Serum level of N-terminal propeptide of type I procollagen in people of various ages and gender

Abstract. Background. The purpose of the study is to determine the serum level of N-terminal propeptide of type I procollagen (PINP) in healthy men and women of various ages. Materials and methods. The study included 1,568 individuals (1,422 females and 146 males, aged 20-89 years (mean age 60.36 ± 13.68 yrs). All patients were divided into 7 groups, by decades and according to the gerontological age classification: young age – 20–44 yrs, middle age 45–59 yrs, elderly 60–74 yrs, and old 75-89 yrs. During the study, we examined the effect of such demographic characteristics, as age and gender, on serum PINP level, using the electrochemiluminescence immunoassay ECLIA on the cobas e 411 analyzer. Results. We have detected no significant effect of age on the serum variability of PINP in females (F = 1.453, p = 0.19). However, we observed a significant decrease of PINP level in the female age groups of 40–49 yrs (47.74 ± 21.31, p = 0.02), 60–69 yrs (49.76 ± 25.75, p = 0.03), 70–79 yrs (50.49 ± 26.71, p = 0.04), compared with the age group of 20–29 yrs (58.67 ± 27.46). The regression analysis revealed a significant decrease of PINP level with age in young women and men (20–44 yrs). When comparing serum PINP level in the oldest age group (80-89 years), we detected its increase in women (55.20 ± 28.38 ng/ml), compared with the 70–79 years group (50.49 ± 26.71 ng/ml), and decrease in men (54.87 ± 28.24 and 39.16 ± 12.46 ng/ml, respectively). In men, we revealed significant effect of age on the serum variability of PINP (F = 3.077, p = 0.007). Conclusions. The regression analysis showed a significant decrease in PINP level with age in men and women of 20-44 years. In men, we detected a significant effect of age on the variability of serum PINP level. A comparison of serum PINP levels in the oldest age group of 80-89 revealed its increase in women, compared to the 70–79 age group, and decrease in men. The obtained results may be used as reference values for PINP level in serum among representatives of the Ukrainian population of various ages and sexes.

Keywords: bone turnover markers; N-terminal propeptide of type I procollagen; age; sex
All across the world, there has been an active study of the BT remodeling markers, singling out the most informative ones, going on for the 30 recent years [4]. There was an important impetus when an anti-osteoporotic therapy was introduced into the clinical practice in the 1990s, and the OP treatment’s efficacy started to be monitored [3]. Previously, the only bone formation marker widely-used was the total alkaline phosphatase, discovered nearly 90 years ago, early in the last century. However, today it is not considered sensitive enough to testify to the BT formation in the laboratory framework [3, 5, 6].

The BT remodeling markers are divided into two groups: those in charge of the BT formation, and others in charge of its resorption. The formation markers are products of osteoblast activity, detected in the blood serum or plasma: [2]: PINP; serum procollagen I carboxyterminal propeptide (PICP), osteocalcin, alkaline phosphatase (ALP) bone isoenzyme. The resorption markers are primarily the products of collagen destruction: β-Ctx, N-terminal telopeptide of Type I collagen (NTX-I), deoxypyridinoline, hydroxyproline, tartrate-resistant acid phosphatase (TRACP), cathepsin K (cat K), receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) [4, 6, 7].

The aim of our study was to determine the PINP blood serum levels of healthy men and women of various ages.

Materials and methods
The study was based at the State Institution “D.F. Chebotaryov Institute of Gerontology by the N AMS of Ukraine” and the Ukrainian Scientific-Medical Center of Osteoporosis by the NAMS of Ukraine. The study included 1,568 individuals (1,422 females and 146 males, aged 20-89 years (mean age 60.36 ± 13.68 years). All patients were divided into 7 groups, by decades and according to the gerontological age classification: young age - 20–44 years, middle age - 45–59 years, elderly - 60–74 years, and old - 75–89 years.

Among the inclusion criteria, there were the first recourse to the medical institutions and signed informed consent. Among the exclusion criteria, there were: comorbidities influencing the bone metabolism, the second PINP assaying (dynamic indexing), a history of osteotropic medications influencing the bone metabolism, the second PINP assaying in the recent year.

During the study, we examined the effect of such demographic characteristics, as age and gender, on serum PINP level, using the electrochemiluminescence immunoassay ECLIA on the cobas e 411 analyzer. The blood serum assays were performed according to 2017 recommendations on the standardized sample processing and patient PINP assay priming [4]: blood collection at fasting, between 7.30 and 10.00 [8]. In the next 2 hours, the blood was centrifuged in the special vacuum test tubes with a distribution gel. The sample analysis was usually made at the day of collection or within the limits of 3 days after (the centrifuged serum was stored at the temperature -20°C). The day before the collection, the patients were to avoid exhausting physical activities.

Statistical analysis was performed with Statistica 6.0 software. The obtained results were presented as: mean values (M) ± their standard deviations (SD). The critical level of significance in terms of statistical hypotheses’ verification was considered to be 0.05 (p < 0.05). The age’s influence on PINP variability was determined by one-factorial (ANOVA) dispersion analysis. The intergroup differences were evaluated by Scheffe’s test. The interaction among age, sex and PINP serum levels was determined by linear regression analysis.

Results
According to the distribution of the examined subjects into 7 groups by decades (Fig. 1), the study was made of: 66 individuals (47 females and 19 males) aged 20-29 years, 101 individuals (80 females and 21 males) aged 30-39 years, 105 individuals (79 females and 26 males) aged 40-49 years, 358 individuals (334 females and 24 males) aged 50-59 years, 526 individuals (496 females and 30 males) aged 60-69 years, 344 individuals (322 females and 22 males) aged 70-79 years and 68 individuals (64 females and 4 males) aged 80-89 years. The patients were distributed by their gerontological characteristics into the following groups: 212 individuals (158 females and 54 males) of young age, 418 individuals (382 females and 36 males) of middle age, 714 individuals (673 females and 41 males) of elderly age, and 224 individuals (209 females and 15 males) of old age.

There was no significant effect of age on the serum PINP variability; F = 1.45, p = 0.19 (Table 1; Fig. 1). While evaluating the intergroup differences, we found a significant PINP decrease in a group of 40-49 years (47.74 ± 21.31, p = 0.02), 50-69 years (49.76 ± 25.75, p = 0.03), 70–79 years (50.49 ± 26.71, p = 0.04), compared with the group of 20-29 years (58.67 ± 27.46) (Fig. 2 A).

By contrast, for men age played a significant role in the serum PINP variability; F = 3.08, p = 0.007 (Table 1). While evaluating the intergroup differences (Fig. 2 B), we found a significant PINP decrease in a group of 30–39 years (50.44 ± 20.10, p = 0.02), 40–49 years (42.93 ± 14.87, p < 0.001), 50–59 years (49.57 ± 25.03, p = 0.01), 60–69 years (42.31 ± 27.95, p<0.001) and 80–89 years.

Fig. 1. PINP levels in males and females according to their ages
Note: PINP - N-terminal propeptide of type I procollagen.
(39.16 ± 12.46, p = 0.03), compared with the group of 20-29 years (69.18 ± 27.92).

The results of regression analysis of PINP level and age (Fig.3) did not reveal any significant differences in women (r = -0.012, t = -0.44, p = 0.66) and men (r = -0.15, t = -1.87, p = 0.06).

According to the female gerontological distribution (Fig. 4a), the serum PINP level was significantly lower in the age group of 75-89 years (54.17 ± 28.84, p<0.05), compared with the age group of 60-74 years (49.26 ± 25.38, Table 2). The males of 60-74 years had the serum PINP level of 43.85 ± 28.08, (p < 0.05), and it was significantly lower than that of the young subjects — 33.30 ± 6.88 (Fig. 4 B).

According to the regression analysis, PINP was reducing with age in young women (p < 0.05) and men (p < 0.05) (Fig. 5 A, C). Among the women of the oldest group (75-89 years), its level increased with age (Fig. 5 B). By contrast, in men of the same age there was a negative correlation observed between PINP and age (p > 0.05) (Fig. 5 D).

**Discussion**

Organic matrix of the bone tissue is made of 90% collagen type I. Synthesized by osteoblasts in the shape of its precursor — procollagen, it guarantees the bone strength. Type I procollagen has C- (carboxy) and N-(amino) terminal fragments, split away by enzymes — proteinases — with a further collagen formation, joined by the bone matrix, and penetration of C- and N-terminal fragments in the intracellular liquid and blood flow. N-terminal propeptide of type I procollagen (PINP) is the most sensitive marker of the BT

![Fig. 2. The serum PINP levels in males and females of various ages with distribution into decades: (A) - females, (B) - males](image)

**Note:** PINP - N-terminal propeptide of type I procollagen; 7.

**Table 1. The serum PINP levels in men and women of various ages**

| Age, years | Women       | Men         |
|------------|-------------|-------------|
|            | Mean age, years | Number of subjects, n | PINP level, ng/ml | Mean age, years | Number of subjects, n | PINP level, ng/ml |
| 20–29      | 25.83 ± 2.50 | 47          | 58.67 ± 27.46 | 25.84 ± 2.69 | 19          | 69.18 ± 27.92 |
| 30–39      | 35.21 ± 2.92 | 80          | 49.54 ± 26.60 | 34.24 ± 3.28 | 21          | 50.44 ± 20.10 |
| 40–49      | 45.28 ± 2.83 | 79          | 47.74 ± 21.31 | 44.42 ± 3.07 | 26          | 42.93 ± 14.87 |
| 50–59      | 55.59 ± 2.72 | 334         | 51.91 ± 26.82 | 54.21 ± 2.64 | 24          | 49.57 ± 25.03 |
| 60–69      | 64.15 ± 2.80 | 496         | 49.76 ± 25.75 | 64.87 ± 2.03 | 30          | 42.31 ± 27.95 |
| 70–79      | 74.02 ± 2.73 | 322         | 50.49 ± 26.71 | 74.59 ± 3.05 | 22          | 54.87 ± 28.24 |
| 80–89      | 82.25 ± 2.46 | 64          | 55.20 ± 28.38 | 82.50 ± 1.73 | 4           | 39.16 ± 12.46 |
| Total      | 61.24 ± 12.99 | 1422       | 50.85 ± 26.25 | 51.73 ± 16.91 | 146         | 50.08 ± 25.51 |

**Note:** PINP - N-terminal propeptide of type I procollagen.
formation and reflects the bone-formation rate, has a greater stability and diagnostic value than C-terminal fragments (CTF), and it is dissolved in 6-8 minutes by the blood flow. The PINP blood rate is directly proportionate to the rate of produced osteoblasts and built-in collagen [2, 3, 12].

While interpreting the results, one should take into account all the available modified and non-modified: age, sex, a recent history of fracture, long-lasting immobilization, pregnancy, comorbidities and a previous history of anti-osteoporotic medications and some other drugs.

**Table 2. The serum PINP levels in men and women of various ages according to the gerontological classification**

| Age, years | Mean age, years | Number of subjects, n | PINP level, ng/ml | Mean age, years | Number of subjects, n | PINP level, ng/ml |
|------------|----------------|-----------------------|-------------------|----------------|-----------------------|-------------------|
| 20–44      | 33.80 ± 6.37   | 158                   | 51.70 ± 25.77     | 33.30 ± 6.88   | 54                    | 55.18 ± 24.38     |
| 45–59      | 54.54 ± 3.78   | 382                   | 51.47 ± 26.38     | 51.89 ± 4.07   | 36                    | 47.21 ± 22.10     |
| 60–74      | 66.18 ± 4.24   | 673                   | 49.26 ± 25.38     | 66.05 ± 4.13   | 41                    | 43.85 ± 28.08     |
| 75–89      | 78.33 ± 3.14   | 209                   | 54.17 ± 28.84     | 78.60 ± 2.78   | 15                    | 55.66 ± 27.32     |

Note: PINP - N-terminal propeptide of type I procollagen.

**Fig. 3. Regression analysis of interaction between the serum PINP level and age: (A) – females, (B) – males.**

Note: PINP - N-terminal propeptide of type I procollagen. A. PINP (ng/ml) = 52.277 – 0.0234 * AGE (years), r = -0.012, t = -0.44, p = 0.66. B. PINP (ng/ml) = 62.087 – 0.2320 * AGE (years), r = -0.15, t = -1.87, p = 0.06

**Fig. 4. The serum PINP levels in males and females of various ages according to the gerontological classification:**

(A) – females, (B) – males.

Note: PINP - N-terminal propeptide of type I procollagen; 1 – subjects of 20-44 years, 2 – 45-59 years, 3 – 60-74 years, 4 – 75-89 years.
For most BT metabolism markers, the acrophase, or peak time of the highest blood rate, is similar for both sexes [13]. The BT formation marker rate in the newly-born and neonates is at its highest, compared to other age groups. By the pubertal age, their level drops down. At the beginning of sexual maturation (by Tanner’s classification - I-III), the rate of markers are higher, and afterwards it starts to decline, earlier for girls, later for boys [4, 14]. According to the reference data, among the juniors the PINP rate is higher in men than in women. It is attributed to a larger BT volume and mass of men (taking into account their height and constitution) and, thus, to a faster formation and resorption of larger bones [15, 14]. Our findings did not reveal a significant PINP rate’s increase in men compared to women (men – 69.18 ± 27.92, women – 58.67 ± 27.46). The absence of significant difference is, to our mind, explained by a small number of the young examined male subjects (n = 19).

With advancing age, the BT metabolism markers increase in men at a more significant rate, than in women [14]. Our findings confirm that among the older women (75-89 years) there was an increase of blood PINP blood rates with age; however, among the similarly-aged men there was an opposite tendency. These results may be associated with a small sample of old men (n = 15) and an “assumed” low average life expectancy of male population in Ukraine. According to the 2018 data by the Statistical Service of Ukraine, an average life expectancy of men is 66.69 years while an average life expectancy of women is 76.72 years [10]. It is lower than the European one: where an average life expectancy of men is 78.3 years and an average life expectancy of women is 83.5 years [11]. J. E. Aaron et al. described the BT histomorphic alterations in men and women. The BT trabecular volume was similar for both sexes; however, it differed by the histological parameters. The men’s principal BT loss factors were attributed to the reduced formation, and the women’s BT loss remained unchanged. These findings enabled an assumption that the BT alterations in women is caused by an amplified resorption. The BT loss in women is primarily associated with the destruction of individual trabeculae, by contrast to the one in men who are subject to a generalized trabecular wearing-down, though it has a lower effect on mineral density and the BT quality [16]. Furthermore,
high BT metabolism markers reveal bone microarchitecture degradation and the BT loss in men less frequently than in women. However, it may be suggested that the BT metabolism marker alterations are no predictors of fracture in men [14].

The blood serum PINP level grows during menopause; however, its inner individual variations in the peri- and post-menopausal women are less than 10%. Our findings reveal an increase of the blood serum PINP level in the age group of 50-59 years (51.91 ± 26.82, p = 0.03), corresponding to the late postmenopausal period in Ukraine; the mean age of menopause being 48.7 years [9]. At the same time, as in case of older men, its level remains stable or increases insignificantly, usually after 70 years [4, 14, 17]. The post-menopausal women have a higher PINP level than men of a similar age.

According to the reference data, the BT formation marker level is often affected by the seasonality. It is claimed that in winter their level is higher, which is attributed to the vitamin D deficiency [13]. The serum PINP level is often affected by the seasonality. It is claimed that in winter their level is higher, which is attributed to the vitamin D deficiency [13].

The PINP level is higher during the lutein stage of the menstrual cycle than during the follicular stage. Szulc P. et al. (2017) recommend that the women of reproductive age should be subject to the blood sampling during the follicular stage, lasting on average from the 1st to 14th day of the menstrual cycle [4].

During pregnancy, the bone formation marker level remains intact during the first two trimesters and rapidly increases during the third trimester. After the labors, their concentration rapidly drops down; however, it remains elevated during the postpartum period, compared to the non-pregnant women of a similar age. The lactating women have a higher PINP level, compared to the non-lactating ones [4, 18].

The serum PINP level grows dramatically during the first weeks after the fracture (by 150 % from the basic level). It reaches its maximum 12 weeks after fracture with a further decrease. The elevated PINP concentration is preserved for more than 1 year. The fractures with a larger scale of destruction and consolidation (for instance, pertrochanteric fracture) are associated with a higher BT formation and resorption markers alike. Immobilization also results in an elevated serum PINP level [4, 14].

The influence of physical activity on the PINP level depends on the sports subjects are involved in, duration and intensity of physical activity. The women who are into swimming did not report any alterations of BT metabolism markers [14], confirming the fact that the physical exercises do not affect the PINP level significantly [19].

The BT metabolism marker rates depend on the body weight. The studies show that the BT formation markers are lower in the obese people, compared to subjects with a normal BMI [14].

Disorders of the BT formation and resorption balance is observed with some endocrine diseases (hypothyroidism, hypoparathyroidism, hypopituitarism, growth hormone deficiency) and HIV-infection. The PINP level is inversely correlated to the fast glomerular filtration rate, or GFR, in patients with a chronic renal insufficiency. The oral and parenteral corticosteroids depending on their doses are suppressing the bone tissue formation, unlike the inhalers affecting the BT metabolism level [4].

The women using oral estrogen-based contraceptives had the BT formation marker levels which were lower by 15-25 %; however, medroxyprogesterone acetate raises the PINP level insignificantly [4].

The PINP's advantage over other BT formation markers is its minimal circadian variability and the fact that unlike others, it is not affected by the food intake, enabling random blood samplings during the day [4].

Our study had a certain limitation: there were few men in the age group of 20-29 (n = 19) and 80-89 years (n = 4), complicating the interpretation of findings.

Conclusions
According to the regression analysis, the PINP level decreased significantly with age, both in men and women of 20-44 years. A considerable age-related effect was revealed on the blood serum PINP’S variability in men. While comparing the PINP’s levels in the oldest group of 80-89 years, we found its reduction in women by comparison with the group of 70-79 years as well as its reduction in men. The obtained findings may be used as reference data on the serum PINP level in the Ukrainian patients of various ages and sexes.

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.

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Рівень N-термінального пропептиду проколагену І типу в сироватці крові в осіб різного віку та статі

Метою дослідження є визначення рівня N-термінального пропептиду проколагену І типу (PINP) у сироватці крові здорових чоловіків та жінок різного віку. Матеріал та методи. У дослідженнях були включені 1568 осіб (1422 особи жіночої та 146 — чоловічої статі) віком 20—89 років (середній вік — 60,36 ± 13,68 року). Усіх обстежених осіб розподілили на 7 груп відповідно до десятиріччя та генеративного пропептиду проколагену I типу (PINP) у сироватці крові (F = 3,08, p = 0,007). Висновки. За результатами регресійного аналізу встановлено зменшення рівня PINP в осіб з віком 70—79 років (54,87 ± 28,41 нг/мл та 39,16 ± 12,46 нг/мл відповідно). У чоловіків доведено вірогідний вплив віку на варіабельність рівня PINP у сироватці крові (F = 3,08, p = 0,007). Резюме. Результати дослідження в жінок не відповідають вірогідному впливу віку на варіабельність PINP у сироватці крові (F = 1,453, p = 0,19). Виявлено збільшення рівня PINP у жінок віком від 70—79 років (47,74 ± 21,31 нг/мл та 50,49 ± 28,74 нг/мл відповідно). В середньому рівень PINP у жінок залежить від віку та статі. Ключові слова: PINP — N-термінальний пропептид проколагену І типу в сироватці крові; вік; статя.
Уровень N-термінального пропептида проколлагена I типу в сыворотці крові у людей разного віку та статі

Резюме. Цілью дослідження є визначення рівня N-термінального пропептида проколлагена I типу (PINP) в сыворотці крові у здорових чоловіків та жінок усіх вікових груп.

Матеріали та методи. В дослідження входили 1568 осіб (1422 жінки та 146 чоловіків) віком від 20 до 89 років (середній вік 60,36 ± 13,68 років). Все населення було розділено на 7 груп, відповідно до десятиліттях та геронтологічної класифікації віку: молодий вік — від 20 до 44 років, середній вік — від 45 до 59 років, пожільний вік — від 60 до 74 років, і старческий вік — від 75 до 89 років. В процесі дослідження його положення в сыворотці крові методом електрохемілюмінесцентного імунометрії ECLIA на аналізаторі cobas e 411.

Результати. В результаті дослідження не було виявлено значної відповідності PINP в сыворотці крові за віком та статтю (F = 1,453, р = 0,19). Виявлено достовірне зниження PINP в групах від 40 до 49 років (47,74 ± 21,31, р = 0,02), 60 до 69 років (49,76 ± 25,75, р = 0,03), та 70 до 79 років (50,49 ± 26,71, р = 0,04), порівняно з групою від 20 до 29 років (58,67 ± 27,46). Застосування регресійного аналізу виявило достовірне зниження PINP з різноманіттям у жінок та чоловіків 20—44 роки.

Висновки. Результати регресійного аналізу виявили достовірне зниження PINP з різноманіттям у жінок та чоловіків 20—44 роки. Дослідження виявило, що зниження PINP зростає у жінок та чоловіків 20—44 роки. Наші результати можуть бути використані як підстави для зміни PINP в сыворотці крові у представників української популіції, різного віку та статі.

Ключові слова: маркери формування та резорбції костної тканини; N-термінальний пропептид проколлагена I типу; вік; статта.