Growth factor deficiencies in diabetic impaired fracture healing

Deficiências de fatores de crescimento na consolidação de fraturas em diabéticos

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INTRODUCTION

In Brazil, over 4.6 million people have been diagnosed with *diabetes mellitus* (DM), and over 170 million people are affected worldwide(1). More than 50% of patients with DM are undiagnosed, and an estimated 11.3 million Brazilians will develop DM over the next 20 years(1). With the advent of improved pharmacologic methods to control blood glucose levels, including insulin and oral hypoglycemics, the diabetic population is now more active and ultimately lives longer. However, the management of fractures in patients with DM remains to be a significant challenge to the orthopaedic surgeon (Figure 1).

The association between DM and impaired osseous healing has been clinically examined. Several retrospective studies have evaluated complications following elective arthrodesis in DM patients(2-4). While the patients in these studies represented a subpopulation of diabetics with neuropathy, the noted increase in incidence of delayed union, nonunion, and pseudoarthrosis was highly significant in DM patients(2-4). Perlman and Thordarson(5)compared the results of ankle fusion in several nonunion risk groups. They found a higher incidence of nonunion in attempted arthrodesis in DM patients compared to non-DM patients. Three of eight non-neuropathic patients undergoing arthrodesis resulted in nonunion. Another study analyzed the predisposing factors contributing to nonunion after ankle arthrodesis. Non-neuropathic patients with major medical problems including renal failure, DM, and alcohol abuse (11 of 13,85%), were noted to have a significantly higher risk of nonunion ($p<0.04$) compared to other patients(6).

Impaired fracture healing has also been described in several cohort studies of acute fractures in patients with DM(7,8). Loder demonstrated a significant delay in fracture healing in DM patients without neuropathy. Although the value of this study was compromised by variability in patient demographics, fracture pattern, and location, diabetic patients experienced a significant delay in time to union,

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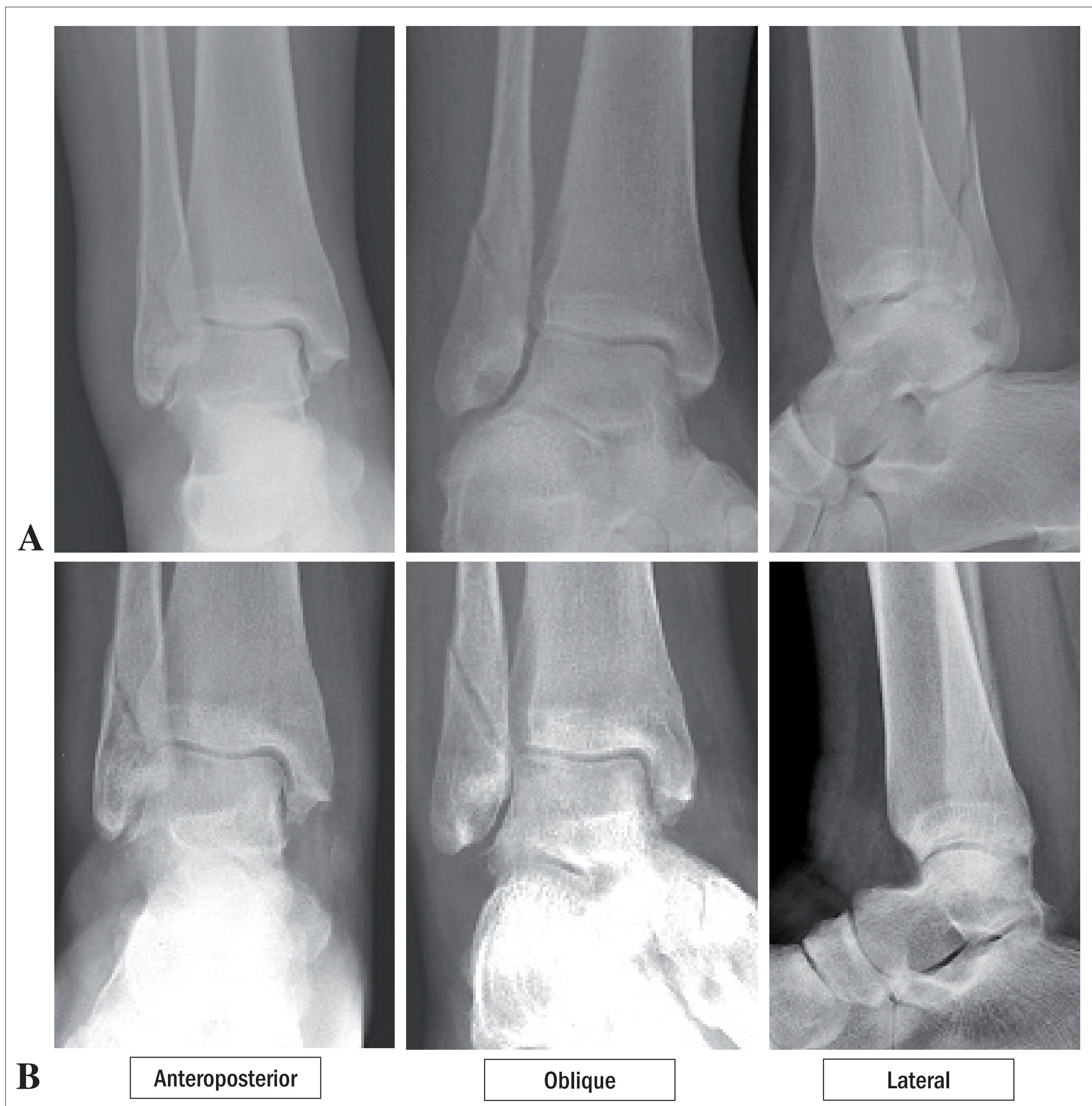

Figure 1 - Anteroposterior, lateral, and oblique views of the ankle in a 39-year-old diabetic female who fells down a flight of stairs; initial injury films; (A): a clinically stable, lateral, malleolar ankle fracture; films obtained three months postfracture; (B): nonunion.

uniting at an average of 187% of the time required for fractures to heal in patients without DM[8]. Cozen, in a comparative cohort study, noted over double the healing time for DM *versus* non-DM with lower extremity fractures (18 patients; 8.2 *versus* 3.6 months with 3 of 9 DM having partial unions)[7].

Similar findings have been obtained in animal experiments that have measured a reduction in the biomechanical strength of the DM fracture callus[9-12]. Wray and Stunkle showed that the breaking strength of a healing fracture in alloxan-induced diabetic animals was significantly less than that of control animals[12]. Herbsman et al.[10]demonstrated a significant reduction in the tensile strength of a fibula fracture in an alloxan-induced DM rat model four weeks post-fracture. Macey et al. showed that the fracture callus from untreated streptozocin-induced DM rats had a 29% decrease in tensile strength and a 50% decrease in stiffness compared to non-DM animals two weeks after the produc-

tion of a closed fracture[11]. Beam et al. showed that at six and eight weeks postfracture, the fracture callus from insulin dependent BB Wistar rats had a decreased torsional rigidity (70 and 58% respectively) and decreased callus stiffness (78 and 71% respectively) compared to non-DM rats[13].

Many inferences can be drawn with reference to fracture healing from data regarding the effect of DM on collagen synthesis and cellular proliferation[14-16]. The synthesis of type *versus* collagen by chondrocytes undergoing hypertrophy is a critical step in the process of endochondral ossification[15-16]. Previous studies have demonstrated a reduction in the synthesis of collagen by articular cartilage and bone cells from diabetic rats[14-16]. Topping et al. showed that type *versus* collagen synthesis was 54 to 70% less in the fracture callus of DM rats compared to non-DM rats.

Macey et al. hypothesized that the decreased mechanical strength in the fracture callus of DM animals during the early stages of repair results from diminished synthesis of collagen secondary to impaired cellular proliferation and/or migration. Between days 4 and 11 postfracture, a significant difference in collagen content was observed between the untreated DM animal fracture calluses (50%) and Control Group (55%). Treatment of DM animals with insulin resulted in restored tensile strength and callus stiffness values similar to the corresponding control values. The DNA content, an indicator of callus cellularity, was decreased 40% in the DM Group, suggesting retarded cellular proliferation. Moreover, a decreased collagen to DNA ratio (representative of collagen synthesis) was documented during the 14 day healing period in DM animals. In comparison, the controlled animals demonstrated a rapid increase in the collagen to DNA ratio as well as a rapid increase in the collagen content of the callus between days 4 and 7. The correlation of decreased mechanical strength and decreased or abnormal collagen synthesis suggests that early events play an important, persistent, and deleterious role in DM fracture healing[11].

Additional experimental studies have supported the hypothesis that DM alters the early stages of fracture healing. Beam et al. showed that insulin-dependent DM BB Wistar rats treated with a low insulin dose to maintain hyperglycemia without ketoacidosis had a decrease in cell proliferation at early time points (two, four, and seven days) compared to non-DM rats[13]. These results parallel an *in vitro* study which demonstrated that an insulin deficient environment, mimicking Type I diabetes, yields delayed cartilage synthesis and reduced ossification in cultured organ explants system[17]. Additionally, DM BB Wistar rats have decreased early vascularity at the fracture site compared to control rats at the same time point, ten days after fracture[18]. These alterations in the early parameters of fracture healing, namely decreased cell proliferation, collagen synthesis, and angiogenesis, ultimately translate to reduced biomechanical properties of the DM fracture callus in healing bones.

Various growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin growth factor-I (IGF-I), and transforming growth factor-beta (TGF-β), have been identified as playing a critical role in the early stages of the musculoskeletal healing process[19-20]. PDGF is released by the alpha granules of platelets and aids the migration, proliferation, and differentiation of osteoprogenitor cells. PDGF also up-regulates VEGF and then stabilizes newly formed capillaries at the site of injury. VEGF is derived from platelets, osteoblasts, and chondrocytes, and is a precursor for angiogenesis. VEGF also plays a role in the conversion of cartilage into bone and in osteoblast proliferation and differentiation. IGF-I is derived from a number of cell types including osteoblasts, chondrocytes, and endothelial cells. IGF-I acts to promote bone matrix synthesis, cell proliferation and differentiation, and resorption during the early stages of fracture healing. TGF-β is released by platelets during the inflammation stage of fracture healing, but affects all stages of the healing process. TGF-β can stimulate undifferentiated mesenchymal stem cells to proliferate during the early stages of healing, and has also been shown to recruit osteoclast precursor cells[19-20], as is summarized in Table 1.

In the present study, we hypothesized that fracture site growth factor deficiencies during the early phase of fracture repair are associated with DM and lead to impaired fracture healing. To test this hypothesis, the above growth factors were measured in fracture callus specimens from an experimental DM model and from DM patients, and compared to control levels.

Table 1 – Growth factors

Growth factors	Source	Function
PDGF	Platelets and osteoblasts	Aids the migration, proliferation, and differentiation of osteoprogenitor cells
TGF-β	Platelets	Stimulate undifferentiated mesenchymal stem cells to proliferate during early healing; recruits osteoclast precursor cells
IGF-I	Osteblasts, chondrocytes, and endothelial cells	Promote bone matrix synthesis, cell proliferation and differentiation, and resorption during early fracture healing
VEGF	Platelets, osteoblasts, and chondrocytes	Precursor for angiogenesis; plays a role in the conversion of cartilage into bone; osteoblast proliferation and differentiation

PDGF: platelet-derived growth factor; TGF-β: growth factor-beta; IGF-I: insulin growth factor-I; VEGF: vascular endothelial growth factor.

METHODS

Animal model – femur fracture

A rat femoral fracture model was used in the present study. All research protocols were approved by the Institutional Animal Care and Use Committee at New Jersey Medical School (UMDNJ). Experiments were conducted on diabetic resistant (non-DM) and diabetic prone (DM) female BB Wistar rats, between the ages of 80 to 120 days, bred from parent strains purchased from Health Canada Animal Research Division (Ottowa, Canada). This animal model was chosen as it represents a close homology to human type I DM. The onset of insulin-dependent *diabetes mellitus* in the BB Wistar rat occurs through the development of insulitis accompanied by a selective, autoimmune destruction of pancreatic β cells[21]. Four to seven days after the onset of glycosuria, the β cells are destroyed, leading to elevated blood glucose (BG) levels.

Animals were housed under pathogen-free conditions and fed *ad libitum*. Metabolic caging was used to house diabetic-prone animals for 2-4 hours, three times per week. The urine from these animals, beginning at the age of 60 days (until 120 days), was tested for glycosuria with urine test strips. Once glycosuria was detected, blood from the tail vein was tested three times per week for BG levels (ACCU-CHEK Advantage, Roche Diagnostics, Indianapolis, IN). When the BG exceeded 250 mg/dL, the animals were considered to be diabetic. In an aseptic environment, diabetic rats were treated with a bovine insulin-releasing palmitic acid implant (Linplant, LinShin Canada, Ontario, Canada) (14% bovine insulin, 86% palmitic acid; weight 26±2 mg/implant; 2 U/day release rate), which was placed subcutaneously in the dorsal neck, providing constant insulin release for approximately 30 days. BG was measured three times per week after the initial insulin treatment. DM BB Wistar rats were treated with 2 U of insulin per day to maintain the DM rats in a state of hyperglycemia without ketoacidosis.

Surgery was performed to produce a closed mid-diaphyseal fracture model in the right femur. General anesthesia was administered prior to surgery by intraperitoneal (IP) injection of ketamine (60 mg/kg) and xylazine (8 mg/kg). The right leg of each rat was shaved and the incision site was prepared with Betadine and 70% alcohol. A one centimeter medial, parapatellar skin incision was made, followed by a smaller longitudinal incision through the quadriceps muscle, just proximal to the quadriceps tendon. The patella was dislocated laterally and the intercondylar notch of the distal femur was exposed. An entry hole was made with an 18 gauge needle and the femoral intramedullary canal was subsequently reamed. A Kirschner wire (316LVM stainless steel, 0.04 inch diameter, Small Parts, Inc., Miami Lakes, FL) was inserted into the intramedullary canal. The Kirschner wire was cut flush with the femoral condyles. After irrigation, the wound was closed with 4-0 vicryl resorbable sutures. A closed midshaft fracture was then created unilaterally with the use of a three-point bending fracture machine. X-rays were taken to determine whether the fracture was of acceptable configuration. Only transverse, mid-diaphyseal fractures were accepted. The rats were allowed to ambulate freely immediately postfracture.

Growth factor quantification – PDGF, TGF-β, IGF-I, and VEGF

On days fourth and seventh postfracture, animals from both the non-DM and DM Groups were sacrificed, and fractured femora were harvested. The fracture callus was flash frozen in liquid nitrogen, pulverized, and total protein was extracted using an acid extraction protocol[22]. Insoluble material was removed from the protein extract by centrifugation (12,000 RPM for 10 minutes) and the resulting supernatant was stored at – 80°C until testing. Growth factor quantification was performed using enzyme-linked immunosorbent assays (ELISA) specific for rat PDGF-AB (R&D Systems, MN), TGF-β1 (Brenzel BioAnalytica, Germany), IGF-I (Brenzel BioAnalytica) and VEGF (Alpco, NH) based on the direct sandwich technique. The assays and analyses were performed according to the instruction of the manufacturers. Growth factor levels were normalized to total protein concentration measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL).

Gene expression analysis

On days fourth and seventh postfracture, fracture calluses from non-DM and DM Groups were obtained as described above. Total RNA was isolated from each specimen as previously described in the literature[23]. Aliquots of total RNA from each fracture callus were converted to cDNA using MMLV reverse transcriptase and an oligo(dT)20 primer under standard reaction conditions[23]. Target cDNAs were quantified by real-time PCR using SYBR Green I fluorescence as a measure of DNA amplification in 25 uL reactions (ABgene, Rochester NY). The amplifications were performed in an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using a two-step protocol that alternated between 95°C for 15 seconds and the annealing temperature for 1 minute. Fluorescence was measured after each of the 40 amplification cycles. Each reaction yielded an

amplification plot and melting curve, which was used to verify the integrity of the qPCR reaction while the amplification plot was used to determine the cycle threshold (Ct) needed to quantify target DNA expression. Relative gene expression was calculated by normalizing to GAPDH mRNA values using the software supplied with the instrument.

Clinical study

An institutional review board approval was obtained for a prospective preliminary study performed at a Level I trauma center (UMDNJ University Hospital). 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 age range of 18 and 72 years old, and (3) the time from injury to surgery was less than 20 days.

Growth factor quantification – PDGF, TGF-β

Fracture hematoma and blood samples were obtained from patients undergoing surgical treatment. One set of blood samples was collected for testing of HbA_{1C} levels, which is an indicator of blood glucose control. Another set of blood samples was collected for serum protein levels. These samples were subjected to centrifugation for 10 minutes at 2,000 RPM, and the supernatant was collected and stored at – 80°C until testing. Fracture hematoma samples were collected at the time of 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.

Total protein concentration in the serum and fracture hematoma extracts was measured using a BCA assay and the concentration was adjusted to 4 mg/mL using cell lysis buffer containing protease inhibitors (Sigma, St. Louis, MO) before storage at – 80°C until testing. PDGF and TGF-β were quantified using Quantikine ELISA kits (R&D Systems, Minneapolis, Minnesota) as per the manufacturer's instructions. Growth factor levels were then normalized to sample total protein.

Statistical analysis

Statistical analysis for the experimental study was performed using a student t-test to identify differences between the diabetic and non-diabetic Groups using SigmaStat (SigmaStat 3.0, SPSS Inc., Chicago, Illinois). Statistical analysis for the clinical study was performed using one-way ANOVA and correlation analyses using StatView (SAS, Kerry, NC). A p-value less than 0.05 was considered statistically significant.

RESULTS

Experimental Study

Growth factor quantification

At four days post-fracture, the mean normalized PDGF concentration within the fracture callus of DM animals was significantly lower (42% less, p=0.030) than that of the non-DM animals. Similar trends, although not reaching significance, were observed for the mean normalized TGF-β, IGF-I, and VEGF concentrations in DM *versus* non-DM fracture calluses; 49% less (p=0.079), 12% less (p=0.874), and 30% less (p=0.183), respectively. The data is summarized in Table 2.

Gene expression analysis

At four days post-fracture, normalized PDGF, TGF-β, and IGF-1 mRNA concentration within the fracture callus of DM animals were significantly lower (75% less, p=0.02; 49% less, p=0.011; and 49% less, p=0.011; respectively) than that of the non-DM animals.

At seven days post-fracture, normalized PDGF, IGF-1, and VEGF mRNA concentration within the fracture callus of DM animals were significantly lower (50% less, p=0.02; 18% less, p=0.016; and 64% less, p=0.01; respectively) than that of the non-DM animals. A similar trend was observed for the mean normalized TGF-β mRNA concentrations in

Table 2 – Normalized growth factor concentration in fracture callus at day four

Four day growth factor quantification [pg/mg total protein]			
Growth factor	Non-DM	DM	% Difference
PDGF	5.6±1.1 (n=4)	3.3±1.4 (n=5)*	42 decrease*
TGF-β	120±40 (n=3)	61±17 (n=3)	49 decrease
IGF-I	1138±894 (n=2)	1003±843 (n=3)	12 decrease
VEGF	88±62 (n=3)	38±21 (n=4)	30 decrease

PDGF: platelet-derived growth factor; TGF-β: growth factor-beta; IGF-I: insulin growth factor-I; VEGF: vascular endothelial growth factor.
The values are expressed as ratios to total protein (pg/mg); *p=0.030 significant decrease in diabetic PDGF levels compared to non-DM.

DM *versus* non-DM fracture calluses (10.6% less, p>0.05). All gene expression data is summarized in Tables 3-6.

Clinical study

DM and non-DM patients were matched in age, time to surgery, fracture type, and fracture location. The non-DM Group consisted of 23 patients with a mean age of 38.6±15.8 years and a mean time to surgery of 12.8±4.0 days. The Diabetic Group consisted of 7 patients with a mean age of 42.8±11.0 years and a mean time to surgery of 17.0±6.1 days. The mean Hb_{A1C} value of the Diabetic Group (7.61±0.90%) was significantly higher ($p<0.05$) than that of the non-DM group (3.15±0.68%).

No significant difference was appreciated between the DM and non-DM Groups with reference to total protein concentration within the fracture hematoma (1,256 and 1,540 mg/mL, respectively) or total plasma protein concentration (513 and 594 mg/mL, respectively). The mean normalized PDGF concentration within the DM fracture hematoma was significantly lower (42% less, p=0.048) than that of the non-DM group (160 *versus* 275 pg/mg). The mean normalized TGF-β concentration was also significantly lower (95% less, p=0.021) in the DM *versus* non-DM fracture hematoma (375 *versus* 8,500 pg/mg). PDGF and TGF-β levels were higher in the blood plasma (p=0.005) and (p=0.047), respectively, in DM patients compared to non-DM patients (Table 7).

Table 3 – Normalized PDGF-BB mRNA levels in healthy and diabetic fracture callus

Time after fracture	PDGF-BB/ GAPDH mRNA		% Decrease
	Healthy (x10^{-4})	Diabetic (x10^{-4})	
Day 4	23.0±7.4	5.8±2.9*	74.8
Day 7	23.5±12	11.8±5.*	49.8

Values are presented as an average value ± standard deviation. The values for PDGF-BB are normalized to GAPDH; PDGF: platelet-derived growth factor; *p=0.020 significant decrease in diabetic mRNA levels compared to healthy mRNA levels at both 4 and 7 days.

Table 4 – Normalized TGF-beta mRNA levels in healthy and diabetic fracture callus

Time after fracture	TGF-β1/ GAPDH mRNA		% Decrease
	Healthy (x10-4)	Diabetic (x10-4)	
Day 4	4.17±1.36	2.11±0.3*	49.4
Day 7	8.58±2.23	7.67±4.77	10.6

Values are presented as an average value ± standard deviation. The values for TGF-â are normalized to GAPDH; *p=0.011 significant decrease in diabetic mRNA levels as compared to healthy mRNA levels at 4 day; TGF-β: growth factor-beta.

Table 5 – IGF-1 mRNA levels in healthy and diabetic fracture callus

Time after fracture	IGF-I/ GAPDH mRNA		% Decrease
	Healthy (x10-4)	Diabetic (x10-4)	
Day 4	2.84±0.45	1.46±0.9*	48.6
Day 7	1.32±0.37	1.08±0.4	18.1

Values are presented as an average value ± standard deviation. The values for IGF-I are normalized to GAPDH; *p=0.016 significant decrease in diabetic mRNA levels compared to healthy mRNA levels at both 4 and 7 days; IGF-I: insulin growth factor-I.

Table 6 – VEGF mRNA levels in healthy and diabetic fracture callus

Time after fracture	VEGF/ GAPDH mRNA		% Decrease
	Healthy (x10-4)	Diabetic (x10-4)	
Day 7	0.37±0.13	0.13±0.07*	64.9%

Values are presented as an average value ± standard deviation. The values for VEGF are normalized to GAPDH; *p=0.010 significant decrease in diabetic mRNA levels compared to healthy mRNA levels at 7 days; VEGF: vascular endothelial growth factor.

Table 7 – Human growth factor levels in fracture hematoma

Growth factor	Normalized growth factor quantification of PDGF and TGF-β [pg/mg]		% Change
	Non-DM (n=23)	DM (n=7)	
PDGF	275	160*	42% decrease
TGF-β	8500	375**	95% decrease

The values are expressed as ratios to total protein (pg/mg); *p=0.048 significant decrease in normalized PDGF levels as compared to non-DM; **p=0.021 significant decrease in normalized TGF-β levels as compared to non-DM.

DISCUSSION

The aim of this study was to explore the etiology of impaired fracture healing that is associated with DM, possibly linked to local growth factor levels within the fracture callus. An *in vivo* experimental study using an animal model was performed in tandem with a clinical study to quantify local growth factor levels within the fracture hematoma of DM and non-DM populations. We hypothesized that as a result of the systemic effects of DM, a local reduction in the synthesis of growth factors that are critical during the early phases of musculoskeletal healing ensues. This local deficiency of critical growth factors, namely PDGF, TGF-β, IGF-I and VEGF, at the fracture site may impede the fracture healing process.

Previous experimental studies have suggested that DM impairs fracture healing. Beam et al. examined the mechanical and histological effects of DM on fracture healing in a femur fracture rat model[13]. At later time points, a significant decrease in torsional strength and stiffness was observed in DM rat femurs compared to non-DM rat femurs[13]. Such decreases in the mechanical properties can be explained by the histological results which revealed decreases in periosteal bone synthesis[13]. Early cell proliferation data showed that poor glucose controlled DM animals had a decreased amount of proliferating cells which may explain the decreased histological and mechanical results in the DM animals[13].

Similarly, clinical studies have also shown that the presence of DM leads to a prolonged healing time in patients with acute fractures or those undergoing elective arthrodesis. DM human fracture healing takes twice the time compared to non-DM fracture healing[7]. Moreover, Kline et al. showed that DM not only leads to increased delayed unions, but also to increased nonunions in tibial pilon fractures compared to non-DM patients[24]. Stuart and Morrey reported that only half of the DM patients who received elective arthrodesis achieved satisfactory results, and over half experienced complications which required subsequent operations[3].

Studies demonstrating an alteration in local growth factor concentrations at the site of fracture have been done. Street et al. evaluated the angiogenic properties within the fracture hematoma by measuring levels of VEGF and PDGF in the fracture hematoma and peripheral blood samples of human patients[25]. A significant increase in the levels of VEGF and PDGF existed in the fracture hematoma was compared to plasma. One critical observation was a significant reduction of PDGF levels in the fracture hematoma of patients over 65 years. Giannoudis et al.[26] theorized that an early local increase of growth factors observed after reaming may have led to new bone formation through increased cell proliferation, differentiation, chemotaxis, migration of osteoblasts, and sustained angiogenesis.

The concept of reduced growth factor levels in DM fracture callus has also been substantiated in the scientific literature[13,18,27-28]. Our growth factor ELISA quantification demonstrated similar trends to levels observed by Tyndall et al. for PDGF quantification[24]. Moreover, Gandhi et al.[28] and Coords et al.[18] demonstrated significant decreases of similar growth factors at comparable time points in the DM fracture callus compared to controls. This reduction may explain both the observed impairment of cell proliferation and the resulting histomorphology of the DM fracture callus[13,27].

In the clinical context, the presence of DM has been shown to reduce the local growth factor concentrations within the fracture hematoma. One recent unpublished study by Verma et al.[29] reported a correlation of decreased growth factors within the fusion bed site in patients with Charcot's arthropathy undergoing elective hindfoot fusion. Patients who did not achieve union had significantly lower levels of PDGF and VEGF at the fusion site than patients who achieved union and did not require surgery. This observation is supported by findings from our present study. While only two growth factors (PDGF and TGF – β) were measured, their levels within DM versus non-DM fracture hematomas corresponded well with the data obtained from our animal study and previously published experimental data[28]. Furthermore, the same findings may explain the high incidence of complications, such as delayed union and nonunion, in DM patients sustaining fractures or those undergoing elective arthrodesis[3-4,7,8,24].

If impaired fracture healing due to DM is attributed to decreased local growth factors, it may be possible then to improve the impaired healing by focusing treatment modalities on increasing growth factor concentrations locally at the fracture site. Treatment with platelet rich plasma (PRP), a bioactive component derived from autologous blood containing a high concentration of platelets, has been shown to ameliorate the deleterious effects of DM on fracture healing both experimentally and clinically. When activated, platelets within the concentrate release a number of growth factors shown to be critical in the early healing and regeneration of musculoskeletal tissue. Several studies, both experimental[22] and clinical[30], have evaluated the potential role of PRP as a bioadjuvant to augment fracture healing in high-risk patients, such as those with DM.

Gandhi et al. studied the effects of percutaneous delivery of PRP locally at the fracture site in a diabetic BB Wistar femoral fracture model[22]. Histomorphometry and mechanical testing results suggested a positive outcome with the utility of PRP, as it normalized the early parameters (cell pro-

liferation and chondrogenesis) while improving the late parameters (mechanical strength) to mediate the DM fracture healing process. In an early prospective preliminary study, Gandhi et al. used PRP in nine human patients who sustained foot and ankle fractures complicated by nonunion[28]. The success of the preliminary study indicated that the application of PRP would help nonunion sites achieve union. Coetzee analyzed the effect of PRP-augmented bone grafting on syndesmotic fusion rates in patients undergoing total ankle replacement with the Agility prosthesis (DePuy, Warsaw, Indiana). Fusion rates in 66 patients receiving PRP-augmented bone grafting was compared to 114 historical control patients receiving non-PRP augmented bone grafting[30]. In the PRP group, a statistically significant difference was observed with respect to fusion rates at 8-week and 12-week time points between the Control Group (61.4 and 73.6%, respectively) and experimental group (76 and 93.9%, respectively) [30]. Moreover, a significantly reduced number of delayed unions and nonunion was observed at six months in the PRP Group as compared to the Control Group. Additionally, Dallari et al. demonstrated that allograft bone chips loaded with bone marrow aspirate containing pluripotent stromal cells combined with PRP significantly improved healing rates in patients undergoing tibial osteotomy[31]. These studies suggest that PRP, when applied locally, delivers a highly concentrated amount of critical growth factors, and seems to be a promising bioadjuvant to facilitate fracture healing in this high-risk patient population.

To the best of our knowledge, this is the first study comparing local growth factor concentrations within the fracture hematoma of DM and non-DM populations. The outcomes from the present study, both experimental and clinical, imply that deficiencies in local growth factors results in impaired fracture healing that has been previously documented in the DM rat model[13], human DM fracture[7,8, 24], and human DM elective arthrodesis[3]. In future experimental studies, it would be valuable to quantify growth factor levels at additional time points such as day seven and day ten. Newer methods, such as MAPx techniques, will enable future investigations to measure more growth factors with less starting material. This will provide additional insight into the effects of DM on fracture site biology. Further clinical investigation is needed to correlate local growth factor levels, at the fracture site, with healing outcomes. Understanding the effect of diabetes on the fracture healing process, and specifically its impact on the concentration of growth factors within the fracture callus, will provide a background to yield future therapies that can enhance local levels to potentially overcome the impaired fracture healing associated with DM.

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