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ORIGINAL RESEARCH

Establishment of Reference Intervals for Bone Turnover Biomarkers in Healthy Populations in Northern China

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Aim: This study was intended to establish the reference intervals of bone turnover markers (BTMs) for healthy populations.

Methods: According to the Clinical Laboratory Standards Institute (CLSI) EP28-A3c, we recruited 774 healthy Chinese and investigated their clinical characteristics and relationships among gender, age, season and BTMs. The reference intervals of BTMs for healthy populations in Hebei of China were established through defining the central 95% range of all observations.

Results: We found that gender were associated with 25(OH)D, OC, β-CTX, and P1NP ($P < 0.05$), but not PTH1-84 (P=0.138). All serum BTMs showed differences among different age groups ($P < 0.01$). The level of 25 (OH) D in winter showed statistical differences with spring, summer, and autumn ($P<0.05$). The OC level showed statistical difference between summer and winter (P=0.000). The P1NP levels showed statistical difference between spring and winter (P=0.019), summer and winter (P=0.000), and summer and autumn (P=0.012), respectively. The PTH1-84 levels in winter showed statistical differences with spring, and summer (all P=0.000), while there was no statistically significant difference in β- CTX levels between seasons.

Conclusion: We have established the reference intervals of several BTMs for healthy individuals in Hebei of China, which have statistical significance across different age groups and genders, and there are also significant differences between different seasons. Therefore, the Chinese medical laboratories in different locations should group individuals according to gender and age groups in different seasons, and establish corresponding biological reference intervals.

Keywords: 25-hydroxyvitamin D, 25(OH)D, osteocalcin, OC, β-carboxy-terminal cross-linking telopeptide of type I collagen, β-CTX, N-terminal procollagen of type I collagen, P1NP, parathyroid hormone 1-84, PTH1-84

Introduction

Bone turnover markers (BTMs) are metabolites or enzymes, usually secreted by osteoclasts and osteoblasts, can be categorized as reflecting either bone resorption or formation and can reflect osteoclast activity and bone resorption status. Detection of BTMs concentration is valuable for the diagnosis and identification of various skeletal diseases, fracture risk prediction, and drug efficacy evaluation,[1,2] which can also provide a reference value for Diagnosis or treatment of osteoporosis, tumor bone metastasis monitoring, and auxiliary diagnosis, monitoring, and efficacy evaluation of non-skeletal diseases.[3,4]

Here, we review some of the most commonly used BTMs, including 25-hydroxyvitamin D (25(OH)D), osteocalcin (OC), β-carboxy-terminal cross-linking telopeptide of type I collagen, (β-CTX), N-terminal procollagen of type I collagen (P1NP), parathyroid hormone 1–84 (PTH1-84), which have been used in the prevention, monitoring and control of many skeletal diseases, according to higher sensitivity and specificity. Chinese medical laboratory usually use electrochemical luminescence method for the detection of BTMs and use the reference range provided in the manual

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directly. But the main problem is that, most of the kits are produced by Roche diagnostics company, which were designed and developed based on European populations.

Recently investigations indicated that the level of BTMs is not only influenced by age, gender, and geographical location, but also by various factors such as racial differences, nutritional status, lifestyle habits, and hormone levels.[5,6] Therefore, the clinical laboratory is faced with different populations, geographical location, etc, only reference to the specification of bone markers reference interval is not sufficient to meet clinical needs. The present study aimed to investigate the relationship of BTMs with gender, age, different seasons in healthy individuals in Hebei of China and establish more valuable reference intervals for measured the level of 25(OH)D, OC, and β-CTX, P1NP, and PTH1-84 to be used in clinical services.

Methods

Subjects

Although the reference interval (RIs) plays an important role in clinical diagnosis, there are significant differences among geographic locations, sex, age and race. The Association for Clinical Laboratory Standards (CLSI) EP28-A3C recommends that clinical laboratories develop reference intervals appropriate for their populations. The direct approach to establish RIs requires clear inclusion and exclusion criteria, and the use of specific criteria to select reference individuals from the reference population to establish reference intervals. We established 25 (OH) D, OC, β-CTX, P1NP, and PTH1-84 reference ranges for local healthy populations by direct approach.

The inclusion criteria were as follows: Age >18; have resided in the local area for at least 5 years; have complete medical examination information. The exclusion criteria were as follows: Osteoporosis or previous use of drugs that affect bone metabolism; Hyperparathyroidism; Having hyperthyroidism and metabolic bone disease; People with malignant tumors, rheumatoid arthritis and other diseases affecting bone or calcium Metabolic disorder; Liver and kidney dysfunction; Neurological or musculoskeletal disorders; Take any form of vitamin D and calcium or anti-osteoporosis drugs (such as Calcitonin and bisphosphonate) within three months; Missing data on biochemical markers of bone turnover. Age, sex and bone metabolic markers were recorded. All the subjects underwent medical examination and were interviewed using standard questionnaires.

A total of 774 participants (aged 46.79 ± 14.01 years), who visited Hebei General Hospital Health Examination Center and completing the clearly defined questionnaire, from January 2022 to December 2022 for health check were included in the analysis. All participants provided informed consent before participation. All enrolled subjects were divided into 2 groups according to gender: 340 men and 434 women; 3 groups according to age: 205 cases in the 20–35-year-old group (92 men and 113 women); 351 cases in the 36–60-year-old group (140 men and 211 women); and 218 cases in the >60-year-old group (108 men and 110 women). There were 4 groups according to seasons: 212 cases in spring, 174 cases in summer, 185 cases in fall, and 203 cases in winter. The study was authorized by the Medical Ethics Committee of the Hebei General Hospital in accordance with the Declaration of Helsinki (No.2021099), and all participants were informed and signed a consent form before registration. Information collected from recruited participants was kept confidential.

Methods

Fasting blood samples were collected by qualified nurse in the early morning (from 6 am to 9 am, 12h of fasting) and collected the serum (2–4 mL) with. Serum 25(OH)D, OC, β-CTX, P1NP, and PTH1-84 were analyzed using Roche COMBAS 8000 autoanalyzer (Roche Diagnostics, Basel, Switzerland) and using 25(OH)D (Elecsys Vitamin D total Roche Diagnostics), OC (Elecsys N-MID Osteocalcin Roche Diagnostics), β-CTX (Elecsys β-CrossLaps/serum Roche Diagnostics), P1NP (Elecsys total P1NP) and PTH1-84 (Elecsys PTH1-84 Roche Diagnostics) detection kits, respectively.

Vitamin D levels were grouped as follows:[7–12] Vitamin D deficiency group was defined as 25(OH)D ≤ 20 ng/mL, vitamin D insufficiency group was defined as 25(OH)D 21–29 ng/mL, and vitamin D sufficiency was defined as 25(OH)

D ≥30 ng/mL. According to the above criteria, the subjects were categorized into vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency groups.

Statistical Analysis

All statistical analysis was carried out with SPSS 19.0 (IBM Corp., Armonk, NY, USA). GraphPad Prism 8.0 for graphical presentation.

A P-value of less than 0.05 was considered statistically significant. Measures that are not normally distributed are denoted by M (P25, P75). For non-parametric analyses to compare the differences between groups, Mann–Whitney-U test for gender, ethnic, or Kruskal–Wallis H-test were used for age and season. If there are statistical differences within seasons in age groups, further comparisons will be made to establish reference intervals for groups, respectively.

Establishment of Reference Intervals

Following the CLSI-EP28-A3c guide, RI were established using the parametric method (x ± 1.96s) as the 95% data distribution range when the data were normally distributed. Reference intervals are established using the non-parametric method with reference limits at the 2.5 and 97.5 percentiles. There were a minimum of 120 samples per group.

Results

General Characteristics of Participants

A total of 774 healthy participants underwent BTM measurements, including serum 25(OH)D, OC, β-CTX, P1NP, and PTH1-84. In all participants, the median value (IQR) of 25(OH)D was 18.14 (14.81–23.19) ng/mL, with 20.20 (15.65–25.96) ng/mL for men, and 16.90 (13.69–21.11) ng/mL for women. Supplementary material Table A shows the results of each item based on different age groups and season groups.

Comparison of Differences Within the Groups

The results for 25(OH)D, OC, β-CTX, P1NP, and PTH1-84 showed a non-normal distribution. We compared gender differences using the Mann–Whitney *U*-test ($P < 0.05$). We observed significant differences ($P < 0.05$) in the levels of 25(OH)D, OC, β-CTX, and P1NP between males and females (Figure 1a–e).

However, we did not find any significant difference ($P = 0.138$) between males and females in terms of PTH1-84. We performed the Kruskal–Wallis H-test on all age and season groups. Significant differences were observed in all BTMs across age groups ($P < 0.05$). Following additional pairwise comparisons, significant differences were observed in 25(OH)D levels, between the age group of 20–35 years and the age groups of 36–60 years or over 60 years (all P=0.000). Similarly, the levels of OC were significantly different in the age group of 36–60 years compared to those of 20–35 and over 60 years of age (P=0.000 and P=0.011, respectively). Furthermore, significant differences in β-CTX levels were found between the age group of 20–35 years and the age group of 36–60 years (P=0.034). In addition, statistically significant differences in P1NP levels were observed between the age group of 20–35 years and the age groups of 36–60 years or over 60 years (P=0.000, P=0.008, respectively). There was a statistically significant difference in PTH1-84 levels between participants aged 36–60 and those over 60 years of age (P=0.001) (Figure 1f–j).

The paired comparison of seasonal groups showed a statistically significant difference in 25(OH)D levels between the winter group and the other seasons ($P<0.05$). There was a statistically significant difference in OC levels between the summer and winter seasons (P=0.000). Significant differences in P1NP levels were observed between spring and winter (P=0.019), winter and summer (P=0.000), summer and autumn (P=0.012). Significant statistical differences (P=0.000 for all) were observed in the levels of parathyroid hormone 1–84 between winter and spring or summer. No significant statistical differences were observed between the seasons for the levels of β-CTX (Figure 1k–o).

During the seasons, 25(OH)D serum levels showed an increasing trend from spring to summer, then a clear decreasing trend from summer to fall to the lowest levels in winter. PTH1-84 levels showed an increasing trend from spring to summer to fall and winter, with the highest levels in the winter. OC, β-CTX, and P1NP serum levels all started to increase from a decrease in the spring to the summer followed by a decrease in the fall to the winter and reached a high level in the winter (Figure 2).

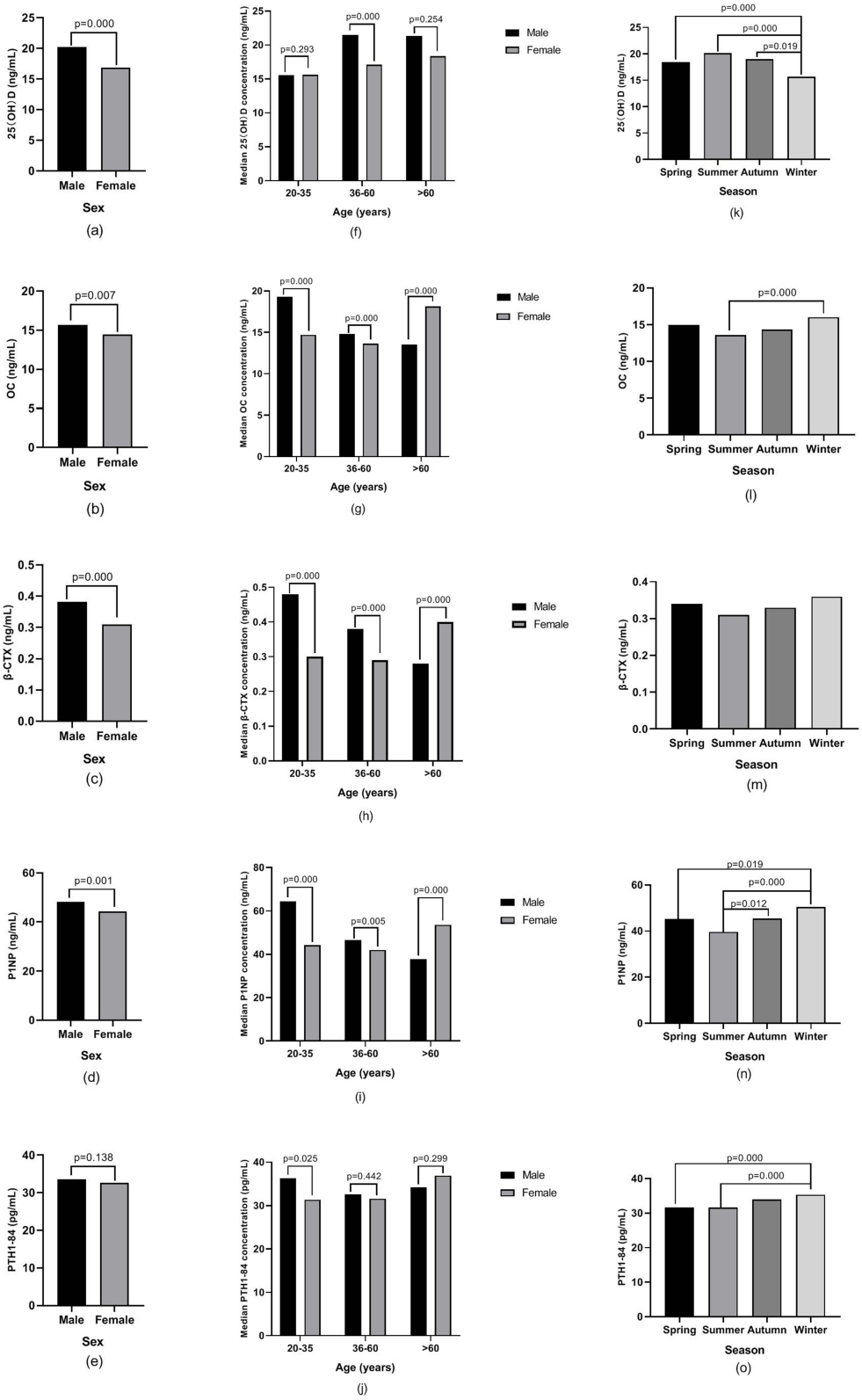

Figure 1 Levels of 25(OH)D and other bone metabolism markers across gender, age and season. (**a**–**e**) Differences in the presence of 25(OH)D, OC, β-CTX and, P1NP and PTH1-84 at different sex levels. (**f**–**j**) are differences in the presence of 25(OH)D, OC, β-CTX and, P1NP and PTH1-84 at different age levels. (**k**–**o**) for differences in the presence of 25(OH)D, OC, β-CTX and, P1NP and PTH1-84 at different seasonal levels.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

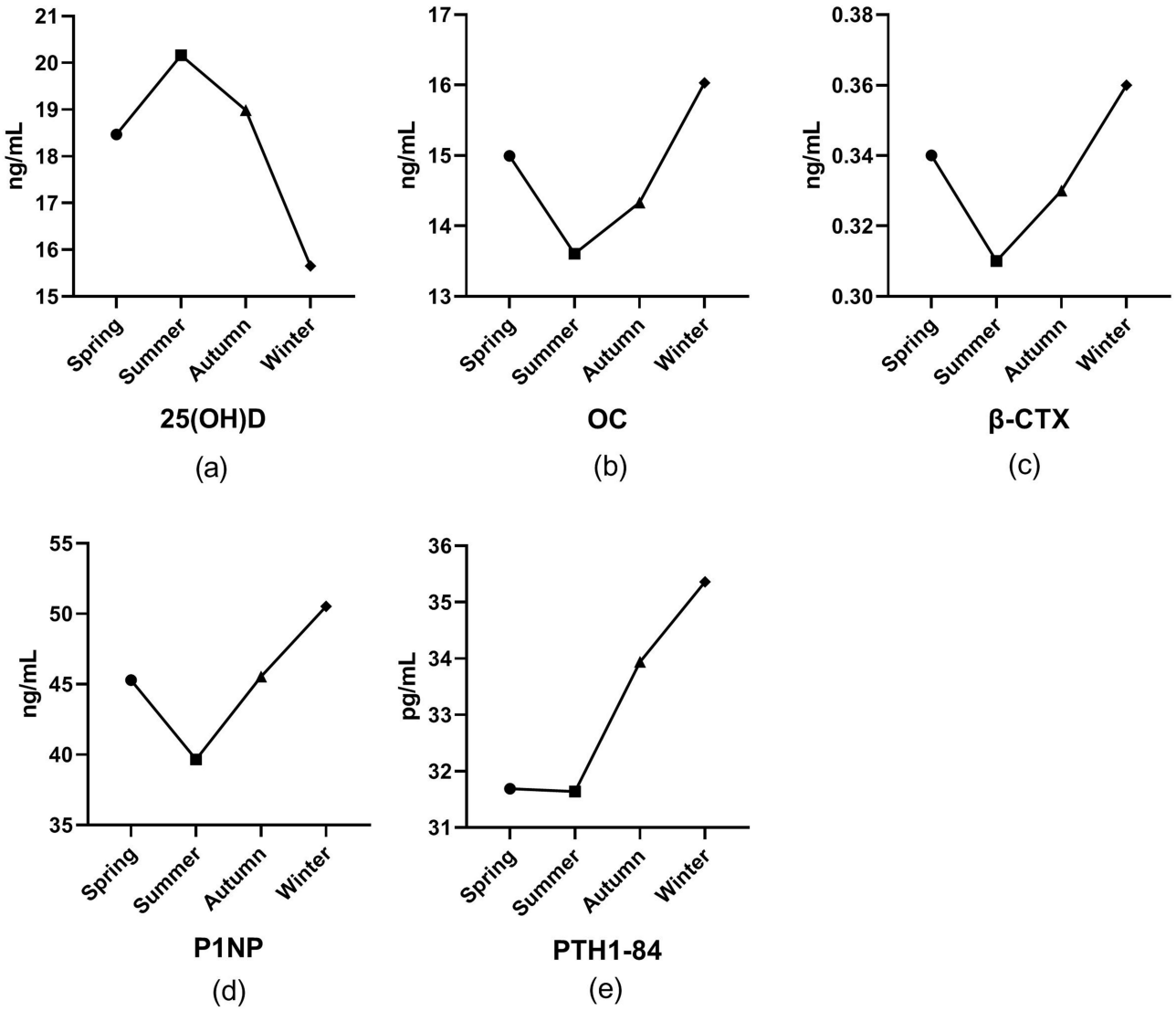

Figure 2 (**a–e**) Plots of trend changes of 25(OH)D, OC, β-CTX and, P1NP and PTH1-84 at different seasonal levels.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

Characterization of bone markers in female before and after menopause, including maximum, minimum, median and quartiles of serum 25(OH)D, OC, β-CTX, P1NP, PTH1-84.25(OH)D, OC, β-CTX, and P1NP except PTH1-84 serum levels were statistically different before and after menopause, and all were greater in the postmenopausal period than in the premenopausal period (Table 1).

Table 1 Characteristics of Female Reference Personnel

	Premenopausal (n=270)			Postmenopausal (n=164)			P
	Maximum	Minimum	Median (P25, P75)	Maximum	Minimum	Median (P25, P75)	
25(OH) D(ng/mL)	43.60	5.54	15.65 (12.55–19.01)	45.82	2.59	19.83 (15.72–25.14)	0.000
OC (ng/mL)	29.88	3.02	13.27 (10.50–15.82)	43.8	7.76	17.8 (14.05–22.60)	0.000
β-CTX(ng/mL)	0.84	0.09	0.26 (0.20–0.34)	1.13	0.05	0.42 (0.30–0.56)	0.000
P1NP (ng/mL)	114.90	17.17	39.54 (32.54–48.72)	121.4	20.00	54.55 (43.57–69.09)	0.000
PTH1-84 (pg/mL)	91.52	8.55	31.81 (26.09–39.77)	58.66	13.18	33.47 (26.66–40.54)	0.396

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

Establishment of Reference Intervals by Direct Method

The CLSI-EP28-A3c guideline requires a minimum of 120 samples in each group that satisfy the inclusion and exclusion criteria. As the data from the subjects' samples were non-normally distributed, non-parametric statistical techniques were utilized. To determine the reference intervals (RIs), a central range of 95% between the 2.5th and 97.5th percentiles of the serum concentrations of 25(OH)D, OC, β-CTX, P1NP and PTH1-84 was used to determine the RIs. The analyses revealed that gender, age, and season groups showed statistically significant differences in the levels of 25(OH)D, OC, and P1NP, gender and age groups display statistically significant differences in the levels of β-CTX, and age and season groups display statistically significant differences in the levels of PTH1-84. Reference intervals for gender, age and season were determined. Due to an insufficient number of male and female participants within the age groups of 20–35 years and over 60 years, the reference intervals were calculated by combining the male and female groups. This was due to non-compliance with the CLSI-EP28-A3c guideline minimum of 120 samples.

The 25(OH)D reference intervals were 9.90–39.52 ng/mL for all participants, 10.88–41.65 ng/mL for men and 9.15–34.95 ng/mL for women, 8.72–32.28 ng/mL for 20–35 years, 11.87–45.15 ng/mL for men aged 36–60 years, 9.43–36.77 ng/mL for women aged 36–60 years, 10.64–38.97 ng/mL for over 60 years. 10.14–42.37 ng/mL for spring, 9.48–41.53 ng/mL for summer, 8.65–41.79 ng/mL for autumn, 9.36–32.82 ng/mL for winter. The reference ranges of 25(OH) D were 8.73 ~ 29.89 ng/mL and 10.20 ~ 39.15 ng/mL in premenopausal and postmenopausal women, respectively. Table 2 shows the reference intervals for each programme by sex, age group and season group.

The Relationship Between Grouping of Different 25(OH)D Levels and PTH1-84 Levels

A total of 774 participants were involved in the study. Among these, 465 individuals (60.1%) were vitamin D deficient, 238 individuals (30.7%) were insufficient, and only 71 individuals (9.2%) were sufficient. The serum PTH1-84 levels of the deficient, sufficient, and insufficient groups displayed significant differences (P<0.01) (Table 3).

Correlation analysis of PTH1-84 levels with 25OHD, OC, β-CTX, and P1NP levels in all patients Statistical results showed that OC (P < 0.01) and β-CTX (P < 0.05) serum levels were positively correlated with PTH1-84, 25(OH)D (P < 0.001) serum levels were negatively correlated with PTH1-84, and P1NP serum levels were unrelated to PTH1-84 (Table 4 and Figure 3).

Table 2 The 25(OH) D, OC, β-CTX, P1NP and PTH1-84 Direct Method Establishes the Reference Interval

	n	25(OH) D (ng/mL)	OC (ng/mL)	*β*-CTX (ng/mL)	P1NP (ng/mL)	PTH1-84 (pg/mL)
Sex						
Female	434	16.90(9.15–34.95)	14.44(7.69–29.32)	0.31(0.12–0.77)	44.25(20.00–91.54)	32.60(18.57–58.28)
Male	340	20.20(10.88–41.65)	15.65(7.87–28.43)	0.38(0.18–0.85)	48.15(23.77–100.55)	33.63(16.48–61.55)
Female						
Premenopausal	270	15.65 (8.73–29.89)	13.27 (7.36–24.84)	0.26 (0.12–0.59)	39.54 (18.82–73.98)	31.81 (18.67–61.60)
Postmenopausal	164	19.83 (10.20–39.15)	17.80 (9.75–33.43)	0.42 (0.15–1.00)	54.55 (23.70–112.09)	33.47 (16.78–56.34)
Age						
20–35	205	15.65(8.72–32.28)	16.34(9.26–34.39)	0.37(0.16–0.83)	51.73(27.82–116.51)	32.68(19.45–62.17)
36–60(female)	161	17.11(9.43–36.77)	13.65(7.42–28.96)	0.29(0.12–0.75)	42.01(19.23–91.61)	31.59(17.74–61.85)
36–60(male)	190	21.51(11.87–45.15)	14.81(7.89–25.66)	0.38(0.17–0.79)	46.52(23.88–90.91)	32.57(15.57–57.98)
36–60	351	18.94(10.26–41.89)	14.10(7.56–27.69)	0.33(0.12–0.75)	43.55(20.54–89.72)	31.86(17.14–59.55)
>60	218	19.90(10.64–38.97)	15.46(7.72–34.78)	0.35(0.16–0.89)	47.29(22.61–91.94)	36.16(16.66–56.25)
Season						
Spring	212	18.47(10.14–42.37)	15.00(7.62–29.48)	0.34(0.14–0.80)	45.30(20.14–102.23)	31.69(19.79–56.67)
Summer	174	20.16(9.48–41.53)	13.61(7.43–27.34)	0.31(0.12–0.76)	39.67(20.30–81.80)	31.64(15.43–57.73)
Autumn	185	18.99(8.65–41.79)	14.33(7.67–28.83)	0.33(0.16–0.83)	45.55(23.39–91.88)	33.94(19.58–65.78)
Winter	203	15.65(9.36–32.82)	16.03(8.58–33.16)	0.36(0.13–0.84)	50.53(22.99–99.15)	35.36(18.83–61.92)
Total	774	18.14(9.90–39.52)	14.88(7.73–28.64)	0.34(0.14–0.82)	45.57(22.16–95.16)	33.11(17.68–59.73)

Notes: Outside the parentheses is the median, inside is the reference interval; Reference intervals were determined as the 2.5th and 97.5th percentiles.

Abbreviations: n, number of sample; BTMs, bone turnover markers; 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

Table 3 The Difference of 25(OH) D Level in Different Groups

Variables	Number	Rank Mean	df	P	Chi-Square value
Lack[25(OH)D ≤ 20 ng/mL]	465	413.83	2	0.000	18.193
Insufficient[25(OH)D 21–29 ng/mL]	238	357.79			
Enough[25(OH)D ≥ 30 ng/mL]	71	314.64			

Table 4 Results of Correlation Analysis of Parathyroid Hormone Levels with 25OHD, OC, β-CTX, and P1NP Levels in All Patients

BTMs(ng/mL)	PTH1-84 (pg/mL)	
	r	P
25 (OH) D	−0.183	0.000
OC	0.109	0.002
β-CTX	0.083	0.021
P1NP	0.034	0.340

Abbreviations: BTMs, bone turnover markers; 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

Discussion

Vitamin D is a fat-soluble vitamin, and 25(OH)D is the main active form of vitamin D in the bloodstream[13,14]. This study shows that 25(OH)D levels are higher in men than in women, which is consistent with reports in the literature.[15] It may be related to women's low outdoor activity, diet and age profile. Levels of 25(OH)D were statistically different between the age groups 20–35 years and 36–60 years or over 60 years (all P=0.000). Individuals aged 36 and above exhibit higher 25 (OH) D levels compared to the 20–35 age group. However, there is no significant difference observed in the 25 (OH) D levels between the 36–60 and above 60 age groups. The reason for the difference in age may be due to the small number of specimens and the higher proportion of females in the <35-year-old group (82 males and 103 females).

Research has confirmed a positive correlation between sunlight exposure and the duration of exposure, and the amount of vitamin D that assists in calcium absorption[16]. According to the findings of this article, the average serum content of 25 (OH) D is higher in spring, summer and autumn than in winter. Currently, there is no defined standard for

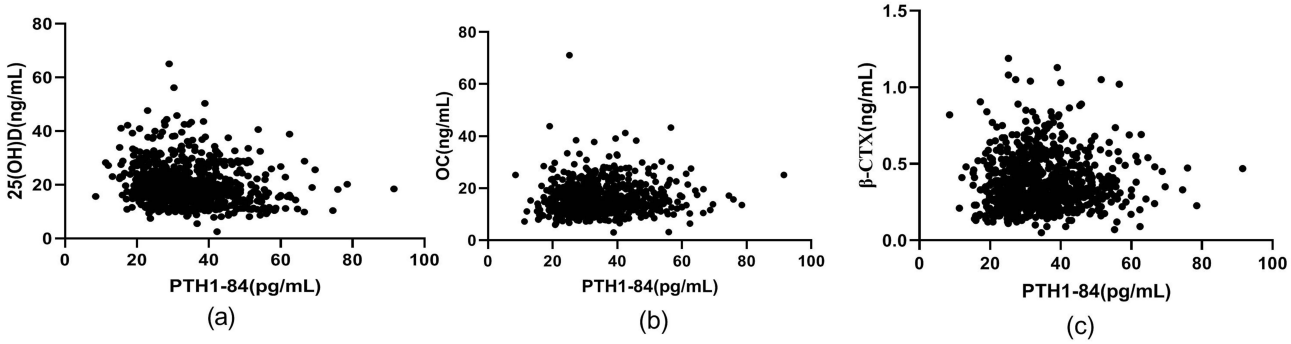

Figure 3 (**a–c**) Correlation distribution plots of 25(OH)D, OC, β-CTX levels and PTH1-84 levels.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OC, osteocalcin; β-CTX, β-carboxy-terminal cross-linking telopeptide of type I collagen; P1NP, N-terminal procollagen of type I collagen; PTH1-84, parathyroid hormone 1–84.

the optimal value of vitamin D. A study investigating vitamin D levels in healthy Caucasians in northern Germany showed a mean value of 20.60 ng/mL, with a reference interval of 5.26–47.00 ng/mL. 6.23–49.90 ng/mL in women and 4.92–42.70 ng/mL in men.[17] According to this study, the reference range for 25(OH)D levels within the overall healthy population ranges from 9.90 to 39.52 ng/mL. The reference range for males is 10.88–41.65 ng/mL, while for females, it is 9.15–34.95 ng/mL. The reference intervals obtained in this study are different from the established intervals for Caucasians.

This study's findings indicate gender-based variations in bone turnover markers (BTMs), specifically OC, β- CTX and P1NP, that show differing degrees of variability with age. Therefore, it is necessary to establish reference intervals that consider both gender and age. Bone conversion markers increase with age and women have higher levels of bone markers than men, speculated to be related to menopause in women.

Osteocalcin is one of the most important specific non-collagenous proteins in the bone matrix. Roche's overall reference range for OC in European populations was 11.00–46.00 ng/mL. According to a study conducted in Shanghai location, the overall reference range for OC is 13.81–16.59 ng/mL, with a range of 5.58–28.62 ng/mL for males and 4.91–22.31 ng/mL for females.[18] According to this study, the overall reference range for OC is 7.73–28.64 ng/mL, with a male range of 7.87–28.43 ng/mL and a female range of 7.69–29.32 ng/mL. The male reference range observed in this study is consistent with the reference range in Shanghai. However, the observed overall reference range differs from the reference range provided in both the manual and the Shanghai region. This article reports a higher reference range for females than for males, which differs from the reference range in the Shanghai location. Different reference ranges are established for OC in each region based on geographical location, population structure, and other factors. Therefore, it is necessary for each laboratory to investigate the range of normal values for the population in their respective regions and establish an appropriate reference range for themselves.

β-CTX is a biomarker for bone resorption as it is intimately linked to the breakdown of type I collagen, which reflects the activity of osteoclasts.[19] According to reference range in the Shanghai region, the normal β-CTX levels for the general population, male and female populations are within the range of 0.22–0.30 ng/mL, 0.10–0.61 ng/mL, and 0.11–0.50 ng/mL, respectively. The Roche manufacturer recommends an overall reference range of 0.00–1.01 ng/mL.[18] This article reports that the reference ranges for β-CTX levels in the general population and in males and females specifically are 0.14–0.82 ng/mL, 0.18–0.85 ng/mL, and 0.12–0.77 ng/mL, respectively. This article proposes a reference range for OC that is different from both the Roche manual's range and the range reported for the Shanghai region. However, the reference range proposed in this article for males is consistent with the range provided by the Shanghai region.

The level of P1NP serves as an indicator for evaluating and monitoring the effectiveness of bone anabolic therapy. Females had statistically significantly lower serum levels of OC, β-CTX, and P1NP compared to males, according to this study. A decrease in the levels of OC can be observed in individuals aged 36–60 when compared to those aged 20–35 years and over 60 years. According to the study, the level of serum β-CTX is higher in the age group of 20–35 when compared to the age group of 36–60. In comparison to both the 36–60 and over 60 age groups, the serum P1NP levels are higher in the age group of 20–35. When grouping OC, β-CTX, P1NP by age and gender, it is observed that all three BTMs differ between genders within each age group. Statistical differences in serum OC and P1NP levels also exist during the season. In China, the available literature on seasonal research in this field is limited. A report states that the general reference range for P1NP in Shanghai ranges from 30.67 to 40.10 ng/mL. Males have a reference range of 16.89 to 65.49 ng/mL, while females have a range of 13.72 to 58.60 ng/mL, as cited in reference.[18] The provided reference range by the manufacturer is 15.13–76.31 ng/mL. In this paper, not only did we establish P1NP reference ranges for different genders but we also examined seasonal differences, and the results for spring and summer were statistically different from winter and summer and fall levels, so we established P1NP reference ranges for different seasons. In the same age group, men were found to have higher β-CTX levels than women in β-CTX level analysis, according to relevant literature[20] which is consistent with the results of the study. The use of β-CTX in clinical practice is becoming increasingly widespread. Therefore, it is essential to determine a precise reference range for this indicator to enable an accurate interpretation of clinical reports.

Parathyroid hormone (PTH) raises blood calcium and lowers blood phosphorus. Our study showed no statistical difference in PTH1-84 levels between genders within the health examination population of this region. However, the levels among individuals aged over 60 were significantly higher than those of individuals aged 36–60 in the population. PTH1-84 levels tend to be higher in spring and summer compared to winter. The median levels of serum PTH1-84 in the vitamin D deficiency group and the sufficient group were lower than those in the vitamin D deficiency group. The outcome establishes a negative association between PTH1-84 and vitamin 25 (OH) D concentrations. A related study indicated that the reference range for PTH1-84 in Shanghai was 14.61–63.22 pg/mL for men and 15.52–66.78 pg/mL for women.[19] The Roche manual provides a reference range of 14.90–56.90 pg/mL for PTH1-84. In this article, PTH1-84 reference range is 17.68–59.73 pg/mL, with separate ranges for males (16.48–61.55 pg/mL) and females (18.57–58.28 pg/mL). Although it is consistent with the Roche manual, the reference range for PTH1-84 differs from that observed in the Shanghai region. The data for vitamin 25(OH) D levels were analyzed by several grouping.

25(OH) D, OC, β-CTX and P1NP serum levels were higher in the postmenopausal period than in the premenopausal period, and the reason for the rise in vitamin D is speculated that this may be due to the fact that older people are more aware of their health care, go outdoors more often, have increased levels of care, and are equally focused on vitamin D supplementation. The increase in OC, β-CTX, and P1NP in postmenopausal women is well known, mainly due to a decrease in estrogen concentrations, which in turn leads to an increase in osteoclast activity.

25(OH)D serum levels increased and then decreased seasonally, with the lowest levels in winter. The reason for this is that due to the geographical location of the region, the light duration is higher in summer than in other seasons, and the light duration is shortest in winter. PTH1-84 levels were flat in spring and summer, increased in fall, and reached the highest level in winter. OC, β-CTX and P1NP showed a trend of decreasing in spring to summer, and then further increased in fall and winter. It was further verified that the decrease in vitamin D levels caused an increase in PTH1-84 levels, which also promoted bone conversion. Similarly, the results of the present study indicated that 25(OH)D serum levels were negatively correlated with PTH1-84. OC ($P < 0.01$) and β-CTX serum levels were positively correlated with PTH1-84.

The advantages of this paper are firstly, we study the reference range of bone markers in the same latitude and the same region of the population in the northern region, which is more well applied to the clinic, and secondly, there is no relevant study on the reference range of bone markers in the northern region in China at present. We are the first time to explore it. This paper also has some limitations, firstly, the results of this paper are only applicable to the population in this region and cannot represent the whole earth; therefore, it is recommended that different regions establish their own regional reference values. Secondly, the sample size of this paper is small, which may bias the analysis results, and we will increase the sample size later to explore further.

Conclusion

This study has established the corresponding biological reference intervals for several BTMs, including 25(OH)D, OC, β-CTX, P1NP and PTH1-84. Manufacturers grouped products primarily on the basis of gender and did not provide age or seasonal reference ranges. His article divides different bone metabolism biomarkers into seasons and calculates the differences. The results show that most bone metabolism markers do indeed have seasonal differences. It is recommended that each laboratory establishes a reference range for this experiment. This action would facilitate the provision of an accurate basis for clinical diagnosis and treatment.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

Written informed consent was obtained from all participants. This study was approved by the Research Ethics Committee of the Hebei General Hospital. All methods were carried out in accordance with relevant guidelines and regulations.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare that they have no competing interests in this work.

References

1. Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*. 2011;22:391–420. doi:10.1007/s00198-010-1501-1
2. Lorentzon M, Branco J, Brandi ML, et al. Algorithm for the Use of biochemical markers of bone turnover in the diagnosis, assessment and follow-up of treatment for osteoporosis. *Adv Ther*. 2019;36:2811–2824. doi:10.1007/s12325-019-01063-9
3. Kasai H, Mori Y, Ose A, et al. Prediction of fracture risk from early-stage bone markers in patients with osteoporosis treated with once-yearly administered zoledronic acid. *J Clin Pharmacol England*. 2021;61:606–613. doi:10.1002/jcph.1774
4. Jain S. Role of bone turnover markers in osteoporosis therapy. *Endocrinol Metab Clin North Am*. 2021;50:223–237. doi:10.1016/j.ecl.2021.03.007
5. Baum E, Peters KM. The diagnosis and treatment of primary osteoporosis according to current guidelines. *Dtsch Arztebl Int*. 2008;105:573–581. doi:10.3238/arztebl.2008.0573
6. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. *Lancet Diabetes Endocrinol*. 2017;5:908–923. doi:10.1016/S2213-8587(17)30184-5
7. Cashman KD. Global differences in vitamin D status and dietary intake: a review of the data. *Endocr Connect England*. 2022;2022:e210282.
8. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab United States*. 2011;96:1911–1930. doi:10.1210/jc.2011-0385
9. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol United States*. 2009;19:73–78. doi:10.1016/j.annepidem.2007.12.001
10. Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmun Rev Netherlands*. 2010;9:709–715. doi:10.1016/j.autrev.2010.06.009
11. Dawson-Hughes B, Heaney RP, Holick MF, et al. Estimates of optimal vitamin D status. *Osteoporos Int*. 2005;16:713–716. doi:10.1007/s00198-005-1867-7
12. Vieth R, Bischoff-Ferrari H, Boucher BJ, et al. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr United States*. 2007;85:649–650. doi:10.1093/ajcn/85.3.649
13. Shoemaker TJ, Mowry EM. A review of vitamin D supplementation as disease-modifying therapy. *Mult Scler*. 2018;24:6–11. doi:10.1177/1352458517738131
14. Kheiri B, Abdalla A, Osman M, et al. Vitamin D deficiency and risk of cardiovascular diseases: a narrative review. *Clin Hypertens*. 2018;24:9. doi:10.1186/s40885-018-0094-4
15. Stöckl D, Sluss PM, Thienpont LM. Specifications for trueness and precision of a reference measurement system for serum/plasma 25-hydroxyvitamin D analysis. *Clin Chim Acta*. 2009;408:8–13. doi:10.1016/j.cca.2009.06.027
16. Biersack MG, Hajdukiewicz M, Uebelhack R, et al. Sustained Increase of 25-Hydroxyvitamin D Levels in healthy young women during wintertime after three suberythemal UV Irradiations-The MUVY Pilot Study. *PLoS One*. 2017;2017:e0169709.
17. Heijboer AC, Blankenstein MA, Kema IP, et al. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. *Clin Chem*. 2012;58:543–548. doi:10.1373/clinchem.2011.176545
18. Hu WW, Zhang Z, He JW, et al. Establishing reference intervals for bone turnover markers in the healthy shanghai population and the relationship with bone mineral density in postmenopausal women. *Int J Endocrinol*. 2013;2013:513925. doi:10.1155/2013/513925
19. Bernotiene E, Bagdonas E, Kirdaite G, et al. Emerging technologies and platforms for the immunodetection of multiple biochemical markers in osteoarthritis research and therapy. *Front Med Lausanne*. 2020;7:572977. doi:10.3389/fmed.2020.572977
20. Jørgensen NR, Møllehave LT, Hansen YBL, et al. Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population. *Osteoporos Int*. 2017;28:2103–2113. doi:10.1007/s00198-017-4026-z