Is Atrophic Nonunion a Misnomer – A Hospital-based Prospective Cross-Sectional Study

Pseudoartrose atrófica é um termo incorreto? – Um estudo hospitalar transversal prospectivo

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Abstract

Objective The present study was conducted to estimate histologically the proportion of avascularity of fracture ends in case of nonunion of long bones.

Methods A total of 15 cases of established quiescent nonunion were operated according to the standard protocol and the fracture ends were evaluated histologically. The biopsied tissue was briefly fixed with formalin, embedded with paraffin (FFPE), and 5-micron sections were stained with hematoxylin and eosin according to standard protocols. Immunohistochemistry with anti-CD31 antibody (JC70A clone, DBS) was performed manually using standard protocols.

Results All cases of quiescent nonunion were included; radiologically, 2 cases were oligotrophic, and 13 cases were of atrophic nonunion. A total of 20% of the patients were females, 40% were in the age group between 31 and 40 years old, and, radiologically, all cases were of atrophic nonunion. All cases showed positivity for CD-31 on immunohistochemistry. The blood vessel density was category I in 13.33% of the cases and category II in 86.67% of the cases. Four cases presented with mild inflammation and two presented with moderate inflammation. The average vessel count was 10 per high power field in the age groups between 20 and 30, 31 and 40, and 41 and 50 years old. The age group between 61 and 70 years old showed an average vessel count of 4 per high power field. The difference in the vessel counts of oligotrophic and atrophic nonunion was not significant. No correlation was observed in the density of vessel count and duration of nonunion.

Keywords
► fractures, ununited
► fractures, bone
► antigen, CD31

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Introduction

The literature has plenty of information on the process of fracture healing.\(^1\) Also, there are studies on the regulations of events that occur during fracture healing. But there is always a lack of consensus and need for further studies. Most fractures unite well, but some do present with complications of delayed union and nonunion. Lower limb fractures account of one-third of all fractures and result in significant morbidity when associated with open injuries and delayed treatment.\(^2\) One of the common morbidities associated with these fractures is nonunion, which accounts for between 5 and 10% of all fractures.\(^3\) Nonunion is described as cessation of any further healing, radiologically, on 3 consecutive x-rays taken in a 1-month interval.\(^4\)

Broadly, these nonunions are described as:

- Infected and noninfected depending on the presence of infection in the fracture ends
- Stiff and mobile depending on the presence of movement at the fracture site\(^5\)
- Hypertrophic, oligotrophic, and atrophic nonunion according to the biological activity observed radiologically\(^6\)

The assessment of biological activity has been predominantly based on radiological parameters and have been followed for a long time. It is a common preconception that hypertrophic nonunions on x-ray are biologically active and bony stabilization is sufficient to attain union. In contrast, atrophic nonunions are considered avascular, acellular, and lack the inherent ability to heal under a correct, stable environment.\(^6\) It has been shown that stability and vascularity of the fracture ends are important factors guiding the callus formation in opposed fractured bones.\(^7\) Although an initial disruption of blood supply may be the cause of nonunion, persistent avascularity may not be a constant factor associated with it.

The aim of the present study is to investigate the histology and find out the incidence of vascularity of the fracture ends of various nonunions.

Materials and Methods

The present study was conducted after obtaining approval from the institutional ethics committee.

Only patients who sustained open fractures with bone loss due to high velocity trauma such as road traffic accident,
fall from height, and gunshot injuries and were infected at some point but now have healed and become quiescent (nondraining) were included in the present study.\(^8\)

The eligibility criteria for the present study were skeletally mature patients presenting with quiescent (nondischarging) nonunion of long bones. All patients with suspected pathological fracture, patients with discharging nonunions of long bones, and patients in the pediatric age group were excluded from the study.

Methodology: All patients fulfilling the inclusion criteria were informed about the methodology of the present study. Informed and written consent were obtained from all patients. All patients had their history taken and were submitted to clinical examination. X rays of each patient were taken and nonunions of long bones were classified according to x ray findings by two independent orthopedic surgeons. The classification was made according to the morphology of the fracture ends and the amount of callus formation.\(^9\) During the operation, intraoperative biopsy samples were taken from the nonunion site (\(\rightarrow\) Figure 1).

Histological Examination: Biopsy samples taken from the nonunion site were fixed with 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E); 5-µm sections stained with H&E were analyzed by light microscopy to see the general morphology and the characteristics of the tissue. CD31 immunohistochemistry was employed to highlight the vasculature (\(\rightarrow\) Figure 2).

The inflammatory infiltrate comprised of lymphocytes and neutrophils was graded as:

- Mild: Inflammatory cells in < 33% of the area of the tissue section
- Moderate: Inflammatory cells in between 33 and 66% of the area of the tissue section
- Severe: Inflammatory cells in > 66% of the area of the tissue section

The distribution of the blood vessels was semiquantitatively graded on 100x magnification according to the following scheme: \(^10\)

Category 0: No positively stained blood vessels present

Category 1: Between 1 and 50% of the field containing blood vessels

Category 2: > 50% of the field containing blood vessels

The density of the blood vessels was quantified by taking the average of 3 noncontiguous 400x fields with the highest blood vessel counts. Each 400x field in the microscope model used corresponds to 0.55 mm\(^2\). \(^10\)

Results with Statistical Analysis

In our series of 15 cases, the mean age of the patients was 40.60 ± 12.99 years old. Most patients were in the age group between 31 and 40 years old (40%). Our series had 12 males and 3 females. The cases of nonunion were in the tibia (66.7%), the femur (13.3%), the forearm bones (13.3%), and the humerus (6.7%). Thirteen cases were atrophic and 2 were oligotrophic.

The duration of nonunion was 16.87 ± 6.59 months. All cases were positive for CD31 (100%) (\(\rightarrow\) Figure 3). The average vessel count was 9.27 ± 4.28. Two cases had blood vessel density in category 1, and the rest were in category 2 (\(\rightarrow\) Figure 4). Most cases did not show any inflammation (60%), with mild inflammation in 4 cases (\(\rightarrow\) Table 1).
There was a moderate negative correlation between average vessel count and age (years old), and this correlation was not statistically significant (\(\rho = -0.4; p = 0.145\)) (Figure 5 and Table 2).

There was a weak negative correlation between duration of nonunion (months) and age (years old), and this correlation was not statistically significant (\(\rho = -0.11; p = 0.691\)) (Figure 6 and Table 3).

On correlating the radiological features with the histological features of the types of nonunion, it was seen that all cases that were radiologically atrophic were histologically hypertrophic. They had good vessel count and density. The two cases that were oligotrophic on x-ray were oligotrophic histologically (Figure 7).

### Discussion

Nonunion of long bone following fracture is a common complication, especially when associated with open injuries and bone loss, leading to high morbidity and clinical burden. The US Federal Drug Administration council defines nonunion as "failure to achieve union by 9 months since the injury, and for which there has been no clinical and radiological signs of healing for 3 consecutive months". Traditionally, x-rays have been used to assess the biological activity in the nonunion fracture site on the basis of which nonunion is classified in three groups hypertrophic,
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Table 2 Correlation between blood vessel count and age of the patients

| Correlation                          | Spearman correlation coefficient | p-value |
|--------------------------------------|----------------------------------|---------|
| Average vessel count versus age (years old) | −0.4                             | 0.145   |

Nonparametric tests (Spearman correlation) were used to explore the correlation between the two variables, as at least one of the variables was not normally distributed. There was a moderate negative correlation between average vessel count and age (years old), which was not statistically significant (rho = −0.4; p = 0.145).

Table 3 Correlation between duration of nonunion and age of the patients

| Correlation                          | Spearman correlation coefficient | p-value |
|--------------------------------------|----------------------------------|---------|
| Duration of nonunion (months) versus age (years old) | −0.1                             | 0.691   |

Nonparametric tests (Spearman correlation) were used to explore the correlation between the two variables, as at least one of the variables was not normally distributed. There was a weak negative correlation between duration of nonunion (months) and age (years old), which was not statistically significant (rho = −0.11; p = 0.691).

In our study, we had 13 (86.7%) cases of atrophic nonunion and 2 (13.3%) cases of oligotrophic nonunion classified radiologically. The mean duration of nonunion (months) was 16.87 ± 6.59. In the study by Reed et al.,12 11 out of 22 patients had hypertrophic nonunions (median time after fracture: 21 months)) and 11 patients had atrophic nonunions (median time after fracture: 24 months). In the study by Vallim et al.,13 only cases of atrophic nonunion were included.

In our study, the histology of all 15 (100.0%) patients were found to be CD31-positive. The mean average vessel count was 9.27 ± 4.28. Nine of the patients had no inflammation, 4 (26.7%) had mild inflammation, and 2 (13.3%) had moderate inflammation. In another study by Reed et al.,12 all of the cases stained for CD 31 were found to be positive and were not different histologically; however, a difference between the densities of the vessels of the different types of nonunion was observed, but it was not significant (p > 0.05).

Since there has been a shift in research from the anatomical level to the molecular level, the present study was conducted to define the molecular characteristics of the nonunion site and its correlation with radiological and anatomical parameters. In the present study, 15 patients with clinically healed quiescent nonunion were included. The mean age (years old) was 40.60 ± 12.99; 12 (80.0%) of the participants were male and 3 (20.0) were female. One similar previous study by Reed et al.12 included 22 patients with a mean age of 47.18 years old, 17 male and 5 females. In another in vitro study conducted by Vallim et al.,13 15 patients with a mean age of 46.4 years old with atrophic nonunion were included, 9 males and 6 females.

In our study, the histology of all 15 (100.0%) patients were found to be CD31-positive. The mean average vessel count was 9.27 ± 4.28. Nine of the patients had no inflammation, 4 (26.7%) had mild inflammation, and 2 (13.3%) had moderate inflammation. In another study by Reed et al.,12 all of the cases stained for CD 31 were found to be positive and were not different histologically; however, a difference between the densities of the vessels of the different types of nonunion was observed, but it was not significant (p > 0.05).

In our study, 11 out of 22 cases (50%) were found to be CD31-positive. Nine of the patients had no inflammation, 4 (26.7%) had mild inflammation, and 2 (13.3%) had moderate inflammation. In another study by Reed et al.,12 all of the cases stained for CD 31 were found to be positive and were not different histologically; however, a difference between the densities of the vessels of the different types of nonunion was observed, but it was not significant (p > 0.05).

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Conclusion

The radiological distinction of nonunions into atrophic, oligotrophic, and hypertrophic has been done for a long time. But there is not much histological difference between atrophic and oligotrophic nonunions in terms of vessel density and activity. Atrophic nonunion, as the name suggests, is not hypo- or avascular. It still has the potential of new bone formation if provided with adequate stabilization and chemotactic factors.

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Conflict of Interests
The authors have no conflict of interests to declare.

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