INTRODUCTION

Bone is a vital, dynamic and specialized connective tissue which forms the metabolically active skeletal system (1). The structure of bone is composed of (a) inorganic component (69%) consisting of hydroxyapatite (b) organic component (22%) as collagen and noncollagenous structural proteins (NCPs) including osteocalcin (gla proteins), osteopontin (sialoproteins), decorin and biglycan (proteoglycans) and osteonectin (2HS-glycoprotein). Osteoid matrix is a crystalline salt (calcium and phosphate in the form of hydroxyapatite) deposited in the organic part of the bone. Due to the presence of mineral and salt in the osteoid matrix these components maintain rigidity of the bone (2, 3).

Bone is a porous mineralized structure composed of four types of cells: osteoblasts, bone lining cells, osteocytes and osteoclasts (4). Mesenchymal lineage and hematopoietic lineage are two different lineages from which these cell types are derived. These cells play a key role in maintaining matrix arrangement and structure-function association. Healthy skeleton required bone tissue renewal throughout the life and process is called bone remodeling (5). Bone remodeling involves bone resorption (osteoclast cells) followed by new bone formation (osteoblast cells) on same location and is a balanced process which helps in maintaining bone mass and bone quality (6-8).

Osteoblasts are derived from mesenchymal stem cells (MSCs) and responsible for the production of bone matrix. These cells are enriched with the higher number of Golgi bodies and endoplasmic reticulum (ER), secrete type I collagen in the extracellular matrix, predominantly in ubiquitous form (9, 10). These cells also produce non-collagenous protein of bone matrix, osteocalcin and the proteoglycans of ground substance (11).

Bone lining cells are the major source of osteoblasts (12), and during bone remodeling these cells help in removing demineralized matrix on the bone surface prior to the bone formation (13, 14).

Similar to osteoblast, osteocytes are also differentiated from mesenchymal lineage under the regulation of Runx2 and Osterix transcription factors (15). Osteocytes are

embedded in the bone matrix and are related to mechanosensory function in the bone. These cells play a key role in maintaining Ca^{+2} homeostasis within the bone, by rapid fluxes of Ca^{+2} between surface lining osteoblast and neighbouring osteocytes (16-18).

Osteoclasts are multinucleated cells which arise by fusion of myeloid hematopoietic precursors formed in the bone marrow (19). These are considered as bone resorbing cells (8); their activity is tightly regulated to prevent osteoporosis (20) conversely, less number of osteoblasts result in osteopetrosis (21). Among the major chemokines, macrophage colony-stimulating factor (MCSF) (22) and receptor activator of NFKB ligand (RANKL) secreted from osteoblast initiate differentiation and resorption by osteoclast (23, 24).

Approximately 90–95% of the organic matrix made up of type I collagen that appears to be the main structural element of bone and consists of three polypeptide chains encoded by 104 exons composed of approximately1000 amino acids in each. Type I collagen is heterotrimer containing two alpha-1(α1[1]) and one alpha-2 (α2[1]) chains, synthesized as a procollagen precursor, with N-terminal propeptide, central collagen domain chains and C-terminal propeptides (**Fig. 1)** (25). Procollagen chains are translocates from the nucleus to the rough endoplasmic reticulum (RER) by a series of post-translational modifications and folding (26). These post-translationally modified procollagen molecules transports through Golgi and secreted out from the cell (27-29). After the secretion, N'- and C'- propeptides are cleaved and collagen monomers self-assembles as fibrils to form collagen triple helix (30). The inter-chain hydrogen bonding throughout the backbone maintains the triple helix form of collagen (31).

The triple helical domain contains glycine, proline and hydroxyproline amino acid respectively with continuous 338 uninterrupted repeats (32). The glycine residue at every 3^{rd} position maintains tightness and stability of the triple helix (33, 34). Proline and hydroxyproline confer the rigidity, while other amino acids form hydrophobic and electrostatic region to assemble the monomers as fibrils (35).

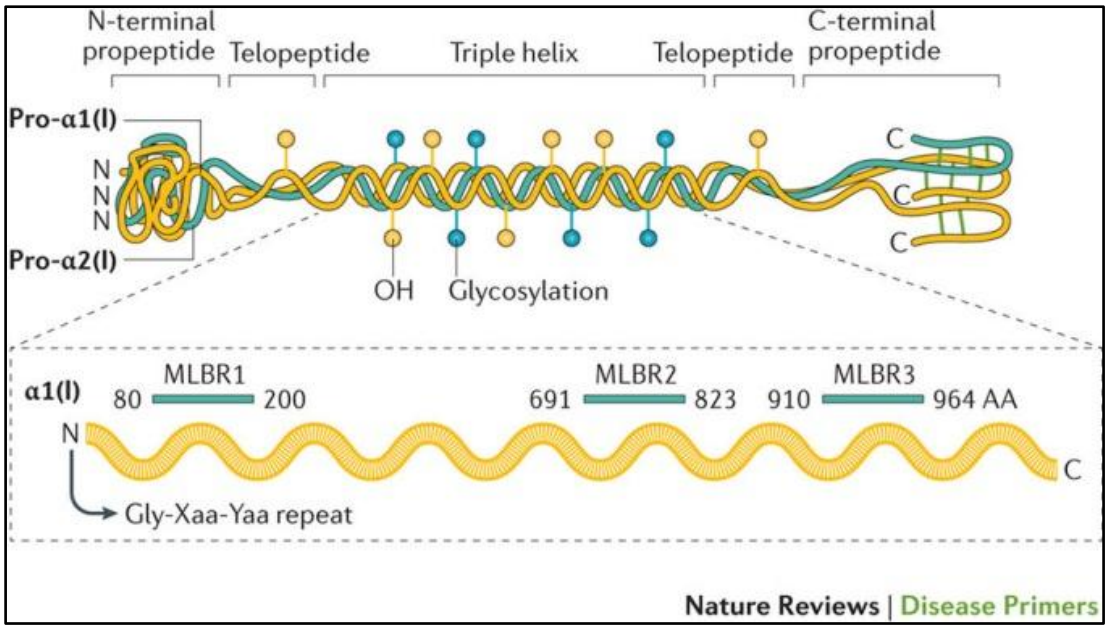

Fig.1: Structure of the collagen

Source: Marini JC,et al. Osteogenesis imperfecta. Nature reviews Disease primers. 2017;3:17052.

The structural abnormalities in the type I collagen triple helix is reported in approximately 80% of osteogenesis imperfecta (OI) cases (36-38). These are mainly autosomal dominant mutations caused by amino acid substitution and lead to quantitative and qualitative defects in the type I collagen (39).

OI is a rare metabolic disorder (40, 41) affecting one in 15-20000 births (42). It is characterized by fragility fractures, blue sclera, short stature and hearing loss. Less commonly observed findings in OI are decreased pulmonary function, valvular heart disease, chest wall deformities, pigeon chest and scoliosis (43). The phenotype of OI has a broad spectrum from mild skeletal deformity (no fracture) to prenatal lethality. Recently, OI has been classified into 16 types, based on phenotypic expression of the cases and molecular data of the gene causing OI (44). Among different types of OI, types II and III are relatively uncommon but are extremely aggressive and lethal (43, 45-47). Recently identified autosomal recessive forms are mainly due to mutations in leucine-and proline-enriched proteoglycan 1 **(**LEPRI), cartilage-associated protein (CRTAP), bone morphogenetic protein1 (BMP1), serine proteinase inhibitor (SERPINH1), peptidylprolyl isomerase B (PPIB), procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 (PLOD2) and FK506 binding protein 10 (FKBP10) gene of OI. These genes are essential to encode type 1 collagen protein (1, 48, 49).

The literature on mutation in OI is limited in Indian population, yet fewer published (47). The studies have reported mutations in autosomal recessive genes (PPIB, WNT1, FKBP10, CRTAP, SERPINF1) in consanguineous families (50, 51) however autosomal dominant gene (COL1A1 and COL1A2) mutations in non-consanguineous families (47). We planned this study to identify the existing and novel mutations in COL1A1 and COL1A2 genes as well as their interacting partner SERPINH1 gene in OI patients.

Bisphosphonates (BPs), the analog pyrophosphate, are the mainstay of therapy for OI cases (52). It blocks the enzymatic activity of farnesyl-pyrophosphate to inhibit the bone resorption. BPs inhibit the–prenylation of intracellular proteins resulting in an increase in osteoclast apoptosis (53). Few studies have reported that BPs act directly on osteoclasts and osteoclast precursors after the inhibition of the mevalonate pathway (53, 54). There may be indirect effect on osteoblasts that secreates the soluble paracrine factors and influence the osteoclast activity (55, 56).

BPs administered to OI patients increase the bone mass and reduce the fracture risk. However, due to mutation the new bone formed may still contain defective collagen (52). BP- Zoledronic acid (ZOL) have the dual function in which either it decreases osteoclastic activity (57) or enhances osteoblastic activity (58) to improve the bone mass in OI patients. Further, ZOL is effective in reducing the fracture risk via a decrease in bone resorption through osteoclasts but not the brittleness which is due to defective collagen fibres (59, 60). ZOL increases osteoclast apoptosis through mevalonate pathway (6).

Recombinant human parathyroid hormone (rhPTH), the only FDA approved anabolic agent, which is commonly used for metabolic bone disorders (61). The dose and duration of exposure time of rhPTH has dual effects on bone- anabolic as well as catabolic (62). It further regulate transcription factors (Runx2 and Osterix) promotes proliferation, differentiation and osteoblast maturation (63). Also, it regulates the osteoanabolic genes such as bone sialoprotein (BSP), type 1 collagen (COL-1A1), osteocalcin (OCN) and osterix (64, 65).

Henceforth, it would be worthwhile to study the catabolic effect of ZOL on precursor osteoclastic cells isolated from OI patients and to investigate the effect of rhPTH and ZOL on osteoblast cell line.