Pergamon

Clinical Biochemistry, Vol. 28, No. 5, 541–545, 1995

0009-9120/95 $9.50 + .00

0009-9120(95)00043-7

Serum Levels of the Pyridinoline Crosslinked Carboxyterminal Telopeptide of Type I Collagen (ICTP) and Osteocalcin in Rachitic Children in Nigeria

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Objectives: We measured the levels of the pyridinoline crosslinked carboxyterminal telopeptide of type I collagen (ICTP) and osteocalcin (OC) in the serum of 12 rachitic and 27 healthy Nigerian children, and compared the performance of these relatively new markers of bone metabolism with established laboratory parameters of skeletal disease.
Design and Methods: Active rickets was diagnosed on the basis of clinical and biochemical criteria. Serum calcium and phosphorus concentration and alkaline phosphatase activity were determined using clinically accepted methods. Radioimmunoassay was performed to quantify parathyroid hormone, 1-,25-dihydroxyvitamin D, OC, and ICTP.
Results: The rachitic children had statistically significant serum elevations of ICTP and osteocalcin as compared with age- and sex-matched controls. Serum levels of ICTP correlated with alkaline phosphate activity.
Conclusions: As a marker of abnormal bone metabolism, ICTP performs at least as well as alkaline phosphate. ICTP and OC are valuable additions to the growing repertoire of bone markers.

KEY WORDS: rickets; bone resorption; collagen metabolism; biological markers; osteocalcin; metabolic bone disease; nigeria; child

Introduction

As part of a comprehensive ongoing biochemical assessment of children's health in northern Nigeria, we took advantage of the availability of two new markers of bone metabolism and sought to compare their diagnostic performance with established clinical laboratory parameters of skeletal disease. Our primary aim was to evaluate the clinical utility of the serum pyridinoline crosslinked carboxyterminal telopeptide of type I collagen (ICTP) determination, a sensitive and specific indicator of bone resorption, and osteocalcin (OC), a somewhat controversial index of osteoblast activity, in populations of rachitic and unaffected West African children between the ages of 10 months and 7 years.

In 1993, Risteli and coworkers introduced a new radioimmunoassay for measuring serum ICTP (1), a trivalent lysinylpyridinoline crosslink which stabilizes type I collagen in bone, cartilage, ligaments, and aorta. ICTP is formed extracellularly only in mature collagen. As bone undergoes remodeling, collagen is proteolyzed; however, being very stable, the lysinyl crosslinks are not further metabolized or degraded but rather are filtered and excreted by the kidneys. Circulating levels of ICTP, therefore, correlate well with rates of bone resorption estimated histomorphometrically and by calcium kinetic studies (2,3). Serum concentrations of ICTP correlate with the urinary excretion of deoxypyridinoline crosslinks and are elevated in conditions associated with increased bone loss, *e.g.*, multiple myeloma, osteolytic metastases, and immobilization (4–6). Estrogen replacement therapy reduces both bone loss and the serum level of ICTP in women with postmenopausal osteoporosis (5). ICTP in serum and deoxypyridinoline in urine arise nearly exclusively from the metabolism of bone. Furthermore, both represent the most specific markers currently available for the clinical assessment of bone resorption (4,6).

Manuscript received February 20, 1995; revised and accepted May 9, 1995.
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The function of the vitamin K-dependent protein OC, or bone Gla protein (BGP) in physiology remains controversial, as does its clinical relevance. This 5.8 Kd polypeptide appears to be synthesized and secreted into the circulation exclusively by osteoblasts and odontoblasts during the matrix mineralization phase of their development (4,7). OC constitutes the major fraction of noncollagenous bone protein, and most likely plays a role in stabilizing hydroxyapatite via complexes between calcium and γ-carboxyglutamate residues. Elevations in serum OC have been reported in Paget's disease, skeletal metastases, primary hyperparathyroidism, and renal osteodystrophy as well as in postmenopausal women (7–11). Although there is evidence to the contrary, it is generally accepted that serum osteocalcin values correlate with parathyroid hormone, vitamin D, and ALP concentrations, and that circulating levels of OC are related to the activity of osteoblasts (4,7,8).

Here, we determined the concentration of calcium, phosphorus, parathyroid hormone (PTH), 1-,25-dihydroxyvitamin D, and alkaline phosphatase (ALP) activity in the serum of 12 children with active rickets, and 27 healthy controls, all who were recruited from villages surrounding the city of Jos in Nigeria.

Materials and methods

Specimens were obtained from children (10 months to 7 years of age) living in five villages located 10–25 km distant from Jos, Nigeria. Procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983, and the study was approved by the Human Research Review Committee at the University of New Mexico School of Medicine. Venous blood was collected into clear, dry 5 mL vacutainer tubes. After 45 min, the tubes were cooled and centrifuged at ×1200 g for 8 min. The serum layer was separated and transferred to Eppendorf microcentrifuge containers which were immediately frozen (−43 °C) for 1–6 weeks until they were transported to Albuquerque, NM, for analysis. The subjects were screened (n = 37) by experienced pediatricians at the Jos University Teaching Hospital (JUTH) using the following clinical criteria: stereotypical skeletal abnormalities such as bowing of the legs, widening of the distal epiphyseal plates of the wrists, cranial bossing, or the presence of a rachitic rosary. Because the technology necessary to perform radiographic confirmation of rickets was unavailable at the site of our study, we defined active disease on the basis of biochemical criteria. In order to meet these criteria a child with the clinical impression of rickets had to have a decreased serum calcium and phosphorus level (≤9.0 mg/dL and ≤4.5 mg/dL, respectively) and serum ALP activity >500 U/L in order to be classified in the experimental group. (1-,25-dihyroxyvitamin D levels were supranormal and not statistically significant between both groups studied). Each subject's medical history was obtained, including physical examination, with particular attention being given to recent infections and a family history of skeletal abnormalities.

The controls (n = 27) were matched according to age, religion (because of differences in diet), and sex, and were recruited from immunization clinics of JUTH and the surrounding villages. All of these children were found to be in good health, and were free of the manifestations of rickets. The salient physical characteristics of the rachitic children and controls are summarized in Table 1.

Serum calcium, creatinine, phosphorus, and ALP activities were measured with the aid of a Kodak Ektachem DT-60 analyzer (Rochester, NY) which monitors rate or end-point colorimetric assays by reflectance photometry. Reagents and calibrating standards were purchased from Physician Sales and

TABLE 1
Summary of the Characteristics of the Study Groups

	Controls (n = 27)		Rickets (n = 12)			
	Mean/SD	Median/(Range)*	Mean/SD	Median/(Range)	p Value	R.I.
Age (yr)	2.9/1.7		3.3/1.8		NS	
Males-Females	19M-8F		9M-3F			
BMI (k/m^2)	16.7/2.3		17.8/1.3		NS	
ICTP (μg/L)	11.0/3.6	11.3/(6.5–21.6)	35.7/27.8	24.8/(15.1–93.4)	<0.0001	age-dependent
Osteocalcin (μg/L)	2.4/1.7		4.6/2.2		<0.01	not established
ALP (U/L)	258/91	251/(124–529)	812/288	742/(511–1319)	<0.0001	<500
PTH (μg/L)	0.6/0.1		1.2/0.3		<0.0001	0.33–0.55
Total calcium (mg/dL)	9.4/0.5		8.2/0.6		<0.0001	9.0–10.8
Phosphorus (mg/dL)	5.1/0.6		3.5/0.6		<0.0001	4.5–6.7
1,25-$(OH)_2$D (ng/L)	195/83	160/(87–332)	256/91	280/(101–372)	NS	18–64

* Median and range are given where data is not normally distributed. R.I. = childhood reference interval (see reference 14), SD = standard deviation, NS = statistically insignificant ($p > 0.05$), BMI = body mass index, ICTP = pyridinoline crosslinked carboxyterminal telopeptide of type I collagen, ALP = alkaline phosphatase, PTH = parathyroid hormone, 1,25-$(OH)_2$ D = 1,25-dihydroxyvitamin D.

Services, (Albuquerque, NM). Quality control materials were a gift from the Clinical Chemistry Section of the University of New Mexico Hospital. The method utilized for ALP was originally described by Bessey, Lowry and Brock and modified by Bowers and McComb (12) and relies on the rate of nitrophenoxide production (pH = 10.5) monitored at 400 nm. Prior to assay, aliquots of sera were incubated for 18–22 h at 22 °C in order to ensure full recovery of ALP activity (13). Calcium was measured by an Arsenazo III dye-binding method (pH = 5.6) monitored at 680 nm and phosphorus by reduction of an ammonium phosphomolybdate complex (pH = 4.2) at 660 nm. Creatinine was quantified by an enzymatic method in selected sera so as to rule out glomerular dysfunction as a cause of elevated ICTP. Rigid analytical performance criteria were met and the overall precision of the determinations ranged from 2–6%.

Radioimmunoassay kits for the determination of ICTP, OC, PTH (mid-molecule), and 1-,25-dihydroxyvitamin D were purchased from INCSTAR Corp., Stillwater, MN. Radioactivity in the osteocalcin and ICTP pellets were counted in a United Technology Packard Crystal Multi Detection System scintillation counter, (Downers Grove, IL). Replicates had a coefficient of variation between 1.3 and 6.0%, and rigid analytical performance criteria were met. To rule out influences stemming from specimen processing, diurnal variation, and racial differences, we also determined serum OC levels in fresh sera obtained from 14 children living in Albuquerque, NM.

All statistical analyses were calculated using the Number Crunching statistical software program (NCSS version 5.X; Kaysville, UT). Group comparisons were made using the Mann–Whitney (nonparametric) Two-Sample Non-Matched test and simple linear regression.

Results

Serum Levels of ICTP are Elevated in Rachitic Children

As shown in Table 1, we observed a highly statistically significant difference in the circulating levels of ICTP between the rachitic subjects and the unaffected children who served as controls ($p < 0.0001$). The median ICTP value of the control group was 11.3 μg/L with values ranging from 6.5 to 21.6 μg/L. The median serum ICTP calculated for children with active rickets was 24.8 μg/L (15.1 to 93.4 μg/L). Elevations ranged upward of ten-fold in some children as compared with controls (Fig. 1).

The serum ICTP concentration and the rate of urinary excretion of lysinylpyridinoline crosslinks are known to be strongly age-dependent, with each parameter decreasing from infancy until the onset of puberty (14). We, therefore, compared our results with 8 separate age intervals established for these

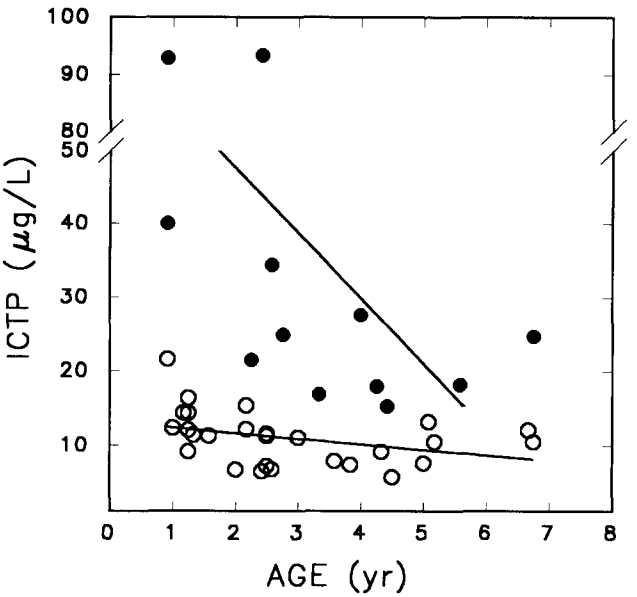

Figure 1 — Serum ICTP values as a function of age in healthy (n = 27) and rachitic (n = 12) Nigerian children. Symbols: ●, rachitic children; ○, healthy children.

healthy British children from 3 months to 8 years of age. Between the ages of 10 months and 4 years, the serum concentration of ICTP in healthy Nigerian children was 25–45% lower than the corresponding values reported for British children.

Serum ICTP Correlates with Alkaline Phosphatase Activity

The serum ICTP value correlated with alkaline phosphatase activity overall (Fig. 3, r = 0.72). The

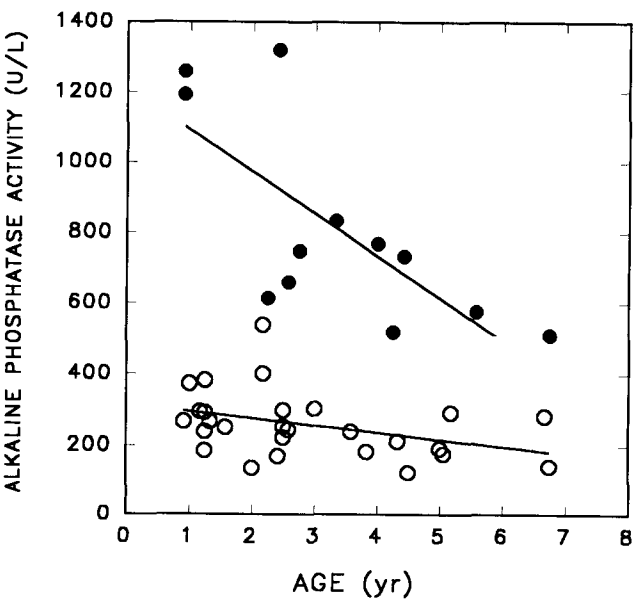

Figure 2 — Serum alkaline phosphatase activity as a function of age in healthy (n = 12) and rachitic (n = 19) Nigerian children. ○, healthy children; ●, rachitic children.

median serum ALP activity was 251 U/L in the control group (124–539 U/L) and 742 U/L (511–1319 U/L) for the rachitic group ($p < 0.0001$, Table 1 & Fig. 2). Healthy children between the ages of 1–12 years have highly variable serum ALP activity; however, the upper limit of the reference interval is widely accepted as being 500 U/L (13). Noteworthy in our study is the fact that the two rachitic children who had the highest serum alkaline phosphatase levels (1319 and 1259 U/L) also exhibited the highest serum ICTP concentrations (93.4 and 92.9 μg/L, respectively), reinforcing the assertion that the serum ICTP value is a sensitive and reliable marker of bone turnover. When these two children are excluded from the regression analysis, the correlation coefficient remains essentially unchanged. Interestingly, the single control (a 2-year-old male) with an ALP activity greater than 500 U/L happened to be the only control who had an elevated ICTP value (15.4 μg/L) as determined by age-matched comparison. Because elevations in both markers are highly indicative of abnormal bone physiology, we question whether this child was assigned incorrectly to the control group.

Osteocalcin

We found a slight but statistically significant difference ($p < 0.01$) in the serum OC levels between the rachitic, (mean = 4.6 μg/L), and African control group (2.4 μg/L). The mean OC concentration in fresh sera obtained from 14 healthy, nonrachitic children living in Albuquerque, NM was 2.5 μg/L and was also significantly different as compared with the rachitic children ($p < 0.05$). The serum OC level did not correlate with serum levels of ALP, ICTP, total calcium, phosphorus nor vitamin D. Tietz has reported a reference interval for serum osteocalcin in children of 10–25 μg/L (15). Circulating OC has been reported to be lower in individuals of African descent (16); however, our results indicate there were no significant differences in serum OC levels between the healthy African populations we studied and 14 children of different races who reside in New Mexico.

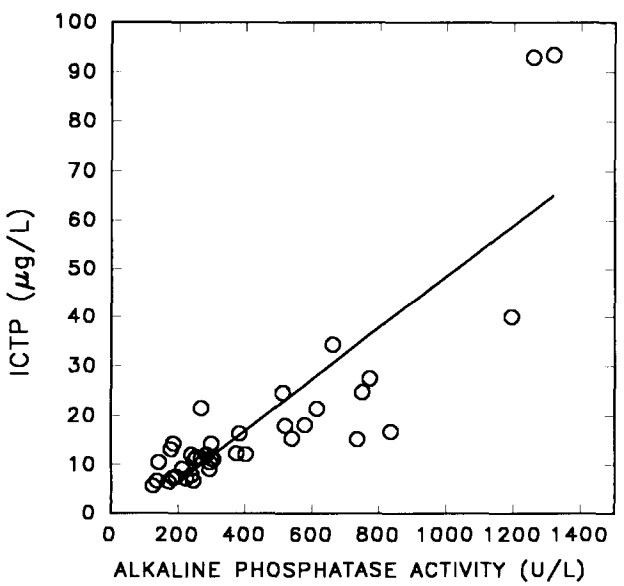

Figure 3 — Correlation of alkaline phosphatase activity and serum ICTP levels in Nigerian children ($r = 0.72$).

Discussion

Specific as the determination of lysinyl crosslinks may be for the laboratory assessment of bone resorption, the question has been raised as to whether it provides a clinician with any more diagnostic information than that conferred by the determination of the established indices of bone disease such as serum alkaline phosphatase activity or urinary hydroxyproline excretion (17,18,19). Our study of Nigerian children with active rickets demonstrates that the serum ICTP determination performs at least as well as ALP as a marker of abnormal bone turnover. Furthermore, ICTP, urinary deoxypyridinoline, and new radiometric assays which specifically measure the bone isoform of ALP may provide the clinician with appreciably more specific tools for diagnosing, evaluating the efficacy of therapy and monitoring the course of metabolic bone disease (2,3,20,21).

The elevations we observed in circulating ICTP seen in rickets may be the result of increased turnover of collagenous matrix resulting from insufficient mineralization of bone, and may also be the result of an increased rate of mature collagen crosslinking which occurs in response to deficient mineralization. There is a five-fold increase in the concentration of bone hydroxypyridinoline in chicks deprived of vitamin D, presumably because deficient mineralization allows more time for crosslink formation (22). Therefore, two factors may account for elevations in serum ICTP concentration which occur in metabolic bone disease: abnormal turnover of bone matrix and pathological changes which alter the extent of crosslinking in type I collagen.

The lower circulating ICTP levels we observed in healthy Nigerian children between the ages of 10 months and 4 years, as compared to their British counterparts, argues strongly for the need to establish appropriate age-dependent ICTP reference intervals for African children. By 5 years of age, however, the healthy Nigerian control children had serum ICTP levels which fell within the defined intervals for British children. In healthy children between 5 years of age and the onset of puberty, serum ICTP is expected to be at a relatively low concentration, indicating that bone remodeling may be somewhat slowed at this age as compared with infancy and puberty. Several factors could account for the decreased serum ICTP levels observed in the younger healthy African children, protein malnutrition being one such factor. A study of the excretion of lysinylpyridinoline crosslinks in the urine of malnourished Jamaican children demonstrated a signif-

icantly reduced turnover of bone matrix, which accelerated when the children's diets were improved (23). Genetic factors that influence the rate of bone turnover could also account for the observed discrepancy in serum ICTP levels between Africans and Caucasians. Because osteoporosis is much less common of a problem in black postmenopausal women (24), bone turnover is surmised to be decreased in individuals of African descent. The present report corroborates this hypothesis.

In the population of children we studied, the most likely cause of rickets is a calcium deficiency induced by the presence of well-characterized antinutrients such as tannins and oxalates in their diet (25). The increased serum vitamin D levels and decreased phosphorus levels we observed were most likely the result of elevated PTH, which is a normal response to calcium deficiency.

Improvements in the measurement of type I collagen metabolites and bone associated peptides in serum and urine offer the clinician a much expanded and advanced choice of clinical laboratory indices of bone resorption. Our report demonstrates that ICTP and OC are valuable additions to the growing repertoire of clinically useful markers of bone metabolism. Future studies should be directed at delineating which of these markers is the best index of bone formation.

Acknowledgements

We wish to express our gratitude to: William Ginn, John Sharpe, and Jeff Ferguson of the Eastman Kodak Company for their generous technical advice and assistance, and to Leila and Juha Risteli of the University of Oulu, Finland, and Premila Trivedi for sharing ideas and results with us. We are also grateful to Carolyn Sanborn of the Reproductive Endocrinology Department of the University of New Mexico School of Medicine for technical assistance with scintillation counting.

This work was supported by a Minority International Research Training (MIRT) grant from the Fogarty International Council of the National Institutes of Health.

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