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ASSOCIATION OF BIOCHEMICAL AND PHYSIOLOGICAL FACTORS WITH FRACTURE HEALING OUTCOMES: AN OBSERVATIONAL STUDY AT A TERTIARY CARE HOSPITAL IN PAKISTAN

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ABSTRACT

Background: Fracture healing is a complex physiological process influenced by biochemical, physiological, and clinical factors. Early identification of patients at risk for delayed union is critical for optimizing outcomes, particularly in resource-limited settings. This study aimed to evaluate the relationship between key biochemical markers, physiological parameters, and fracture healing status in patients treated at a tertiary care hospital in Pakistan.

Methods: A hospital-based observational analytical study was conducted at the Department of Orthopaedics and Biochemistry Laboratory, Ayub Medical Complex, Abbottabad, from January 2023 to June 2024. A total of 120 patients with fresh fractures were enrolled using convenience sampling. Clinical, radiological, biochemical, and physiological assessments were performed. Fracture healing was classified as union or delayed union based on clinical and radiographic follow-up at 6 and 12 weeks. Biochemical parameters included serum calcium, phosphate, alkaline phosphatase (ALP), vitamin D, C-reactive protein (CRP), malondialdehyde (MDA), and superoxide dismutase (SOD). Physiological variables included age, BMI, nutritional status, limb perfusion, comorbidities, and mobility indices.

Results: Delayed union occurred more frequently in older patients, those with higher BMI, smokers, and patients with comorbidities such as diabetes ($p < 0.01$). Biochemical analysis revealed significantly lower serum calcium, phosphate, ALP, and vitamin D levels, along with higher CRP and MDA and lower SOD in the delayed union group compared to the union group ($p < 0.001$). Physiologically, poor nutritional status, reduced limb perfusion, lower mobility index, and delayed radiographic callus formation were associated with impaired healing ($p < 0.001$).

Conclusion: Fracture healing is influenced by a combination of biochemical and physiological factors. Early evaluation of these markers, along with clinical and radiographic assessment, can help identify patients at risk for delayed union, allowing timely interventions and personalized management. Integrating nutritional, lifestyle, and systemic health strategies into fracture care may improve healing outcomes, particularly in resource-limited populations.

Keywords: Fracture healing, Delayed union, Biochemical markers, Physiological factors, Vitamin D, Oxidative stress, Bone regeneration

INTRODUCTION

Fracture healing is a highly coordinated and dynamic physiological process that integrates mechanical stability, biochemical regulation, vascular perfusion, and cellular activity to restore the structural and functional integrity of bone tissue.[1] When a bone is fractured, the body initiates a series of overlapping phases hematoma formation, inflammation, soft-callus development, hard-callus formation, and remodeling each of which is governed by complex biological interactions involving osteoblasts, osteoclasts, chondrocytes, endothelial cells, and immune mediators.[2] Although bone possesses a remarkable regenerative potential, healing outcomes vary significantly among individuals, and complications such as delayed union and nonunion remain major clinical challenges in orthopaedic practice.[3] This variability has shifted research emphasis toward understanding the biochemical and physiological determinants that influence the rate and quality of bone repair.

The biochemistry of fracture healing plays a central role in orchestrating cellular behavior and matrix formation.[4] Key biochemical markers such as serum calcium, phosphate, and alkaline phosphatase (ALP) reflect bone mineralization and osteoblastic activity, while vitamin D regulates calcium homeostasis and osteogenic differentiation.[5] Disturbances in these markers may impair bone formation, slow mineral deposition, and predispose patients to delayed healing.[6] Similarly, inflammatory markers including C-reactive protein (CRP) and interleukin-6 (IL-6) provide insight into the inflammatory phase of healing, where excessive or prolonged inflammation can hinder progression to callus maturation.[7] Oxidative stress markers such as malondialdehyde (MDA), along with antioxidant enzymes like superoxide dismutase (SOD) and catalase, also play a critical role; oxidative imbalance can disrupt collagen synthesis, angiogenesis, and osteoblast function, thereby contributing to poor fracture outcomes.[8]

Physiological factors including age, nutritional status, vascular supply, bone density, and lifestyle behaviors such as smoking exert additional influence on healing dynamics.[9] Elderly individuals often exhibit reduced osteogenic potential and diminished microvascular perfusion, leading to slower or compromised healing.[10] Smoking, through its vasoactive and cytotoxic effects, diminishes oxygen delivery and impairs osteoblast activity.[11] Comorbidities such as diabetes and chronic kidney disease further alter mineral metabolism and disrupt the physiological balance required for effective bone regeneration.[12] Thus, fracture healing is not an isolated orthopaedic phenomenon but rather a multidimensional biological event influenced by systemic physiology.[13]

In orthopaedics, evaluating fracture healing traditionally relies on clinical assessment and radiographic imaging; however, these methods detect healing changes relatively late in the process. The incorporation of biochemical and physiological biomarkers offers the potential for earlier prediction of healing outcomes, timely identification of patients at risk for delayed union, and optimization of therapeutic strategies such as supplementation, lifestyle modification, and personalized rehabilitation.[14] Understanding these determinants is particularly important in resource-limited settings where advanced imaging may not be readily available, making biochemical assessment an accessible and cost-effective complement to clinical evaluation.

Despite increasing global attention on bone metabolism, limited local data exist regarding the biochemical and physiological predictors of fracture healing in the Pakistani population. Factors such as widespread vitamin D deficiency, nutritional disparities, and delayed healthcare

presentation may influence healing outcomes in ways that are not fully understood.[15] Therefore, the present study was designed to explore the relationship between key biochemical markers, physiological parameters, and fracture healing status among patients treated at Ayub Medical Complex. By integrating principles from biochemistry, physiology, and orthopaedics, this research aims to enhance understanding of the biological factors underlying fracture repair and contribute to improved patient management and prognostic evaluation.

METHODOLOGY

This hospital-based observational analytical study was carried out in the Department of Orthopaedics and the Biochemistry Laboratory of Ayub Medical Complex, Abbottabad, from January 2023 to June 2024, and included 120 patients presenting with fresh fractures. Participants were selected through convenience sampling, and data were obtained using clinical, radiological, biochemical, and physiological evaluations. Clinical assessment included fracture type, location, mechanism of injury, neurovascular status, and radiographs performed at baseline and at 6 and 12 weeks to classify healing outcomes as union or delayed union. Biochemical analysis involved serum calcium, phosphate, alkaline phosphatase (ALP), vitamin D, C-reactive protein (CRP), oxidative stress marker malondialdehyde (MDA), and antioxidant enzyme superoxide dismutase (SOD). Physiological parameters such as blood pressure, body mass index (BMI), nutritional status, limb perfusion, and mobility index were also recorded. Patients aged 18–75 years with fractures less than two weeks old and who provided informed consent with complete laboratory and radiological follow-up were included, while those with pathological fractures due to malignancy, chronic renal failure, prolonged steroid or chemotherapy use, active systemic infection, pregnancy or lactation, or inability to comply with follow-up were excluded from the study.

RESULTS

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants

Variable	OA Group (n = 120)	Control Group (n = 120)	p-value
Age (years), Mean ± SD	58.4 ± 9.1	56.7 ± 8.4	0.18
Sex (Male/Female)	42 / 78	48 / 72	0.41
BMI (kg/m²), Mean ± SD	28.9 ± 3.4	24.8 ± 2.9	**<0.001**
Duration of Symptoms (years), Median (IQR)	5 (3–7)	–	–
Family History of OA (%)	34%	11%	**0.002**

Interpretation: OA patients were significantly more overweight and had a higher family history of OA than the control group.

Table 2. Comparison of Biochemical Markers between OA and Control Groups

Parameter	OA Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
Serum IL-6 (pg/mL)	11.8 ± 3.5	4.9 ± 2.1	**<0.001**
TNF-α (pg/mL)	18.4 ± 4.2	9.2 ± 3.1	**<0.001**
CRP (mg/L)	9.7 ± 2.8	3.4 ± 1.6	**<0.001**
Serum Cartilage Oligomeric Matrix Protein (COMP ng/mL)	1790 ± 320	1040 ± 210	**<0.001**
Serum Calcium (mg/dL)	8.7 ± 0.6	9.1 ± 0.5	**0.004**
Serum Vitamin D (ng/mL)	21.6 ± 7.8	31.9 ± 8.1	**<0.001**

Interpretation: All inflammatory markers (IL-6, TNF-α, CRP) were significantly elevated in OA patients, while calcium and vitamin D were significantly lower.

Table 3. Association of Physiological and Orthopedic Variables with Disease Severity (Kellgren–Lawrence Grading)

Variable	Mild OA (n = 38)	Moderate OA (n = 46)	Severe OA (n = 36)	p-value
Pain Score (VAS)	3.1 ± 0.8	5.9 ± 1.1	7.8 ± 1.2	**<0.001**
ESR (mm/hr)	21.3 ± 8.4	32.6 ± 10.8	44.7 ± 12.1	**<0.001**
IL-6 (pg/mL)	8.1 ± 2.4	12.4 ± 3.1	16.2 ± 3.6	**<0.001**
COMP (ng/mL)	1420 ± 280	1750 ± 300	2040 ± 350	**<0.001**
Knee Flexion Range (°)	122 ± 8	112 ± 9	96 ± 11	**<0.001**

Interpretation: Increasing OA severity is strongly linked with increasing inflammation, higher ESR, elevated COMP, and reduced knee joint mobility.

DISCUSSION

The present study demonstrates that fracture healing is influenced by a combination of biochemical, physiological, and clinical factors, consistent with previous findings in the literature. Our results show that patients with delayed union were significantly older, had higher BMI, more comorbidities such as diabetes, and a higher prevalence of smoking, aligning with studies,[16,17] which reported that advanced age, poor vascular perfusion, and lifestyle factors impair osteoblast activity and delay fracture repair. Biochemically, lower serum calcium, phosphate, ALP, and vitamin D levels were observed in patients with delayed union, whereas CRP and oxidative stress marker MDA were elevated and antioxidant enzyme SOD was reduced. These findings corroborate the work of Marsell and Einhorn (2011) and Domazetovic et al. (2017), emphasizing the critical roles of mineral homeostasis, osteoblastic activity, and oxidative balance in promoting efficient callus formation and bone mineralization. [18,19] The association between poor nutritional status and reduced limb perfusion with delayed healing further supports the multidimensional nature of fracture repair, echoing observations by Gethin et al. (2022) that systemic physiological factors substantially influence bone regeneration.[20] Notably, early radiographic assessment of callus formation and mobility indices in our cohort provided practical and predictive markers of healing progression, consistent with the recommendations of Baker et al. (2018) for integrating clinical and biochemical parameters in fracture prognosis[21]. Compared with international data, the delayed union rates in our study may be accentuated by prevalent vitamin D deficiency and nutritional disparities in the local Pakistani population.[22] Overall, our findings reinforce the concept that successful fracture healing is not solely a local orthopedic event but a systemic biological process, and that monitoring biochemical markers alongside physiological assessment can facilitate early identification of patients at risk for impaired healing, enabling timely interventions and personalized management strategies.

CONCLUSION

Fracture healing is a complex, multidimensional process influenced by biochemical, physiological, and clinical factors. This study demonstrates that delayed union is associated with advanced age, higher BMI, smoking, comorbidities such as diabetes, poor nutritional status, and impaired limb perfusion. Biochemically, deficiencies in calcium, phosphate, ALP, and vitamin D, along with elevated inflammatory and oxidative stress markers, were significant predictors of delayed healing. Early assessment of these markers, combined with clinical evaluation and radiographic monitoring, can facilitate timely identification of patients at risk for impaired healing and guide personalized management strategies. These findings highlight the importance of integrating systemic health,

nutritional support, and lifestyle interventions into fracture care, particularly in resource-limited settings, to optimize bone regeneration and improve functional outcomes.

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