Nutritional Deficiency & Impact on Health

Chapter 1

Iron Deficiency Anaemia: the Link Between Oxidative Stress and Bone Turnover

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Abstract

Iron-deficiency anaemia (IDA), one of the most common and widespread health disorders worldwide, affects fundamental metabolic functions and has been associated with deleterious effects on bone. On the other hand, oxidative stress results from an imbalance between the formation and neutralization of reactive oxygen species (ROS) and it is imposed on cells as a result of one or more of the following factors: an increase in oxidant generation, a decrease in antioxidant protection, or a failure to repair oxidative damage. During IDA oxidants are increased and antioxidants decreased, so the oxidative/antioxidative balance is shifted toward the oxidative side. Fe status is directly correlated with mineral bone density and Fe deficiency diminishes the mineral bone content, the bone mass and mechanical resistance. Fe deficiency diminishes bone matrix formation, reducing the amount of procollagen type I N-terminal propeptide released to the serum under these conditions. Bone resorption process increases in Fe deficiency because osteoblast function and bone formation are strongly oxygen-dependent. Hypoxic condition (a consequence from decreased oxygen delivery in Fe deficiency anaemia) diminishes bone formation. The inhibitory effects of hypoxia are due to decreased osteoblast proliferation and differentiation and evoked oxidative stress. In addition, hypoxia stimulates osteoclast activity in favor of pathological resorption. In conclusion, Fe deficiency anaemia has a significant impact upon bone, affecting bone mineralization, decreasing the matrix

formation and increasing bone resorption, therefore it is of great interest to assess bone status in situation of Fe deficiency anaemia.

1. Introduction

1.1. Iron Deficiency Anaemia

Iron-deficiency anaemia (IDA), one of the most common and widespread health disorders worldwide, affects fundamental metabolic functions and has been associated with deleterious effects on bone. To more fully understand iron deficiency anemia, consideration must be directed toward concepts of iron supply and demand for the production of erythrocytes. Erythropoiesis-related demands for iron are created by three variables: tissue oxygenation, erythrocyte turnover, and erythrocyte loss from hemorrhage. Tissue oxygenation requirements and erythrocyte production generally remain stable during adulthood in the absence of hemorrhage, disease, or altered physical activity.

The most affected groups are women in fertile age, especially pregnant women and children, during the stage of growth. Anaemia takes place when the levels of Fe decrease or the requirements overcome the contribution of the intake that is provided in the diet, so the Fe storages of the organism are depleted. There is no doubt that the Fe deficiency is the major cause of the great majority of anaemias. The Fe-deficiency anaemia is characterized by low levels of serum Fe and haemoglobin (Hb), reduction of the haematocrit and increased levels of platelets, low percentage of transferrin saturation, decrease of serum ferritin and a drastic increase in total Fe binding capacity (TIBC) [1].

In addition, the chemical properties of Fe render it a potential hazard within the organism in that ferrous ion Fe (II), in small non-protein shielded chelates, can catalyze the production of reactive oxygen species (ROS), which in turn can lead to peroxidation and radical chain reactions with molecular damage [2,3]. On the other hand, regulation of ROS levels and oxidative stress is extremely important in erythropoiesis. Starting at the basophilic erythroblast stage, erythroid precursors synthesize large amounts of Hb, which require haem as a prosthetic group. Thus, Fe uptake for haem biosynthesis also increases, potentially generating ROS through the Fenton reaction [4].

Oxidative stress results from an imbalance between the formation and neutralization of ROS and it is imposed on cells as a result of one or more of the following factors: an increase in oxidant generation, a decrease in antioxidant protection, or a failure to repair oxidative damage [2,3]. Disturbance of the pro-oxidant/antioxidant balance is also considered to be a causative factor underlying oxidative damage to cellular molecules, such as DNA, causing strand breaks. There is controversy about the susceptibility of cells to lipid peroxidation in Fe deficiency anaemia: some investigators have claimed there is no difference in lipid peroxidation among patients with Fe deficiency anaemia compared with controls [5,6], but others have

reported that among patients with IDA oxidants are increased and antioxidants decreased, so the oxidative/antioxidative balance is shifted toward the oxidative side [7-9]. In a study by Díaz-Castro et al., [10], the authors reported that Fe deficiency anaemia does not affect DNA stability or lipid peroxidation in rats and suggest that there is enough compensatory capacity to keep antioxidant defenses high. This apparent discrepancy may be due to different concentrations of ROS and antioxidant enzymes in the tissues studied, the subjects of the study, the severity of the Fe-deficiency and the methods used for the assessment of the oxidative stress. However, in humans, were the degree of Fe-deficiency is not very high, it is accepted that Fe-deficiency increases lipid peroxidation, fact that can also be attributed to the repletion process with several sources of Fe.

1.2. Oxidative Stress

Oxidative stress results from an imbalance between the formation and neutralization of oxygen-derived pro-oxidants, which can cause damage to biological targets such as lipids, DNA, and proteins, and on the defending systems of the cell, which are composed of enzymes and reducing equivalents, or antioxidants. In general these pro-oxidants are referred to as reactive oxygen species (ROS) that can be classified into 2 groups of compounds, radicals and nonradicals. The radical group, often incorrectly called free-radical (the term is not accurate, because a radical is always free.), contains compounds such as nitric oxide radical (NO•), superoxide ion radical ($O_2^{\cdot -}$), hydroxyl radical (OH•), peroxyl (ROO•) and alkoxyl radicals (RO•), and one form of singlet oxygen ($^1O_2$). These species are radicals, because they contain at least 1 unpaired electron in the shells around the atomic nucleus and are capable of independent existence [3,11].

Most of the transition metals contain unpaired electrons and can, therefore, with the exception of zinc, be considered radicals by definition [3]. They can participate in the chemistry of radicals and convert relatively stable oxidants into powerful radicals. Among the various transition metals, copper and especially iron are most abundant, present in relatively high concentrations, and are major players in the Fenton reaction [12] and the metal-mediated Haber-Weiss reaction [13]. The metal ions participating in this reaction are those bound to the surface of proteins, DNA, and other macromolecules or chelates. These particular ions can still undergo the reduction-oxidation process, interact with oxygen derivatives, and are often called “loosely bound metals” or “removable metals” [14]. Metals that are hidden in proteins, as in catalytic sites and cytochromes, or storage complexes; are not exposed to oxygen radicals; or are kept under 1 oxidation state cannot participate in this chemistry.

1.3. Bone Turnover

Bones is a metabolically active tissue that undergoes continuous remodelling to cope with the body’s Ca requirements and to repair microscopic damage in a dynamic process where

osteoblasts are responsible for bone formation and osteoclasts for its resorption. These processes rely on the activity of osteoclasts (resorption), osteoblasts (formation) and osteocytes (maintenance). Under normal conditions, bone resorption and formation are tightly coupled to each other, so that the amount of bone removed is always equal to the amount of newly formed bone. The entire skeleton is replaced every 10 years in adults, and around 10% of the skeleton is involved in bone remodelling at any one time. This balance or turnover is achieved and regulated through the action of several hormones (PTH, vitamin D, osteocalcin, alkaline phosphatase…) and local mediators (cytokines, growth factors…). In contrast, growth, ageing, metabolic bone diseases, states of increased or decreased mobility, therapeutic interventions, nutritional deficiencies and many other conditions are characterized by imbalances in bone turnover. The results of such uncoupling in bone turnover are often changes in bone structure, strength, mineralization and mass.

Osteoblasts are specialized mesenchymal cells that undergo a process of maturation where genes like core-binding factor a1 (Cbfa1) and osterix (Osx) play a very important role. Moreover, it was found recently that the Wnt/b-catenin pathway plays a part on osteoblast differentiation and proliferation. Osteoblasts have also a role in the regulation of bone resorption through receptor activator of nuclear factor-kB (RANK) ligand (RANKL), that links to its receptor, RANK, on the surface of pre-osteoclast cells, inducing their differentiation and fusion. On the other hand, osteoblasts secrete a soluble decoy receptor (osteoprotegerin, OPG) that blocks RANK/RANKL interaction by binding to RANKL and, thus, prevents osteoclast differentiation and activation. Therefore, the balance between RANKL and OPG determines the formation and activity of osteoclasts [15].

Osteoclasts are the cells that degrade (resorb) bone during normal bone remodeling and in pathologic states in which bone resorption is increased. Osteoclasts form microscopic trenches on the surfaces of bone trabeculae in the spongy bone by secreting hydrochloric acid and proteases, such as cathepsin K, into an extracellular lyzosomal compartment beneath a ruffled part of their basal cell membrane to dissolve the mineral and matrix components of bone simultaneously. Precursors of osteoblasts, the cells that form bone, are recruited to these trenches from the adjacent bone marrow stromal cell population and differentiate into osteoblasts, which lay down new matrix and mineralize it [16]. Bone remodeling can be increased in response to many influences, including mechanical strain, cytokines, hormones, growth and dietary factors.

A number of nutrients have crucial roles in the development and maturation of bone. Ca in particular has received a great amount of interest because of its impact on the bone disease osteoporosis. This is a lifelong disease in which peak bone mass in the late teens can lessen the risk of development later in life. Development of osteoporosis is also dependent upon other factors and nutrients, such as vitamin D, estrogen and weight-bearing exercise. Other nutrients

that may have an impact on bone physiology include proteins, ascorbate, Cu and Fe [17].

2. Iron Deficiency Anaemia and Bone Turnover

2.1. Iron Deficiency and Bone Mineralization

There is a growing body of evidence from animal research, epidemiologic, and clinical studies indicating an association between osteopenia and anaemia. The relation between Fe and the bone metabolism, has received attention, revealing that Fe intake is directly correlated with the mineral bone density in women postmenopausal women. Several authors [17-25] have demonstrated that Fe deficiency diminishes the mineral bone content, the bone mass and mechanical resistance. Kipp et al. [26] reported that, when iron-deficient rats were compared with a weight matched control group, cortical and cancellous bones were significantly decreased, supporting an independent effect of iron upon bone morphometry.

Campos et al., [24] indicated that the anaemia produces a high degree of bone demineralization, with a lower Ca and P storage in femur of anaemic rats. After the consumption of a diet with a normal Fe content, Ca and P concentrations increases in sternum, but not in femur, revealing that bone demineralization was kept even after the recovery of the anaemia. In addition, when severe Fe deficiency occurs, a feedback effect becomes established, in which the lower degree of metabolism results in less haemoglobin being produced, and thus less oxygen is transported, resulting, in turn, in a lower degree of metabolic reactions. Furthermore, there is an increase in the number of leucocytes and platelets, and also in the concentration of cortisol in serum. This fact is explained by the reduction in the activity of monoaminooxidase (MAO) in Fe deficiency, with a low level of aldehyde oxidase activity. These compensatory mechanisms produce an increase in the levels of circulating catecholamines, which increase the releasing of adrenocorticotrophic hormone, which in turn increases the output of glucocorticoids [20]. Therefore, Fe deficiency induces hormonal changes, such as an increase in serum levels of PTH and cortisol, indicating an increase in the velocity of bone resorption. In this sense, Diaz-Castro et al. [25] reported an increase in PTH serum levels in Fe deficient animal models, revaling that this increase contributes to the lower Ca and P deposit in femur of anaemic rats found previously. Moreover, the weakness of the collagen type I fibres lead to a decrease in the mineralization process, because hydroxyapatite crystals might not be deposited properly when the collagen fibres does not bring together the suitable characteristics of rigidity, flexibility and resistance.

In a large Italian cohort with elder subjects [27], they studied the relationship between bone mass and hemoglobin levels in 420 men and 530 postmenopausal women in this cohort (there was 56 anemic females). The authors found that hemoglobin levels and anaemia were negatively and independently associated with bone mass and density and they suggested that the bone loss associated with Hb levels occurs mainly in the cortical bone. An explanation for

the relationship of anaemia and hemoglobin levels with bone density might be provided by a study conducted by Fujimoto et al. [28], in which, by combining results from animal and human models, they suggested that hypoxemia can affect mineral density and might be a risk factor for bone loss. In fact, the authors showed that patients with low PaO2 presented a decreased bone mineral density. Furthermore, to exclude the hypothesis that limited exercise was influencing their results, they conducted an animal experiment and showed that a significantly lower bone density was found in hypoxemic rats than in normoxemic rats.

Cesari et al. [27] have reported a strong association of hemoglobin levels with the cortical bone mineralization and density. Age-related bone remodeling and bone loss occur mainly in the cortical bone that becomes ‘‘trabecularized’’ due to an increased porosity. This might provide an explanation for the particularly strong association between hemoglobin levels and bone density in that site. Cesari et al. (2005) also reported a small but significant difference in trabecular bone density between women with anaemia and those with high hemoglobin levels. Several diseases characterized by low hemoglobin levels or Fe deficiency anaemia have been associated with an increased risk of bone loss or osteoporosis. In fact, pernicious anaemia is directly correlated with increased risk of osteoporotic fractures [29,30].

Skeletal manifestations are common in both sickle cell anaemia and thalassaemia. In these conditions, bone marrow hyperplasia leads to widening of the medullary cavity; expansion of the medullary space of the skull in combination with orientation of the trabeculae perpendicular to the cortical surface produces the characteristic ‘hair-on-end’ appearance. There is also disturbance of bone growth and reduction in both cancellous and cortical bone mass. In sickle cell disease, microcirculatory disturbances and bone infarction may result in episodes of bone pain; other manifestations include the characteristic H-shaped vertebral bodies, caused by infarction of bone in the centre of the vertebral body and the resulting disturbance in bone growth, patchy osteolysis and sclerosis in cancellous bone of long bones, osteonecrosis and osteomyelitis [31]. A reduced bone mass associated with low hemoglobin levels has also been reported in hemodialysis patients [32]. Hens et al. [33] have shown that, as chronic obstructive pulmonary disease becomes more severe, the prevalence of osteoporotic patients increases [34]. Patients with thalassemia, often present skeletal morbidity [35]. Moreover, it has also been demonstrated that the degree of bone loss is lower in thalassemic patients receiving more blood transfusions [36].

Another factor that should be taken into account for the bone affectation during Fe deficiency is the vitamin D. Renal 25-hydroxyvitamin D 1-hydroxylase, which converts 25-hydroxyvitamin D into the active form of vitamin D, is a system that involves a flavoprotein, an iron-sulphur protein, and a cytochrome P-450. Therefore, in Fe deficiency anaemia, these Fe-dependent enzymes might become inactive and abnormal metabolism of vitamin D might occur, leading to low bone mineralization [25].

Fe might act as a toxin to bone cells and contribute to osteoporosis or other bone diseases in people with impaired Fe metabolism and Fe overload. Most typical such cases are in hemochromatosis, hemosiderosis, chronic renal diseases (including renal osteodystrophy) and any case of Fe overload with prolonged and repeated Fe therapy or hemotransfusion. It is not always clear whether the insult to bone comes from iron itself, Fe overload-induced hypovitaminosis C or both [37]. Conte et al. [38] compared bone mass density and bone histomorphometric analyses among patients with primary hemochromatosis, alcoholic cirrhosis and controls. Densitometric and histomorphometric results indicated impairment of trabecular bone in both patient groups compared with controls, while cortical impairments were limited only to hemochromatotic patients. Similar findings resulted from the study of osteoporosis in African hemosiderosis patients [37].

An important step in the bone formation process is synthesis of type I collagen, which is the major organic component in bone matrix. During collagen synthesis, propeptides are released from both the terminal parts of the procollagen molecule. The noticeably decrease in this bone formation biomarker revealed that anaemic rats had an important bone mineralization impairment induced by Fe deficiency. Fe exerts its influence on bone turnover by affecting type I collagen synthesis and maturation [17-19]. Fe is a cofactor for prolyl and lysyl hydroxylases, enzymes that catalyse an ascorbate-dependent hydroxylation of prolyl and lysyl residues, essential steps prior to crosslinking by lysyl oxidase. Therefore, Fe deficiency diminishes the amount of Fe available, leading to a diminishing in crosslinking of type I collagen which could result in decreased crosslinking activity and, subsequently, weaker collagen fibres [25].

Bone remodelling is a series of complex processes of bone matrix formation, mineralization, and resorption performed by the bone cells. High amount of tartrate-resistant acid phosphatase (TRACP) is expressed by bone-resorbing osteoclasts and activated macrophages. TRACP 5b is derived from osteoclasts and TRACP 5a from inflammatory macrophages. Osteoclasts secrete TRACP 5b into the blood circulation as an active enzyme that is inactivated and degraded before it is removed from the circulation, with a functional correlation of the TRAP activity in osteocytes with osteocytic osteolysis. Diaz-Castro et al. [25] reported that TRACP 5b indicates the number of osteoclasts rather than their activity, therefore the increase in TRAPC 5b found in anaemic animal models indicates an increase in the number of osteoclasts, accelerating the increase of the resorption process.

2.2. Hypoxia and Bone Density

Reduced oxygen to tissues is referred to as hypoxia, and is a consequence from decreased oxygen delivery in Fe deficiency anaemia. In normal body tissues, the partial pressure of oxygen (pO_2) varies greatly. The mean pO_2 of bone marrow aspirated from healthy subjects

is 51.8-54.9 mm Hg (or 6.8 – 7.2% O2 v/v) [39]. In pathological lesions of osseous tissues, including inflammation, fracture, and tumors, pO_2 is evidently lower [40].

Low O_2 can alter bone homeostasis, leading to osteolysis. Patients exposed to long-term hypoxic states are at risk for accelerated bone loss. Vascular flow to the lower extremities is directly linked with bone mass density (BMD). A decrease in blood flow to the lower extremities (measured as a decrease in the ankle/arm index) is associated with an increase in the annual rate of bone loss at the hip and calcaneus. Annual bone loss at the calcaneus was increased about 30% in this group of women. Women with increased blood flow to the lower extremities have a higher bone mass at the hip and in the appendicular skeleton. This association is independent of estrogen use, pattern of fat distribution, history of diabetes, ability to walk, and exercising [41].

Furthermore, hypoxia has been determined to be a risk factor for osteoporosis in animal and human models. Previously, several studies have reported on associations between anaemia or hemoglobin levels and bone density in selected conditions, such as sickle-cell anaemia, chronic inflammatory conditions, or renal failure [42].

2.2.1. Hypoxia and bone cells

The organic matrix of bone consists of approximately 90% type 1 fibrillar collagen. Collagen is a heterotrimer consisting of two α1 subunits and one α2 subunit; these are synthesized as propeptides that undergo a variety of posttranslational modifications to create mature, fibrillar collagen. The initial modification is the hydroxylation of several proline residues, performed by procollagen prolyl 4 hydroxylase (P4OH), the resultant hydroxyproline residues being essential for stable triple helix formation. Like its HIF-modifying counterparts, P4OH also requires molecular oxygen for enzymatic activity. Further hydroxylations are then performed on by the enzymes procollagen- lysine, 2-oxoglutarate, 5-dioxygenase 1–3 (PLOD1–3), in preparation for secretion into the extracellular space and subsequent cleavage of propeptides which renders the collagen triple helix insoluble, and it spontaneously assembles into fibrils, which are then acted upon by lysyl oxidase (LOX) to create covalent cross-links between adjacent lysine and hydroxylysine residues. This binds the fibrils and provides the tensile strength to the collagen fibers in bone. The PLOD and LOX enzymes are also dependent on molecular oxygen for their activity [43].

2.2.1.1. Osteoblasts

Osteoblast function and bone formation are strongly oxygen-dependent. Hypoxic condition diminishes bone formation. The inhibitory effects of hypoxia are due to decreased osteoblast proliferation and differentiation [43,44]. Mineralized bone nodule formation by cultured osteoblasts was strongly inhibited when pO_2 is <5% and almost completely prevented when

pO_2 is <1%. Bone formation in vivo normally occurs in environments where pO_2 is between 12% and 5% (corresponding to arterial and venous blood, respectively). Thus, atmospheric oxygen levels (i.e., 20% O_2) correspond to hyperoxia; our findings indicate additionally that bone formation by osteoblasts in 20% O_2 (which may be considered as hyperoxia) is stimulated by about 50% relative to the physiological 5–12% O_2 range. Hypoxia inhibits the proliferation of immature osteoblast precursors, leading to failure to achieve the 'critical mass' of differentiated cells needed for bone formation in vitro. It also prevents the production of mineralized matrix by disrupting collagen formation and alkaline phosphatase activity [43]. Delayed osteoblastic differentiation associated with hypoxia has been reported elsewhere; this effect has been ascribed to decreased expression and activity of the transcription factors, BMP2 and Runx2 [45]. In addition, delayed osteoblast differentiation in hypoxia can be attributed to the inhibition of alkaline phosphatase gene expression and protein activity and of osteocalcin gene expression [43].

The inhibitory response of osteoblasts to hypoxia is reciprocal with the powerful stimulatory action of hypoxia on osteoclast formation (and thus, bone resorption). It is noteworthy that even in severe, chronic hypoxia (0.2% O_2), mouse or human osteoclast formation is increased 2- to 3- fold compared with 20% O_2 [46,47].

2.2.1.2. Osteoclasts

Oxygen is both an essential metabolic substrate in numerous enzymatic reactions, including mitochondrial respiration, and a regulatory signal that controls a specific genetic program. An important component of this program is the transcription factor HIF-1α, which is a key-mediator of cellular adaptation to low O_2 tension (hypoxia). A central role for hypoxia and the hypoxia-inducible transcription factor (HIF) is emerging in bone biology. HIF comprises a hypoxia-inducible α subunit and a constitutively expressed β subunit. Under normoxia, HIFα is post-translationally hydroxylated by the prolyl hydroxylase domain (PHD) enzymes, targeting it for proteasomal degradation. A limitation of PHD enzyme activity under hypoxia allows stabilization of HIFα and transactivation of genes involved in processes such as angiogenesis, apoptosis, and metabolic adaptation. Hypoxia and the hypoxia-inducible factor (HIF) transcription factor regulate angiogenic-osteogenic coupling and osteoclast-mediated bone resorption [48].

A major role for HIF in regulation of osteoclast activity it has been also demonstrated. The role of HIF is mediated, at least partially, by ANGPTL4. Angiopoietin-like 4 (ANGPTL4) is a recently identified adipokine, which is predominantly expressed in adipose tissue, liver, lung, kidney, and placenta. Hypoxia-inducible expression has been described in adipocytes, endothelial cells, heart, and articular chondrocytes. ANGPTL4 is overexpressed in critical leg ischemia and in the hypoxic, perinecrotic regions of tumors. An effect of ANGPTL4 to stimu-

late osteoblast differentiation at low local concentrations and osteoclast activity at higher concentrations might tip bone homeostatic mechanisms in favor of pathological resorption under conditions of severe local hypoxia and/or inflammation. One of the few cytokines to affect osteoclast activity directly, rather than indirectly enhancing resorption via stimulation of osteoclast differentiation, is RANKL. ANGPTL4-mediated induction of osteoclast activity could be achieved, albeit with reduced total levels of resorption, in the absence of RANKL. ANGPTL4 is activating distinct intracellular signaling pathways to stimulate osteoclast activity. Given the effects of ANGPTL4 on the osteoblast phenotype, this could represent a mechanism whereby HIF coordinates osteoclastic and osteoblastic components of the osseous niche. If we take into account other effects of ANGPTL4, these results suggest a tripartite role for the adipokine in mechanisms coupling the regulation of bone, fat, and angiogenesis [48].

3. Oxidative Stress and Bone Turnover

Bone remodeling is a tightly controlled mechanism in which osteoblasts (OB), the cells responsible for bone formation, osteoclasts (OC), the cells specialized for bone resorption, and osteocytes, the multifunctional mechanosensing cells embedded in the bone matrix, are the main actors [49]. The remodeling process is highly active throughout the life and perturbation of this process can lead to many pathologies including osteoporosis. This pathology, due to an imbalance favoring bone resorption over formation, is characterized by increased OB apoptosis as well as an enhanced OC number and activity [50]. Although age-related estrogen deficiency has long been considered to be the major cause of osteoporosis, the oxidative stress is now also proposed to be a key factor leading to this pathology [51]. In osteoblasts, oxidative stresses may result in lipid peroxidation, protein damage, DNA lesions, and inflammatory responses, finally leading to apoptosis. Nowadays, it is widely accepted that aging increases oxidative stresses and osteoblast apoptosis. Oxidative stresses may induce osteoblast apoptosis by activating c-Jun N-terminal kinase (JNK) pathway, which causes cell injuries and reduces the number and function of osteoblasts, thereby inhibiting bone formation [52-54]. However, forkhead box O- (FoxO-) dependent oxidative defense might provide a mechanism to handle the oxygen free radicals constantly generated by the aerobic metabolism of osteoblasts and is thereby indispensable for bone mass homeostasis [55].

Although there are other forms of ROS, such as hydroxyl radicals, the hydroxyl ion, and peroxide, the majority of the osteoclast-ROS literature revolves around superoxide and H_2O_2. In addition to signaling, ROS production also serves as a beneficial function in mediating the oxidative burst process by hematopoietic cells in the innate immune system to protect the body from foreign invaders [56]. It comes as no surprise then, that ROS, particularly superoxide and H_2O_2, are important in another cell of the hematopoietic lineage—the osteoclast.

The superoxide radical rather than H_2O_2 or hydroxyl was important in mediating the

enhanced bone resorption. Subsequent studies from other groups also found that superoxide production was present within the osteoclast and suggested that it may be derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase localized to the bone–osteoclast interface within the osteoclast ruffled border [57,58]. Further evidence for the involvement of NADPH oxidase was found when addition of a specific inhibitor of NADPH oxidase, diphenylene iodonium (DPI), resulted in a reduction in superoxide production and bone resorption [59]. Other studies, however, indicated that rather than superoxide, H_2O_2 was the primary ROS responsible for promoting osteoclast formation and activity [60].

Antioxidant enzymes are the major defense system of cells in normal aerobic reactions. Although, erythrocytes possess highly efficient antioxidant enzymes, such as CuZn–SOD and GPx compared to other cell types [8], though as the results showed that women with Fe deficiency anaemia have lower CuZn–SOD activity than healthy control. The results of Diaz-Castro et al. [10] are in accordance with earlier such reports [61]. Decreased SOD activity in Fe deficiency anaemia may be linked to increased oxidative stress, because it is well known that ROS, especially hydrogen peroxide (H_2O_2), inhibit SOD activity [6]. CAT and SOD are metaloproteins and accomplish their antioxidant function by enzymatically detoxifying the peroxides. CAT has been suggested to provide important pathway for H_2O_2 decomposition into H_2O and O_2. Acharya et al. [5] who reported decreased CAT activity in patients with Fe deficiency anaemia. CAT is an iron-dependent enzyme and is not unexpected to be decreased in iron deficiency. Similarly, GPx activity in IDA groups was decreased when compared with controls. This finding is in accordance with the finding of Yetgin et al. [62], who reported decreased GPx activity in children with iron deficiency. Decreased antioxidant activity in IDA may be due to perturbed pentose phosphate pathway, as IDA may have restricted the availability of NADPH, a co-factor for GPx functioning [8]. GSH plays a pivotal role in protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interactions of SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a coenzyme [63]. Therefore the impairment in antioxidant status caused by Fe deficiency has a clear effect on bone turnover and mineralization (**Figure 1**).

4. Conclusion

In conclusion, Fe-deficiency anaemia oxidants are increased and antioxidants decreased, so the oxidative/antioxidative balance is shifted toward the oxidative side. On the other hand, Fe deficiency also diminishes the mineral bone content, the bone mass and mechanical resistance. Hypoxic condition (a consequence from decreased oxygen delivery in Fe deficiency anaemia) and the evoked oxidative stress also diminishes bone formation. In conclusion, Fe deficiency anaemia has a significant impact upon bone, affecting bone mineralization, decreasing the matrix formation and increasing bone resorption, therefore it is of great interest to assess bone status in situation of Fe deficiency anaemia.

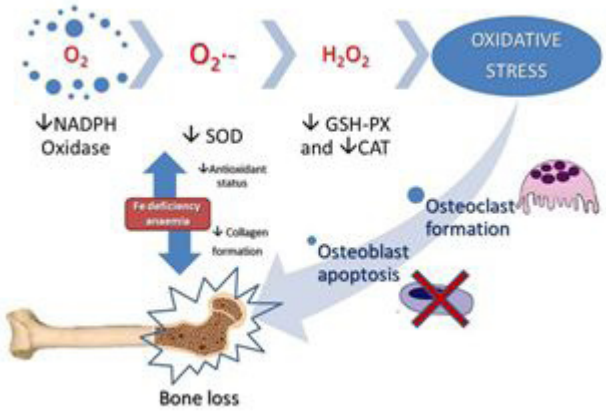

Figure 1: Effects of Fe deficiency on bone turnover.

5. References

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