

Hydroxyproline for the Assessment of Fracture Union

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Abstract

Determining whether a bone fracture is healed is one of the most important and fundamental clinical determinations made in orthopaedics. However, there are currently no standardized methods of assessing fracture union, which in turn has created significant disagreement among orthopaedic surgeons in both clinical and research settings. Here we try to focus on urine biomarker-hydroxyproline for clinical assessment of fracture union, because the urinary hydroxyproline reflect the actual status of bone resorption and bone formation over a short time frame. Urinary hydroxyproline is the commonly known as biomarker of bone resorption. During bone resorption, hydroxyproline may release either free or with fragments of the collagen molecule that will not reutilize in collagen synthesis. Urinary total and free hydroxyproline ratio increased in all patients after fracture however decreased after treatment in nonunion patients. Thus, the hydroxyproline can be used as an adjunct to clinical evidence of fracture healing. Its use may identify patients at high risk of impaired fractures healing as well as monitoring therapeutic efficacy, because they constitute a relatively inexpensive non-invasive measurement, its use should be used to predict the fracture healing outcome early.

Keywords: Hydroxyproline; Bone mass density; Fracture union; Osteoclast; Osteoblast

Introduction

Fractures are common orthopaedic problem and it occurs most in long bones. Amongst all long bones, shaft of tibia is one of the commonest bones that are prone to fractures. Such fractures have relatively higher incidence of impaired healing as the fracture site has lesser soft tissue coverage, because of subcutaneous bone on anterior aspect. The mentioned reasons account for a high rate of tibial non-unions amounting to 2 - 10% of all tibial fractures which leads to significant patient morbidity. Non-union fracture is a dreaded complication with devastating outcomes for the patients. Most of the time, a complex and expensive treatment are required involving multiple surgical procedures, pain, morbidity, prolonged hospital stay and functional and psychosocial disability. The implementation of treatment protocols for such clinical situation can be complex and long-lasting. Moreover, the consequent socio-economic burden on patients and then families should not be underestimated. Ability in prompt identification of patients who are at high risk of non-union will enable early appropriate targeted treatment intervention leading to a successful outcome. Such approach would benefit not only the patient's wellbeing but also the health care system in terms of the cost implications associated with long lasting treatment interventions and prolonged hospital stay.

There are no methods till date to measure rates of these processes and quantify the healing process. Currently, clinical and radiological methods are most commonly used to assess the healing of fractures. A study on radiological evaluation of the stage of union in fractures of tibia found that the radiographic assessment is not a most suitable method to assess fracture healing.

The biochemical markers of bone-turnover have long been used to complement the radiological assessment of patients with metabolic bone disease. Since they are derived from both cortical and trabecular bones, they reflect the metabolic activity of the entire skeleton rather than that of individual cells or the process of mineralization. Quantitative changes in skeletal - turnover can be assessed easily and

non-invasively by the measurement of bone-turnover markers. Among the several bone markers, hydroxyproline is one of them which is a modified amino acid that is metabolic product of collagen breakdown. And as the type I collagen is a major product of osteoblastic cells, during bone resorption, hydroxyproline may be released either free or with fragments of the collagen molecule attached. Thus they reflect the bone turnover in real time. In the present review article, we just focused the silent feature of hydroxyproline and its role in assessment of fracture healing.

Physiology of Fracture Healing

Fracture healing, is a proliferative and physiological process in which the body facilitates the repair of a bone fracture [1]. Fracture healing restores the tissue to its original physical and mechanical properties [2]. Healing occurs in three distinct but overlapping stages [3], first is reactive phase, second one is reparative phase and third is remodeling phase.

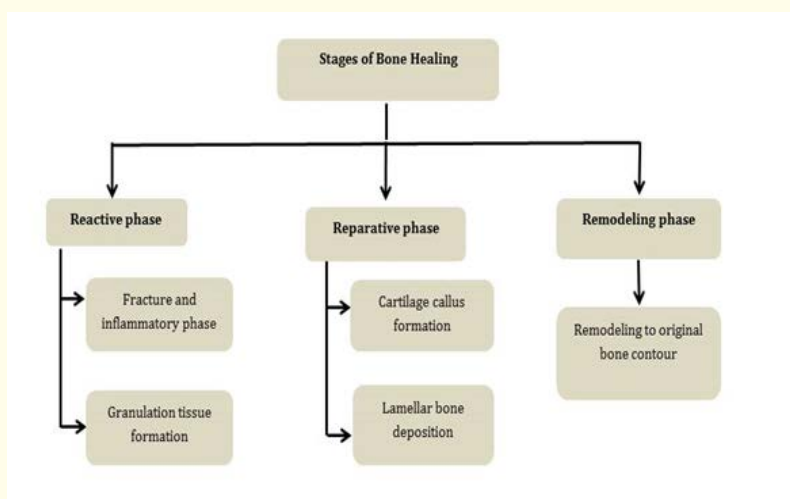


Figure 1: Representing Phases of bone healing and their subdivisions.

In reactive phase, the first change seen is the presence of blood clots within the tissues adjacent to the injury site just after fracture in which the blood vessels constrict immediately and stops any further bleeding [4]. The extravascular blood clot within a few hours of fracture, known as a hematoma in which the blood cells release the cytokines that increases blood capillary permeability. However, the remaining blood cells clot degenerate and die [5,6]. Within the same area, the fibroblastic cells survive and replicate to form a loose bunch of cells, interspersed with blood vessels known as granulation tissue [7]. Furthermore, the Osteoclasts moves to reabsorb dead bone ends and other necrotic tissues are removed [6]. The periosteal cells (proximal) in the fracture gap develop into chondroblast cells which form hyaline cartilage. The periosteal cells (distal) to the fracture gap develop into osteoblasts which form woven bone [8].

In remodeling process, trabecular bone replaced with compact bone. In this, "Howship's lacuna" (shallow resorption pit) created by resorption of trabecular bone by osteoclasts and then in resorption pit, osteoblasts deposited in form of compact bone. After some time, the fracture callus is remodelled into a new shape forms the bone's original shape and strength. Depending on age or general conditions the remodeling phase takes 3 to 5 years [9,10].

Factors affecting fracture healing

Bone is unique in its inherent capability to completely regenerate without scar tissue formation. This characteristic is central to skeletal homeostasis, fracture repair, as well as bone graft incorporation. However, there are several factors which affect bone healing e.g.,

bone type, age, preexisting bone pathology and drug therapies etc. [11,12].

S. No.	Factors	Factors affecting the rate of fracture healing
About the patient		
1.	Age	In young children fractures heal faster than other age groups, that means the younger the child the faster the healing (may be expected to heal up until the mid-late teenage years) after attaining full size the rate of healing of fractures becomes low.
2.	General health	Good general health may expect fractures to heal faster than those have chronic health issues.
About the bone		
1.	Type of bone	It depends on the type of bone affected either Spongy bone or Compact bone.
2.	Pathology of bone	Sometimes, due to common causes healing may delay (reducing the rate of healing = increasing healing time).
About other aspects of the fracture		
1.	Mobility at the fracture site	If the structure and their surrounding tissues are mobile causes delayed healing.
2.	Separation of bone surfaces	Separation of the bone ends also causes delayed healing until that broken parts are not firmly reconnected.
About other aspects of the injury		
1.	Joints near the fracture	If a fracture occurs at a joint the rate of healing sometimes reduced because these sites are the mobile areas and most of the times it is very difficult to immobilize.
2.	Blood supply	Interrupted blood supply increases healing time. Because the affected tissues require sufficient blood supply to function correctly.
3.	Infection	Infection in the vicinity of fractures can delay healing (i.e. decreasing the rate of healing of the fracture = increasing the healing time).

Table 1: Table representing factors affecting the rate of fracture.

Assessment of fracture healing

Healing is continuous process to achieve Union [13]. Thus, healing should be measured. No clinically validated method to measure healing over time is available to date. According to [14], a valid measurement for union should be measurable at each point of time during the union process. Thus, the values yielded by measurement should be on a continuous numeric scale. However, till now researchers have used an end point for completely healed fracture at a point of time without documenting the values signifying progress to union before that point. For them union is when normal weight bearing becomes possible. According to [15], none of the measures of union will help early detection of problems with healing that lead to problems with union. The end point will not help the clinician to identify delayed and non-unions early while starting the treatment. Thus the patient will have to suffer for a larger period of time. The below section followed the basic assessment tools to measure the fracture healing progression.

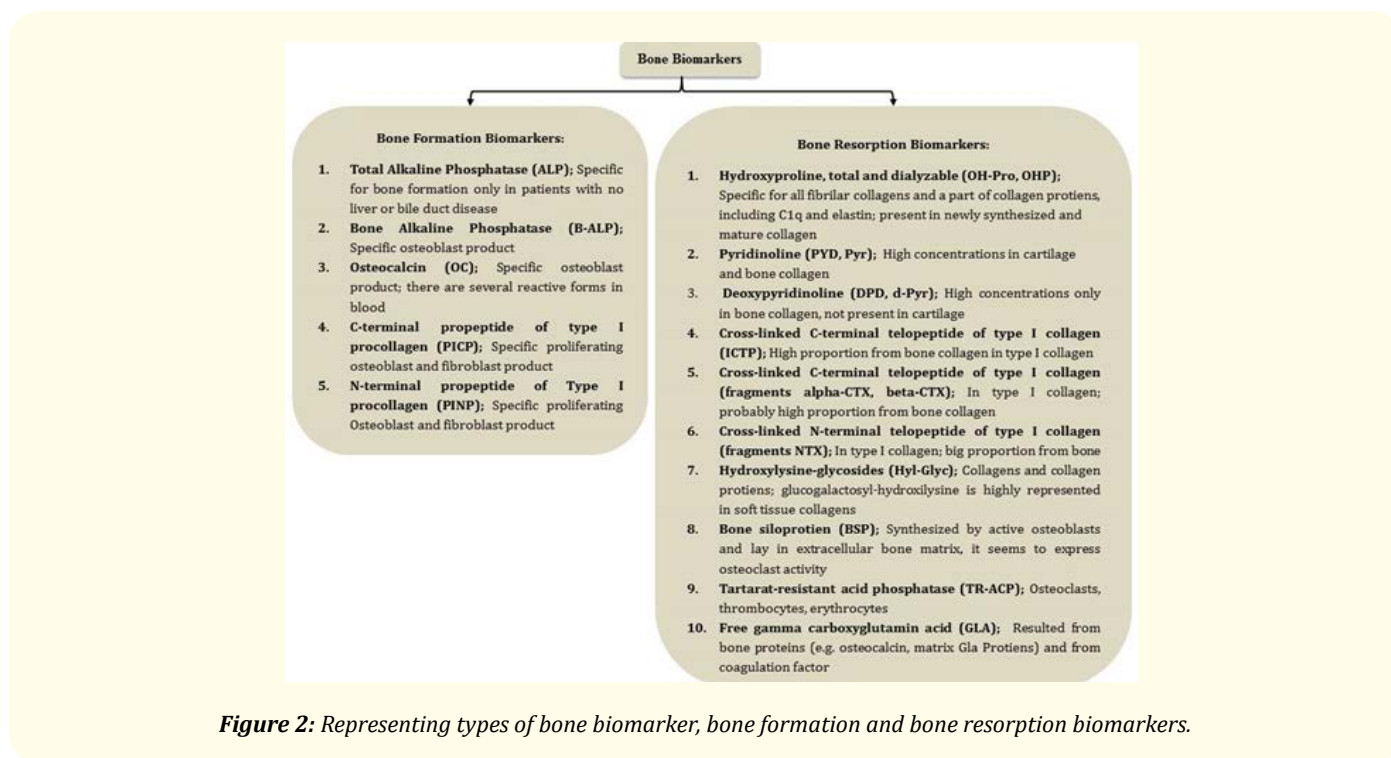
Clinico-radiological Assessment

Currently, clinical and radiological methods are most commonly used to assess the healing of fractures. The most common clinical criteria are ability to bear weight, ability to walk and perform activities without pain or tenderness on examination no motion and no pain at fracture site and also full range of motion at adjacent joint without pain during weight-bearing [16]. The X-rays, computed tomography,

and ultrasound are the used in the radiological assessment of fracture healing, in which the X-ray was used most commonly. However, according to [17], the probability of correct radiological evaluation of the stage of union in fractures of tibia has been shown to be only about 50%. The radiographic assessment is not a very good method to asses' fracture healing, a fact borne out by a study on radiological evaluation of the stage of union in fractures of tibia [18]. However, due to lack of objectivity, now-a-days for long bones, especially for tibia, Radiographic Union Score for Tibial fractures (RUST) scoring was used [19]. But instead of this, till now need of some complementary fracture healing assessing tool are still in demand.

Biochemical Assessment

A close supervision is required for fracture union progress. The actual status of bone resorption and bone formation may define with biochemical markers over a short duration of time. Biochemical markers of bone resorption reflect degradation of collagen, osteoclast activity, and also may provide a new potentiality for the assessment and monitoring of bone metabolism [20]. Commonly markers are classified into bone formation biomarkers (connected with osteoblastic activity) and of bone resorption biomarkers (connected with osteoclastic activity) [21].



Among the different types of biomarkers, urinary hydroxyproline is one of the osteoclastic biomarker that plays a vital role in bone pathogenesis and metabolic bone diseases. Although they provide additional and complementary information about bone mass density in the study of fractured patients [22]. Few studies have evaluated either laboratory-based methods or imaging methods for prediction of normal and delayed healing. Biological markers may facilitate the earliest diagnosis of delayed union. Study shows, biological markers may represent the release of extracellular matrix components; either they may produce or degraded during the remodeling phase. Also they may enhance the bony growth, either systemically or locally, which may influence the rate of bony healing process [23].

Biogenesis of Hydroxyproline

Hydroxyproline present in urine in three forms (Figure 3). First, free hydroxyproline (nearly all reabsorbed by tubules and degraded

by the liver). Second, small hydroxyproline (containing peptides that are dialyzable and constitutes 90% of urinary hydroxyproline excretion) and third, no dialyzable polypeptides (containing hydroxyproline which are formed from the breakdown of newly synthesized collagen). As most of the endogenous urinary hydroxyproline is derived from the breakdown of collagen, the urinary excretion of hydroxyproline in fractured patients may better to assess its healing progression [24].

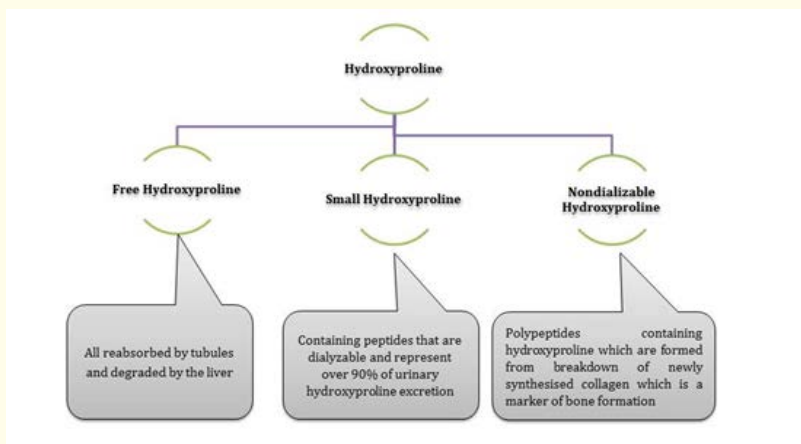


Figure 3: Above chart showing types of hydroxyproline as free hydroxyproline, small hydroxyproline and nondializable hydroxyproline.

After post translational modification an enzyme prolyl hydroxylase action on amino acid proline followed by its hydroxylation produces hydroxyproline. This enzymatic reaction occurs in the lumen of the endoplasmic reticulum [25]. Hydroxyproline is a major component of the protein collagen [26] and along with proline they play key roles for collagen stability [27].

Hydroxyproline is released by the breakdown of collagen in the tissues, especially during bone resorption, it degraded into free amino acid that circulates in plasma, and is almost entirely reabsorbed by the kidney. At last it completely oxidized in the liver and degraded to carbon dioxide and urea [28,29]. As free hydroxyproline released during degradation of collagen cannot be reutilized in collagen synthesis [30]. The hydroxyproline excreted in the urine is a definitive indicator of bone matrix turnover [31,16].

Hydroxyproline in fracture union

Bone turnover is probably faster than in soft tissues, whereas nearly half of human collagen remains in bone. Excretion of hydroxyproline in urine is regarded as a marker of bone resorption. Approximately 50% of urinary hydroxyproline is derived from bone collagen breakdown [32]. Increased production of collagen is allied with increase in the hydroxyproline [33-35].

Increase in the hydroxyproline also found soon after fracture that associated with an increased production of collagen. These modifications represent the changes in levels of total and free hydroxyproline excretion in urine and ALP in plasma [36-40]. In a recent study by Das., *et al.* [22], in patients with long bone fractures, significant differences were observed between normal union and nonunion groups in case of serum ALP, urinary total and free hydroxyproline levels after treatment with a positive correlation between serum ALP and urinary total hydroxyproline in normal united group. These study observations conducted by Das., *et al.* found to be same as previous study carried out by Mukhopadhyay., *et al.* [31]. Thus, they concluded that serial monitoring of urinary hydroxyproline, serum ALP reflect the actual status of bone turnover in real time.

Significance of Urinary Hydroxyproline

Nearly all of the hydroxyproline of the body is found in collagen, that's why [41,42] the urinary excretion of this amino acid may be an

important index of collagen metabolism. Although ingestion of large amounts of gelatin was found to increase urinary hydroxyproline, its urinary excretion did not decrease when hydroxyproline was eliminated from the normal diet, or even if subjects were placed on a protein-free diet, that means in scarcity it may provide as oral diet. Furthermore, no diurnal variation in urinary hydroxyproline was found. The hydration or dehydration process also did not alter excretion of hydroxyproline. Ingestion of large amounts of hydroxyproline as the free imino acid resulted in an increased excretion of free, but not of bound hydroxyproline. Also urinary excretion of hydroxyproline did not decrease when subjects were changed from a normal to a low hydroxyproline diet [43]. As well as there are several factors e.g. larger body size, diet, collagen synthesis, collagen degradation, serum proteins which contribute variations in hydroxyproline [44].



Figure 4: Above figure representing variations in urinary hydroxyproline due to several factors [44].

Conclusion

Serial monitoring of biochemical marker of bone turnover like urinary hydroxyproline, reflect the actual status of bone turnover rate over a short period. Furthermore, because of no diurnal variation or alteration due to dietary or other physiological process make them ideal for a biomarker. Thus, it can be used as a complementary assessment tool to assess the fracture healing progression in parallel to clinic-radiological examination. By this way we can better assess the healing progression and may early predict the indications of any healing impairment, which could be helpful in performing early interventional procedures. Thus, this approach would benefit not only the patient's wellbeing but also the health care system in terms of the cost implications associated with long lasting treatment interventions and prolonged hospital stay.

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