Growth Factors and Cytokines in Head Injury Patients with Concomitant Long Bone Fractures

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Abstract

In this prospective controlled study, blood samples were withdrawn from 52 patients with head injury, 50 patients with head injury and associated long bone fractures, 60 patients with long bone fractures only, and 50 healthy subjects. Samples were collected: one day, three days, one week, two weeks, and three weeks after the injury and tested for growth factors Insulin growth factor- II (IGF-II); platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), Activin-A transferring growth factor β, and Cytokine Interleukin-1 (IL-1). The results of the study showed that Long bone fractures with head injury heal more expectedly, faster and with exuberant callus (P=0.001) and demonstrated that levels of PDGF, VEGF, Activin-A were persistently high with statistical significance during the 3 weeks of follow-up in head injury patients with associated long bone fractures (P=0.004). IGF-II showed statistically significant subnormal level along the whole follow-up period in the same patients (P=0.001). IL-1 cytokine showed initial elevation in the first two weeks then, declined to normal levels at the end of the period of follow-up (P=0.003). We concluded that long bone fractures in head injury patients heal more expectedly, faster, and with exuberant and florid callus formation and growth factors IGF-II, PDGF, VEGF, Activin-A, and cytokine IL-1 have roles as mediators, molecular events and by-products of the subtle mechanism of accelerated osteogenesis in head injury patients with associated long bone fractures and may represent therapeutic potentials to serve as agents to enhance bone repair.

Keywords: Head injury; Long bone fractures; Acceleration of bone healing; Growth factors; Cytokines

Introduction

The increased rate of fracture healing and abundant callus formation in patients of head injury and long bone fractures is currently, well established through many studies in the orthopedic literature. In spite of numerous efforts aimed at clarifying the way in which severe head injury can influence osteogenesis at a distant site, this phenomenon is still not understood. Various studies stated growth factors and cytokines as a possible cause to that phenomenon [1-20]. 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 head injury and concomitant long bone fractures on the serum level of growth factors and cytokines, rather than bone morphogenetic protein growth factor (BMP).

Materials and Methods

Non-smoker 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, endocrine diseases, and patients on corticosteroids for bronchial asthma, rheumatoid arthritis, other inflammatory arthritis, and autoimmune diseases were excluded.

From 19/09/2011, blood samples were collected from: 52 group (A) patients with severe head injury (defined as patients admitted to the Intensive Care Unit ICU with Glasgow Coma Scale GCS of 8 or less) without long bone fractures, 50 group (B) patients with severe head injury and long bone diaphyseal fractures (humerus, femur, and tibia), 60 group (C) patients with long bone fractures only, and 50 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.

Assessment of radiological healing of fractures is difficult and controversial, but mostly, radiological union is defined by the presence of bridging callus, disappearance of fracture line or the continuity of cortex in at least three of the four bone cortices appear in the antero-posterior and the lateral X-ray views, so a score of 3-4 points of basically bridging callus defines fracture union [4-7]. The healing of long bone fractures in this study has been followed up by radiological assessment of the fracture in antero-posterior and lateral X-ray views weekly and once the plain X-ray showed fracture union according to the aforementioned radiological criteria, we use Computed Tomography CT scan to assess the amount of union bridging callus in millimeters as observed in Computed Tomography CT scan, divided by the time to healing in weeks. In patients with head injuries, the GCS was determined to assess the severity of head injury.

Blood samples were withdrawn from the injured patients at: a) 24 hours, b) 72 hours, c) one week, d) two weeks, and e) three weeks from the time of injury. 10 ml of blood was withdrawn each time. These blood samples were processed by centrifugation and separation of the sera which were preserved at -850 C.

The blood samples from different patients’ groups were used to measure the level of Insulin growth factor (IGF- II); platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), Activin-A transferring growth factor β, and Cytokine Interleukin1 (IL-1).

Statistical Analysis: Results were analysed with Statistical Package for the social sciences 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.

Results

52 patients have been recruited in group (A). Patients’ age, gender, and type of accidents were mentioned in table (1). The mean GCS in the patients of this group was 6/15, range (3-8)/15. The findings of head CT scan in the patients of this group have been mentioned in table (2).

Table 1. Patients’ biodata and characteristics of injuries.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. recruited</td>
<td>52</td>
<td>50</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>Mean Age (Years)</td>
<td>32</td>
<td>31.4</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>Age Range (Years)</td>
<td>18-60</td>
<td>18-59</td>
<td>18-60</td>
<td>20-60</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>49</td>
<td>48</td>
<td>55</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Mean (Range) GCS*</td>
<td>6</td>
<td>7</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Cause of Injury</td>
<td>RTA**</td>
<td>36</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>Fallen object</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fall from height</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Blast</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Industrial trauma</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Head injury</td>
<td>yes</td>
<td>yes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Types of fracture</td>
<td>Humerus</td>
<td>0</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Femur</td>
<td>0</td>
<td>21</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Tibia or fibula</td>
<td>0</td>
<td>19</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Status of Patient</td>
<td>Alive / Dead</td>
<td>52/7</td>
<td>50/1</td>
<td>60/0</td>
</tr>
</tbody>
</table>

*GCS = Glasgow coma scale
**RTA = road traffic accident
50 patients have been recruited in group (B). Their biodata have been mentioned in table (1). The mean GCS in this group of patients was 6.15, range (3-8)/15. The findings of head CT scan in the patients of this group have been shown in table (2). 58 long bones were fractured in these 50 patients, 46 (79.3%) closed fractures and 12 (20.7%) open fractures. There were 18 (31%) fractured shafts of the humerus, 21 (36%) fractured shafts of the femur, and 19 (33%) fractured shafts of the tibia as shown in table (1). All long bone fractures have been treated surgically by closed or open reduction and skeletal stabilization by plates or interlocking intra-medullary nails.

The mean time to union in this group was 6.9, range (3-20) weeks. There are no cases of non-union of long bones in this group. The mean maximal thickness of union bridging callus as shown in X-rays or CT scan was 26.3, range (4-48) mm, as shown in figure(1). The mean healing rate, which is defined as the maximal thickness of union bridging callus in mms as evident in X-rays or CT scan, divided by the time to healing in weeks, was 4.5, range (0.2-10.6) mm/week, as shown in table (3).

60 patients were recruited in group (C) and their biodata were shown in table (1). There were 69 fractured long bones in this group, closed in 47 (68%) and open in 22 (32%). Among the 69 fractured long bones in this group, there were 17 (25%) fractured shaft of humerus, 22 (32%) fractured shaft of femur, and 30 (43%) fractured shaft of tibia & fibula as shown in table (1). All fractures of long bone in this group were managed surgically, as well and skeletally stabilized by plates or nails.

Among the 69 fractured long bones in the 60 patients of this group, 60 fractures (86.9%) united and 9 (13%) went into atrophic nonunion. The mean healing time in this group of patients was 22.4, range (13-41) weeks. The mean maximal thickness of callus in the united fractures in this group was 8.1, range (2-20) mm. The mean healing rate was 0.38, range (0.11 – 1) mm/week, as shown in table (3).

IGF-11 was in statistically significant subnormal level in patients of all groups, but was the lowest and sustained along the 3 weeks of follow-up, in group (B) patients (P=0.001).
Figure 2. X-ray of femur with accelerated fracture healing and abundant callus formation in a group (B) patient with severe head injury and long bone fracture.

Table 3. Comparison of healing indicators of long bone fractures in patients in groups B and C.

<table>
<thead>
<tr>
<th>Patients group</th>
<th>No of patients finished follow-up</th>
<th>No of long bones fractures</th>
<th>No of fractures non-union</th>
<th>Mean (range) of healing time in weeks</th>
<th>Mean (range) of maximal thickness of union callus in mm</th>
<th>Mean (range) of healing rate in mm/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>50</td>
<td>58</td>
<td>0</td>
<td>6.9° (3-20)</td>
<td>26.3° (4-48)</td>
<td>4.5° (0.2-10.6)</td>
</tr>
<tr>
<td>C</td>
<td>60</td>
<td>69</td>
<td>9 (13 %)</td>
<td>22.4° (13-41)</td>
<td>8.1° (2-20)</td>
<td>0.38° (0.11- 1)</td>
</tr>
</tbody>
</table>

*p = 0.001 , 2p = 0.01 , 3p = 0.0001, 4p =0.01, 5p =0.001, 6p= 0.001

Figure 3 a&b. Graph of values of PDGF in patients’ groups (A) to (D) in pg/ml.

Figure 4 a&b. Graph of values of VEGF in patients’ groups (A) to (D) in pg/ml.
PDGF started to elevate 24 hours after injury in all groups and remained elevated along the 3 weeks of the follow-up. The highest levels of PDGF were in group (A) and (B) patients with statistical significance (P=0.001), as shown in figure (3).

All groups showed increase in VEGF level till the end of the second week and then they declined at the end of the third week to their normal levels as in healthy subjects, with the highest increase was in group (B) patients which was statistically significant (P=0.001), as shown in figure (4).

Groups (A) and (B) patients showed statistically significant elevated levels of Activin-A growth factor along the 3 weeks of follow-up (P=0.004). Group (C) patients showed subnormal levels of Activin-A which returned to normal levels at the end of the three weeks of follow-up (P=0.006).

IL-1 Cytokine was found elevated in patients of group (A) and group (B) (P=0.003). The elevation in group (A) patients remained persistent during the whole period of the 3 weeks of the follow-up. Although the patients of Group (B) showed the highest increase till the end of the second week, they declined at the end of the third week reaching the normal levels of healthy subjects. Patients from group (C) showed normal levels of IL-1 cytokine persistent along the three weeks of follow-up (P=0.01).

**Discussion**

The possible association between traumatic brain injury and accelerated fracture healing has long been recognized [4-6]. 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]. In the last two decades many published articles showed statistically significant evidence supporting accelerated osteogenesis of long bone fractures in patients with traumatic brain or spinal cord injuries [4-6].

Newman et al., 1987 [4], Giannoudis et al., 2006 [5], Yang et al., 2012 [6], and others demonstrated accelerated long bone fractures healing mainly in femoral diaphyseal fractures with significant shorter union time and enhanced abundant union callus in patients with concomitant severe head injuries.

The results of this study showed that long bone diaphyseal fractures, not only of femur as shown in the aforementioned previous studies, but also of humerus and tibia, in severe head injury patients 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 severe head injury united more expectedly and all healed without a single case of nonunion or delayed union. However, 9 (13%) long bone fractures in group (C) patients had atrophic nonunion. Furthermore, 5 (7.2%) long bone fractures in group (C) had delayed union. Long bone fractures in group (B) patients united with the mean time to union was 6.9, range (3-20) weeks compared to 22.4 (range 13-41) weeks in group (C) patients, a statistically significant difference (p=0.001). Another important finding of our study is that long bone fractures in head injury patients group (B) healed with more exuberant and florid callus formation compared with patients with long bone fractures only group (C) patients. The mean maximal thickness of union bridging callus as shown in CT scan was 26.3, range (4-48) mm in group (B), compared with 8.1 (range 2-20) mm in group C (p=0.001). The mean healing rate was also faster, statistically significant, in group B compared to group C (4.5, range 0.2-10.6) mm/week versus 0.38 (range 0.11-1) mm/week). These data, which all were statistically significant, indicate that patients with severe brain injuries have accelerated bone healing of concomitant long bone fractures with 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 severe head 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 (CNS) 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 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 volunteers group and the lowest levels were in group (B) patients with head 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 its role in enhancing bone formation, especially the intramembranous ossification or to localization and utilization of abundance of IGF-II at the sites of healing fractures 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. Radiographic analysis at two and four 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 endo-
osteally and periosteally in the animals that had been treated
with PDGF than in the controls.

The statistically significant high level of PDGF in head injury
patients with or without long bone fractures in our study may
confirm its possible osteogenic effect as reported by previous
studies and suggest that its secretion in abundance in head in-
jury 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] concluded that VEGF stimulate the for-
mation of competent bone in an environment deprived of its
normal vascularization and osteoprogenitor cell supply and
could be used to enhance the healing of fracture predisposed
to nonunion.

The results of this study showed statistically significant high
levels of VEGF in head injury patients with or without long
bone fractures and their decline after two weeks from injury.
This could be explained that in the initiation of the bone repair
process a crucial factor is the restoration of the blood flow to
the fracture site. VEGF is a potent angiogenic stimulator that
plays an important role in early processes of fracture healing.
Previous studies have demonstrated large amounts of VEGF
in the fracture hematoma in early stages of bone repair. Oth-
er 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 undiffer-
entiated mesenchymal stem cells (MSCs) into osteoblasts.

Sakai et al. [20] investigated the effects of local application
of Activin-A, a member of transforming growth factor-B super
family, on fracture healing using rat fibula fracture models.
Activin-A was injected locally to the fracture once a day for 2
weeks. Callus formation in a dose-dependent manner and both
callus volume and weight were found significantly increased.
Histologically, it was found that Activin-A promoted endochon-
dral bone formation. These findings suggest that Activin-A ex-
pressed during fracture healing promotes the healing process.
The finding of our study that the Activin-A growth factor was
found persistently elevated with statistical significance in head
injury patients with or without long bone fractures, groups (A)
and (B) may reveal its secretion in abundance in the damaged
brain which crosses blood brain barrier to systemic circulation
and produces secondary osteogenic effect, accelerating heal-
ing of associated long bone fractures.

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. The per-
sistent three weeks elevation of IL-1 Cytokine with statistical
significance in group (A) patients with head injury only and its
decline after the initial two weeks of the highest rise in group

(B) patients with head injury and concomitant long bone frac-
tures in our study can be explained that it is needed mostly in
early stages of fracture healing to produce in abundance the
pro-inflammatory cytokines and the inflammatory mediators
to initiate inflammation stage of bone repair. Intense inflam-
ination after fracture may enhance bone healing [21-25].

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 severe head injury and concomitant long bone frac-
tures suggest their mechanism of action as mediators or-
chestrating different molecular, cellular, and mechanical events
to enhance bone repair.

By all means, we do not believe that growth factors and cyto-
kines, we tested can explain the process of accelerated frac-
ture healing in head injury patients with long bone fractures.
They are step in the process not initiating it, the by-products of
subtle mechanism releasing them, the effect and not the initial
primary cause [26-30].

The therapeutic management of new bone formation remains
one of the key issues in orthopedic and this is what is interesting
in researching to understand the accelerated bone healing in
head injury patients, with the inspiration that such understand-
ing will 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,
accordingly, we thought that these growth factors, we tested
may serve as potential therapeutic agents to enhance the re-
pair of bone.

Conclusion

According to the results of this study, we conclude that long
bone fractures in head injury patients heal more expected-
ly, faster, and with exuberant and florid callus formation and
growth factors including: PDGF, VEGF, IGF- II, Activin-A, and
Cytokine IL-1 have coordinated roles in this enhanced fracture
healing, as mediators and molecular events, which were re-
leased 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 re-
generation.

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References


