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Original Article

Correlation between Bone Turnover Markers and Bisphosphonates Treatment in Postmenopausal Osteoporosis

Adnan Khan, Muhammad Hanif, Akhtar Ahmed, Salman Habib and Shahid Kamal
Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN)

Abstract

Objective: To find out the significance of bone turnover markers in management of osteoporotic patients and correlation to bisphosphonate treatments.

Design: Comparative, prospective, managerial

Setting: Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN), Karachi.

Material and Methods: A total of 180 post-menopausal women were selected for the study by taking the verbal consent. Test patients were divided into five groups of 30 patients each on the basis of medicines they receive. Bisphosphonates (alendronates and risedronates) were prescribed to the patients under different brand names as fosamax, drate, osto, dronate and actonel. Serum osteocalcin, alkaline phosphatase and c-telopeptidase were selected as bone turnover markers and were assessed quarterly to find out the correlation between these markers and different treatment groups.

Results: The selected bone turnover markers i.e, alkaline phosphatase, osteocalcin & c-telopeptide were found to drop continuously at different rates in all patients undergoing treatment while in controls these markers remained same with no significant changes throughout the year. The maximum fraction drop was found in telopeptide levels and minimum drop in alkaline phosphatase, while osteocalcin levels were lying in between.

Conclusion: Results showed that ALP is a good marker for indicating drug compliance but is weak for response prediction, at 3 and 6 months. Osteocalcin has slightly less sensitivity but better specificity therefore considered as moderate value marker. However, c-telopeptide showed high sensitivity and specificity as early as 3 months and thus can be considered as an excellent and high value marker for predicting treatment response.

Keywords: Postmenopausal osteoporosis, bone turnover markers, bisphosphonates.

Introduction

Postmenopausal osteoporosis (PO) occurs as a common ailment with a spectrum ranging from asymptomatic bone loss to immobilize hip fracture. PO is reported to affect one third of the women as ageing population increases in many countries around the globe. The disease is generally characterized by reduction in bone mass, impaired bone strength and microscopic architectural changes in bones, resulting in bone fragility and subsequent increase in risk of fractures.

In the United States alone there are an estimated 1.5 million fractures per year because of osteoporosis[1].

Correspondence:
Mr. Adnan Khan
Jr. Scientist, Clinical and Molecular labs, Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN)
adnan.dinar@gmail.com

Furthermore, it was predicted that the fracture occurrence in osteoporotic women will augment by the year 2025, yet very few have received appropriate treatment because of the lack in reliance on diagnostic markers of clinical relevance[2, 3]. The development of bone turnover markers (BTMs) has influenced the assessment and treatment of osteoporosis. A number of BTMs can now be determined clinically, and are generally divided into two categories as markers of bone formation and markers of bone resorption[4]. Bone formation markers reflect different phases of osteoblast in bone formation, for instance alkaline phosphatase, a bone formation marker is reported to be involved in osteoid formation and mineralization. Similarly, osteocalcin is a protein product of osteoblast, odontoblast and hypertrophic chondrocytes and its rate increases with bone formation rates[5]. Conversely, most bone resorption

markers are degradation products of collagen such as c-telopeptide[6]. High levels of bone turnover markers are associated with 2-fold increased risk of osteoporotic fracture as well as suggestive to other metabolic bone diseases. BTMs are significant in management of osteoporosis treatment and thus identification of optimum treatment for an individual is of primary importance, which could be achieved by estimating bone metabolism[5]. Interestingly BTMs predict changes in bone metabolism more rapidly than bone mineral density and therefore BTMs could be used as an indicator of response to osteoporosis treatment[6].

The primary goal of treatment for osteoporosis is to reduce the risk of bone fragility for which clinicians generally recommends bisphosphonates, the most common pharmacological treatment of postmenopausal osteoporosis, which are the analogs of naturally occurring pyrophosphate[7, 8]. It could be deduced from many experimental and clinical studies that biophosphonates increases bone turnover and bone resoprtion which subsequently helps in increased bone mass and mineralization however exact mechanism of action of biophosphonates remains to be resolved[8]. Clodronate and etidronate were evaluated as the first-generation bisphosphonates but had not been approved as a therapeutic agent for osteoporosis. The second generation nitrogen-containing bisphosphonates (alendronate and risedronate) showed an increase bone mineral density in postmenopausal women with osteopenia or osteoporosis in randomized trials[7]. They have been reported to reduce the incidence of hip, vertebral, and nonvertebral fracture by nearly 50 percent particularly during the first year of treatment[8]. A recent study showed that alendronate increased spine and hip bone mineral density slightly more than risedronate, although the clinical significance of this finding is uncertain[9]. There are many studies that reported the use of BTMs as a derived endpoint of treatment value in clinical research and clinical trials of therapies[10], however, the significance of BTMs in management of osteoporotic patients is not clearly defined and is debated in the present study.

Materials & Methods

The study was carried out at Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN) Karachi for a period of one year. All the experiments were conducted as per guidelines under declaration of Helsinki. Patients visiting outpatient department were cautiously segregated by taking into account patients' history of thyroid disorder, osteoporosis in the family and some other factors which may affect the bone turnover markers. A total of 180 numbers of post-menopausal women were selected for the study by taking the verbal consent out of which 150 patients were selected for therapy called as test while remaining 30 patients were selected as controls. Test patients were divided into five groups on the basis of medicines they receive. Each patient received single drug such that 30 numbers of patients were grouped together. Altogether 6 groups of 30 patients were intended wherein 5 groups were on different treatments and one was designated as control.

Drugs & Dosing

Bisphosphonates (alendronates and risedronates) were prescribed to the patients under different brand names as fosamax, drate, osto, dronate and actonel. The dosage was set according to the indications by Peters et al.,[11]. Alendronates i-e fosamax, drate and osto were prescribed at a dose of 70mg weekly while risedronates, dronate and actonel, were given 35 mg weekly. The dosing was prescribed in fasting condition and patients were told to remain at fasting for 45 minutes after taking the medicine. Both alendronate and risedronate are profoundly well tolerated and were found effective in placebo controlled trials for the treatment of osteoporosis. The number of side effects of the bisphoshonate therapy included but not limited to ulceration of oesophagus, infrequent skin rashes, rare osteonecrosis of the jaw, rare cases of auditory hallucinations and visual disturbances etc[11].

Serum Osteocalcin

Osteocalcin ELISA kit supplied by Bioscience diagnostic Denmark was used for determination of osteocalcin in serum of patients. The test was carried out as per methodology described earlier[12]. Two highly specific monoclonal antibodies against human osteocalcin were used as a principle. One antibody is specific for mid region present between 20-29 amino acids while the other recognizes N-terminal region between amino acids 10-16, is a peroxidase conjugated antibody for detection. Samples, controls and standards were added into the streptaviden coated microtitre wells. A mixture of antibodies specific to

mid and N terminal region were added followed by incubation for two hours. After incubation chromogenic substance was added and finally sulphuric acid was put in to stop the reaction. The concentration was measured at 450 nm.

Serum Alkaline Phosphatase (ALP)

Alkaline phosphotase consist of group of enzymes responsible for the catalytic hydrolysis of phosphate esters. Predominantly a change in ALP levels than normal is associated with various bone diseases however the specificity is yet to determine. A DGKC/kinetic method was used for the detection of AP via kit supplied by bioscience. The kinetic determination is done by a reaction in which p-nitrophenyl phosphate was catalyzed by ALP into p-nitrophenol and phosphate. The procedure used as per instructions by the supplier and the reference range was considered between 98-279 U/L.

C-terminal Telopeptide (CTX)

The test was based on the principle described earlier[13]. ELISA Kits supplied by Bioscience Diagnostics were used for quantification of degradation products of C-terminal telopeptides of type 1 collagen in the serum of subjects. The procedure was adopted as per instructions inside the supplied kit. Briefly, Standards, samples and control were added into the apt striptavidin coated microtitre wells. After that a mixture of a biotinylated antibody and a peroxidase conjugated antibody is pipetted in the coated wells. A colored complex was formed as the crosslaps antigens, biotinylated antibody and peroxidase-conjugated antibody combined and attached to the streptavidin surface via the biotinylated antibody. The complex was then incubated for the hours at room temperature followed by washing of wells. A chromogenic substrate was then added further sulfuric acid was added to stop the colour reaction. The absorbance was measured at 450 nm.

Statistical Analysis

Statistical analysis was carried out using SPSS for windows version 12.0. The graphs were plotted in Microsoft Excel.

Results

Three selected bone turnover markers i.e, alkaline phosphatase, osteocalcin & c-telopeptide were found to drop continuously at different rates in all patients undergoing treatment while in the control group or untreated group these markers remained more or less same with no significant changes throughout the year. In five treatment groups ALP showed a significant and steady reduction. Osto treatment group showed a reduction in ALP by –16.18%, -27.64%, -35.32% and –43.82% at 3, 6, 9 and 12 months respectively. Treatment with drate, showed a decrease by –15.16%, -24.56%, -33.15% and –41.54% at 3, 6, 9 and 12 months respectively. Patients receiving actonel exhiubited a fall in ALP levels by –18.17%, -27.49%, -35.90% and –44.69% at 3, 6, 9 and 12 months respectively. Fosamax showed a decrease in ALP by –20.16%, -30.35%, -40.00% and –49.33% at 3, 6, 9 and 12 months respectively. Similarly Dronate treatment showed decrease in ALP levels as the treatment progresses by –14.97%, -23.30%, -31.32% and –36.46% at 3, 6, 9 and 12 months respectively (Table 1 and Figure 1).

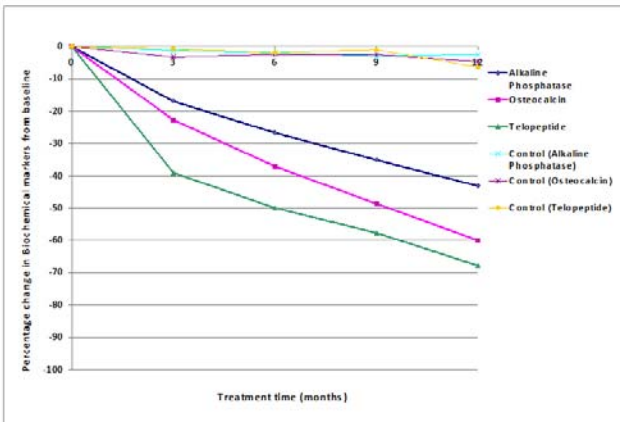

Figure-1: Percentage drop in biochemical markers in therapy and control groups

Altogether, maximum decrease in ALP was observed in Fosamax treated group while osto, drate and actonel showed comparable results. Dronate treatment showed less decrease in ALP as compared to all treatment groups. It was noted that control group exhibited non-significant change at all.

Osteocalcin exhibited a significant reduction in five treatment groups. In patients receiving osto the levels

dropped by –19.94%, -35.71%, -46.27% and –56.86% at 3, 6, 9 and 12 months respectively. Drate treated patients showed a decrease by –16.06%, -30.10%, -43.26% and –54.86% at 3, 6, 9 and 12 months respectively. Actonel treatment exhibited a fall in osteocalcin by –28.75%, -43.67%, -55.65% and –66.51% at 3, 6, 9 and 12 months respectively. Fosamax showed a decrease in osteocalcin by –29.38%, -44.53%, -57.54% and –72.79% at 3, 6, 9 and 12 months respectively. Dronate treatment showed reduction in oesteocalcin levels by –19.27%, -31.42%, -40.46% and –49.07% at 3, 6, 9 and 12 months respectively (Table 1and Figure 1). It can be deduced that the fall was maximum for fosamax. Osto, drate and actonel showed comparable levels of osteocalcin while the least drop was observed in case of Dronate. Control patients exhibit non-significant change in osteocalcin levels at all. In the present study telopeptide showed a significant reduction with all of the treatment groups. For instance in the osto group it showed a decrease by –40.71%, -54.39%, -63.26% and –72.14% at 3, 6, 9 and 12 months respectively.

In Drate treated patients it was dropped by –39.59%, -50.54%, -58.05% and –68.42% at 3, 6, 9 and 12 months respectively. Actonel treatment exhibited a fall by –39.13%, -48.41%, -54.94% and –66.95% at 3, 6, 9 and 12 months respectively. Treatment with fosamax showed a decrease in telopeptide levels by –44.35%, -55.91%, -65.17% and –76.63% at 3, 6, 9 and 12 months respectively. Lastly, dronate treatment showed the fall by –31.62%, -40.40%, -46.80% and –55.0% at 3, 6, 9 and 12 months respectively (Table 1 and Figure 1.). It can be seen that fosamax showed maximum decrease. Results are somewhat comparable in case of osto, drate and actonel, while minimum decrease in osteocalcin was observed in dronate treated group. The control showed non-significant change.

The graphical representations of the observations were presented in Figure. 1 which demonstrate maximum fraction drop in telopeptide levels and minimum drop in alkaline phosphatase while osteocalcin lying in between. It is important to note that telopeptide levels dropped very instantly at 3 months and then moderately in the remaining period of treatment. The behaviors of BTMs in different treatment groups are summarized in Table 1.

Table. 1: Average percentage change in biochemical markers from baseline in treatment & control groups

	Percentage change in Alkaline Phosphatase from baseline after treatment					
Time (months)	Osto	Drate	Dronate	Actonel	Fosamax	Control
3	-16.18	-15.16	-14.97	-18.17	-20.16	-1.22
6	-27.64	-24.65	-23.3	-27.49	-30.35	-1.96
9	-35.32	-33.15	-31.13	-35.90	-40.00	-3.00
12	-43.82	-41.54	-36.46	-44.69	-49.33	-2.46
	Percentage change in Osteocalcin after treatment					
Time (months)	Osto	Drate	Dronate	Actonel	Fosamax	Control
3	-19.94	-16.06	-19.27	-28.75	-29.38	-3.29
6	-35.71	-30.10	-31.42	-43.67	-44.53	-2.45
9	-46.27	-43.26	-40.46	-55.65	-57.54	-2.48
12	-56.86	-54.86	-49.07	-66.51	-72.79	-4.64
	Percentage change in Telopeptide after treatment					
Time (months)	Osto	Drate	Dronate	Actonel	Fosamax	Control
3	-40.71	-39.59	-31.62	-39.13	-44.35	-0.74
6	-54.39	-50.54	-40.4	-48.41	-55.91	-1.88
9	-63.26	-58.05	-46.8	-54.94	-65.17	-0.99
12	-72.14	-68.42	-55.00	-66.95	-76.63	-6.29

Discussion

In postmenopausal osteoporosis the dose dependent bone turnover generally decreases with a range of bisphosohonates treatment[14]. It was reported that bisphosphonates treatment induces significant decrease in bone resorption markers after one month while the decrease in bone formation markers is reported to be delayed, reaching at the peak post 6-12 months of therapy[15]. The decrease in bone markers under antiresorptive therapy is strongly related to the increase in bone mass density (BMD)[16]. Alendronate belongs to the group of bisphohonates, reported to be used for the treatment of PO, showed a strong association between the changes in BTMs and the increase in BMD. There is plethora of evidence suggesting that BTMs can be used to scrutinize treatment effectiveness within the first six months

from the start of treatment when BMD alteration are still too small to be used clinically[15]. Present study addresses this critical issue in a precise manner.

Present study showed a sound drop in alkaline phosphatase (ALP) by –16.93%, -26.69%, -35.10% and –43.17% at 3, 6, 9 and 12 months respectively in the treatment group. In the control group there was little and non-significant change at all, i.e., -2.46%, during one year of the treatment. ALP is one of the BTMs, used as a marker of bone metabolism for examining patients during treatment. For example in the management of patients with metabolic bone disease such as Paget's disease, osteoporosis, hyperparathyroidism and metastatic bone disease ALP might help to guide treatment choices for antiresorptive therapies and is also been used to confirm patient compliance in taking medications[17]. ALP levels can also determine an earlier signal of response to treatment efficacy than bone density measurements[18]. However there can be a few limitations in using ALP as a marker of bone turnover and these needs to be bear in mind. Many bone diseases influence a demineralization process and therefore many treatments are aimed at inhibiting bone resorption. Measurement of a bone resorption marker is thus more pertinent to the assessment of augmented bone loss with ailment. Moreover, the earliest changes in bone markers with antiresorptive therapy were seen with biochemical markers of bone resorption. It can be seen that changes in ALP may thus lag by a few weeks[19].

In our study a significant drop can be observed in osteocalcin levels by -22.68%, -37.09%, -48.63% and -60.02% at 3, 6, 9 and 12 months respectively in the treatment group. In control group there was little and non-significant change at all, i.e., -4.64%, in one year of treatment with bisphoshonates. A number of reasons make serum osteocalcin as a marker of bone formation. First and foremost is that serum osteocalcin is correlated with bone formation which was shown via histomorphometry or calcium kinetics. Secondly the synthesis of osteocalcin usually increases with mineralization and with progressive osteoblastic differentiation. Third reason could be deduced from early animal studies which reported that circulating osteocalcin ameliorated from new bone formation and not from breakdown of bone matrix[20]. However, few advantages in using osteocalcin as a clinical index of bone turnover are its tissue specificity, its broad availability, and relatively small person to person difference[21]. In general, serum levels of osteocalcin are elevated in patients with disease characterized by high bone turnover rate, and reflect the expected changes in bone formation following surgical or therapeutic intervention.

Present study reported that the degradation products of type I collagen has been the most promising BTM among three selected biomarkers. At present, C-telopeptide is divided into two categories depending on the choice of methods. These are CTX and ICTP, both immunoassays. The difference is due to recognition of different domains of the C-terminal telopeptide region of the α1 chain of type I collagen[22]. Although there are few contrary reports in the literature, preliminary data from prospective reports indicate that certain bone markers, such as CTX, predict the risk of fracture as well as bone mineral density[23]. It is quite possible that contradictory reports may have arose from studies that do not state well-defined sampling procedures, such as fasting status, time of collection, sample processing and storage conditions. Furthermore, the analytical methods have been improved with time and the more specific and precise automated methods have become available[24]. Therefore to discover the clinical value of the markers in treatments which may not produce as large a change as seen, it is essential to reduce the least significant change of the marker. With added improvements of the assays a more accurate clinical picture will emerge. In the current study BTM telopeptide showed a significant and steady drop by -39.08 %, -49.93 %, -57.65 % and -67.83 % at 3, 6, 9 and 12 months respectively in the treatment group. In the control group there was a non-significant change i.e., -6.29 % during one year of treatment.

In a nut shell BTMs ALP, Osteocalcin and C-Telopeptide (CTX) were studied on a quarterly basis and it was revealed that ALP has a high sensitivity but a low specificity especially at 3 and 6 months. This showed that the ALP is a good for indicating drug compliance but is weak for response prediction, at 3 and 6 months. On the other hand osteocalcin has

slightly less sensitivity but better specificity. Therefore the osteocalcin might be considered as moderate value marker which predicts the patients' response to drugs.It is to be noted that the BTM telopeptide showed high sensitivity and specificity as early as 3 months and thus can be considered as an excellent and high value marker for predicting treatment response.

The BTMs can be used for convincing prediction of response in bone mass during antiresorptive therapy as early as 3 months. It was established in our study that the marker telopeptide most specific and sensitive among the three selected BTMs for prediction of individual response to treatment. BTM telopeptide exhibited a pronounced response to therapy with a low minimum significant change and values were showed as change from baseline at month 3 or 6 for optimal individual prediction of BMD response to treatment.

Conflict of interest

Authors declare no competing interest

References

1. G. Russell, G. Mueller, C. Shipman, P. Croucher Clinical disorders of bone resorption Novartis Found Symp, 232: 2001; pp. 251–267 (discussion 267–71)
2. Liberman UA, Weiss SR, Broll J. et al. Alendronate Phase III Osteoporosis Treatment Study Group. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 1995; 333: 1437-1443
3. Black DM, Cummings SR, Karpf DB. et al. Fracture Intervention Trial Research Group. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 1996; 348: 1535-1541
4. Lee J, Vasikaran S. Current Recommendations for Laboratory Testing and Use of Bone Turnover Markers in Management of Osteoporosis. Ann Lab Med 2012; 32(2): 105-112.
5. Ebeling PR, Atley LM, Guthrie JR, Burger HG, Dennerstein L, Hopper JL, Wark JD. Bone turnover markers and bone density across the menopausal transition. J ClinEndocrinMetabol 2011; 81:9.
6. Seibel MJ. Biochemical Markers of Bone Turnover Part I: Biochemistry and Variability 2005; 26(4): 97-122
7. Cummings SR, Black DM, Thompson DE. et al. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. JAMA. 1998;280:2077-2082
8. Papapoulos SE, Quandt SA, Liberman UA, Hochberg MC, Thompson DE. Meta-analysis of the efficacy of alendronate for the prevention of hip fractures in postmenopausal women. Osteoporos Int. 2005;16:468-474
9. Gabriel SE, Tosteson AN, Leibson CL, et al. Direct medical costs attributable to osteoporotic fractures. OsteoporosInt 2002; 13:323-30.
10. Rosen CJ, Hochberg MC, Bonnick SL, et al. Treatment with once-weekly alendronate 70 mg compared with once-weekly risedronate 35 mg in women with postmenopausal osteoporosis: a randomized double-blind study. J Bone Miner Res 2005;20:141-51Bahl S, Coates PS, Greenspan SL. The management of osteoporosis following hip fracture: have we improved our care? OsteoporosInt 2003;14:884-8.
11. Peters ML, Leonard M, Licata AA. Role of alendronate and risedronate in preventing and treating osteoporosis. Cleve Clin J Med 2001; 68(11): 945-951.
12. Rosenquist C, Qvist P, Bjarnason N, Christiansen C. Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. ClinChem 1995; 41(10): 1439-1445.
13. Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, Rosenquist C, Qvist P. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone. 2001;29:209–15.
14. KraenzlinEM, Seibel MJ. Measurement of biochemical markers of bone resorption. In: Dynamics of Bone and Cartilage Metabolism, Seibel MJ, Robins SP, Bilezikian JP eds. Academic Press, San Diego; 1999, 11–426.
15. Iwamoto J, Takeda T, Sato Y. Efficacy and safety of alendronate and risedronate for postmenopausal osteoporosis. Curr Med Res Opin 2006; 22(5): 919-928.
16. Iwamoto J, Takeda T, Sato Y.Effects of antifracture drugs in postmenopausal, male and glucocorticoid-induced osteoporosis--usefulness of alendronate and risedronate. Expert OpinPharmacother 2007;8(16):2743-2756.
17. Rosalki SB, Foo AY. Lectin affinity electrophoresis of alkaline phosphatase for the differentiation of bone hepatobiliary disease. Electrophoresis 1987;10:604–611.
18. Woitge H, Seibel MJ, Ziegler R. Comparison of total and bone-specific alkaline phosphatase in patients with nonskeletal disorders or metabolic bone disease. Clin Chem. 1996;42:1796–804.
19. Van Straalen JP, Sanders E, Prummel MF, Sanders GTB. Bone alkaline phosphatase as indicator of bone formation. ClinChimActa 1991;201:27–34.
20. Taylor AK, LinkhartSG, Mohan S, Baylink DJ. Development of a new radioimmunoassay for human osteocalcin: evidence for a midmolecule epitope. Metabolism. 1988;37:872–877.
21. Chen JT, Hosoda K, Hasumi K, Ogata E, Shiraki M. Serum N-terminal osteocalcin is a good indicator for

estimating responders to hormone replacement therapy in postmenopausal women. J Bone Miner Res1996;11:1784–1792

22. EriksenEF, Charles P, Meisen F, et al. Serum markers of type 1 collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. J Bone Miner Res 1993;8:127–132.
23. Charles P, Mosekilde L, Risteli L, et al. Assessment of bone remodeling using biochemical indicators of type I collagen synthesis and degradation: relation to calcium kinetics. Bone Miner 1994;24:81–94.
24. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay of the carboxyterminalpropeptide of human type I procollagen. ClinChem 1990;36:1328–1332.

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CONTRIBUTION OF AUTHORS	
Author	**CONTRIBUTION**
Adnan Khan	B – C
Muhammad Hanif	A – B
Akhtar Ahmed	A
Salman Habib	C
Shahid Kamal	A – C

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception, Synthesis and Planning of the research
B. Active Participation in active methodology
C. Interpretation, analysis and discussion