Reference intervals of serum carboxy-terminal telopeptide of type I collagen in the Ukrainian residents of various ages and sexes

Abstract. Background. The purpose of the study is to determine the reference intervals of carboxy-terminal telopeptide of type I collagen (β-CTX) in the blood serum of healthy males and females of various ages, residents of Ukraine. Materials and methods. The study included 4754 individuals aged 20–89 years (4123 women (mean age 59.6 ± 13.6) and 631 men (mean age 49.5 ± 16.3)). All participants were divided into 7 groups by the age decades (from 20 to 89 years), and three groups of 40–49, 50–59 and 60–69 year-olds were additionally divided into the subgroups by five years. Furthermore, the patients were divided according to the gerontological classification of age into 4 groups: young — 20–44 years, middle-age — 45–59 years, elderly — 60–74 years, and old — 75–89 years. The blood serum β-CTX concentration was determined by ECLIA electrochemiluminescence immunoassay on the cobas e 411 analyzer. Results. We have detected significant effect of age on serum β-CTX variability: a significant increase in β-CTX levels in women (F = 15.48, p < 0.00001), and a decrease in men (F = 11.73, p < 0.00001) with age. The significantly lower levels of β-CTX were found in the women aged 20–29 (F = 35.68, p < 0.00001) and 30–39 years (F = 19.47, p = 0.00001) compared to the men of similar ages. However, the women of 65–69 years (F = 10.50, p = 0.001) had a significantly higher serum β-CTX levels compared to men of the same age group. Conclusions. The serum β-CTX levels have been associated with age: in men they are decreasing significantly with age, in women — significantly increasing. In the young men aged 20–44 years and women aged 65–69 years, the β-CTX concentration was significantly higher compared with that of the opposite sex subjects of a similar age. The findings on β-CTX serum levels in the practically healthy men and women of various ages, who are residing in this country, may be used as the reference intervals for the Ukrainian population.

Keywords: bone turnover markers; carboxy-terminal telopeptide of type I collagen; age; sex
At the same time, determining the BT remodeling markers enables us to detect the significant alterations much earlier, starting at three months from the beginning of treatment [8], and to reduce the necessity of a repeated DXA [1], which is especially relevant for those regions where the BMD measurements are limited.

The positive aspect of BT metabolism marker use is the fact that blood and urine sampling is an easy and accessible process, while its findings boost the informative DXA value [6]. However, while using the markers in one’s practice, one should consider certain limitations influencing their results and analysis. Among them, there are age, sex, circadian rhythms, food intake, medication use, comorbidities and even the seasonality of study [6, 9, 10].

In order to reduce the pre-analytical, analytical and interlab fluctuations of the BT resorption and formation indices, the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry Bone Marker Standards Working Group (IOF–IFCC) offered standardization of the patients prepared for the blood sampling and processing the samples while determining the \textit{β}-CTX and procollagen type I N propeptide (PINP) concentrations in the blood serum [10]. The above mentioned markers are recommended by the IOF as the benchmarks of the BT metabolism [2].

The recent reference literature data reveal that the level of blood serum BT formation and resorption markers may vary dramatically among the residents of some countries or even regions of one country even though they belong to one and the same age group, proving the effect of genetic, epigenetic and environmental factors on the BT metabolism [1]. The importance of correct clinical interpretation of the findings requires determining the reference intervals for each BT metabolism marker in various populations and ethnic groups [2]. In Ukraine, there are no reference data on the BT resorption markers, namely \textit{β}-CTX, for various sexes and age groups, emphasizing the topicality of this study.

The aim of this study is to determine the reference levels of carboxyterminal cross-linking telopeptide of Type I bone collagen in the blood serum of healthy Ukrainian men and women of various ages, residing in Ukraine.

Materials and methods

The study was performed at the SI “D.F. Chebotaryov Institute of Gerontology by the NAMS Ukraine” and the Ukrainian Scientific-Medical Center of Osteoporosis by the NAMS Ukraine, recruiting 4754 people, aged 20-89 years, among them 4123 women (mean age 59.6 ± 13.6) and 631 men (mean age 49.5 ± 16.3). The study included the subjects attending the above-mentioned healthcare institutions for the first time, on condition they signed the inform consent for the study participation. The exclusion criteria consisted in the attending comorbidities, affecting the BT metabolism, a history of osteotropic medications (excluding calcium and Vitamin D), bone fractures of any localization during the recent year. Furthermore, the pregnant and lactating women were also excluded.

The blood sampling analysis of subjects was performed after their distribution into decades, and 7 groups were made (from 20 to 89 years). In order to interpret the \textit{β}-CTX variations in the blood samples of men and women more precisely and detect the postmenopause-related changes in women, three age groups of 40-49, 50-59 and 60-69 years were further subdivided into the subgroups of five years (Table 1). The patients were also distributed into 4 groups according to the gerontological age classification: young age - 20-44 years (255 men and 591 women), middle age - 45-59 years (187 men and 1248 women), elderly age - 60-74 years (142 men and 1730 women) and old age – 75-89 years (47 men and 554 women).

The blood serum \textit{β}-CTX concentration was determined by the \textit{ECLIARIA} electrochemiluminescence immunoassay at the cobas e 411 analyzer. The blood serum studies were performed according to the 2017 IOF and IOF-IFCC recommendations [10] on the standardized sample processing and preparation of subjects for the \textit{β}-CTX assays: blood sampling was performed after the overnight sampling, in the time span from 7.30 to 10.00, by means of standard test tubes with a sample distribution gel. The blood was centrifuged for 30 minutes after the sampling. The blood samples were analyzed at the day of sampling or within the limit of 3 days after sampling (storing the centrifuged serum at -20°C). The day before sampling subjects were advised to avoid any great physical strains.

The statistical analysis of findings was performed by means of Statistica 6.0. software. In order to check verify the normalcy of data distribution, we used the Kolmogorov–Smirnov and Shapiro–Wilks test. By means of one-way ANOVA analysis, we detected the age and sex effect on \textit{β}-CTX variance. By means of Scheffe’s method, we evaluated the intergroup variances. The interconnections of age, sex and \textit{β}-CTX concentration were determined by linear regression analysis. The findings obtained were presented as M ± SD, where M – mean value, SD – standard deviation. The difference was considered significant if p < 0.05.

Results

Our study has revealed a significant influence of age on the \textit{β}-CTX level variance in the blood serum of women (F = 15.48, p < 0.00001), as well as men (F = 11.73, p < 0.00001) (Fig.1, Table 2).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>20-29 years</th>
<th>30-39 years</th>
<th>40-49 years</th>
<th>50-59 years</th>
<th>60-69 years</th>
<th>70-79 years</th>
<th>80-89 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20-29 years</td>
<td>30-39 years</td>
<td>40-49 years</td>
<td>50-59 years</td>
<td>60-69 years</td>
<td>70-79 years</td>
<td>80-89 years</td>
<td>Total</td>
</tr>
<tr>
<td>Women</td>
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<td>156</td>
<td>281</td>
<td>154</td>
<td>208</td>
<td>389</td>
<td>651</td>
<td>678</td>
<td>580</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td>81</td>
<td>115</td>
<td>59</td>
<td>68</td>
<td>57</td>
<td>162</td>
<td>57</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1. Age distribution of subjects into decades

While evaluating the women’s intergroup variance according to Scheffé’s method (Fig. 2 A), we found a significantly lower β-CTX level in the age groups of 30-39 years (0.42 ± 0.24, p < 0.00001), 40-44 years (0.42 ± 0.22, p < 0.000001), 45-49 years (0.47 ± 0.25, p = 0.0005), 50-54 years (0.51 ± 0.26, p = 0.02), 55-59 years (0.52 ± 0.27, p = 0.05) and 60-64 years (0.51 ± 0.27, p = 0.01) compared with the age group of 80-89 years (0.62 ± 0.34). Furthermore, β-CTX level was significantly lower in the age group of 30-39 years (0.42 ± 0.24) and 40-44 years (0.42 ± 0.22) compared with the following age groups: 50 – 54 years (0.51 ± 0.26, p = 0.05), 55 – 59 years (0.52 ± 0.27, p = 0.001) and 60 – 64 years (0.51 ± 0.27, p = 0.01), 65 – 69 years (0.53 ± 0.28, p = 0.0003), 70 – 79 years (0.54 ± 0.28, p = 0.000002) and 80 – 89 years (0.62 ± 0.34, p < 0.000001).

The male subjects (Fig. 2 B) aged 20-29 years (0.72 ± 0.27) had a significantly higher level of β-CTX compared with the age groups of 30-39 years (0.54 ± 0.26, p = 0.004), 40-44 years (0.49 ± 0.18, p = 0.001), 45-49 years (0.46 ± 0.19, p = 0.00001), 50-54 years (0.50 ± 0.23, p = 0.0003), 55-59 years (0.51 ± 0.24, p = 0.04), 60-64 years (0.49 ± 0.30, p = 0.001) and 65-69 years (0.39 ± 0.17, p < 0.000001). In the subjects aged 65-69 years (0.39 ± 0.17), β-CTX level was significantly lower compared with subjects aged 70-79 years (0.58 ± 0.27, p = 0.049).

While studying the effect of age on β-CTX level of various age groups, we found a significantly lower β-CTX level in women of 20-29 (p < 0.000001) and 30-39 years (p = 0.00001) compared with men of the same age (Fig.3). However, women of 65-99 years (p = 0.001) had significantly lower β-CTX levels compared with men of similar age group.

Our findings on the examined subjects, distributed according to the gerontological age classification, are presented in Table 3.

According to the distribution of subjects by the gerontological age classification (Fig.4), β-CTX level in the blood serum was significantly higher in the men aged 20-44 years (p = 0.00002) in comparison with women of the same age. The difference was 25%.

According to the regression analysis, β-CTX levels of women revealed a significant age-related increase (p < 0.00001). At the same time, there was a significant negative correlation observed in men, confirming the age-related β-CTX decrease (p = 0.0004) (Fig. 5).

**Discussion**

Under optimal physiological conditions, the bone resorption lasts about 10 days, while bone formation – about 3 months [2]. The reference literature contains a lot of data...
Fig 2. Blood serum β-CTX levels in men and women of various ages, distributed into decades: (A) – women, (B) – men
Note: β-CTX - carboxyterminal cross-linking telopeptide of Type 1 bone collagen.

Fig 3. Blood serum β-CTX levels in men and women of various ages, distributed into decades: (1) – women, (2) – men
Note: β-CTX - carboxyterminal cross-linking telopeptide of Type 1 bone collagen; A – subjects aged 20-29 years, B - 30-39 years, C - 65-69 years.
Fig 4. Blood serum β-CTX levels in men and women of various ages, according to the gerontological age classification: (A) – women, (B) – men

Note: β-CTX - carboxyterminal cross-linking telopeptide of Type 1 bone collagen.

Fig. 5. Correlation and regression analysis of age and blood serum β-CTX level correlation for: (A) -  women, (B) -  men

Note: β-CTX - carboxyterminal cross-linking telopeptide of Type 1 bone collagen: A. β-CTX (ng/ml) = 0.364 + 0.003 * Age (years), (r = 0.13, t = 8.24, p < 0.00001); B. β-CTX (ng/ml) = 0.645 – 0.002 * Age (years), (r = -0.14, t = -3.56, p = 0.0004).

Table 3. β-CTX level in the blood serum of men and women according to their gerontological classification

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age, years</td>
<td>Number of subjects, n</td>
<td>β-CTX level, ng/ml</td>
<td>Mean age, years</td>
<td>Number of subjects, n</td>
<td>β-CTX level, ng/ml</td>
</tr>
<tr>
<td>20–44</td>
<td>34.50 ± 6.56</td>
<td>591</td>
<td>0.44 ± 0.24</td>
<td>33.24 ± 6.72</td>
<td>255</td>
<td>0.59 ± 0.26</td>
</tr>
<tr>
<td>45–59</td>
<td>54.98 ± 3.94</td>
<td>1248</td>
<td>0.50 ± 0.26</td>
<td>51.72 ± 4.40</td>
<td>187</td>
<td>0.48 ± 0.22</td>
</tr>
<tr>
<td>60–74</td>
<td>66.23 ± 4.25</td>
<td>1730</td>
<td>0.52 ± 0.27</td>
<td>66.13 ± 4.29</td>
<td>142</td>
<td>0.49 ± 0.27</td>
</tr>
<tr>
<td>75–89</td>
<td>78.46 ± 3.16</td>
<td>554</td>
<td>0.59 ± 0.31</td>
<td>79.13 ± 3.59</td>
<td>47</td>
<td>0.59 ± 0.29</td>
</tr>
</tbody>
</table>

Note: β-CTX - carboxyterminal cross-linking telopeptide of Type 1 bone collagen.
confirming the significant influence of modified and non-modified risk factors on β-CTX blood serum levels.

The β-CTX blood serum concentration has a great circadian variance, preserved across the ethnic groups and nationalities. The daily fluctuation rate may account for 40% - 66%, reaching its peak in the morning hours: between 2:00 and 5:00 AM, and its bottom between 11:00 and 2:00 PM [10].

The elevated β-CTX blood serum concentration is observed during 2 weeks after the fracture occurrence, while its decrease takes about 2-3 months afterwards. After 6 months, it returns to its stable level, which is close to normal, reference level; although a slight increase is preserved during the first follow-up year [11, 12].

While interpreting the β-CTX data, one should take into account the seasonal effect on its concentration. The highest values are observed in the spring, while the lowest are observed in winter [13], which may be attributed to the Vitamin D effect on the BT metabolism, as the former’s concentration is season-dependent [14]. The results of our previous studies on the Ukrainian residents show that the highest 25(OH)D level is observed in August (28.6 ± 11.4 ng/ml) and in September (28.6 ± 11.6 ng/ml), while the lowest one is observed in February (23.1 ± 11.6 ng/ml) [15]. In the Central Europe, the winter 25(OH)D concentration varies from 11 ng/ml in Poland to 18 ng/ml in Estonia, while the summer 25(OH)D concentration varies from 18 ng/ml in Ukraine to 35 ng/ml in Hungary [16]. In Ukraine, 81.8% of residents are afflicted by the Vitamin D deficiency [17], while in the Western Europe, 40.4% of residents have it [18]. The mean β-CTX level in the blood serum is higher in the smoking subjects (approximately by 8.3%) compared with the non-smoking subjects of similar ages [19].

The food intake the day before the blood sampling provokes the decrease of blood serum β-CTX level of up to 40 %. This food intake effect may be attributed to one of the incretin hormones (GLP-2 - Glucagon-like peptide-2), whose concentration grows after the food intake by 5-10 times [9]. The BT resorption intensifies in the night and drops in the day following the food intake, and this fact is confirmed by the reference data on the correlation of BT homeostasis and gastrointestinal (GI) activity, which is mediated by the intestine-secreted hormones in response to the food intake [20].

The oral contraceptive use reduces the β-CTX concentration by 15-25%, while the sports activities - by 8 % [10] [19]. The women, who at the moment of blood sampling, were experiencing the ovulatory phase of menstrual cycle (MC) (6-18th day of the MC), had the β-CTX concentrations which were lower than the concentrations of those who were experiencing the initial or final phase of menstrual cycle (0–5 and 19–28th day of the MC) [19].

The level of BT metabolism markers of the young men is inversely related to age, being significantly higher than the reference intervals for the reproductive age women described in the reference sources [21]. During their lifetime, the β-CTX blood serum concentration drops by 31-58 % - from 0.12 to 0.83 ng/ml at the age of 25-29 years to 0.05-0.58 ng/ml at the age of 75-79 years [22]. According to our research findings, the men reported a significant negative regression correlation between the age and β-CTX level, confirming its age-related decrease (p = 0.0004). The β-CTX level of the men aged 20–29 (0.72 ± 0.27) and 30–39 years (0.54 ± 0.26) is definitely higher in comparison with the one of similarly-aged women (0.51 ± 0.24, p < 0.000001 and 0.42 ± 0.24, p < 0.000001 respectively). The BT turnover remains elevated in men after their reaching peak bone mass and reaches its bottom only during the 5-6th decades [21]. In the population of the Ukrainian men, the lowest concentration of blood serum β-CTX is revealed in the men of 65-69 years (0.39 ± 0.17).

The population of young men demonstrates a strong family resemblance of the BT metabolism irrespective of age [21]. There is a significant correlation among the BT metabolism markers (apart from the bone alkaline phosphatase) of brothers unlike the group of males unrelated by the family ties. The level of BT turnover markers correlates with the one of a twin irrespective of their sex, confirming the significant effect of heredity on the rate of BT metabolism in twins. The strong correlation was also found in the grown twins, residing in various localities [23].

The reference literature presents the data on the β-CTX blood serum concentration varies significantly among countries. The women of 30–39 years, residing in various European countries, the highest β-CTX level was registered in the female residents of France (0.34 ng/ml) while the lowest one – in the UK female residents 0.29 ng/ml (p = 0.01). The obtained results may be attributed to the fact that the French women had a significantly lower mean body mass index (BMI), while the ratio of smokers was the highest among the women of Europe [19]. In the Asian countries, the premenopausal women of Japan had the lowest mean β-CTX level – up to 0.099 ng/ml and the women of Thailand had the highest – 0.26 ng/ml [11].

The Ukrainian female residents of similar ages had the mean β-CTX levels which were higher than the ones of the upper average level of the European residents (namely, the French ones), which, to our mind, is the reflection of lower menopause onset in the European residents. Thus, the mean age of menopause in the European female residents is 54 years [22], which is more advanced than the one in Ukraine – 48.7 years [24]. The reference intervals of women aged 30-54 years [22], experiencing the perimenopausal period, are from 0.05 to 0.67 ng/ml [22]. With the menopausal onset, the healthy women report the growth of blood serum BT turnover marker concentration compared to the women of reproductive age [9, 22], and further increases with an advancing age [14]. The women of elderly and old age (over 70 years) demonstrate the CTX concentrations which are significantly higher than the ones of women aged 60-69 years [25]. However, there are studies which observe the β-CTX level diminishing in women after 70 [22, 26]. Our studies show that β-CTX concentration increases in the women of 50-54 years (0.51 ± 0.26), compared with the younger age groups, and grows some more in the ages of 65 – 69 years (0.53 ± 0.28), 70 – 79 years (0.54 ± 0.28) and 80 – 89 years (0.62 ± 0.34).

The estrogen deficiency bringing forth the menopause also leads to a generalized imbalance between the BT for-
The obtained results confirm that the blood serum β-CTX levels in the practically healthy subject of various ages, who resided at the territory of this country, may be used as the reference values for the Ukrainian population.

Conflicts of interests. Authors declare the absence of any conflicts of interests and their own financial interest that might be construed to influence the results or interpretation of their manuscript.

References

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Владикова В.В., Заверуха Н.В., Солоненко Т.Ю., Мусієнко А.С.
ДУ “Інститут генетології імені Д.Ф. Чеботарьова НАМН України”, м. Київ, Україна
Український науково-медичний центр проблем остеопорозу, м. Київ, Україна
Поворознюк В.В., Заверуха Н.В., Солоненко Т.Ю., Мусієнко А.С.
Український науково-медичний центр проблем остеопорозу, м. Київ, Україна

Оригінальні дослідження / Original Researches

10–44 роки, середній — 45–59 років, літній — 60–74 роки та старчий вік — 75–89 років. Концентрацію β-CTX у сироватці крові у практично здорових чоловіків і жінок різного віку, які проживають на території України, можуть бути використані як референтні показники для населення України. Вони можуть бути використані як референтні показники для населення України.

Резюме. Метою дослідження є визначення референтного гранду карбокситермінального телопептиду колагену I типу (β-CTX) в сироватці крові у практично здорових чоловіків і жінок різного віку, які проживають на території України. Матеріал та методи. У дослідженні були включені 4754 особи віком 20–89 років (4123 жінки (середній вік — 50 ± 39 років) та 631 чоловік у червень о 1997–2019. Усіх учасників розподілили за віком на 7 груп за десятиріччями (від 20 до 89 років), та три вікові групи — 40–49, 50–59 та 60–69 років — додатково розподілили на підгрупи за п’ятиріччями. Також розподілили віком 20–29 років (p < 0,00001) порівняно з чоловіками цього ж віку. Проте у жінок 65–69 років (p < 0,001) виявили вірогідно вищий рівень β-CTX у сироватці крові порівняно з чоловіками цього ж віку. Проте у молодих чоловіків віком 20–29 років (p < 0,001) виявили вірогідно вищий рівень β-CTX у сироватці крові порівняно з чоловіками цього ж віку. Вони можуть бути використані як референтні показники для населення України. Ключові слова: маркери метаболізму кісткової тканини; карбокситермінальний телепептид колагену I типу; вік; стать; ней підвищення рівня β-CTX з віком у жінок (p < 0,00001) та зниження у чоловіків (p < 0,00001). Вірогідно нижній рівень β-CTX виявили у жінок віком 20–29 років (p < 0,00001) та 30–39 років (p < 0,00001) порівняно з чоловіками цього ж віку. Проте у жінок 65–69 років (p = 0,001) виявили вірогідно вищий рівень β-CTX у сироватці крові порівняно з чоловіками цього ж віку. Вони можуть бути використані як референтні показники для населення України.

Ключові слова: маркери метаболізму кісткової тканини; карбокситермінальний телепептид колагену I типу; вік; стать
Резюме. **Цель** исследования является определение референтного уровня карбокситерминального телопептида коллагена I типа (β-CTX) в сыворотке крови у практически здоровых мужчин и женщин разного возраста, проживающих на территории Украины. **Материалы и методы.** В исследование были включены 4754 лица в возрасте 20–89 лет (4123 женщины (средний возраст — 59,6 ± 13,6 года) и 631 мужчина (средний возраст — 49,5 ± 16,3 года)). Всех участников распределили по возрасту на 7 групп по десятилетиям (от 20 до 89 лет), и три возрастные группы — 40–49, 50–59 и 60–69 лет — дополнительно разделили на подгруппы по пятилетиям. Также распределение пациентов проводили по геронтологической классификации возраста на 4 группы: молодой возраст — 20–44 года, средний — 45–59 лет, пожилой — 60–74 года и старческий возраст — 75–89 лет. Концентрацию β-CTX определяли в сыворотке крови методом электрохемилюминесцентного иммуноанализа ECLIA на анализаторе Cobas e 411. **Результаты.** Определено достоверное влияние возраста на вариабельность β-CTX в сыворотке крови: достоверное повышение уровня β-CTX с возрастом у женщин (p < 0,00001) и снижение у мужчин (p < 0,00001). Достоверно более низкий уровень β-CTX обнаружили у женщин в возрасте 20–29 (p < 0,00001) и 30–39 лет (p < 0,00001) по сравнению с мужчинами данного возраста. Однако у женщин 65–69 лет (p = 0,001) выявили достоверно более высокий уровень β-CTX в сыворотке крови по сравнению с мужчинами этой же возрастной группы. **Выводы.** Обнаружили связь уровня β-CTX в сыворотке крови с возрастом: у мужчин он достоверно снижается с возрастом, у женщин — достоверно повышается. У молодых мужчин в возрасте 20–44 года и женщин в возрасте 65–69 лет концентрация β-CTX была достоверно выше по сравнению с лицами противоположного пола этого же возраста. Полученные нами результаты в отношении уровня β-CTX у практически здоровых лиц разного возраста, проживающих на территории нашей страны, могут быть использованы как референтные показатели для населения Украины. **Ключевые слова:** маркеры метаболизма костной ткани; карбокситерминальный телопептид коллагена I типа; возраст; пол