

CORRELATION WITH VEGF LEVEL FOR CLINICAL IMPROVEMENT IN BUERGER'S DISEASE PATIENTS AFTER ILLIZAROV SURGERY

Shailendra Kumar, Ambrish Kumar & Archana Mishra

Department of Cardio Thoracic and Vascular Surgery

King George's Medical University, Lucknow.

ABSTRACT

Buerger's disease also called thromboangiitis obliterans (TAO), is widely recognized as a specific disease characterized by onset of distal extremity ischemic symptoms at early age in absence of an underlying proximal embolic source, trauma, autoimmune disease, diabetes, or hyperlipidemia.1 The disease is far more prevalent in the middle, near, and far east regions than in North America.2 It occurs predominantly in men, but recent evidence indicates that the incidence rate in women is increasing.3 Treatment of Buerger's disease is primarily medical. Surgical treatment in non responders to medical management including lumbar sympathectomy has been tried without promising result. Various orthopaedic procedures have been tested in various studies for the treatment of peripheral vascular diseases with variable successes. We have used Ilizarov surgery to distract and then compress to produce stimulus for angiogenesis. Vascular Endothelial Factor (VEGF) is an angiogenic factor which stimulates endothelial cell growth, angiogenesis and capillary permeability.4 In this study we have measured the clinical improvement parameters and VEGF level after ilizarov procedure in peripheral arterial vascular disease.

Key words: Buerger's disease, autoimmune disease, Ilizarov surgery, VEGF

INTRODUCTION

In 1951, Ilizarov began to use distraction osteogenesis to treat acute fractures. Over the years, the methods and devices have evolved and its indications have been extended to treat fractures and associated complications: non-union, chronic osteomyelitis, shortened extremity, joint contracture, and deformity.(1) The lower limb bone (tibia) is vertically osteotomised into two bone fragments and is slowly distracted to induce neo-angiogenesis leading to effective collateral circulation to salvage the limb.(2) Only osteotomy with periosteal stripping.(3) revascularising osteotripanation(9-4)and vascular gene therapy, (5) are alternative methods.

VEGF (also called vascular promoting factor) is primarily active on vascular endothelial cells. Analysis of VEGF transcripts by PCR, DNA cloning or RT-PCR identified 5 isoforms with 121, 141, 145, 165 and 189 amino acids. VEGF controls blood vessel formation and permeability by interacting with 2 endothelial tyrosine kinase receptors FLT1 and KDR/FLK1. VEGF165 seems to be the predominant form and has a specific receptor (VEGF165R) identical to neuropilin-1 VEGF expression is regulated by the level of glycaemia and

oxygen, both hypoglycaemia and hypoxia being strong inducers of VEGF synthesis. Reduced tissue level of nutrients and oxygen are common features of peripheral ischaemic disease, thus providing a rationale for using VEGF in the treatment of chronic lower limb ischaemia.

Fokin AA et al. (5-6) used the Ilizarov method in 28 patients with occlusion of lower extremity arteries, mainly of distal localization with III and IV degrees of ischemia. Osteotomy of mainly the tibial bone and distraction of the free osseous spit was performed during 31-36 days. The immediate positive result was noted in 20 patients. The patients were followed up from 2 to 15 months, good results were observed in 18 patients. The use of Ilizarov procedure was most justified in patients with the III and IV degrees of ischemia.(7)

Milind Chaudhary et al. (13) used Ilizarov technique in 31 patients from 1991 to 2001. Pain relief was dramatic and prolonged, claudication distance improved in these patients to more than 5 kilometers. Gangrene did not spread up in any of the 29 patients in whom the treatment succeeded. Two patients had infective complications and underwent amputation.

Address for correspondence

Shailendra Kumar

Department of Cardio Thoracic and
Vascular Surgery,
King George's Medical University,
Lucknow

Ph : 9415017353

Email: skkgmu@gmail.com

They concluded that bone widening using distraction by the Ilizarov method is a simple, safe, and inexpensive, albeit reliable technique to improve limb vascularity in patients suffering from peripheral arterial occlusive diseases and may prevent an amputation. (8)

Tsung-Ming Lee, Sheng-Fang SU (14), assessed the potential role of VEGF in the improvement in exercise tolerance, they investigated plasma levels of VEGF in 50 patients with intermittent claudication who were allocated randomly to groups receiving cilostazol ($n=17$), pentoxifylline ($n=17$) or placebo ($n=16$). Patients given either cilostazol or pentoxifylline showed a significant improvements in maximal walking distance compared with the placebo group (34 m and 33 m respectively, compared with 5 m; both $P=0.05$). Circulating VEGF levels were increased (from 116-29 to 169-45 pg/ml; $P=0.002$). (9)

Andrei Anghel, Bogdan Mut-Vitcu, Lorand Savuet al. (15) recently (2004) focused on the application of a therapeutic strategy in patients with chronic severe lower limb ischaemia using a plasmid vector encoding the vascular endothelial growth factor (phVEGF165). The clinical evolution has been monitored by angiography and estimated by walking time on the rolling carpet (Gardner protocol). Two months after therapy, all three patients showed complete relief of rest pain, improvement of ischaemic ulcer lesions and increased walking distance on the rolling carpet most probably due to appearance of newly formed collateral vessels. (10)

MATERIALS AND METHODS

We conducted the present study to assess the clinical improvement and level of VEGF after Ilizarov application in patients of Buerger's disease. This study included 32 patients of peripheral arterial occlusive diseases admitted to the department of General Surgery, King George's Medical University, Lucknow from August 2010 to July 2011. Clinical evaluation and VEGF level assessment were done pre-operatively and post-operatively at 1 and 3 months interval. (11)

Clinical assessment

Four clinical parameters, *i.e.* pain score, temperature variation between two limbs, skin changes and ABPI were considered. (12)

Determination of plasma VEGF levels

Plasma VEGF levels in each sample were measured in duplicate using a human VEGF ELISA kit (Quantikine human VEGF; R&D system, Minneapolis, MN) according to the manufacturer's instructions. The detection limit was 9 pg/ml. The interassay variability was <5%. (13)

OBSERVATION AND RESULTS

All the four clinical parameters, *i.e.* pain score,

temperature variation between two limbs, skin changes and ABPI showed significant improvement following treatment ($p<0.001$). Pain score by VAS scale decreased from 7.61 ± 0.29 to 2.52 ± 0.32 ($p<0.001$). Temperature of affected side and skin changes (Pre-gangrene / gangrene) improved post-operatively ($p<0.001$). Ankle brachial pressure index (ABPI) was increased from 0.45 ± 0.03 to 0.84 ± 0.03 . ($p<0.001$).

Post-treatment assessment of Color Doppler Flowmetry revealed an increment in flow velocity all three arteries of lower limb which was also found to be significant statistically. Anterior tibial artery flow velocity increased from 23.30 ± 3.16 to 35.10 ± 2.60 ($p<0.001$). Posterior tibial artery flow velocity increased from 23.80 ± 2.35 to 34.90 ± 2.23 ($p<0.001$). Dorsalis Pedis artery flow velocity increased from 4.50 ± 0.73 to 25.10 ± 2.28 ($p<0.001$). Pre and post-operative assessment of CT angiography showed increase in number of collaterals from 2.80 ± 1.32 to 6.20 ± 1.32 which is significant statistically ($p<0.001$). 6 months after procedure dorsalis pedis artery post contrast enhancement increased from 60% to 100% thus showing statistically significant change ($p=0.025$).

Pre-operative VEGF level was 119.90 ± 5.43 pg/ml which was increased post-operatively to 243.80 ± 6.51 pg/ml at 1 month and 259.70 ± 21.95 pg/ml at 3 month interval with p value <0.001 which is very significant. (14)

DISCUSSION

This study is a non-randomized pilot study based on the concept of histiogenesis after historic Ilizarov application for bone lengthening which was monitored with VEGF level. This conceptual study has already been conducted by various investigators at different places. Our study is an additive effort to test the hypothesis of this histiogenesis in the very troublesome disabling arterial occlusive diseases. In this study rest pain was present in all patients with mean pain score of 7.61 ± 0.29 before the procedure. Pain reduced significantly at 1 month. This reduced further up to 2.52 ± 0.32 by 3 months which is also statistically significant ($p<0.001$). Patient was able to walk significant distance without pain, claudication distance increased significantly and patient was able to do his routine work as previously he could not. Pre-gangrenous changes were measured in the form of their presence or absence. Gangrenous changes were present in 6 patients preoperatively, which was debrided. In further follow ups upto 3 months, there was no development or progression of disease pathology was observed. Skin temperature of affected limb was low as compared to opposite limb pre-operatively and

Table 1: Pre and Post-Operative Assessment of VEGF Level at 1 Month and 3 Months Interval

S.No.	Time interval	VEGF value		Significance of change	
		Mean	SD	"t"	"p"
1.	Pre-treatment	119.90	5.43	—	—
2.	At 1 month	243.80	6.51	42.334	<0.001
3.	At 3 months	259.70	21.95	18.298	<0.001

Assessment of Clinical Findings Pre and Post-Operative

S.No.	Variables	Pre-op.		Post-op.		Significance of Change	
		No.	%	No.	%	"χ ² /t"	"p"
1.	Pain score (VAS) (Mean±SD)	7.61±0.29		2.52±0.32		40.177	<0.001
2.	Temperature of affected side lower than the opposing side	32	100	0	0	20.00	<0.001
3.	Skin changes (Pregangrene/gangrene)	32	100	0	0	20.00	<0.001
4.	Ankle brachial pressure index (Mean±SD)	0.45±0.03		0.84±0.03		28.462	<0.001

gradually increased after Ilizarov procedure to become nearly same of opposite limb at 3 months follow-up. Ulcer over the affected limb was present in 7 patients at the time of Ilizarov application which gradually healed up in one month follow-up and no new ulcer noticed at 3 months follow-up. ABPI ratio of ankle and brachial blood pressure showed increment from 0.45 ± 0.03 to 0.84 ± 0.03 after 3 months of Ilizarov application ($p < 0.001$). Color-Doppler were done in all patients preoperatively and at 3 month postoperatively. Pre-operative VEGF level was 119.90 ± 5.43 pg/ml which was increased post-operatively to 243.80 ± 6.51 pg/ml at 1 month and 259.70 ± 21.95 pg/ml at 3 month interval with p value < 0.001 which is very significant. (15)

CONCLUSION

All the 32 patients under this study had significant improvement in symptoms involving lower limbs and significant rise in VEGF levels after ilizarov procedure. This concludes that ilizarov procedure can be a good surgical option in peripheral arterial occlusive disease and Combinations of VEGF with other growth factors (such as with angiopoietin-1, hepatocyte growth factor) can be tested as alternative strategies to promote new vessel growth. Nevertheless, VEGF therapy for lower limb ischaemia is still controversial and very few data are known about the level of expression necessary in order to achieve a therapeutic effect and, even more, about the related side effects.

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