

# REDUCTIONS IN GROWTH FACTORS IN THE FRACTURE HEMATOMA OF DIABETIC PATIENTS

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**Introduction:** The association between diabetes mellitus (DM) and impaired bone healing has been documented in both clinical and experimental settings. Although there is a paucity of literature concerning diabetic fracture healing, previous work suggests that the effects of diabetes begin at a very early stage in the fracture healing process. Cellularity in the callus during the proliferative phase of healing is reduced by 40 percent in untreated diabetic animals due to impairment in proliferation, migration or both. Despite these advances, little work has been done to describe the critical factors affecting cellular proliferation in fracture healing, such as mitogenic growth factors and/or inflammatory cytokines. Growth factors, which are locally produced within the fracture environment, play a critical role in cellular chemotaxis, cellular proliferation, extra-cellular matrix production and angiogenesis. Two of these growth factors are platelet-derived growth factor (PDGF) and transforming growth factor- $\beta$  (TGF- $\beta$ ). The purpose of this study was to determine whether the expression of growth factors, PDGF and TGF- $\beta$ , are altered in DM versus non-DM fracture hematoma.

**Materials and Methods:** Patient Population: At this Level I trauma center, patients undergoing open reduction internal fixation (ORIF) surgery were enrolled in an IRB-approved study. To minimize variability in this study the following inclusion criterion were used: 1. closed fracture requiring ORIF, 2. patient's age between 18 and 72 years old, and 3. the time from injury to surgery < 20 days. Sample Collection/Processing: Fracture hematoma and blood samples were obtained from patients undergoing surgical treatment. One set of blood samples were collected for testing of HbA<sub>1c</sub> levels which is an indicator of blood glucose control. The other blood samples were centrifuged for ten minutes at 2,000 RPM, and the supernatant was collected and stored at -80° C until testing. The fracture hematoma samples were collected during surgery, flash frozen, and then homogenized using a biopulverizer to release the cellular contents. These contents were then diluted by 4 mg/ml with a protease inhibitor cocktail (Sigma, St. Louis, MO) and stored at -80° C until testing. Growth Factor and Total Protein Quantitation: For both PDGF and TGF-B quantitation, Quantikine ELISA kits (R&D Systems, Minneapolis, MN) were used. The ELISA assay used a quantitative sandwich enzyme technique. Using a spectrophotometer and a standard curve, the reflected wavelength of each sample was correlated to a specific concentration. These growth factor levels were then normalized using a total protein assay (Pierce St. Louis, MO). Statistical Analysis: Statistical analysis was performed using 1-way ANOVA and correlation analyses using StatView (SAS, Kerry, NC).

**Results: Patient Population:**

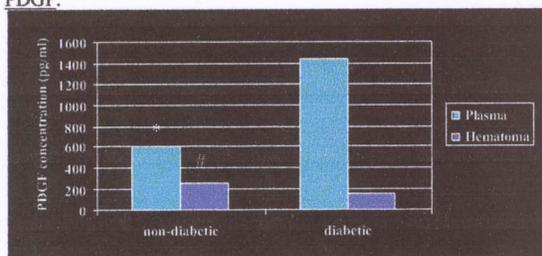
	Avg Age (yrs)	Time to Surgery (days)	HbA <sub>1c</sub>
Non-diabetic (n=23)	38.6 ± 15.8	12.8 ± 4.0	3.15 ± 0.68
Diabetic (n=7)	42.8 ± 11.0	17.0 ± 6.1	7.61 ± 0.97*

Table 1. \* indicates significance (p < 0.05)

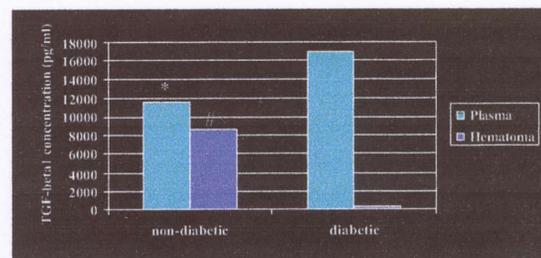
The diabetic and non-diabetic groups were matched for age, time to surgery and fracture type and location. The diabetic group has a significantly higher HbA<sub>1c</sub> levels indicating poor glucose control.

**Growth Factor Quantitation:**

**PDGF:**



**TGF- $\beta$ :**



Figures 1 and 2. \* and # indicates statistical significance (p < 0.05) between the diabetic and non-diabetic plasma and hematoma groups, respectively.

**Protein Content:**

The protein content in the fracture hematoma (314 Vs. 385 mg/ml) and plasma (513 Vs. 594 mg/ml) were not statistically different between the DM and non-DM groups.

**Statistics:**

	PDGF Plasma	PDGF Hematoma	TGF- $\beta$ Plasma	TGF- $\beta$ Hematoma
DM vs non-DM	0.005	0.048	0.047	0.021

Table 2. Summary of p-values

**Discussion:** Little work has been done to describe the role of the inflammatory phase, specifically the fracture hematoma, in the normal and DM fracture healing process. Grundness et al removed the fracture hematoma in a Sprague-Dawley rat fracture model during stabilization, which impaired the initial phase of fracture healing. Removal of the fracture hematoma at days 2 and 4 significantly decreased bending moment, bending rigidity and fracture energy, compared to control fractures. However, the removal of the fracture hematoma 30 minutes following fracture had less of an impact on the mechanical properties. They concluded that removal of an organized fracture hematoma two or four days after fracture impairs fracture healing more acutely than immediate removal. It is inferred that the early fracture hematoma develops critical factors or role in the development of the fracture callus. Analysis of the non-DM human fracture hematoma confirm this observation with the presence of two critical factors TGF- $\beta$  and PDGF. While both growth factors, PDGF and TGF- $\beta$ , are released from platelets during the earliest phase of fracture healing leading to the hematoma formation, the growth factor levels quantitated in this study (over 12 days after injury) probably reflect local production and non-platelet derived etiology.

Growth factors PDGF and TGF- $\beta$ , which are locally produced within the fracture environment, play a critical role in cellular chemotaxis, cellular proliferation, extra-cellular matrix production and angiogenesis. Andrew et al demonstrated that PDGF is expressed during normal human fracture repair and likely to be an important local regulator in this process. Our recent study using DM BB Wistar rat femur fracture model, confirmed decreased PDGF and TGF- $\beta$  levels using immunohistochemical and quantitative mRNA methods at days 2, 4, and 7 post-fracture.

This study noted similar findings with significant reduction in the early growth factor levels (PDGF, TGF- $\beta$ ) in the human DM compared to non-DM fracture hematoma. However, circulating levels of these growth factors were significantly higher in the DM group compared to the non-DM group. The clinical implication of our findings lies within its confirmation of decreased human growth factor levels in DM fracture hematoma. This reduction of critical growth factors may contribute to the delayed healing documented in diabetic patients. The increased levels of circulating growth factors may be related with complications associated with DM. This study justifies the potential use of growth factors localized to the fracture site in the amelioration of impaired DM fracture healing.

**References:** 1. Andrew JG et al *Bone* 16:455-60, 1995; 2. Grundness et al *Acta Orthop Scand*, 64:340-42, 1993; 3. Loder *Clin Orthop*, 232:210-16, 1988; 4. Joyce ME et al *Prog Clin Biol Res*, 365:391-416, 1991.