Study of Serum Vascular endothelial growth factor level in Patients with Simple Diaphyseal Fractures healing

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Abstract

Background & Objectives: Vascular endothelial growth factor (VEGF) plays an important role in the bone repair process by acting as a potent mediator of angiogenesis. In the present study, we aim to analyse the temporal expression of serum VEGF during the early phase of fracture healing and further correlate it with the fracture healing outcomes.

Methods: In this longitudinal cohort study, as per inclusion- exclusion criteria, total 76 adult patients with simple, fresh traumatic diaphyseal fractures of both bones of leg managed conservatively; were enrolled. The VEGF examination was carried out by taking venous blood (2ml) at 4th, 7th, 10th, 15th and 20th day after trauma and clinico-radiological examination was done at 6th, 10th 16th, 20th and 24th week after post trauma. As per clinico-radiological outcomes, these patients were divided into normal healing and impaired healing groups. These healing outcomes then were correlated with serum VEGF level in both groups.

Results: Difference between the demographic variables of both groups was statistically insignificant. The mean RUST Score in each follow up except the baseline value shows statistically significant difference. The serum VEGF level seem to higher in impaired healing patients (Group-II) as compared to the normal healing patients (Group-I) in each follow-ups, however stastically, they shows insignificant difference. Further the correlation between healing outcomes with serum VEGF level was also found to be insignificant.

Interpretation & Conclusions: The serum VEGF act as an potent mediator of angiogenesis during the early phase of healing and can be used as a biomarker to predict the progress of fracture healing outcomes.

Key Word: Vascular endothelial growth factor, Diphyseal tibial fracture, simple fracture

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Introduction

Fractures occurring as a result of trauma are common in long bones. Tibia owes a special consideration due to its subcutaneous position, making it more prone to fracture as a result of trauma. Due to lack of soft tissue coverage anteriorly, fracture of this bone shows a higher incidence of impaired healing and non union and also there are higher incidences of open fractures in this bone.[1] Non-union of tibia in fracture both bone leg amounts to 2-10% of all tibial fractures.[2] This delayed or non-union of fracture shaft of tibia leads to prolonged disability with substantial morbidity incurring an additional socioeconomic burden on the suffering of the patient.[3]

Vascular endothelial growth factor (VEGF) seems to play an important role in the bone healing process by acting as a potent mediator of angiogenesis.[4-6] In vitro studies suggest that, VEGF couples angiogenesis in the formation of bone through an intimate interplay with bone morphogenetic proteins (BMPs) and direct activation of osteoblasts.[7] VEGF deposition at the site of bone damage has been shown to enhance the formation of bone in burr hole defects[8] of murine femoral fractures and in critical-sized defects of the radius in rabbits.[9] Exogenous VEGF application for the treatment of tibial non-unions has been shown to be as useful as autologous bone-grafting in a rabbit model.[10] Further, VEGF also has a direct influence on osteoblast differentiation.[9,11] Inhibiting VEGF not only suppresses angiogenesis, but also bone formation and callus mineralization in rabbits and mice.[9] Results from these experimental evidences suggest that VEGF is involved in mineralization of the cartilaginous callus during fracture repair.

Despite of many studies focusing on the growth factors that play a vital role in bone repair,[12-16] to our knowledge only limited data exist so far in literature, on serum VEGF in correlation with impaired fracture union process in human beings. The aim of present study is to analyse the temporal expression of serum VEGF during the early phase of fracture healing and further to establish its correlation, if any with the fracture healing outcomes.

Materials and Methods

After obtaining ethical clearance (Ref. Code: 57 E.C.M. IIA/P4) from institutional ethical review committee, a total of 92 adult patients in the age group between 18 to 45 years were included in this

longitudinal cohort study. All the enrolled patients were having fresh (< 7 days), closed traumatic diaphyseal fractures (42-A1, A2 & A3, as per as AO muller classification) of both bones leg managed conservatively by above knee POP cast. However, patients having age less than 18 yrs and more than 45 yrs, osteoporotic fractures, polytrauma, pathological fractures, open or fractures with infection, chronic alcoholic or tobacco smokers, immune-compromised patients, patients with intact fibula, uncontrolled diabetes, patients with bile duct obstruction and chronic inflammatory bowel disease, patients on prolonged drugs like anabolic steroids, thiazides, diuretics, hormonal therapy, NSAIDs, calcium, fluorides and immunosuppressive drugs, and those not willing for getting enrolled in the study were excluded from our study.

After obtaining informed consent from all enrolled patients, the peripheral blood sample(2ml) was collected into plain vials by standard aseptic technique at the time of management. This was done for the estimation of nutritional status of enrolled patients by measuring haemoglobin, total leukocyte count (TLC), serum albumin and serum ferritin levels. The serum VEGF level estimation (Thermo Fisher Scientific, KHG0111) was done at 4th, 7th, 10th, 15th, and 20th day following the day of trauma as per protocol. All the patients were managed conservatively (reduction setting of fracture both bones leg and above knee plaster was applied under regional/ general anesthesia). All the enrolled and managed patients were discharged with a standard advice written on the discharge card.

The clinico-radiological evaluation of these patients were done at 6th, 10th, 16th, 20th and 24th week. Clinical examination of the fracture site was done for the assessment of skin condition, abnormal mobility, bony tenderness and transmitted movements. Radiological assessment was done using RUST score[17] by taking standard plain radiographs of leg including knee and ankle joint (AP and Lateral views). The radiographic scoring for each patient was done independently by an orthopaedic surgeon and a radiologist. The average of two scores obtained was taken as the final score for that radiograph.

Based on above clinico-radiological evaluation, we divided the study population into 2 groups. Group-A comprised of patients having clinico-radiological evidence of bone healing with RUST score ≥ 7 by the end of 6th month (normal healing) and Group-B comprised of patients having clinico-radiological evidence of bone healing with RUST score <7 by the end of 6th month (impaired healing). Clinically, bone union is defined as the stage during healing process when the fracture site becomes painless (i.e., there is no tenderness), motionless (i.e., has no abnormal mobility at the fracture site) along with the presence of transmitted movements across the fracture site. Radiographic bone healing is defined as is evidenced by the presence of bridging callus across the fracture site in at least three cortices on standard AP and Lateral views and with a RUST score of ≥ 7.[17]

Statistical analysis

Statistical analysis was performed using SPSS software for Windows program (15.0 version). The continuous variables were analysed with mean (±SD) or range value when required. For comparison of the means between patient groups, unpaired t-test with its 95% confidence interval and Pearson correlation coefficient was used. A p value less than 0.05 or 0.001 was considered as significant.

Results

In the present study, a total of 92 patients were enrolled out of which 76 patients were analysed, rest of the patients were lost in follow-up. The baseline characteristics of all 76 patients are described in **Table 1**. Based on the clinico-radiological outcomes of each patient, they were divided into two groups, with group-A (N = 65) patients having normal bony union and group-B (N = 11) patients having impaired bony union. The two groups were comparable on considering the baseline characteristics, i.e., the difference was statistically insignificant. The baseline serum sampling as done on second day (range 1-3) to investiagate serum albumin, serum ferritin, TLC and haemoglobin was 3.77±0.23, 105.19±36.9, 6426.92±2290.23 and 10.60±1.23 respectively in group-A patients and 3.65±0.16, 93.11±30.33, 6386.36±2547.38 and 10.10± 0.53 respectively in Group-B patients. In the present study, the mode of injury, limb involved (right/ left), gender or the pattern of injury had no affect on the fracture healing process and were found to be statistically insignificant **(Table 1)**.

The mean RUST Score in each follow up except the baseline value shows a statistically significant difference **(p <0.0001; Table 2, Fig.1)**. The mean time for fracture healing in group-A patients was 12.49±2.21 weeks and had a RUST score of ≥ 7. The serum VEGF levels were higher in group-B patients as compared to group-A patients at each follow-up, however statistically, the difference was insignificant **(Table 3, Fig. 2)**. The ANOVA multiple comparison test for serum VEGF level within the groups also reveals an insignificant difference **(Table 4)**. Further, the correlation between healing outcomes based on the RUST score and serum VEGF levels at 24th week of follow-up was found to be insignificant **(Table 5)**.

Table 1: Comparison of baseline characteristics between normal and impaired healing patients

Characteristic	Group I Normal Union (*n*=65) (85.52%)	Group II Impaired Union (*n*=11) (14.47%)	Significance of difference
Mean age±SD (range) in years	30.86±9.11 (18–45)	32.18±8.51 (19–45)	*t*=0.4483; *P*=0.6552
Gender			
Male	61 (93.84%)	9 (81.81%)	*P*=0.2066 (Fisher exact test)
Female	4 (6.16%)	2 (18.19%)	
Site			
Left	29 (44.61%)	4 (36.36%)	*P*=0.7474 (Fisher exact test)
Right	36 (55.39%)	7 (63.63%)	
Mode of injury			
Fall from height	15 (23.08%)	03 (27.27%)	χ^2=6.784; *P*= 0.0791
RTA	42 (64.61%)	05 (45.45%)	
Simple fall	8 (12.31%)	2 (18.18%)	
Slip	0 (0%)	1 (9.09%)	
AO type			
A1	14 (21.54%)	3 (27.27)	χ^2=.5759; *P*=0.7498
A2	35 (53.85%)	5 (45.45)	
A3	27 (41.53)	3 (27.27)	
Mean Hb±SD (range) g/dl	10.60±1.23 (8.8–12.9)	10.10±0.53 (9.4–11.3)	*t*=0.322; *P*=0.1903
TLC±SD cells/µL	6426.92±2290.23 (4200-1050)	6386.36±2547.38 (4300-9800)	*t*=0.5270; *P*=0.9581
Mean albumin level±SD (range) g/dl	3.77±0.23 (3.5–4.3)	3.65±0.16 (3.5–4.0)	*t*=1.479; *P*=0.149
Mean ferritin level±SD (range) ng/mL	105.19±36.9 (30–163.2)	93.11±30.33 (25–131)	*t*=1.011; *P*=0.3154

SD – Standard deviation, **RTA** – Road traffic accident, **Hb** – Hemoglobin, **TLC**- Total Leukocyte Count

Table 2: Mean RUST Score of normal and impaired healing patients

Follow-up	RUST Score		95% confidence interval	p-value (two-tailed)
	Group I Normal Healing (N=65)	Group II Impaired Healing (N=11)		
Baseline	4±0.0	4±0.0	-	-
06th Week	6.3±0.47	4.3±0.38	-2.289 to -1.675	t= 12.86; p <0.0001*
10th Week	7.89±0.47	4.59±0.41	-3.610 to -2.993	t= 21.34; p<0.0001*
16th Week	8.45±0.59	5.09±0.41	-3.743 to -2.983	t= 17.65; p<0.0001*
20th Week	10.24±0.90	5.54±0.44	-5.257 to -4.129	t= 16.58; p<0.0001*
24th Week	11.10±0.86	5.81±0.57	-5.828 to -4.736	t= 0.4359; p<0.0001*

Table 3: Mean serum VEGF level of normal and impaired healing patients

Follow-up	VEGF Level (pg/mL)		95% confidence interval	p-value (two-tailed)
	Group I Normal Healing (N=65)	Group II Impaired Healing (N=11)		
04th Day	698.4±308.32	721.9±289.37	-177.7 to 224.8	t= 0.2332; p= 0.8162
07th Day	855.2±341.39	911.9±375.94	-171.5 to 284.9	t= 0.4952; p= 0.6219
10th Day	960.3±405.83	1056±456.85	-176.1 to 368.5	t= 0.4952; p= 0.6219
15th Day	986.3±438.80	1070±390.85	-200.9 to 368.1	t= 0.5855; p= 0.5600
20th Day	754.2±354.54	806.8±424.63	-188.0 to 293.3	t= 0.4359;p= 0.6641

Table 4: Mean serum VEGF level within normal and impaired healing patients

ANOVA(Tukey's multiple comparisons test)	Group I Normal Union (N=65)		Group II Impaired Union (N=11)	
	95% CI of diff.	P value (two-tailed)	95% CI of diff.	P value (two-tailed)
4th day vs. 7th day	-337.6 to 23.90	ns	-685.6 to 305.6	ns
4th day vs. 10th day	-442.7 to -81.16	***	-830.2 to 161.1	ns
4th day vs. 15th day	-468.7 to -107.2	***	-843.6 to 147.6	ns
4th day vs. 20th day	-236.6 to 124.9	ns	-580.5 to 410.7	ns
7th day vs. 10th day	-285.8 to 75.69	ns	-640.2 to 351.1	ns
7th day vs. 15th day	-311.9 to 49.64	ns	-653.6 to 337.6	ns
7th day vs. 20th day	-79.72 to 281.8	ns	-390.5 to 600.7	ns
10th day vs. 15th day	-206.8 to 154.7	ns	-509.1 to 482.2	ns
10th day vs. 20th day	25.34 to 386.8	*	-246.0 to 745.3	ns
15th day vs. 20th day	51.39 to 412.9	**	-232.5 to 758.7	ns

Table 5: Correlation of fracture outcomes (RUST Score at 24th week) with serum VEGF level

	24th day X-ray vs. 4th day	24th day X-ray vs. 7th day	24th day X-ray vs. 10th day	24th day X-ray vs. 15th day	24th day X-ray vs. 20th day
Pearson r	-0.02247	-0.04515	-0.03043	-0.02090	0.0009694
95% confidence interval	-0.2467 to 0.2040	-0.2679 to 0.1822	-0.2541 to 0.1964	-0.2452 to 0.2055	-0.2245 to 0.2264
P value (two-tailed)	0.8472	0.6986	0.7941	0.8578	0.9934

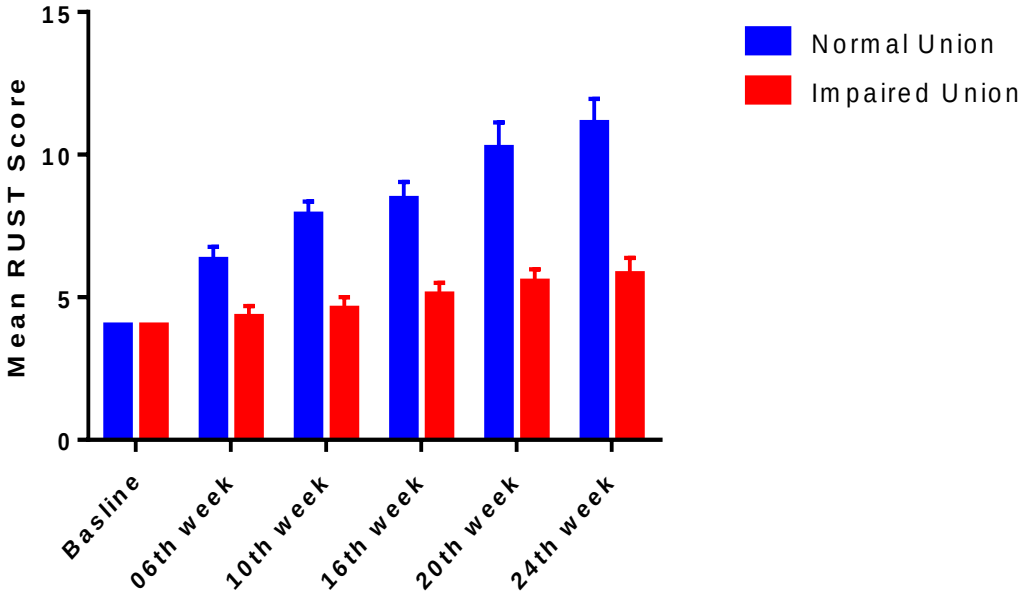

Fig. 1: Mean RUST Score of normal and impaired healing patients

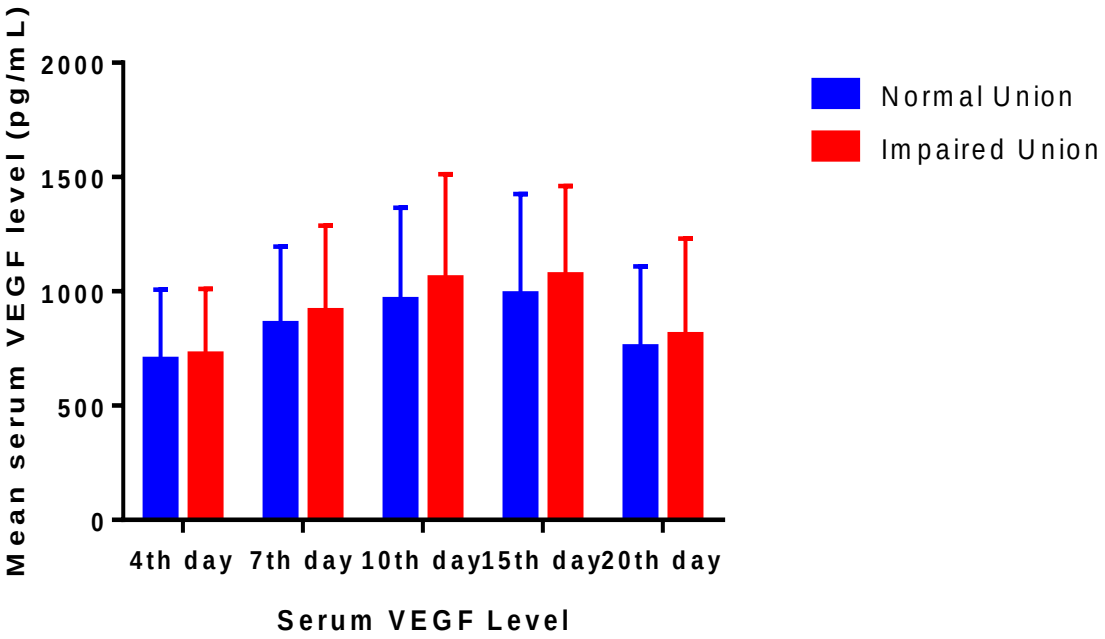

Fig. 2: Mean serum VEGF level in normal and impaired healing patients

Discussion

The formation of new blood vessels (angiogenesis) is an essential biological process which is required for fracture healing, and angiogenic cytokines play a vital role in the formation and growth of these new blood vessels.[18] VEGF is one of the dimeric heparin-binding glycoprotein regulating the process of angiogenesis. Present study demonstrates the temporal systemic expression pattern of VEGF in patients with fracture both bones leg. It further compares the differences in the systemic expression of VEGF between patients with normal fracture healing pattern and patients with impaired fracture healing pattern. High levels of serum VEGF in the initial stage of trauma shows that VEGF is an integrated part of the angiogenic response in the fractured bone which is responsible for creating high demands for growth of new blood vessels at the fracture site during inflammatory phase.

Our study findings are in concordance with those of Weiss et al.[19] who have reported a high serum VEGF expression in early stages of fracture healing(1-2 weeks) in patients of distraction osteogenesis for limb length correction or in patients who underwent elective osteotomies for axis correction of the lower limb. Later on, the serum VEGF level was found to decrease, which reflected the ongoing remodelling of callus tissue in which the soft callus remodells into hard callus.

Again, similar finding were reported by Weiss et al.[20] who reported a higher VEGF serum concentrations in patients having long bone fracture with failed fracture healing as compared to the controls in a cohort of 30 patients; nevertheless, no statistically significant differences were observed.[20]

Our hypothesis of increased VEGF levels at the fracture site where there is ongoing process of healing, is supported by the study conclusion of Street et al.[21] who demonstrated highly elevated VEGF concentrations in fracture hematoma and peripheral blood of 32 patients immediately after trauma. Further, Grad et al.[22] also observed highly elevated VEGF concentrations in 55 polytraumatized patients within the first week after trauma, with the peak level in second week. Sarahrudi et al.[23] also find the similar temporal serum VEGF expression results in patients with long bone fracture.

Our results are nearly identical and confirm the previously reported data by Weiss et al.,[19,20] Street et al.,[21] Grad et al.[22] and Sarahrudi et al.[23] in the present study, serum VEGF level in patients with impaired fracture healing of long bones were significantly higher than in patients with normal fracture union. A continuous increase of VEGF serum concentration is observed immediately after fracture and it continued till the highest VEGF concentrations is reached at about 15 days after trauma and then it tends to decline.

Although the differences were not statistically significant but there appears to be a general increase in serum VEGF level in patients with impaired fracture healing as compared to those having normal healing process. While we observed a significant decline of VEGF concentration after 15 days of injury in both of the groups, we also concluded that higher serum VEGF levels in patients with impaired fracture healing might reflect the effort to enhance vascularization at the

fracture site. However, due to multicausal association of impaired fracture healing, the reason for development of non-union or impaired fracture healing cannot be explained alone by the present study. Although significantly elevated serum VEGF level at the initial healing phase in patients with tibial fractures indicate the vital role of serum VEGF in the fracture healing process, however due to small sample size, lesser number of patients with impaired fracture healing and lack of data regarding the VEGF concentration at the site of fracture, was our study limitation.

Conclusion

The serum VEGF act as a potent mediator of angiogenesis and therefore plays a vital role during the early phases of fracture healing process. Thus it can be used as a potential biomarker to predict the fracture healing outcomes. This knowledge can open new dimensions in the arena of management of these fractures with more specific indications of early surgical intervention and more advanced treatment options. However, authors recommend further multicentric clinical studies in this area in order to establish the role of VEGF in fracture healing.

Conflict of Interest: None
Source of Support: Nil

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