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Research Article

Comparative Study of Histomorphometric Analysis of Fracture Healing in Controlled Vs Uncontrolled T2DM Patients

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Abstract
Introduction: Type 2 Diabetes Mellitus (T2DM) is known to adversely affect bone metabolism and fracture healing. However, the differential impact of glycemic control on histomorphometric and vascular parameters remains underexplored.
Aim: To compare fracture healing outcomes in controlled versus uncontrolled T2DM patients using biochemical, histological, and vascular indices.
Methods: A prospective observational study was conducted on 120 patients with long bone fractures, divided into controlled (HbA1c ≤ 7.0%) and uncontrolled (HbA1c ≥ 8.5%) T2DM groups. Serum bone remodeling markers (osteocalcin, P1NP, CTX, ALP, RANKL/OPG), histomorphometric indices (osteoblast/osteoclast surface, mineral apposition rate, bone formation rate), and vascularization parameters (CD31+ vessel density, VEGF expression, perfusion index) were assessed at six weeks post-fracture.
Results: Uncontrolled T2DM patients showed significantly lower osteocalcin, P1NP, and ALP levels, and higher CTX and RANKL/OPG ratios ($p<0.001$). Histomorphometry revealed reduced osteoblast surface and bone formation rate, with elevated osteoclast activity. Vascularization was markedly impaired, with reduced VEGF expression and perfusion indices in the uncontrolled group.
Conclusion: Poor glycemic control in T2DM patients is associated with delayed and compromised fracture healing. Optimizing metabolic status should be a key component of fracture management in diabetic populations.

Keywords: Type 2 Diabetes Mellitus; Fracture Healing.

INTRODUCTION

Fracture healing is a complex biological process involving inflammation, cellular proliferation, matrix deposition, and remodeling. In patients with Type 2 Diabetes Mellitus (T2DM), this process is often compromised due to chronic hyperglycemia, impaired angiogenesis, and altered bone metabolism[1]. Poor glycemic control has been associated with delayed union, increased risk of nonunion, and suboptimal functional recovery[2]. While the systemic effects of diabetes on bone health are well-documented, the differential impact of controlled versus uncontrolled T2DM on histomorphometric parameters of fracture healing remains inadequately explored[3].Histomorphometric analysis offers a quantitative assessment of bone remodeling by evaluating osteoblast and osteoclast activity, mineral apposition rate, and bone formation indices[4]. Additionally, serum biomarkers such as osteocalcin, P1NP, CTX, and RANKL/OPG ratio provide insights into the dynamic balance between bone formation and resorption[5]. Vascularization, a critical determinant of fracture repair, is often impaired in diabetic patients due to reduced VEGF expression and microvascular density[6].This study aims to compare the histomorphometric and biochemical profiles of fracture healing in patients with controlled versus uncontrolled T2DM. By integrating cellular, molecular, and vascular parameters, the research seeks to elucidate the pathophysiological differences that influence healing outcomes and inform targeted therapeutic strategies.

Aims and Objectives

Aim

To compare the histomorphometric and biochemical parameters of fracture healing in

patients with controlled versus uncontrolled Type 2 Diabetes Mellitus.

Objectives

1. To evaluate and compare serum bone remodeling markersincluding osteocalcin, P1NP, CTX, ALP, and RANKL/OPG ratioin controlled and uncontrolled T2DM patients at six weeks post-fracture.
2. To assess histomorphometric indices such as osteoblast surface, osteoclast surface, mineral apposition rate, and bone formation rate in both groups.
3. To analyze vascularization parametersincluding CD31+ vessel density, VEGF expression, and perfusion indexusing immunohistochemistry and imaging techniques.

To determine the association between glycemic control (as measured by HbA1c levels)and the quality of fracture healing across cellular, biochemical, and vascular domains.

MATERIALS AND METHODS

Study Design

This was a prospective, observational, comparative study conducted over a 12-month period at the Department of Orthopaedics and Department of Medicine.

Study Population

A total of 120 adult patients (aged 40–70 years) with radiologically confirmed long bone fractures were enrolled. Patients were stratified into two groups based on glycemic control:

A. Controlled T2DM group (n=60): HbA1c ≤ 7.0%

B. Uncontrolled T2DM group (n=60): HbA1c ≥ 8.5%

Inclusion Criteria

1. Diagnosed cases of Type 2 Diabetes Mellitus for ≥1 year
2. Closed long bone fractures managed surgically
3. Willingness to participate and provide informed consent

Exclusion Criteria

1. Type 1 Diabetes Mellitus or secondary diabetes
2. Open fractures, pathological fractures, or polytrauma
3. Chronic kidney disease, malignancy, or immunosuppressive therapy
4. Non-compliance with follow-up protocol

Data Collection

Clinical and demographic data were recorded at baseline. Blood samples were collected at week 6 post-fracture to assess serum bone remodelling markers: osteocalcin, P1NP, CTX, ALP, and RANKL/OPG ratio. Histomorphometric analysis of callus tissue was performed during routine follow-up using standardized biopsy protocols. Parameters included osteoblast surface (% BS), osteoclast surface (% BS), mineral apposition rate (μm/day), and bone formation rate (%/year). Vascularization was evaluated using CD31 immunohistochemistry, VEGF expression scoring, and CT angiography–based perfusion index.

Statistical Analysis

Data were analysed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation and compared using independent t-tests. Categorical variables were analyzed using chi-square tests. A p-value < 0.05 was considered statistically significant

OBSERVATION AND RESULT

Table 1: Baseline Demographics and Clinical Characteristics

Sr No	Characteristic	Controlled T2dm (N=60)	Uncontrolled T2dm (N=60)	P-Value
1	Age (years)	58.2 ± 7.6	59.1 ± 8.1	0.48
2	Male (%)	38 (63.3%)	36 (60.0%)	0.71
3	BMI (kg/m²)	27.4 ± 3.2	28.1 ± 3.5	0.22
4	Duration of T2DM (years)	6.8 ± 2.5	7.1 ± 2.9	0.53
5	HbA1c (%)	6.9 ± 0.4	9.2 ± 0.6	<0.001

The study enrolled 120 patients with long bone fractures, evenly divided into controlled (n=60) and uncontrolled (n=60) Type 2 Diabetes Mellitus (T2DM) groups. The mean age was comparable between groups (58.2 ± 7.6 vs 59.1 ± 8.1 years, p=0.48), with a similar male predominance (63.3% vs 60.0%, p=0.71). Body mass index (BMI) and duration of diabetes were also statistically similar (BMI: 27.4 ± 3.2 vs 28.1 ± 3.5 kg/m², p=0.22; duration: 6.8 ± 2.5 vs 7.1 ± 2.9 years, p=0.53). However, glycemic control differed significantly, with the uncontrolled group exhibiting markedly higher HbA1c levels (9.2 ± 0.6%) compared to the controlled group (6.9 ± 0.4%, p<0.001), confirming effective stratification.

Table 2: Bone Remodelling Markers (Serum Levels at Week 6 Post-Fracture)

Sr No	Marker	Controlled T2DM (n=60)	Uncontrolled T2DM (n=60)	p-value
1	Osteocalcin (ng/mL)	18.4 ± 3.2	12.7 ± 2.9	<0.001
2	P1NP (ng/mL)	56.1 ± 7.8	41.3 ± 6.5	<0.001
3	CTX (ng/mL)	0.42 ± 0.08	0.58 ± 0.10	<0.001
4	ALP (U/L)	92.5 ± 14.6	78.3 ± 12.9	0.002
5	RANKL/OPG Ratio	0.72 ± 0.11	1.03 ± 0.14	<0.001

Serum biomarkers assessed at week 6 post-fracture revealed significant differences in bone turnover. Patients with controlled T2DM had higher levels of osteocalcin (18.4 ± 3.2 ng/mL vs 12.7 ± 2.9 ng/mL, p<0.001) and P1NP (56.1 ± 7.8 ng/mL vs 41.3 ± 6.5 ng/mL, p<0.001), indicating more active bone formation. Conversely, CTX levels—a marker of bone resorption—were elevated in the uncontrolled group (0.58 ± 0.10 ng/mL vs 0.42 ± 0.08 ng/mL, p<0.001). Alkaline phosphatase (ALP) was also significantly lower in uncontrolled T2DM (78.3 ± 12.9 U/L vs 92.5 ± 14.6 U/L, p=0.002). The RANKL/OPG ratio, a key indicator of osteoclast activation, was notably higher in the uncontrolled group (1.03 ± 0.14 vs 0.72 ± 0.11, p<0.001), suggesting enhanced bone resorption

Table 3: Osteoblast and Osteoclast Activity (Histomorphometric Indices)

Sr No	Parameter	Controlled T2DM (n=60)	Uncontrolled T2DM (n=60)	p-value
1	Osteoblast surface (% BS)	22.6 ± 4.1	14.2 ± 3.7	<0.001
2	Osteoclast surface (% BS)	8.3 ± 2.2	12.5 ± 2.9	<0.001
3	Mineral apposition rate (μm/day)	1.45 ± 0.21	0.98 ± 0.17	<0.001
4	Bone formation rate (%/year)	28.9 ± 5.3	17.4 ± 4.8	<0.001

Histomorphometric analysis of fracture callus tissue demonstrated impaired cellular activity in uncontrolled T2DM. Osteoblast surface coverage was significantly reduced (14.2 ± 3.7% vs 22.6 ± 4.1%, p<0.001), while osteoclast surface was elevated (12.5 ± 2.9% vs 8.3 ± 2.2%, p<0.001). The mineral apposition rate—a measure of new bone deposition—was lower in uncontrolled T2DM (0.98 ± 0.17 μm/day vs 1.45 ± 0.21 μm/day, p<0.001). Similarly, the bone formation rate was markedly reduced (17.4 ± 4.8%/year vs 28.9 ± 5.3%/year, p<0.001), indicating compromised regenerative capacity**.**

Table 4: Vascularization Parameters (Immunohistochemistry & Imaging)

Sr No	Parameter	Controlled T2DM (n=60)	Uncontrolled T2DM (n=60)	p-value
1	CD31+ vessel density (/mm²)	34.7 ± 6.2	21.8 ± 5.4	<0.001
2	VEGF expression score (0–3 scale)	2.4 ± 0.5	1.6 ± 0.4	<0.001
3	Perfusion index (CT angiography)	0.82 ± 0.07	0.61 ± 0.09	<0.001

Vascularization, assessed via immunohistochemistry and imaging, was significantly impaired in uncontrolled T2DM. CD31+ vessel density was lower (21.8 ± 5.4/mm² vs 34.7 ± 6.2/mm², p<0.001), and VEGF expression scores were reduced (1.6 ± 0.4 vs 2.4 ± 0.5 on a 0–3 scale, p<0.001). Perfusion index measured by CT angiography also showed diminished vascular supply in the uncontrolled group (0.61 ± 0.09 vs 0.82 ± 0.07, p<0.001), suggesting that poor glycaemic control adversely affects angiogenesis and tissue perfusion during fracture healing.

DISCUSSION

The present study demonstrates that patients with uncontrolled Type 2 Diabetes Mellitus (T2DM) exhibit significantly compromised fracture healing compared to those with controlled glycemic status. This is evident across serum bone remodeling markers, histomorphometric indices, and vascularization parameters.Biochemical markers of bone turnover showed a clear divergence between groups. Osteocalcin and P1NP, both indicators of bone formation, were significantly lower in uncontrolled T2DM patients. These findings are consistent with Forner and Sheu's review, which highlighted suppressed osteoblast activity and reduced bone formation in poorly controlled diabetes due to chronic hyperglycemia and oxidative stress[5]. Elevated CTX and RANKL/OPG ratios in the uncontrolled group suggest enhanced bone resorption, corroborating Marin et al.'s findings that hyperglycemia promotes osteoclastogenesis and disrupts bone remodeling[1].Histomorphometric analysis revealed reduced osteoblast surface and mineral apposition rate, alongside increased osteoclast activity in uncontrolled T2DM. Follak et al. demonstrated similar trends in diabetic rat models, where poor metabolic control led to diminished bone formation and increased resorptive surfaces[7]. Cai et al. further confirmed that diabetes impairs biomechanical integrity and cellular regeneration, contributing to delayed union and increased nonunion rates[8].Vascularization parameters were markedly reduced in uncontrolled T2DM, with lower CD31+ vessel density and VEGF expression. Wang et al. showed that insulin therapy in diabetic rats restored VEGF levels and improved angiogenesis, underscoring the role of glycemic control in vascular regeneration[9]. Schall et al. identified that osteoblast-derived VEGF is essential for coupling angiogenesis with osteogenesis, and its downregulation in diabetes impairs both vascular and bone healing[10].The underlying mechanisms are multifactorial. Chronic hyperglycemia induces the formation of advanced glycation end products (AGEs), which bind to RAGE receptors and trigger inflammatory cascades that inhibit osteoblast differentiation and promote osteoclast activity[1]. Additionally, diabetes-associated microvascular dysfunction limits nutrient delivery and impairs neovascularization, further delaying callus maturation and remodeling[9].Taken together, these findings reinforce the hypothesis that glycemic control is a critical determinant of fracture healing outcomes. The integration of biochemical, histological, and vascular data provides a comprehensive understanding of the pathophysiological disruptions in uncontrolled T2DM and highlights the need for targeted metabolic and orthopedic interventions.

CONCLUSION

Present study demonstrates that uncontrolled Type 2 Diabetes Mellitus significantly impairs fracture healing across biochemical, histomorphometric, and vascular domains. Patients with poor glycemic control exhibited reduced osteoblast activity, elevated bone resorption markers, diminished mineral apposition, and compromised vascularization. These findings underscore the critical role of glycemic regulation in promoting effective bone regeneration. Integrating metabolic control into orthopedic management protocols may improve healing outcomes and reduce complications in diabetic fracture patients.

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