Original Research Article

Bone metabolism assessment in hemodialysis patients by using Carboxy-terminal cross-linked telopeptide of type I collagen

ABSTRACT

Aims: To evaluate the carboxy terminal telopeptide of type I collagen (CTX I) as a serum bone metabolism marker in haemodialysis patients
Study design: Cross-sectional observational.
Place and Duration of Study: Hemodialysis unit of Tanta University Hospital, between October 2018 and March 2020.
Methodology:80 male patients aged from (18-65y) on regular haemodialysis were included. All patients were subjected to: history taking, full clinical examination, laboratory investigations including: Serum calcium, phosphorus,Serum albumin, Alkaline phosphatase, Intact para thyroid hormone, Serum carboxy terminal cross linking telopeptide and dual energy X ray absorbtiometry scan of the lower third of the right fibula to evaluate bone mineral denesity (BMD) and patient were divided according to bone mineral density T score into two groups (normal – osteopenic)
Results: There is significant difference between normal &oesteopenic groups according to PTH (p value 0.001*) and alkaline phosphatase(ALP) (p value 0.001*) and (CTX I) (p value 0.001*), but there is non-significant difference between normal &oesteopenic groups according to calcium (p value 0.239), phosphorus (p value 0.672), albumin (p value 0.749) and corrected calcium (p value 0.314).There was negative significant correlation between CTXI and DEXA scan, and between DEXA scan and PTH & ALP. There was positive significant correlation between CTX I and PTH, ALP & albumin and there was positive significant correlation between PTH & ALP. At cutoff 2.0 ng/ml CTX I can significantly diagnose osteopenia in hemodialysis patients with 93% sensitivity, 95% specificity and accuracy of 92%. It had PPV of 95% & NPP of 83%, in multiple regression analysis the increased in PTH and CTX I was the significant predictor of osteopenia.

Conclusion: This study showed high association between CTX I and other established markers of bone metabolism and BMD by DEXA demonstrating the potential utility of CTX I as marker of bone resorption in renal bone disease

Keywords: Hemodialysis, bone metabolism,DEXA scan, carboxy terminal telopeptide of type I collagen (CTX I)

1. INTRODUCTION

Chronic kidney disease (CKD) is currently a public health problem. More than 60 million worldwide people lose their lives annually due to the risk of kidney failure [(1)]

Metabolic bone disease is a common complication of CKD and is part of abroad spectrum disorders of mineral metabolism that occur in this clinical setting during dialysis [(2)].

In the course of chronic renal failure most of the metabolic bone diseases are characterized by alteration in the bone resorption / formation balance as secondary hyperparathyroidism, osteoporosis, mixed bone diseases, osteomalacia, a dynamic osteopathy, and extra skeletal calcifications [3].

There are biochemical markers currently available for the assessment of bone turnover include enzymes and non-enzymatic peptides derived from the cellular and non-cellular compartments of bone metabolism, these markers which are formed during the bone resorption phase of bone remodeling include products of osteoclasts activity released during bone resorption [4].

The present study evaluate the carboxy terminal telopeptide of type I collagen (CTX I) as a serum bone metabolism marker in HD patient [5].

2. METHODOLOGY

The study carried on 80 male patients of chronic kidney disease on regular hemodialysis between October 2018 and March 2020. Awritten informed consent was obtained from all participants before inclusion in the study, explaining the value of the study, plus the procedures that was commenced.Approval from Ethical Committee of Tanta Faculty of Medicine was obtained before starting the study.Confidentiality and personal privacy was respected in all levels of the study.Participants are free to withdraw from the study at any time without any consequences.Collected data were not and will not be used for any purpose.

2.1.Inclusion criteria:

Adult male patients who are on regular hemodialysis due to chronic kidney disease.

2.2.Exclusion criteria:

1. Patients suffer from chronic inflammatory disease.
2. Patients with past history of renal transplantation.
3. Patients with past history of parathyrodictomy.
4. Patients with past history of fracture.
5. Patients with acute illness.
6. Female patients due to hormonal effect on bone metabolism.
7. Medication that affect calcium metabolism.

All patients were subjected to the following:

1. Through history taking.
2. Complete clinical examination.
3. Laboratory investigations including:

A- Routine laboratory investigation: Serum calcium, phosphorus,Serum albumin, Alkaline phosphatase (ALP), Intact para thyroid hormone (iPTH) ,

B- Specific laboratory investigation: Serum carboxy terminal cross linking telopeptide.

dual energy X ray absorbtiometry scan (DEXA) of the lower third of the right fibula to evaluate bone mineral denesity (BMD) and patient were divided according to bone mineral density

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4.Imaging:

Abdominal and pelvic ultrasonography, DEXA: Dual-energy X-ray absorptiometry scan.Bone mineral density (BMD) of the distal third of right fibula was assessed by dual energy x ray absorptiometry (DEXA). BMD T and Z scores were classified according to World Health Organization criteria and T-score <−1, which was below the expected range for age, was indicative of the diagnosis of ostropenia. In the T-score scale, 0 represents normal, healthy bone density of T-scores above 0 and slightly below 0 are within the normal range. T-score of -2.3 shows lower bone density than a score of -1.8. The T-score is a radiographic diagnosis, meaning it is an X-ray diagnosis and doesn't imply anything about the cause of osteoporosis. T-scores mean different things on the different DEXA scans.

- A T-score of -1 to 0 and above is considered normal bone density.
- A T-score between -1 and -2.5 is diagnosed as osteopenia.

A score of -2.5 or below is diagnosed as osteoporosis

2.3.Statistical Analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 22.0 Quantitative data were expressed as mean± standard deviation (SD). Mean value, Standard student "t test", The Mann-Whitney U test, Linear Correlation Coefficient [r] (z test), ROC-curve, The subject of multivariate analysis deals with the statistical analysis of the data collected on more than one (response) variable.

3. RESULTS AND DISCUSSION

Table (1) showed that: There is significant difference between Normal &Oesteopenic groups according to PTH with p.value (0.001) and ALP with p.value (0.001).

No significant difference between two groups as regard Ca with p.value (0.239), PO4 with p.value (0.672), Albumin with p.value (0.749) and Corrected Ca with p.value (0.314).

Table 1: Comparison between Normal &Oesteopenic groups according to Calcium, phosphorus, parathyroid hormone, albumin, alkaline phosphatase, Corrected Calcium

		Range	Mean ± S. D	t. test	p. value
Ca	Group A	7.5 – 10	8.40 ± 0.75	1.407	0.239
	Group B	6.8 – 10	8.61 ± 0.73		
PO4	Group A	3.3 – 6.9	5.08 ± 1.14	0.180	0.672
	Group B	2.5 – 7.7	5.21 ± 1.21		
PTH	Group A	170 – 612	309.48 ± 126.16	16.513	0.001*
	Group B	45 – 2500	643.68 ± 385.30		
ALP	Group A	214 – 720	376.26 ± 137.45	14.476	0.001*

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	Group B	350 – 2900	769.77 ± 486.96		
Albumin	Group A	2.2 – 3.4	2.86 ± 0.32	0.103	0.749
	Group B	2.1 – 3.9	2.89 ± 0.46		
Corrected Ca	Group A	8.14 – 10.5	9.30 ± 0.82	1.027	0.314
	Group B	7.08 – 10.96	9.50 ± 0.77		

Ca =calcium. PO4= phosphorus. PTH= parathyroid hormone. ALP= alkaline phosphatase.

Table (2) showed that: There is difference between Normal (group A) &Oesteopenic (group B) groups according to BMD.

Table 2:Comparison between Normal &Oesteopenic groups according to BMD by using T score obtained by DEXA scan.

		Range	Mean ± S. D	t. test	p. value
BMD	Group A	-1 – 0.90	0.72 ± 0.40	30.949	0.001
	Group B	-2.40 – -1.20	-2.04 ± 0.34		

Table (3) showed that: There is difference between Normal &Oesteopenic groups according to CTX I with mean± S. D (1.85±0.19) and median (1.8) for normal group and mean± S. D (42.04±42.71) and median (25.9) for oesteopenic groups with P. value (0.001).

Table 3: Comparison between Normal &Oesteopenic groups according to CTX I

		group A	group B	U. test	p. value
CTX I	Range	1.6 – 2.50	1.86 – 174	6.798	0.001*
	Mean ± S. D	1.85 ± 0.19	42.04 ± 42.71		
	Median	1.8	25.9		

Table (4) show that :

Negative significant correlation between CTX I and DEXA scan with r (-520).p (0.001).

- Negative significant correlation between DEXA scan and PTH with r (-0.444) p(0.001) &Alp with r (-0.417) p(0.001).
- Positive significant correlation between CTX I and PTH with r (0590) p (0.001), ALP with r (0575) and p(0.001) & Albumin with r (0339) and p(0.002).
- Positive significant correlation between PTH & ALP with r (0.930) and p (0.001).

Table 4: Correlation between CTX I, DEXA scan & parathyroid hormone and other items

	CTX I	DEXA scan	PTH

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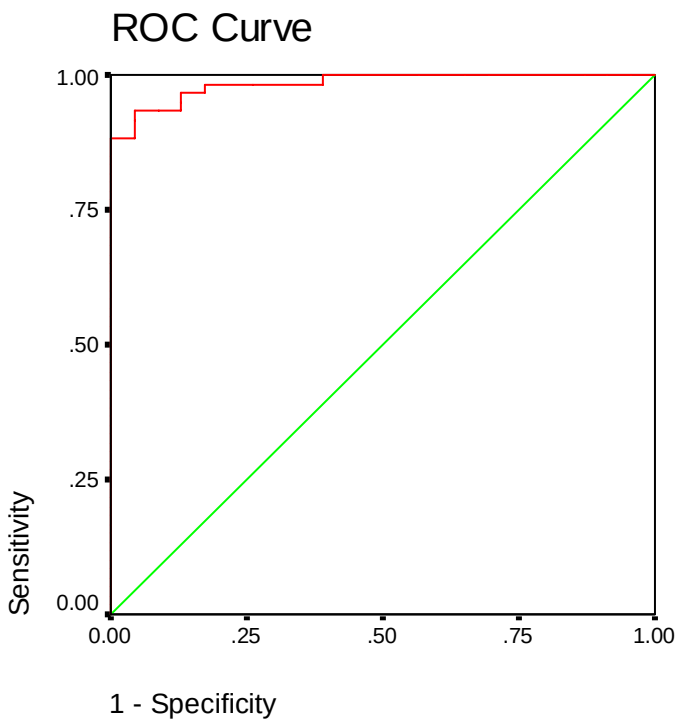
	r	p	r	p	r	p
DEXA scan	-0520	0.001*			-0.444	0.001*
Age	0.020	0.854	-0.054	0.627	-0.163	0.141
Ca	0.096	0.390	-0.157	0.157	0.162	0.143
PO4	0.105	0.343	-0.014	0.901	0.180	0.104
PTH	0590	0.001*	-0.444	0.001*		
Alp	0575	0.001*	-0.417	0.001*	0.930	0.001*
Albumin	0339	0.002*	-0.101	0.362	0.148	0.183
Corrected Ca	-0.058	0.599	-0.108	0.332	0.077	0.487

Ca = calcium. PO4= phosphorus. PTH = parathyroid hormone ALP = alkaline phosphatase.

Figure (1): ROC Curve to CTX I for prediction of osteopenia



PPV: positive predictive value, NPV: negative predictive value, AUC area under curve

Discussion:

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In current study, there is no significant difference between normal &oesteopenic groups according to age. This may be due to the limited number of young adults (i.e., those with peak bone mass) in our study population compared with the studies from the general population.

In harmony with our study ***Barreto et al.*** and ***Park et al***. found that age was not significantly different in relation to BMD changes [6,7].

However, ***Slouma et al.,*** found that patients with osteoporosis were older than patients without osteoporosis [8].

Also, in disagreement with current study, ***Avramovski and Sikole,*** detected a high prevalence of osteoporosis in a relatively young hemodialysis patient population. Bone loss likely begins much earlier and progresses more rapidly in hemodialysis patients [9].

In our study, there was no significant difference between osteopenic and non-osteopenic groups regarding calcium, phosphorus and albumin levels.

Similarly, ***Malluche et al.,*** found no significant relation between baseline BMD and serum calcium or phosphorus [10]

Also, another study done by ***Lai et al.,*** showed that there was no relationship between serum albumin, alkaline phosphatase, phosphate levels and femoral neck BMD in dialysis patients and albumin was not a useful predictor of BMD[11].

Another similar result was reported by studies found that hypoalbuminemia independently associated with bone loss as serum albumin is a marker of systemic inflammatory status among dialysis patients [11, 12].

In disagreement with our study, ***Huang et al.,*** found a positive correlation between serum albumin levels and femoral neck BMD in dialysis patients[13].

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Moreover, ***Polymeris et al.,*** investigated the BMD and bone metabolism in HD patient and found serum phosphorus levels was high in cases with lower BMD [14].

In current study, PTH was significantly higher in oesteopenic groups than normal cases (643.68±385.30 versus 309.48±126.16 respectively, p=0.001).

These results were in harmony with***Slouma et al.,***study which included total of 90 patients and reported that PTH levels were significantly increased in patients with osteoporosis[8].

Also, ***Taal et al.***, showed that the BMD had a negative correlation with PTH[15].

Similarly, BMD was significantly and negatively correlated with serum intact PTH as reported by ***Okuno et al.,***[16].

This results was supported by ***Brunerová et al.,***study revealed that, serum markers of bone resorption and formation were high in the majority of patients with low BMD and in almost 70% of them secondary hyperparathyroidism was present[17].

In contrast to these results, ***Ueda et al.,*** compared between HD patients with and without reduction in radius BMD in serum PTH and bone metabolic markers, they found serum PTH was not significantly different between the two groups (p = 0.4603) [18].

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In current study, ALP was significantly higher in oesteopenic groups than normal cases (769.77±486.96 versus 376.26±137.45 respectively, p=0.001).

This was in accordance with a study done by ***Ueda et al.,*** which included 137 HD patients, and reported that, serum bone ALP was significantly higher in those with BMD reduction than in those without. [(18)].

Moreover, according to study by ***Malluche et al.,*** baseline BMD correlated with PTH and bone-specific ALP [(8)].

In line with our study, ***Ureña and de Vernejoul,***reported that, uremic patients usually exhibit high plasma intact PTH and high serum concentration of biochemical markers of bone metabolism such as ALP [(19)].

In harmony with current work, ***Nybo et al.,*** reported that, BMD correlated with levels of PTH and ALP [(20)].

Also, ***Park et al.,*** found that serum ALP was negatively correlated with bone mineral density assessed by dual-energy X-ray absorptiometry in HD patients [(7)].

In a single-center cohort study, ***Iimori et al.,*** investigated 485 HD patients, and their results also showed that higher values of serum ALP are a predictor of fragility in HD patients [(21)].

However, ***Barreto et al.,*** study data revealed no correlation between BMD and ALP in HD patients [(6)].

In current study, there was Positive significant correlation between PTH & ALP.

In agreement with the present study, ***Barreto et al.,*** found a positive correlation was found between PTH and ALP levels (r=.0408, P=.001) [(6)].

Similarly, ***Polymeris et al.,*** found that, serum PTH correlated significantly in a positive manner with serum ALP (R = 0.690; p <0.001) [(14)].

In current study, there is negative significant correlation between CTX I and DEXA scan, positive significant correlation between CTX I and PTH, Alp & albumin.

In agreement with our study, ***Reichel et al.,*** found that, serum CTX I was significantly correlated with various PTH assays (r >0.56) and with alkaline phosphatase (r = 0.404)[(21)].

Also, ***Pagani et al.,*** found the positive correlation of serum CTX I with serum intact PTH clearly indicates that the increase in CTX I also results from enhanced bone turnover due to secondary hyperparathyroidism [(22)].

Moreover, ***Maeno et al.,*** found that serum NTX I and CTX I, but not other bone markers, correlated significantly with the rate of forearm bone loss during a subsequent 2-year period in HD patient [(23)].

Also, ***Okuno et al.,*** found that, serum CTX I showed significant and negative correlation with BMD change, Serum CTX I was also found to have significant and positive correlations with PTH (r=0.60, P< 0.01) and alkaline phosphatase (ALP) (r=0.66, P< 0.01)[(16)].

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Moreover, ***Herrmann and Seibel,*** have also demonstrated that CTX I concentrations are raised in patients with CKD-5D and correlate well with BMD measurements [(24)].

However, in contrast to our results, another study by ***Ueda et al.,***showed that serum CTX I levels were not different between patients with and without loss of BMD at the distal radius[(18)].

Another study done by ***Slouma et al***., disagreed with our results as it found that there was no significant difference between patients with osteoporosis, those with osteopenia, and those with normal T-scores regarding bone turnover markers including serum CTX I levels[(9)].

In current study, multiple regression analysis for predictor of osteopenia the parameters shows that, the increase in PTH and CTX I was the significant predictor of oesteopenia in hemodialysis patients. Otherwise, age, Ca, PO4, ALP, albumin were not significantly predicted osteopenia in hemodialysis patients. CTX I is significantly higher in oesteopenic groups than normal cases (42.04±42.71versus 1.85±0.19 respectively, p=0.001).

We reported that, at a cutoff 2.0 and AUC of 0.984, CTX I significantly diagnosed oesteopenia in hemodialysis patients with 93% sensitivity, 95% specificity, and accuracy of 92%. It had PPV of 95% & NPP of 83%.

These results were supported by many studies reported that, high sensitivity and specificity as a bone resorption marker is provided by a newly developed assay for degradation fragments of the C-terminal telopeptide of type I collagen that contain the b-isomerized octapeptide (CTX I) [(26, 27)].

Moreover, in study by ***Maeno et al.,*** The sensitivity of the highest quartiles as cutoff points for identification of those HD patients who had lost bone mass in the distal third of the radius was 45% for CTX I, the specificity was 82%, the positive predictive values was 46% for CTX I, and the negative predictive values was 81% for CTX I Although its value was lower than our study [(23)].

Okuno et al., shows the sensitivity of the highest quartile as a cut-off point for identifying those hemodialysis patients who had lost bone mass was 41% for CTX I, The specificity was high at 83% for CTX I, The positive predictive value of serum bone marker values was 55% for CTX I &the negative predictive value of serum marker values was 73% for CTX I [(16)].

4. CONCLUSION:

We found a significantly increase CTX I in osteopenic group and high association between CTX I and other established markers of bone metabolism demonstrating the potential utility of CTX I as marker of bone resorption in hemodialysis patients

CONSENT (WHEREEVER APPLICABLE)

Any unexpected risks appeared during the course of the research will be cleared to the participants, their parents and the ethical committee on time. There are adequate measures to maintain the privacy of participants and confidenality of the data: A code number to every patient with the name and address will be kept in a special file. The patient name will be

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hidden when using the research. The results of the study will be used only in a specific manner and not to use in any otheraims. Informed consent will be obtained from patients 18 years old or older. "All authors declare that „written informed consent was obtained from the patient (or other approved parties) for publication of this research and accompanying images".

ETHICAL APPROVAL

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

REFERENCES

1. **Mohammed FA, Farhood HF and Abdul-Atheem M (2015):** Prediction of malnutrition using Modified Subjective Global Assessment-dialysis Malnutrition Score in patients on chronic Haemodialysis. Candian Open Medical Sciences and Medicine Journal; 1(1): 1-20.
2. **Martin KJ and González EA (2006):** Metabolic bone disease in chronic kidney disease. Division of Nephrology, Saint.; 18(3):875-885.

3.Levey AS and Coresh J **(2012):** Louis University Medical Center. A position statement from Kidney Disease: Improving Global Outcomes. Kidney Int.; 69:165-18.

4.Garnero P, Bauer DC, Mareau E, et al. (2008): Effects of PTH and alendronate on type I collagen isomerization in postmenopausal women with osteoporosis: the PaTH study. J Bone Miner Res.; 23(9):1442-8.

5.Okuno S, Inaba M, Kitatani K, et al. (2005): Osteoporosis International. Serum levels of C-terminal telopeptide of type I collagen: a useful new marker of cortical bone loss in hemodialysis patients. Osteoporos Int.; 16(5):501-9.

6.Barreto FC, Barreto DV, Moyses RMA, et al. (2006): Osteoporosis in hemodialysis patients revisited by bone histomorphometry: A new insight into an old problem. Kidney International; 69(10):1852-7.

7.Park JC, Kovesdy CP, Duong U, et al. (2010): Association of serum alkaline phosphatase and bone mineral density in maintenance hemodialysis patients. Hemodialysis International; 14(2):182-92.

8.Slouma M, Sahli H, Bahlous A, et al. (2020): Mineral bone disorder and osteoporosis in hemodialysis patients. Advances in Rheumatology; 60(1):15.

9.**Avramovski P, Avramovska M, Sotiroski K, et al. (2019):** Acute-phase proteins as promoters of abdominal aortic calcification in chronic dialysis patients. Saudi J Kidney Dis Transpl.; 30(2):376-386.

10.Malluche HH, Davenport DL, Cantor T, et al. (2014): Bone mineral density and serum biochemical predictors of bone loss in patients with CKD on dialysis. Clinical Journal of the American Society of Nephrology; 9(7):1254-62.

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11. **Lai MN, Hsu IS, Chen YY, et al. (2002):** Osteoporosis and associated risk factors for chronic hemodialysis patients. ActaNephrologica; 16(1):25-30.

12. Afshinnia F, Wong KK, Sundaram B, et al. (2016): Pennathur S. Hypoalbuminemia and osteoporosis: reappraisal of a controversy. The Journal of Clinical Endocrinology; 101(1): 167-75.

13. Huang GS, Chu TS, Lou MF, et al. (2009): Factors associated with low bone mass in the hemodialysis patients–a cross-sectional correlation study. BMC Musculoskeletal Disorders; 10(1):60.

14.Polymeris A, Doumouchtsis K, Grapsa E (2012): Bone mineral density and bone metabolism in hemodialysis patients. Correlation with PTH, 25OHD3 and leptin.Nefrología (English Edition): 32(1):73-8.

15.Taal MW, Masud T, Green D, et al. (2000): Risk factors for reduced bone density in haemodialysis patients. Nephrology Dialysis Transplantation; 14(8):1922-8.

16.Okuno S, Inaba M, Kitatani K, et al. (2005): Serum levels of C-terminal telopeptide of type I collagen: a useful new marker of cortical bone loss in hemodialysis patients. Osteoporosis International; 16(5):501-9.

17.Brunerová L, Ronová P, Verešová J, et al. (2016): Osteoporosis and Impaired Trabecular Bone Score in Hemodialysis Patients. Kidney and Blood Pressure Research; 41(3):345-54.

18.Ueda M, Inaba M, Okuno S, et al. (2005): Serum BAP as the clinically useful marker for predicting BMD reduction in diabetic hemodialysis patients with low PTH. Life Sciences; 77(10):1130-1139.

19.Ureña P, de Vernejoul MC (1999): Circulating biochemical markers of bone remodeling in uremic patients. Kidney International; 55(6):2141-56.

20.Nybo M, Jespersen B, Aarup M, et al. (2013): Determinants of bone mineral density in patients on haemodialysis or peritoneal dialysis--a cross-sectional, longitudinal study. BiochemiaMedica; 23(3):342-50.

21.Reichel H, Roth HJ, Schmidt-Gayk H (2004): Evaluation of Serum β-Carboxy-Terminal Cross-Linking Telopeptide of Type I Collagen as Marker of Bone Resorption in Chronic Hemodialysis Patients. Nephron Clinical Practice; 98:c112-8.

22.Pagani F, Bonetti G, Stefini F, et al. (2000): Evaluation of a fully automated assay to measure C-telopeptide of type I collagen in serum. Clinical Chemistry and Laboratory Medicine (CCLM); 38(11):1111-3.

23.Maeno Y, Inaba M, Okuno S, et al. (2005): Serum Concentrations of Cross-Linked N-Telopeptides of Type I Collagen: New Marker for Bone Resorption in Hemodialysis Patients. Clinical Chemistry; 51(12):2312-7.

24.Herrmann M, Seibel M (2008): The amino-and carboxyterminal cross-linked telopeptides of collagen type I, NTX-I and CTX-I: a comparative review. ClinicaChimicaActa; 393(2):57-75.

352 **26.Fledelius C, Johnsen AH, Cloos PA, et al. (1999):** Characterization of Urinary Degradation
353 Products Derived from Type I Collagen Identification of A β-isomerized ASP-GLY sequence
354 within the C-terminal telopeptide (α1) region. Journal of Biological Chemistry; 272(15):9755-63.

355 **27.Bonde M, Qvist P, Fledelius C, et al. (1995):** Applications of an enzyme immunoassay for a
356 new marker of bone resorption (CrossLaps): follow-up on hormone replacement therapy and
357 osteoporosis risk assessment. The Journal of Clinical Endocrinology & Metabolism; 80(3):864-8.

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