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Evaluation and comparison of serum biomarkers in Patients after lower limb fractures

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Abstract

Background: Immediately after the bone injury and trauma, the changes in the concentrations of serum biomarkers initiates the cascading effects on the process of bone remodelling, but these cascading effects directly related to fracture size and time of healing of the bone fracture. Our study highlights the interplay between various serum biomarkers and healing of lower limb fractures during a study period of six months. This study aimed to prospectively investigate changes in serum inflammatory biomarker levels from the normal healthy control group and patient group.

Materials and Methods: In our study, the normal individuals accompanying orthopaedic patients who were visiting/admitted in the OPD and Emergency departments were formally enrolled for this study. A total number of thirty lower limb fracture patients who demonstrate progressive improvement during 12 consecutive weeks (3 Months) were enrolled in this study. These patients also provided their serum samples for biochemical estimation. The estimation and comparison in serum biomarkers' levels have been analysed in concern with their ability to predict impaired fracture healing at an early stage. All this consolidated data was analysed using SPSS software.

Results: The observations and estimations of this study highlight the statistically significant correlations between TNF-α, IL-1β, CRP, Calcium serum levels in Control, Fracture with Mild disability and Fracture with Moderate/Severe Disability groups.

Conclusions: In routine bone healing, serum biomarkers' changes were primarily dependent on the severity of bone damage and fracture size. The delay in healing large-sized fractures with Moderate/Severe Disability warrants massive cascading changes in bone remodelling.

Keywords: serum biomarkers, CRP, interleukin, calcium, lower limb fracture

Introduction

A fracture's anticipated outcome is bone remodelling and healing, which is explained with the proper functional stage of bone repair and regrowth immediately after a trauma. This enables the bone to regain the normal strength with typical load-carrying capacity without additional assistance [1]. The likely time-line process needed for a complete and adequate fracture healing depends on various biochemical and metabolic factors and biomarkers. The delay in fracture healing results in persistent pain and functional disability, so making the early diagnosis of such crucial biomarkers must be compulsory to avoid further complications [1-3].

Recent studies have highlighted that certain growth factors, including transforming growth factor-beta (TGF-β1) and the bone morphogenetic proteins (BMPs), play a significant role in fracture healing. These factors are synthesised at the site of trauma or bone injury; estimation of their serum levels have also been considered non-invasive tools to assess the bone remodelling and healing process [4-6]. Growth factors play a crucial role in regulating the various steps of cellular mechanisms, which leads to normal bone alignment and union, from the initial haematoma to the final remodelling stage [7]. Therefore, unusual expression and synthesis of these growth factors in the systemic blood circulation must be associated with the impaired fracture healing process. The measurement of bone turnover markers (BTMs) during the fracture healing process could enhance the bone healing stage's accurate assessment. By allowing the early detection in patients at risk of developing fracture complications and enabling advanced treatment of these complications prevents prolonged patient distress and disability [8].

In the present study, we investigated different serum biomarkers such as TNF-α, IL-1β, CRP, Calcium serum levels in Control, Fracture with Mild disability, and Fractured with Moderate/Severe Disability groups. This study has highlighted the necessity to estimate serum biomarkers' clinical effectiveness in monitoring the fracture healing process and carefully identifying patients at risk of developing impaired fracture healing processes.

Our study aimed to study prospective changes in inflammatory serum biomarker levels from day one of lower limb fracture to 3 months post lower limb fracture. We have also established the differences in inflammatory biomarker levels between mild and moderate severity of fracture patients who have reported some disability during these three months of lower limb fracture.

Materials and Methods

In our study, the normal individuals accompanying orthopaedic patients who were visiting/admitted in the OPD and Emergency departments were formally enrolled for this study. A total number of 30 lower limb fractured patients

and 30 healthy individuals considered as control subjects, from both ender between the age of 22 and 65 years, were included in this study. All participants were briefed and adequately advised in the local language, and their written informed, voluntary consent was obtained. All the enrolled subjects were subjected to a careful history, general and systemic physical examination. The questionnaire recorded information on gender, age, height, weight and comorbidity status (such as hypertension, diabetes mellitus and any other metabolic disorder) and medication use, including and nutritional supplementation. The height and weight of the individuals were used to calculate the body mass index (BMI). Our study was a prospective longitudinal study; this was designed to study persons who underwent lower limb fracture, and these patients were routinely followed up to 3 months post lower limb fracture.

Biochemical Estimation: 10 ml of blood obtained from every individual in this study. These samples of blood samples were processed and separated using a serum separator tube, which allowed to clot the blood samples within 30 minutes and then centrifuged for 15 minutes (1000 g). Serum samples were stored in a laboratory deep-freezer at -80°C, by using commercially available kits; different ELISA assays performed for estimation of serum CRP, TNF-α and IL-1β levels.

Statistical Analysis: All these consolidated data were analysed using SPSS software. The Student t-test and the Mann-Whitney test used for comparative analyses of serum biomarkers. The p-value of <0.05 was taken to be statistically significant.

Results

Table 1: General Demographic and clinical features of healthy control group and lower limb fractured patients' group.

Parameters	Control Group (n=30)	Patient Group (n=30)
Age (Years)	41±20.5	44±22.8*
Gender		
Male	18 (60%)	14 (46.67%)
Female	12 (40%)	16 (53.33%)
BMI	24.3±3.5	25.3±2.9**
Alcohol Consumption (Daily)	0	17 (56.67%)
Smoking (Frequently)	9 (30%)	20 (66.67%)
Analgesics and/or NSAID	0	30 (100%)***
Hypertensive	0	19 (63.33%)
Hypovitamin D	0	3 (10%)
Hypothyroidism	0	5 (16.67%)
Hypercholesterolemia	0	8 (26.67%)
Cardiovascular ailments	0	13 (43.33%)
Pulmonary ailments	0	10 (43.33%)
Earlier history of factures	6 (20%)	21 (70%)

*P Value 0.005, **P Value 0.007, ***P Value <0.001

Table 1 shows, the statistically significant correlation between BMI and Analgesics and/or NSAID when compared with the control and patient group respectively.

Table 2: Comparison of Serum Biomarkers between Control Group and at the time of lower limb fracture in patient group.

Parameters	Control Group (n=30)	Patient Group (n=30)	
		Fracture with Mild disability	Fracture with Moderate/Severe Disability
Age (Years)	41±20.5	39±8.42	42±20.9
Gender			
Male	18 (60%)	7 (23.33%)	7 (23.33%)
Female	12 (40%)	11 (36.67%)	5 (16.67%)
BMI	24.3±3.5	23.49±1.7	26.1±1.2
CRP (mg/l)	1.4±0.7	1.8±0.3	2.6±1.1
IL-1β (pg/ml)	0.9±0.4	1.3±0.6	1.8±0.4
IL-6 (pg/ml)	0.8±0.1	0.8±0.1	1.02±0.7
TNF-α (pg/ml)	3.6±1.7	2.9±0.9	4.1±1.7
Phosphate (mmol/L)	1.2±0.3	0.8±0.1	1.1±0.4
Calcium (mmol/L)	2.8±0.2	3.1±0.4	3.2±0.1
TSH (mU/L)	1.4±0.7	0.9±0.3	1.3±0.1
PTH (ng/L)	39±3.1	42.3±7	44.5±6.9

Data presented as Mean SD

The results depicted in Table 2 shows the statistically significant correlation between TNF-α, IL-1β, CRP, Calcium serum levels in Control, Fracture with Mild disability and Fracture with Moderate/Severe Disability.

Table 3: Comparison of Serum Biomarkers between Control Group and at the end of 3 months post lower limb fracture in patient group.

Parameters	Control Group (n=30)	Patient Group (n=30)	
		Fracture with Mild disability	Fracture with Moderate/Severe Disability
Age (Years)	41±20.5	39±8.42	42±20.9
Gender			
Male	18 (60%)	7 (23.33%)	7 (23.33%)
Female	12 (40%)	11 (36.67%)	5 (16.67%)
BMI	23.9±2.7	24.17±1.3	25.8±2.4
CRP (mg/l)	1.3±0.8	1.5±0.1	1.9±0.8
IL-1β (pg/ml)	0.8±0.6	1.2±0.3	1.6±0.6
IL-6 (pg/ml)	0.9±0.2	1±0.2	1.1±0.6
TNF-α (pg/ml)	2.5±0.9	3.2±1.2	3.9±0.9
Phosphate (mmol/L)	1.3±0.7	0.9±0.4	1.1±0.4
Calcium (mmol/L)	3±0.5	2.7±0.4	3.44±0.7
TSH (mU/L)	1.3±0.4	1.1±0.3	1.5±0.3
PTH (ng/L)	40.1±2.8	41.2±4.7	42.9±3.5

Data presented as Mean SD

The results depicted in Table 3 shows the statistically significant Corelation between Age, BMI, CRP, IL-1β and Phosphate serum levels in Fracture with Mild Disability and Fracture with Moderate/Severe Disability.

Discussion

The anatomical and physiological process of bone formation is influenced by various nutritional and biochemical factors which are acting locally to control the active mechanism of osteoblasts' proliferation, differentiation. The Transforming Growth Factor, i.e., TGFα and TGFβ and bone morphogenic proteins (BMPs) are, of course, very well-known effectors of signalling in osteoblast, bone formation, skeletal development and homeostasis [1-4].

The various endocrine hormones, such as thyroid hormone, the parathyroid hormone, can also play a crucial role in bone formation. As per the literature survey and our knowledge, our present study which the first of its kind study investigating the influence of serum biomarkers on the healing of lower limb fractures [5, 6].

On the first day of lower limb fracture and within 11-12 weeks post-fracture, both patient groups (i.e., Fracture with Moderate/Severe Disability and Fracture with Moderate/Severe Disability) expressed significantly elevated serum levels of CRP compared to the control group. C-reactive protein (CRP) is a protein synthesised by the human liver, an acute-phase marker of low-grade inflammation [8-10]. The increased CRP levels observed in this study indicate the severity of bone injury and local soft tissues, which causes inflammation. The elevated levels of serum CRP, which is considered an acute phase respondent, stayed elevated for 24 hours of post bone injury. These observations from our study pointed out the significance of the measurement of IL-6 levels in the first few hours of the trauma with bone fractures may indicate the extent of the bone injury. At the end of 3 months post-fracture, the serum CRP levels almost matched in facture recovered patients and those with no fracture (control) group [11].

On the first day of lower limb fracture and within 11-12 weeks post-fracture, both patient groups serum TNF-α levels were significantly elevated in both patient groups (i.e., Fracture with Moderate/Severe Disability and Fracture with Moderate/Severe Disability). The White Blood Cells, mainly monocytes and macrophages, are the prominent synthesisers of TNF-α, which activate the secretion of different types of pro-and anti-inflammatory cytokines, including IL-6, IL-8 and IL-10. Our findings highlight the apparent relationship between higher serum TNF-α levels signifying the rapid bone modelling and healing rate [12-15].

Our study's results highlighted the significant differences in serum cytokines and CRP levels between controls and the patient groups. These results from our study corroborating with other research studies of bone injury, spine ache and musculoskeletal pain conditions.

Our study draws several limitations; one of such limiting parameters is the sample sizes were too small. In addition to this, our study data of BMI also indicates that a large number of participants were from the overweight range. Further, a detailed cohort study must be needed to determine the co-relationship between serum biomarkers and their role in bone modelling and healing.

Conclusions

Humans are blessed with a natural homeostatic mechanism where human bones exhibit a lifelong capacity for remodelling, healing, regenerate and repair. Remodelling and healing of bone healing in adult humans depend on a stimulatory process this enhances the inflammatory response from osteoblasts and the innate immune system.

In conclusion, our study demonstrates the relationship between initially higher levels of serum CRP following a bone injury that persist in those patient group of Fracture with Moderate/Severe Disability and Fracture with Moderate/Severe Disability. This study also highlights the significant role of inflammatory biomarkers in the bone remodelling and healing of fractures.

Further extensive study is need of the hour to determine the role of various other biochemical and pathological biomarkers in the healing of injured bone by analysing their magnitude and timing of inflammation in critically ill patients.

References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet,2017:390(10100):1211-1259. PMID: 28919117.
2. Nguyen C, Poiraudeau S, Rannou F. From Modic 1 vertebral-endplate subchondral bone signal changes

detected by MRI to the concept of 'active discopathy'. Ann Rheum Dis,2015:74(8):1488-94. PMID: 25977562.
3. Sainoh T, Orita S, Miyagi M, Inoue G, Kamoda H, Ishikawa T, *et al*. Single Intradiscal Administration of the Tumor Necrosis Factor-Alpha Inhibitor, Etanercept, for Patients with Discogenic Low Back Pain. Pain Med,2016:17(1):40-5. PMID: 26243249.
4. Tang P, Zhu R, Ji WP, *et al*. The NLRP3/Caspase-1/Interleukin-1β Axis Is Active in Human Lumbar Cartilaginous Endplate Degeneration. Clin Orthop Relat Res,2016:474(8):1818-1826. PMID: 27146654
5. Teboul-Core´ S, Roux C, Borderie D, Kolta S, Lefèvre-Colau M-M, Poiraudeau S, *et al*. Bone mineral density and bone remodeling markers in chronic low back pain patients with active discopathy: A case-control exploratory study. PLoS ONE,2018:13(6):e0196536.
6. Miller RR, Cappola AR, Shardell MD, Hawkes WG, Yu-Yahiro JA, Hebel JR, *et al*. Persistent changes in interleukin-6 and lower extremity function following hip fracture. J Gerontol A Biol Sci Med Sci. 2006; 61(10):1053-8. PMID: 17077198.
7. Matheny ME, Miller RR, Shardell MD, Hawkes WG, Lenze EJ, Magaziner J, *et al*. Inflammatory cytokine levels and depressive symptoms in older women in the year after hip fracture: findings from the Baltimore Hip Studies. J Am Geriatr Soc,2011:59(12):2249-55. PMID: 22188073.
8. Saribal D, Hocaoglu-Emre FS, Erdogan S *et al*. Inflammatory cytokines IL-6 and TNF-α in patients with hip fracture. Osteoporos Int,2019:30:1025-1031.
9. Khallaf FG, Kehinde EO, Mostafa A. Growth factors and cytokines in patients with long bone fractures and associated spinal cord injury. Journal of Orthopaedics,2016:13(2):69-75.
10. Jiao J, Wang JW, Xiao F, Huang YC. The association between the levels of CRP, IL-10, PLA2, Fbg and prognosis in traumatic fracture of lower limb. Exp Ther Med,2016:12(5):3209-3212. PMID: 27882139
11. Wright EH, Khan U. Serum complement-reactive protein (CRP) trends following local and free-tissue reconstructions for traumatic injuries or chronic wounds of the lower limb. J Plast Reconstr Aesthet Surg,2010:63(9):1519-22. PMID: 19758848.
12. Bhosale JP, Deshpande S. CRP for diagnosis and IL-6 for monitoring of infection in lower limb fracture patients. Journal of Trauma and Orthopaedic Surgery,2018:13(4):13-17.
13. Yao P, Bennett D, Mafham M, *et al*. Vitamin D and Calcium for the Prevention of Fracture: A Systematic Review and Meta-analysis. JAMA Netw Open,2019:2(12):e1917789.
14. Ban ZN, Li ZJ, Gu QS *et al*. Correlation of serum PTH level and fracture healing speed in elderly patients with hip fracture. J Orthop Surg Res,2019:14:361.
15. Waring AC, Harrison S, Fink HA, *et al*. A prospective study of thyroid function, bone loss, and fractures in older men: The MrOS study. J Bone Miner Res,2013:28(3):472-479. PMID: 23018684.