, history of the present illness and history of systemic diseases as diabetes, hypertension, liver diseases … etc.

Thorough clinical examination: with emphasis on peripheral vascularity.

Full Laboratory investigations: Complete blood count (CBC), coagulation profile, liver function test, renal function tests and blood glucose level.

Routine radiological investigations: Plain X-ray, duplex ultrasonography, CT angiography and

The procedure: The patients were subjected to autologous injection of bone marrow stem cells concentrates.

Outcome: The outcome was monitored in regards to achieving tissue regeneration, relieving symptoms, improving clinical signs, saving limbs and reducing the level of amputation in cases of critical limb ischemia.

Statistical analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

In this study, age ranged from 55-76 years with mean value 66.10 ± 5.967 years. 24 patients (80%) were over 60 years. In this study, 17 patients (56.7%) were male while 13 patients (43.3%) were female. In this study, 18 patients (60%) had urban residency while 12 patients (40%) came from rural areas. All patients were diabetic as an inclusion criterion of the study. The duration of DM ranged from 7 to 31 years. 5 patients (16.6%) had Type I while 25 patients (83.3%) had Type II DM **(Table 1)**.

Table (1): Demographic data (N=30)

Demographic Data	Number of patients (N=30)
Age	
≤ 60 years	6 (20%)
> 60 years	24 (80%)
Age (Years)	
Mean± SD	66.1 ± 5.97
Range	55-76
Sex	
Male	17 (56.7%)
Female	13 (43.3%)
Residency	
Urban	18 (60%)
Rural	12 (40%)
Type of DM	
Type I	5 (16.7%)
Type II	25 (83.3%)
Duration of DM (Years)	
Mean± SD	20.43 ± 6.709
Range	7-31

Data in N (%), mean±SD

In this study, Hb ranged between 6.9 – 12.10 g/dl with a mean value of 9.30 ± 1.301 g/dl. Platelet ranged between 110 – 277 × 109/L with a mean value of 146 ± 36.194 × 109/L. MCV ranged between 55 – 84 fL with a mean value of 62.80 ± 7.172 fL. Prothrombin Time ranged between 13.0 – 14.7 seconds with a mean value of 13.81 ± 0.593 seconds. PTT ranged between 48.5 – 94 seconds with a mean value of 76.09 ± 14.384 seconds. Total Bilirubin ranged between 0.31 – 0.90 g/dl with a mean value of 0.63 ± 0.182 g/dl. Direct Bilirubin ranged between 0.14 – 0.24 g/dl with a mean value of 0.18 ± 0.034 g/dl. Total proteins ranged between 6.28 – 9.27 g/dl with a mean value of 7.64 ± 0.966 g/dl. Albumin ranged between 3.75 – 6.54 g/dl with a mean value of 4.89±0.702 g/dl. AST ranged between 11 – 61 U/L with a mean value of 44.12 ± 11.149 U/L. ALT ranged between 10 – 46 U/L with a mean value of 35.31 ± 8.945 U/L. Alkaline Phosphatase ranged between 107 – 490 IU/L with a mean value of 353.50 ± 82.871 IU/L. Urea ranged between 26 – 34 mg/dl with a mean value of 28.90 ±2.404 mg/dl. Creatinine ranged between 0.6 – 1.3 mg/dl with a mean value of 1.06 ± 0.231 g/dl **(Table 2)**.

Table (2): Laboratory investigations (N=30)

Laboratory investigations		Number of patients (N=30)
Haemoglobin (g/dl)	Mean± SD	9.3 ± 1.301
	Range	6.9-12.1
Platelets (× 10^9/L)	Mean± SD	146 ± 36.194
	Range	110-277
MCV (fL)	Mean± SD	62.8 ± 7.172
	Range	55-84
Prothrombin Time (sec)	Mean± SD	13.81 ± 0.593
	Range	13-14.7
Partial Thromboplastin Time (sec)	Mean± SD	76.09 ± 14.384
	Range	48.5-94
Total Bilirubin (g/dl)	Mean± SD	0.63 ± 0.182
	Range	0.31-0.9
Direct Bilirubin (g/dl)	Mean± SD	0.18 ± 0.034
	Range	0.14-0.24
Total proteins (g/dl)	Mean± SD	7.64 ± 0.966
	Range	6.28-9.27
Albumin (g/dl)	Mean± SD	4.89 ± 0.702
	Range	3.75-6.54
Aspartate aminotransferase (U/L)	Mean± SD	44.12 ± 11.149
	Range	11-61
Alanine aminotransferase (U/L)	Mean± SD	35.31 ± 8.945
	Range	10-46
Alkaline phosphatase (IU/L)	Mean± SD	353.5 ± 82.871
	Range	107-490
Urea (mg/dl)	Mean± SD	28.9 ± 2.404
	Range	26-34
Creatinine (mg/dl)	Mean± SD	1.06 ± 0.231
	Range	0.6-1.3

Data in mean±SD

In this study, 17 patients (56.7%) had ischemic ulcer while 13 patients (43.3%) had neuroischemic ulcer. Ulcer presented in forefoot in 25 patients (83.3%), midfoot in 3 patients (10.0%) and hindfoot in 2 patients (6.7%). Mean size of ulcer 2.31 ± 0.649 cm2 with a range of 1.5-3.5 cm2. **Table 3).**

Table (3): Ulcer characteristics (N=30)

Ulcer characteristics	Number of patients (N=30)
Type of ulcer	
Ischemic	17 (56.7%)
Neuroischemic	13 (43.3%)
Site of ulcer	
Forefoot	25 (83.3%)
Midfoot	3 (10%)
Hindfoot	2 (6.7%)
Size of ulcer (cm^2)	
Mean± SD	2.31 ±0.649
Range	1.5-3.5

Data in N (%), mean±SD

In this study, bone marrow concentrates injection achieved tissue regeneration in 24 patients (80%), relieved symptoms in 25 patients (83.3%), improved clinical signs in 26 patients (86.7%), saved limbs in 28 patients (93.3%) and reduced the level of amputation in 28 patients (93.3%) **(Table 4)**.

Table (4): Study outcome (N=30)

Outcome	Number of patients (N=30)
Injection results	
Achieving tissue regeneration	24 (80%)
Relieving symptoms	25 (83.3%)
Improving clinical signs	26 (86.7%)
Saving limbs	28 (93.3%)
Reducing the level of amputation	28 (93.3%)
Patients' Satisfaction	
Unsatisfied	3 (10%)
Satisfied	27 (90%)

Data in N (%)

In this study, 27 patients (90%) were satisfied with the results while 3 patients (10%) were not **(Figure 1)**.

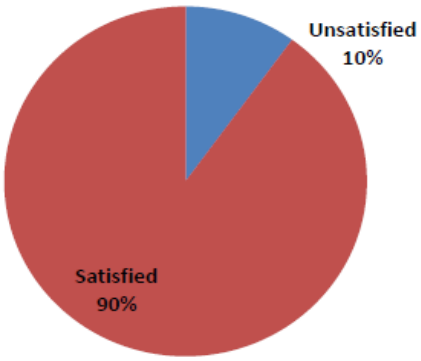

Figure (1): Patients' satisfaction.

DISCUSSION

Critical limb ischemia (CLI) represents the most severe and probably an end-stage manifestation of peripheral arterial disease (PAD) and is still considered an orphan disease with no effective medical treatment. It constitutes a considerable social and economic burden and is associated with a dismal prognosis *(Sharma et al., 2019).*

It may develop from many fundamentally distinct pathophysiological processes, including, more commonly, advanced atherosclerosis and, less commonly, thromboembolism, in situ thrombosis, and the arthritis. It is associated with high short-term mortality, as well as adverse cardiovascular events *(d'Alessandro et al., 2020)*.

Revascularization, wherever feasible, is the cornerstone of therapy; however, major amputations and death remain the most frequent complications. Considerable major amputation rates in the range of 10–40% have been seen in these patients, particularly with failed revascularization or in those with ―no-option CLI *(Sharma et al., 2019).*

Exploring newer approaches for revascularization of these ischemic limbs is therefore of prime importance. Cell-based therapies have come into view as a new frontier in this direction and bone marrow-derived stem cells (BM-SC) are currently seen as a prospective and possible newer therapeutic option in this regard *(Soria-Juan et al., 2019).*

Many studies have shown the effectiveness of stem cell therapy in CLI patients, including randomized trials, nonrandomized trials, and noncontrolled studies. However, owing to the heterogeneity among various studies, acceptance of this mode of therapy as the standard of care is still a matter of debate Transplantation of autologous BM-SC has also been evaluated in terms of different approaches for the implantation, viz. IM injection, IA injection, or combined, and has shown nearly similar results in this aspect *(Sharma et al., 2019).*

Bone marrow stimulation using an injection of the recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) has also shown to be advantageous in terms of higher concentration of mononuclear cells (MCs) requiring lesser aspirations with satisfactory short- term effects *(d'Alessandro et al., 2020)*.

Moreover, a comparative study on autologous injection using peripheral blood stem cells (PB-SCs) or BM-SC has also shown similar efficacy in treating lower limb ischemia *(Soria-Juan et al., 2019).*

The present study illustrated that injection of BMMSC had achieved tissue regeneration in 24 patients (80%), relieved symptoms in 25 patients (83.3%), improved clinical signs in 26 patients (86.7%), saved limbs in 28 patients (93.3%) and reduced the level of amputation in 28 patients (93.3%). These results indicated the efficiency of BMMSC in critical limb ischemia.

The successful outcome was supported by the improvement of ABI and/or TBI, complete ulcer and/or toe stump healing and the evidence of CTA improvement with increased collateral circulation and/or the recanalization in the distal artery.

However, there was no improvement of distal circulation in two patients (6.7%) who had dyspnea due to fluid overload after the injection. This clinical result was also confirmed by no change of ABI and TBI and no change in the degree of collateral circulation and recanalization in CTA.

The compromised cardiac status may play a role of the proliferation of injected progenitor cells. In addition, those patients had the most severe ischemia in this group suffering from both ischemic ulcer and digital gangrene.

The ABI and TBI of these patients were also the lowest in this group. It is worth identifying the level of ischemia at which BMMSC is unable to improve the circulation. There was no abnormality in the laboratory study of hematology, kidney, and liver function in all patients indicating that the injection of BMMSC was safe for patients with critical limb ischemia.

CONCLUSION

Injection of BMMSC may provide safety, and feasibility for the enhancement of limb salvage in patients with critical ischemic limb. The real efficacy of BMMSC for ischemic limb requires randomized control trials in a larger series of such patients.

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دور الخلايا الجذعية في علاج القصور الدموي الحرج الغير قابل للتصليح بالطرف السفلي في مرضى السكر

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خلفيـــة البحـــث: تتمتـــع الخلايـــا الجذعيـــة بإمكانيـــة رائعـــة لتجديـــد نفســـها. يمكـــن أن تتطـــور إلـــى العديـــد مـــن أنـــواع الخلايـــا المختلفـــة فـــي الجســـم خـــلال الحيـــاة المبكـــرة والنمـــو. يـــدرس البـــاحثون أنواعًـــا مختلفـــة مـــن الخلايـــا الجذعيـــة. هنـــاك عـــدة فئـــات رئيســـية: الخلايـــا الجذعيـــة "متعـــددة القـــدرات" (الخلايـــا الجذعيـــة الجنينيـــة والخلايـــا الجذعيـــة المســـتحثة متعـــددة القـــدرات) والخلايـــا الجذعيـــة غيـــر المضـــغية أو الجســـدية (تســـمى عـــادةً الخلايـــا الجذعيـــة "البالغـــة"). تتمتـــع الخلايـــا الجذعيـــة متعـــددة القـــدرات بالقـــدرة علـــى التمـــايز إلـــى جميـــع خلايـــا الجســـم البـــالغ. توجـــد الخلايـــا الجذعيـــة البالغـــة فـــي نســـيج أو عضـــو ويمكـــن أن تتمايز لإنتاج أنواع الخلايا المتخصصة لهذا النسيج أو العضو.

الهـــدف مـــن البحـــث: تقيـــيم فاعليـــة الحقـــن الـــذاتي للخلايـــا الجذعيـــة لنخـــاع العظـــم التـــي تركـــز فـــي تحقيـــق تجديـــد الأنســـجة وتخفيـــف الأعـــراض وتحســـين العلامـــات الســـريرية وإنقاذ الأطراف وتقليل مستوى البتر في حالات نقص تروية الأطراف الحرجة.

المرضـــى وطـــرق البحـــث: فـــي الدراســـة الحاليـــة، تـــم تضـــمين 30 مريضًـــا يعـــانون مـــن أمـــراض الشـــرايين الطرفيـــة غيـــر القابلـــة للعـــلاج الســـكري. أجريـــت الدراســـة فـــي مستشفى الحسين الجامعي في الفترة من يناير 2019 إلى يونيو 2020.

نتـــائج البحـــث: فـــي هـــذه الدراســـة ، كـــان لـــدى 30 مريضًـــا يعـــانون مـــن أمـــراض الشـــرايين الطرفيـــة غيـــر القابلـــة للتصـــحيح الســـكري مركـــزات الخلايـــا الجذعيـــة للنخـــاع العظمـــي. تـــراوح العمـــر بـــين 55-76 ســـنة بمتوســـط قيمـــة 66.10 ± 5.967 ســـنة. 24 مريضـــا (80٪) تجـــاوزت أعمـــارهم 60 عامـــا. 17 مريضـــا (56.7٪) كـــانوا مـــن الـــذكور و 13 مريضـــا (43.3٪) مـــن الإنـــاث. 18 مريضـــا (60٪) لـــديهم إقامـــة فـــي الحضـــر بينمـــا 12 مريضـــا (40٪) جـــاءوا مـــن منـــاطق ريفيـــة. تراوحـــت مـــدة مـــرض

السكري من 7 إلى 31 سنة. كان لدى 5 مرضى (16.6٪) النوع الأول بينما كان لدى 25 مريضاً (83.3٪) مرض السكري النوع الثاني.

في هذه الدراسة، كان 17 مريضاً (56.7٪) مصابين بقرحة إقفارية بينما أصيب 13 مريضاً (43.3٪) بقرحة عصبية. تم عرض القرحة في مقدمة القدم في 25 مريضاً (83.3٪)، ووسط القدم في 3 مرضى (10.0٪)، والقدم الخلفية في 2 مريض (6.7٪). متوسط حجم القرحة 2.31 ± 0.649 سم 2 بمدى يتراوح من 1.5 إلى 3.5 سم 2.

تم تجديد الأنسجة في 24 مريضًا (80٪)، وتخفيف الأعراض في 25 مريضًا (83.3٪)، وتحسين العلامات السريرية في 26 مريضًا (86.7٪)، وحفظ الأطراف في 28 مريضًا (93.3٪) وتقليل مستوى البتر في 28 مريضًا. المرضى (93.3٪). في هذه الدراسة ، كان 27 مريضًا (90٪) راضين عن النتائج بينما لم يكن 3 مرضى (10٪) راضين.

الاستنتاج: قد يوفر حقن الخلايا الجذعية الوسيطة للنخاع العظمي الأمان والجدوى لتعزيز إنقاذ الأطراف في المرضى الذين يعانون من الأطراف الإقفارية الحرجة.

الكلمات الدالة: العلاج بالخلايا الجذعية، السكري، أمراض الشرايين الطرفية.