Estimation of the rate of osteogenesis by tetracycline double labeling in intertrochanteric fractures of the femur in different age groups and its correlation with radiological grading of osteoporosis

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Abstract  Our aim was to estimate the rate of osteogenesis by using tetracycline double labeling and to correlate it with the radiological grading of osteoporosis in patients of intertrochanteric fracture of the femur in different age groups. Thirty-two cases of intertrochanteric fracture of the femur were included in the study. The patients were divided into three age groups: group I, <45 years; group II, 45–60 years; and group III, >60 years. Osteoporosis was graded in all groups on plain X-rays by Singh’s index (SI) and metacarpal index (MI). The patients were labeled with a single large dose (1.5 g) of tetracycline orally on day 1 and day 8. An iliac crest bone biopsy specimen was obtained from each of the patients during surgical intervention. Thin undecalcified sections were made by the hand grinding technique. The sections were studied under UV light for presence of fluorescence, and rate of osteogenesis (mineral appositional rate, MAR) was calculated for each of the groups. Only 7 patients were found to have radiological osteoporosis (by Singh’s index). The average MI and MAR were found to be 46.5% and 1.48 µm/day, respectively. The single-dose labeling schedule has shown good fluorescent bands, comparable to the conventional dose schedule. No correlation was found between Singh’s index and metacarpal index in group I and III, whereas in group II it was significant. There is no significant correlation between the rate of new bone formation and the radiological grading of osteoporosis.

Key words  Osteoporosis · Intertrochanteric fracture femur · Tetracycline double labeling

Introduction

Bone is a highly vascular and dynamic tissue and is in a constant process of resorption at some areas and formation at other sites, a process termed bone remodeling or turnover. Bone turnover continues to occur throughout life. The terms bone formation rate and resorption rate signify the anabolic and catabolic activities, respectively. The balance between the two activities determines the amount of bone present at any given period of time. The amount of bone present determines the strength of bone directly, so that low bone mass is a primary determinant of fracture.1 A decrease in bone mass and mechanical failure of the skeleton that results in the occurrence of fracture with minimal trauma is defined as osteoporosis. The public health impact of osteoporosis stems from its association with fractures of the hip, spine, and forearm.9

Histological heterogeneity in bone has been reported in patients with osteoporosis.16 Some patients have increased resorption alone, some have increased resorption and formation, and some have decreased bone formation. The distinction of low, high, or normal turnover is of interest for the selection of the best therapeutic agent. Ten years ago, bone histomorphometry was used as a routine technique for the diagnosis of osteoporosis and other metabolic bone diseases, but bone mass is now measured by noninvasive methods such as dual energy X-ray absorptiometry (DEXA); the latter, however, do not provide information on bone quality or microarchitecture.2 Bone histomorphometry consists of measuring parameters reflecting bone structure and turnover. It remains the only method that allows the study of bone at the tissue or the cell level to enable measurements at intermediary levels of organization of bone. Any currently available tetracycline antibiotic administered in vivo by any acceptable route will be deposited in newly formed and mineralizing bone, cartilage, and teeth. All the mineral ever deposited in lamellar bone matrix is laid down in the initial 4 days of mineralization in the zone of demarcation.3 The histological measurement of bone formation with a tetracycline tissue marker is a direct measurement of bone tissue metabolism and also provides dynamic param-
The paucity of literature on the rate of bone formation in the Indian population, on correlation of osteoporosis with intertrochanteric fracture of the femur in different age groups, and on correlation of osteoporosis with the rate of new bone formation was the motivating factor for the present study.

**Materials and methods**

This study was conducted in the Department of Orthopaedics, in conjunction with the Department of Anatomy and the Department of Radiodiagnosis, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. Thirty-two patients of various ages, both males and females, with intertrochanteric fracture femur who were admitted to the orthopaedics wards for surgical procedures and were otherwise medially fit constituted the clinical material for the study.

The patients were divided into three groups by age: group I, <45 years; group II, 45–60 years; and group III, >60 years. Informed consent was obtained from each patient. X-rays of the pelvis including both hip joints by anteroposterior (AP) view in 20° internal rotation were taken to grade osteoporosis according to Singh’s index (SI)14 (Fig. 1). Osteoporosis was also graded for each patient by determining the combined cortical thickness of the second metacarpal (metacarpal index, MI) of the dominant hand for which an X-ray hand — AP view was taken (Fig. 2).

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\text{MI} = \frac{\text{Combined width of layers of cortex on each side}}{\text{Total width of shaft}} \times 100
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The foregoing values were calculated at the midpoint of the shaft of the second metacarpal and expressed in percentages.

Patients selected for the study were given a single large dose (1.5 g) of tetracycline orally under direct supervision on day 1, followed by an interval of 6 days. The same dosage was repeated on day 8 while preparing the patient for surgery. The patients refrained from taking food, especially dairy products, 1 h before and 2 h after intake of the tetracycline capsules. Care was taken that there was no delay in the treatment of the patients by labeling them with tetracycline.

A 5-mm-diameter modified Patiala core biopsy needle was used to obtain the iliac crest bone biopsy specimens from the patients. These samples were taken at the time of surgical intervention, which was at least 48 h after the last dose of tetracycline. The samples thus obtained were kept in 70% ethanol at 4°–8°C in a domestic refrigerator until they were further processed.

Thin undecalcified sections were made from the samples by hand grinding them on sandpaper. The sections were ground until they became almost transparent, so that the print was visible through them when held over a newspaper. Sections were air dried and transferred to a grease-free slide, fixed with DPX mounting medium, and then covered with a coverslip. They were then left to dry at room temperature after mounting. The sections were studied under ultra-