LIST OF FIGURES

S6-59 ^{O}C, S7-58 ^{O}C, S8-57 ^{O}C). S7 (58°C) showed specific PCR product amplification.

Fig. 16(a): Agarose gel (1.5%) pictures of COL1A1(1-38) Exons.

Fig. 16(b): Agarose gel (1.5%) pictures of COL1A1 (39-52) Exons.

Fig. 17 (a): Agarose gel (1.5%) pictures of COL1A2(1-21) Exons.

Fig. 17 (b): Agarose gel (1.5%) pictures of COL1A2 (22-40) Exons.

Fig 17 (c): Agarose gel (1.5%) pictures of COL1A2 (41-52) Exons.

Fig.18: Representative image of purified PCR products of exon 41-42 of COL1A1 before sequencing.

Fig.19: Mutation shown in exon 34 of COL1A1 gene. Electrophoretogram of COL1A1 gene exon 34 sequencing a) healthy control, b) OI patient confirmed c.2321delC mutation.

Fig. 20: Electrophoretogram showing c.233 T>C p. Leu78Pro mutation in Exon 1 of SERPINH1 gene.

Fig.21: (a) The inter-chain hydrogen bonding pattern among the triple helix of type I collagen A(blue), B(green) and C (Red) chains (b) indicates the position and spatial arrangement of the cysteine residue substituting glycine.

Fig. 22: Clashes observed in the triple helix of COL1A2 due to substitution of Gly by Cys residue: (a-d) steric hindrance observed by the overlapping of the atoms (Red regions indicates the steric hindrance observed due to clashing of cystine36B and proline34C and glycine33C, chains.

Fig. 23: Ramachandran plot of mutation in exon 49. The mutation G1102 C when observed in Ramachandran plot shows that the Cys residue lies within the allowed conformational region.

Fig.24: Bioinformatic analysis of COL1A2 c.3304 C >T (Exon 49) mutation using PolyPhen-2 a) Output from PolyPhen-2 mutation prediction software predicts the mutation to be highly damaging to triple helix structure as shown by the high score for the c.3304 C >T in Exon 49

Fig. 36: Proline Hydrogen bonding pattern in the triple helix. Mutation restricted Φ angle of about −60° and demolish the polar hydrogen. Yellow dots indicate the H bonding pattern, Pro78 represented in sticks with Nitrogen atom (blue) Oxygen atom (red), Carbon atoms(C) and β-sheets indicated by green.

Fig. 37: Confocal images showing distribution of type I collagen in cultured fibroblast of patient with OI-IV. a-b) Cells stained with FITC labelled proα1 (1) antibody. Fibroblasts obtained from OI-IV patient showed retention of Type I collagen in the Golgi bodies. c-d) Golgi was stained e-f) Merged images showing that type I collagen was diminished in the ER and accentuated in the Golgi in OI-IV patients.

Fig. 38: Confocal images showing distribution of HSP47 in cultured fibroblast of patient with OI-IV a-b) Cells stained with HSP47 antibody. Fibroblasts obtained from OI-IV patient showed severe reduction in HSP47 in OI-IV. c-d) ER marker protein disulfide isomerase (PDI) e-f) Merged images showing normal ER distribution of HSP47 in control cells and severe reduction in HSP47 in cells obtained from OI-IV patient.

Fig. 39: Immunocytochemistry of HSP47 protein (a) wild type fibroblast (b) mutant fibroblast. Brown color (in wild type- a) due to chromogen DAB represents the positive expression of HSP47. This figure showed loss of protein due to mutation in HSP47 mutant (b) as compared to the wild type (a).

Fig. 40: Relative expression of HSP47 in wild type vs HSP 47 mutant cells (a) mRNA expression using real time PCR gene expression (b) protein expression western blotting using HSP47 antibody. Lane 1 mutant clone 1, Lane 2 mutant clone 2, Lane 3 wild type, Lane 4 HSP-/-KO13, Lane 5 mutant clone 3.

Fig. 41: Percentage of Caspase-3 of osteoclast cells in each group. The results are expressed as the mean ±standard error of the mean (n=5 for each group. *$P<0.016$ (control vs high dose), $P<0.55$ (control vs low dose) and **$P<0.005$ (Low dose vs high dose).

Fig. 49(a): Relative expression of IHF in response to hPTH (1µM and 5µM)

Fig. 49(b): Relative expression of IHF in response to ZA (1µM and 5µM)

Fig. 49 (c): Amplification curves IHF gene treated with rhPTH(5µg and 10µg) and ZOL (1µM and 5µM).

Fig. 50: Effect of rhPTH and ZOl alone or sequential treatments on U2OS cells to establish the dose. The gene expression levels of ALP and IGF-1 on 48 hours using RT-qPCR analysis normalized to 18S. The effective dose-5µg of rhPTH was determined, The results are expressed as the mean ± standard error of the mean (n=3 for each group) triplicates ALP, Alkaline phosphatase: IGF-1, Insulin-like growth factor 1.

Fig. 51: Effect of rhPTH and ZOl, alone or sequential treatments on the viability of U2OS.Cell viability was analyzed using a MTT assay. U2OS cells were seeded in 96 well plate and cultured at 37C° incubated with DMEM-F12 medium in the presence 5µg of rhPTH, 1µmol of ZOL, alone or sequential treatments for 0,24,48 and 72 hours. The results are expressed as the mean ± standard error of the mean (n=3 for each group) triplicates. No significant change was found in each group. ZOL, Zoledronic acid, rhPTH recombinant human parathyroid hormone, MTT (3-(4, 5- Dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide.

Fig. 52: Effect of rhPTH and ZOl, alone or sequential treatments on the ALP activity of U2OS. U2OS cells were seeded in 96 well plate and cultured at 37C° incubated with DMEM-F12 medium in the presence 5µg of rhPTH ,1µmol of ZOL ,alone or sequential treatments for 48 hours. The results were expressed as the mean ± standard error of the mean (n=3 for each group) triplicates.*$P<0.05$ compared with untreated group. ZOL, Zoledronic acid, rhPTH recombinant human parathyroid hormone.

Fig. 53: Light microscopic images shows Calcium ion form precipitate with alizarin. The results are expressed as the mean ± standard error of the mean (n=3 for each group). The maximum effect of mineralization was achieved with rhPTH followed by ZOL treatment group, as

demonstrated by increased quantification of Alizarin Red-S, No statistical difference was found.

Fig. 54: Effect of rhPTH and ZOl, alone or sequential treatments on the Mineralization of U2OS. U2OS cells were seeded in 96 well plate and cultured at 37C° incubated with DMEM-F12 medium in the presence 5µg of rhPTH, 1µmol of ZOL, alone or sequential treatments for 48 hours. Graph showing quantitative analysis of Alizarin stain.

Fig.55: Effect of rhPTH and ZOl, alone or sequential treatments on U2OS cells. The gene expression levels of a) ALP, b) COL1A1 and c) OCN for 48 hours using RT-qPCR analysis normalized to 18S. The results are expressed as the mean ± standard error of the mean (n=3 for each group). *P<0.021,**P<0.008 ***P<0.001.ALP ,Alkaline phosphatase: OCN, osteocalcin: COL1A1, collagen type 1.

Fig.56: Immunocytochemistry of Osteocalcin protein in response to rhPTH and ZOL in Osteoblast cells. (a) Untreated cells (b) rhPTH treated cells (c) ZOL treated cells (d) rhPTH followed by ZOL treated Cells (e) ZOL followed by rhPTH treated cells.