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Original Article

Growth factors and cytokines in patients with long bone fractures and associated spinal cord injury

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ABSTRACT

Background: The aim of the study was to test the effect of acute traumatic spinal cord injury of quadriplegia or paraplegia on bone healing in patients with associated long bone fractures and to investigate the molecular and cellular events of the underlying mechanism for a possible acceleration.

Methods: Healing indicators of long bone fractures and growth factors, IGF-II, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), Activin-A, and cytokine I-L-1, in the patients' blood were calculated and measured for 21 patients with spinal cord injuries and associated long bone fractures in prospective controlled study and compared to 20 patients with only spinal cord injuries, 30 patients with only long bone fractures, and 30 healthy volunteers.

Results: The study results showed that long bone fractures in patients with associated acute traumatic spinal cord injury of quadriplegia or paraplegia heal more expectedly, faster, and with exuberant florid union callus ($P > 0.001$) and show statistically significant higher levels of growth factors like PDGF, VEGF, Activin-A, and cytokine I-L-1, along the 3 weeks of follow-up ($P > 0.005$). I-IGF-II showed statistically significant subnormal level along the whole follow-up period in the same patients ($P > 0.005$).

Conclusion: We concluded that long bone fractures in spinal cord injury patients heal more expectedly, faster, and with exuberant and florid callus formation; growth factors like IGF-II, PDGF, VEGF, Activin-A, and cytokine I-L-I have roles as mediators, in molecular events and as byproducts of the subtle mechanism of accelerated osteogenesis in these patients and may represent therapeutic potentials to serve as agents to enhance bone repair.

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1. Introduction

The increased rate of fracture healing and abundant callus formation of long bone fractures in patients with concomitant

head or spinal cord injury (nervous tissue damage) is a well-known orthopedic phenomenon.^{1–10} In spite of numerous attempts to establish the mechanisms involved whereby severe head or spinal cord injury influences osteogenesis at a distant site, the phenomenon remains poorly understood.^{11–15}

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There is also a well-established clinical relationship between spinal cord injuries and heterotopic ossification.⁹ Recent researches in bone healing speculate the cause of these clinical observations to be due either to circulating humoral factors, which are released in the damaged brain or spinal cord, or through direct nerve signaling pathways. Neurohormonal or neurohumoral mechanisms were also suggested.^{16–30}

Growth factors and cytokines are proteins that serve as signaling molecules for cells. They influence critical functions as cell division, matrix synthesis, and tissue differentiation. The results of experimental studies have established that growth factors and cytokines play an important role in fracture healing and repair of musculoskeletal tissue.^{7–25}

The objective of this prospective controlled study was to investigate the effect of spinal cord injury and concomitant long bone fractures on the serum level of growth factors and cytokines, rather than bone morphogenetic protein (BMP) growth factor.

2. Patients and methods

Nonsmoker patients in the age group of 18–60 years, and without history of chronic ill health or systemic diseases, were included in this study. Patients on permanent medications and therapy for diabetes mellitus, ischemic heart diseases, chronic renal failure, and endocrine diseases and those on corticosteroids for bronchial asthma, rheumatoid arthritis, other arthritides, and inflammatory and autoimmune diseases were excluded.

Patients were started to be recruited prospectively in this study from 19/09/2011 and were categorized according to their clinical data into the following groups: 20 group (A) patients with spinal cord injuries of quadriplegia or paraplegia without long bone fractures, 21 group (B) patients with spinal cord injuries of quadriplegia or paraplegia and closed lower limb long bone diaphyseal fractures (femur and tibia), 30 group (C) patients with closed lower limb long bone fractures only, and 30 group (D) healthy subjects. All long bone fractures in patients of group (B) and (C) were treated surgically, by closed or open reduction, and internal fixation by interlocking intramedullary nails or by DCP and screws, and also, the spin fractures or fracture-dislocation have been reduced and internally fixed, whether cervical or dorsolumbar spine.

The time of radiological healing of the fractures was assessed using 4-point scales with the presence of bridging callus in at least one side of the anteroposterior X-ray view and one side in the lateral view. A score of 2–4 points defines fracture union; while a score less than 2 defines nonunion. Radiological assessment of the amount of callus formed at the fracture sites was performed using plain X-ray views and CT scan of the affected bones. Delayed union was defined as no traces of callus formed 3 months after the long bone fracture occurred, while nonunion was defined as no bridging callus and radiologically visible fracture line 6 months after the injury with atrophic or hypertrophic fracture ends. The mean healing rate of long bone fracture was defined as the maximal thickness of union bridging callus in millimeters as observed in X-rays or CT scan, divided by the time to healing in weeks.

Blood samples were withdrawn from the injured patients at: (a) 24 h, (b) 72 h, (c) 1 week, (d) 2 weeks, and (e) 3 weeks from the time of injury, and only once from the healthy volunteers. 10 ml of blood was withdrawn each time. These blood samples were processed by centrifugation and separation of the sera, which were preserved at -85°C .

The blood samples from different patients' groups were used to measure the levels of growth factors: insulin-like growth factor (IGF-II), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), Activin-A, and cytokine interleukin-1 (I-L-1).

2.1. Statistical analysis

Results were analyzed with SPSS for Windows (version 16). Means and standard deviations were determined. Mean scores between the two groups of patients were compared using chi-square and the Student t-test. P value <0.05 was considered statistically significant.

3. Results

20 patients have been recruited in group (A). The mean age of the patients in this group was 32.7 years; range (18–49) years. These 20 patients included 16 males and 4 females in the ratio of 4:1. All the patients in this group have been involved in high-energy trauma with 12 patients (60%) in RTA accidents and 8 patients (40%) in falling from height accidents.

Cervical spine injuries of fracture-dislocation have occurred in 9 patients (45%) and burst vertebral body fracture or fracture-subluxation of dorsolumbar spine has occurred in the remaining 11 patients (55%). Regarding the neurological deficits in this group of patients: 9 patients (45%) had quadriplegia and 11 patients (55%) had paraplegia. In all group (A) patients, spine surgery procedures of open reduction, decompression, cage, plate fixation, transpedicular screws posterior fixation, and/or fusion have been done in cervical and dorsolumbar spine injuries.

21 patients have been recruited in group (B). The mean age in this group was 33.5 years; range (22–48) years. There were 18 men and 3 women in the ratio of 6:1. All the patients of this group had been involved in high velocity trauma: 17 patients (81%) in RTA and four (19%) in falling from height accidents. In this group, 8 patients had cervical spine injuries with quadriplegia (38%) and 13 patients sustained dorsolumbar spine injuries with paraplegia (62%). These injuries included burst vertebral body fracture, fracture-dislocation, or subluxation of cervical or dorsolumbar spine.

In all group (B) patients, spine surgery procedures have been done. In this group, there were 22 closed diaphyseal long bone fractures, which were treated surgically by fixation with DCP or static reamed interlocking intramedullary nail.

The mean time to union in this group was 6.3 weeks; range (3.7–7.5) weeks. There are no cases of nonunion of long bones in this group. The mean maximal thickness of union bridging callus as shown in X-rays or CT scan was 29 mm; range (10–48) mm. The mean healing rate was 4.7 mm/week; range (2.6–7.5) mm/week.

Table 1 – Patients' biodata and characteristics of injuries in all patients' groups.

Groups	A	B	C	D
No. recruited	20	21	30	30
Mean age years	32.7	33.5	33	39
Age range years	18–48	22–48	18–60	20–60
Sex				
M	16	18	25	22
F	4	3	5	8
Cause of injury				
Road traffic accident (RTA)	12	17	27	0
Fall from height	8	4	3	0
Type of spine injury				
Cervical	9	8	0	0
Dorsolumbar	11	13	0	0
Neurological spinal cord injury				
Quadriplegia	9	8	0	0
Paraplegia	11	13	0	0
Type of fracture				
Femur	0	12	22	0
Tibia and fibula	0	10	14	0
Status of patient				
Alive/dead	20/0	21/0	30/0	30/0

30 patients finished their follow-up in group (C); the mean of their age was 33 years; range (18–60) years. There were 25 men and 5 women in the ratio of 5:1. The type of accident was high-energy trauma, RTA in 27 (90%), and falling from height in 3 (10%). There were 36 long bone diaphyseal fractures in this group of humerus, femur, and tibia, which all have been treated surgically by closed or open reduction and skeletal stabilization. Patients' biodata and characteristics of injuries in all the patients' groups are shown in Table 1.

Among the 36 fractured long bones in the 30 patients of this group, 30 fractures (83.3%) united and 6 (16.7%) went into atrophic nonunion. The mean healing time in this group of patients was 22.5 weeks; range (14–42) weeks. The mean maximal thickness of callus in the united fractures in this group was 8 mm; range (2–20) mm. The mean healing rate was 0.41 mm/week; range (0.25–1) mm/week. Comparison of healing indicators between groups (B) and (C) of patients is shown in Table 2.

Measuring the levels of growth factors and cytokines showed that insulin-like growth factor-II (IGF-II) were in statistically significant subnormal levels in the samples taken from the patients of all the groups, which were sustainable and the lowest in group (B) patients with spinal cord injuries and associated long bone fractures along the 3 weeks of follow-up ($P > 0.005$). In groups (A) and (C) patients, IGF-II returned to normal level at the end of 3 weeks (Figs. 1 and 2).

PDGF started to elevate 24 h after injury in all groups and remained elevated along the 3 weeks of the follow-up, in

Fig. 1 – X-ray of right femur with accelerated union and remodeling of ipsilateral femoral neck fracture and comminuted diaphyseal fracture after 7.5 weeks of the injury with abundant callus formation in the shaft fracture in a group B patient with dorsolumbar spine fracture-dislocation and paraplegia.

comparison to its levels in healthy volunteers; this was statistically significant ($P < 0.001$). The highest levels of PDGF were in group (A) and (B) patients with spinal cord injury alone and with concomitant long bone fractures with statistical significance ($P > 0.005$).

Table 2 – Comparison of healing indicators between groups (B) and (C) of patients.

Patients' groups	Union percent (%)	Healing time	Thickness of maximal union callus	Healing rate
B	100	Mean 6.3 weeks Range (3.7–7.5)	Mean 29 mm Range (10–48)	Mean 4.7 mm/week Range (2.6–7.5)
C	83.3	Mean 22.5 weeks Range (14–42)	Mean 8 mm Range (2–20)	Mean 0.41 mm/week Range (0.25–1)

Fig. 2 – X-ray of left femur with accelerated union of fracture of lower third of the diaphysis extending to the supracondylar area with abundant callus formation in 6 weeks from the time of injury in a group B patient with cervical spine fracture-dislocation and quadriplegia.

Patients with spinal cord injuries with or without long bone fractures in group (B) and group (A), respectively, showed statistically significant increase in the VEGF levels till the end of the follow-up after 3 weeks in comparison to their normal levels in healthy subjects ($P < 0.005$), as shown in Fig. 3. Groups (A) and (B) patients showed statistically significant elevated levels of Activin-A growth factor in all groups in comparison to the healthy subjects of group (D) and they started to decline to normal or subnormal levels from the end of the second week in group (C) patients and the end of the third week in groups (A) and (B) patients ($P < 0.001$), as shown in Fig. 4. Human interleukin-1 (I-L-1) cytokine was found elevated statistically significant in group (A) patients with spinal cord injury and in group (B) patients with spinal cord injury and concomitant long bone fractures, and they declined to subnormal levels at the end of the 3 weeks of the follow-up with highest elevation in group (B) patients ($P < 0.005$). Patients from group (C) showed normal levels of I-L-1 cytokine persistent along the 3 weeks of follow-up.

4. Discussion

Patients with central nervous tissue damage have been noticed to have increased incidence of heterotopic ossification and this phenomenon has been extensively described.¹⁻³ The idea that acceleration of fracture healing occurs in long bone fractures in patients with spinal cord injury remains a controversial subject. Research on this topic has been mostly by studies and authors who denied the presence of this relationship. The only abnormality of bone formation that was detected was an unusually high incidence of heterotopic ossification without an effect on clinical or radiological fracture union.¹⁻⁹

The results of this study showed that long bone fractures of humerus, femur, and tibia in spinal cord injury patients of

Fig. 3 – Values of VEGF in patients of groups (A) to (D) in pg/ml.

Fig. 4 – Values of Activin-A growth factor in patients of groups (A) to (D) in pg/ml.

group B healed faster and united within a shorter period of time than in patients from group C with long bone fractures only. The study also showed that long bone fractures in patients with spinal cord injury united more expectedly and all healed without a single case of nonunion or delayed union. However, 6 (16.7%) long bone fractures among the 36 fractures in the 30 patients of group C had atrophic nonunion. Long bone fractures in group B patients united, with the mean time to union as 6.3 weeks, of range (3.7–7.5) weeks, compared to 22.5 weeks, of range (14–42) weeks, in group C patients; this was a statistically significant difference ($P < 0.001$). Another important finding of our study is that long bone fractures in spinal cord patients of group B healed with more exuberant and florid callus formation compared with patients with long bone fractures only in group C. The mean maximal thickness of union bridging callus, as shown in X-rays or CT scan in group B patients, was 29 mm, with range (10–48) mm, compared to 8 mm, with range (2–20) mm, in group C patients; it is a statistically significant difference ($P < 0.001$). The mean healing rate was also faster, and statistically significant in group B patients compared to group C patients [4.7, range (2.6–7.5) mm/week versus 0.41, range (0.25–1) mm/week]. These data, which were statistically significant, indicate that in all probabilities, patients with spinal cord injuries have accelerated bone healing of concomitant long bone fractures; but the underlying mechanism causing it remains to be revealed.

Researching the underlying mechanism causing accelerated bone healing of diaphyseal long bone fractures in patients with concomitant spinal cord injuries, several studies have addressed the issue of released growth factors in addition to BMP, and they suggested that trauma to the central nervous system may increase the release of, or decrease uptake of,

bone formation mediators that can enter the systemic circulation and enhance fracture healing.^{7–9}

Two insulin-like growth factors (IGFs) have been identified: IGF-I and IGF-II. Although IGF-II is the most abundant growth factor in bone, IGF-I has been found to be more potent and has been localized in healing fractures in rats and humans. Therefore, studies evaluating the role of IGFs in fracture healing have concentrated on IGF-I.¹⁰

Our results showed persistent statistically significant subnormal level of IGF-II in all groups of injuries in comparison to the healthy volunteer group and the lowest levels were in group (B) patients with spinal cord injuries and associated long bone fractures. This could be explained either due to less production of IGF-II in favor of more production of IGF-I, which many studies suggested because of its role in enhancing bone formation, especially the intramembranous ossification, or the localization and utilization of abundance of IGF-II at the sites of healing fractures that reduces its peripheral circulation level; to confirm either it needs further research.^{11–13}

Nash et al.¹⁴ evaluated the efficacy of PDGF in the healing of unilateral tibial osteotomies in seven rabbits. Each osteotomy site was treated with either 80 µg of PDGF in a collagen sponge or with a collagen sponge alone. The animals were killed after 28 days. Radiographic analysis at 2 and 4 weeks demonstrated an increase in callus density and volume in the animals that had been treated with PDGF compared with the controls. Histological analysis demonstrated a more advanced state of osteogenic differentiation, both endosteally and periosteally, in the animals that had been treated with PDGF than in the controls.

Although the histological findings suggested that PDGF has a beneficial effect on fracture healing, only a small number of animals were analyzed and the mechanical testing data were

equivocal. Moreover, the small size of the study does not support robust statistical criteria. At the present time, the therapeutic role of PDGF in fracture healing remains unclear.¹⁴⁻¹⁷

The statistically significant high level of PDGF in acute traumatic spinal cord injury patients with or without long bone fractures in our study may confirm its possible osteogenic effect as reported by previous studies and suggests that its secretion in abundance in spinal cord injury patients may play a role in acceleration of bone healing in patients with associated long bone fractures and may suggest a therapeutic role of PDGF in fracture healing.

Eckardt et al.^{18,19} studied three groups of eight rabbits, which underwent a standard nonunion operation. This was followed by interfragmentary deposition of rh-VEGF in hyaluronic acid carrier, carrier alone, or autograft. After 7 weeks, torsional testing and callus size confirmed that VEGF-treated osteotomies had united whereas the carrier-treated osteotomies failed to unite. They concluded that VEGF stimulated the formation of competent bone in an environment deprived of its normal vascularization and osteoprogenitor cell supply. It could be used to enhance the healing of fracture predisposed to nonunion.

The results of this current study of statistically significant high levels of VEGF in spinal cord injury patients with or without long bone fractures could be explained as that a crucial factor of the bone repair process is the restoration of the blood flow to the fracture site and VEGF is a potent angiogenic stimulator that plays an important role from the early processes of fracture healing. Previous studies have demonstrated large amounts of VEGF in the fracture hematoma in early stages of bone repair. Other studies reported that osteoblastic cells have receptors for VEGF, which may indicate that besides its role in angiogenesis at the fracture site, VEGF may affect differentiation of undifferentiated mesenchymal stem cells into osteoblasts. The abundance of secretion of VEGF in spinal cord injury patients may reveal a role in acceleration of bone healing of associated long bone fractures.

Sakai et al.²⁰ investigated the effects of topical application of Activin-A, a member of transforming growth factor- β super family, on fracture healing using rat fibula fracture models. Activin-A (0.4-10 μ g/day) was injected locally to the fracture once a day for 2 weeks. Callus formation in a dose-dependent manner and callus volume weight were found significantly increased. Histologically, it was found that Activin-A promoted endochondral bone formation. Immunohistochemical examination revealed that Activin-A was localized to osteoblasts and chondrocytes in the region ossified both endochondrally and intramembranously. These findings suggest that Activin-A expressed during fracture healing promotes the healing process through an autocrine/paracrine mode of action.

The finding of our study was that the Activin-A growth factor was found elevated with statistical significance in spinal cord injury patients with or without long bone fractures; groups (A) and (B) may reveal its secretion in abundance in comparison of its normal levels in healthy subjects and may indicate an osteogenic role for Activin-A in acceleration of long bone fractures healing in patients of spinal cord injury.

IL-1 is known to regulate both bone resorption and formation. However, the outcomes of different studies on the effects of IL-1 on osteoblasts function are rather divergent. On the one hand, both IL-1 α and IL-1 β have been shown to inhibit osteoblast proliferation and enhance bone formation, as demonstrated by enhanced alkaline phosphatase activity and bone nodule formation. On the other hand, depending on the differentiation stage of the cell, prolongation of the culture period, and concentration of the cytokines, IL-1 α and IL-1 β may stimulate osteoblast proliferation, and inhibit bone formation, osteocalcin, and type I collagen production. Interleukin-1 can also stimulate osteoblasts to produce other proinflammatory cytokines, such as IL-6, IL-7, tumor necrosis factor- α (TNF- α), prostaglandin E₂, and nitric oxide.^{21,24-26}

The persistent 3 weeks elevation of human interleukin-1 (IL-1) cytokine with statistical significance in group (B) patients with spinal cord injury and concomitant long bone fractures in our study can be explained as it is needed mostly in fracture healing to produce in abundance the proinflammatory cytokines and the inflammatory mediators to induce the initial inflammatory stage of bone repair. Intense inflammation after fracture may enhance bone healing.²¹⁻²⁵ The results of this current study confirm that bone healing is directed by the coordinated expression of many molecules, including growth factors and cytokines, which utilize signals to induce cellular and molecular stimuli to guide cellular commitment and differentiation in the proper spatial and temporal sequence.

The finding of this study that PDGF, VEGF, Activin-A growth factors, and IL-1 cytokine are found in abundance in the sera of patients with spinal cord injury and concomitant long bone fractures suggests their mechanism of action as mediators orchestrating different molecular, cellular, and mechanical events to enhance bone repair.

By all means, we do not believe that growth factors and cytokines, which we tested, and the others, which were not tested in this current study, can explain the process of accelerated fracture healing in spinal cord injury patients with long bone fractures. They are rather a step in the process and not initiating it, the by-products of a subtle mechanism, the effect and not the cause.

Management of fracture union complications remains one of the key issues in orthopedics and this is what is interesting in researching to understand the accelerated bone healing in spinal cord injury patients, with the inspiration that such understanding may lead to the development of novel biologically based therapies for the management of bone regeneration, not only in orthopedic surgery but also for many other disciplines, such as maxillofacial and craniofacial surgery; accordingly, we thought that these growth factors may serve as potential therapeutic agents to enhance the repair of bone. Among these growth factors the set which was tested in this study was namely IGF, PDGF, VEGF, and Activin-A member of TGF- β .²⁶⁻³¹

5. Conclusion

According to the results of this study, we can conclude that long bone fractures in spinal injury patients heal more

expectedly, faster, and with exuberant and florid callus formation, and growth factors like IGF, PDGF, VEGF, and Activin-A and cytokine IL-1 have coordinated roles in this enhanced fracture healing, as mediators and molecular events, which were released by a subtle mechanism to stimulate cellular and tissue processes of accelerated osteogenesis, and accordingly, they may serve as potential therapeutic agents to enhance bone regeneration.

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Conflicts of interest

The authors have none to declare.

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