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RESEARCH ARTICLE

Li et al: Diagnostic value of 5-HT precursors for PMOP

Plasma serotonin precursors and metabolites as diagnostic and therapeutic biomarkers for osteoporosis in postmenopausal women

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ABSTRACT

This study aims to evaluate the diagnostic and therapeutic potential of plasma 5-hydroxytryptamine (5-HT) precursors and metabolites in postmenopausal osteoporosis (PMOP). A total of 287 consecutive postmenopausal women were retrospectively enrolled. Data including age, body mass index (BMI), serum calcium, serum phosphorus, menopausal duration, and bone mineral density (BMD) of the lumbar spine and femoral neck, as well as serum and plasma samples were collected. Based on BMD measurements, participants were categorized into normal, osteopenia, and osteoporosis (OP) groups. Serum β-CTX and PINP, along with plasma levels of 5-HT precursors

and metabolites, were measured using ELISA. Receiver operating characteristic (ROC) curve analysis, multivariate analysis, and Kaplan-Meier curves were employed to assess the predictive value of 5-HT precursors and metabolites in PMOP and to evaluate the association between their expression levels and PMOP risk. Plasma levels of 5-hydroxytryptophan (5-HTP), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were elevated in PMOP patients and showed correlations with bone turnover markers and BMD. These biomarkers were identified as independent risk factors for PMOP. Combined analysis of the three biomarkers demonstrated greater predictive value than individual markers. Elevated levels were particularly pronounced in women with ≥ 12 years since menopause (YSM), and were associated with a higher risk of developing PMOP. In summary, 5-HT precursors and metabolites are significantly associated with bone turnover and BMD in postmenopausal women. They serve as independent risk factors and show strong predictive value for PMOP, suggesting their potential as plasma biomarkers for diagnosis and treatment. Furthermore, their relationship with YSM highlights their promise as therapeutic targets to delay the onset of osteoporosis in postmenopausal women.

Keywords: Postmenopausal women; plasma; 5-hydroxytryptophan; 5-HTP; 5-hydroxytryptamine; 5-HT; 5-hydroxyindoleacetic acid; 5-HIAA; osteoporosis; diagnosis; target.

INTRODUCTION

Osteoporosis (OP) is a metabolic bone disorder associated with compromised bone strength and an increased risk of fracture, particularly in postmenopausal females [1]. Hormone levels, aging, genetic susceptibility and immune microenvironment are critically involved in the occurrence of postmenopausal osteoporosis (PMOP), among which, estrogen deficiency has long been considered to be the major cause [2-4]. Of note, fragility fracture, especially hip fracture, represents the most frequent and severe complication of PMOP, which brings great pain and substantial economic burden to patients [5]. With the increase in the aging population, the incidence of PMOP has risen considerably worldwide [6]. Accordingly, further exploration of novel therapeutic targets in PMOP for the development of more effective treatment strategies is imperative.

5-hydroxytryptamine (5-HT) represents a crucial monoamine neurotransmitter, which can be allocated into peripheral 5-HT and central 5-HT dependent on its production site [7]. Recently, a growing number of studies have documented the negative impacts of peripheral 5-HT on osteogenesis, such as containment of osteogenesis, and facilitation of bone resorption [8-10]. Nevertheless, early relevant studies mainly concentrated on the roles of peripheral 5-HT in respiratory function, vascular tone, gastrointestinal inflammation, and hemostasis until a breakthrough was made in the pathogenesis of osteoporosis-pseudoglioma syndrome (OPPG) [11]. OPPG, a condition manifested as blindness and OP, is an event triggered by the dysfunction of low-density lipoprotein receptor-related protein 5 (LRP5) gene [12]. In a similar vein, gain of function mutations in LRP5 results in an increase of bone mass [13]. These two disorders lead to completely converse skeletal phenotypes, but both suggest a pivotal role of LRP5 in the modulation of bone metabolism [14]. Experiment findings in animals have previously validated that the LRP5 loss of function-caused OP is a consequence of elevated peripheral 5-HT levels. After knockout of two alleles of LRP5 in mice, there is a tendency to increase in the tryptophan hydroxylase 1 expression in the intestinal tissues of mice, and the level of peripheral 5-HT; conversely, bone mineral density (BMD) is reduced, and bone microarchitecture is disrupted, along with decreased proliferation and differentiation rates of mouse primary osteoblasts [15]. We thus hypothesized that peripheral 5-HT might act on the relevant receptors on the bone tissues directly to restrain osteogenesis and contribute to bone resorption.

The 5-HT signaling has two distinct functional effects on osteoblasts: on one hand, brain 5-HT serves as a neurotransmitter to stimulate bone formation and suppress bone resorption; on the other hand, peripheral 5-HT acts as a hormone to boost bone remodeling and repress bone formation [16]. Notably, 5-HT plays an inhibitory role in cell proliferation in mouse osteoblasts via the transcription factor cyclic adenosine monophosphate responsive element-binding protein and 5-HT receptor 1B

[17]. 5-hydroxytryptophan (5-HTP) is an aromatic amino acid produced from the essential amino acid L-tryptophan by tryptophan hydroxylase [18]. As the precursor of 5-HT, 5-HTP is accepted as a dietary supplement frequently used to elevate the level of 5-HT [19]. Furthermore, 5-hydroxyindole acetic acid (5-HIAA) is the major 5-HT metabolite [20] and has a negative correlation with femur stiffness in rats with chronic kidney disease [21]. However, to date, there are no available data on the involvement of 5-HT precursors and metabolites in PMOP. This study included postmenopausal women who had normal bone mass, osteopenia and OP, with the aim to investigate the changes in plasma levels of 5-HT precursors and metabolites among these women, as well as their correlations with the bone turnover and their diagnostic value for the occurrence of PMOP.

MATERIALS AND METHODS

Sample size estimation

The sample size estimation was performed using GPower 3.0.10 software (Dusseldorf University, Dusseldorf, Germany). The following parameters were used: power = 0.80, α = 0.05, effect size = 0.25, number of groups = 3. F-tests [analysis of variance (ANOVA): fixed effects, omnibus, one-way) were used to calculate the sample size. The results indicated that the minimum sample size required was 159 (Supplementary figure 1).

Study subjects

This was a single-center, retrospective cohort study on 329 consecutive PM women admitted to The Tenth Affiliated Hospital, Southern Medical University (Dongguan People's Hospital) between June 2020 and April 2024. After applying the inclusion and exclusion criteria, 287 PM women were finally included. Following the BMD measurement outcomes, they were assigned to the normal group (n = 79), osteopenia group (n = 93) and OP group (n = 115). A BMD T-value of ≥ -1.0 was seen as normal bone mass, -2.5 to -1.0 as osteopenia, and ≤ -2.5 as OP [22].

Inclusion and exclusion criteria

Inclusion criteria were as below: 1) diagnosed as PM women; 2) meeting OP-associated diagnostic criteria [23] and diagnosed with OP for the first time [based on the results of BMD measurement by dual-energy X-ray absorptiometry (DXA), where the T-value/Z-value of the BMD determined by DXA was ≤ -2.5]; 3) complete clinical data.

Exclusion criteria were as follows: 1) comorbidities of other chronic disorders or endocrine disorders influencing bone metabolism, comprising malignant tumors, hyperthyroidism and diabetes mellitus; 2) receiving relevant treatments affecting bone turnover within 3 months; 3) administration of medications influencing bone metabolism, such as calcium-containing medications, bisphosphonates, androgen, estrogen, vitamin D and glucocorticosteroids; 4)

dysfunctions of important organs such as the kidney and liver.

Clinical data collection

The basic characteristics of the enrolled subjects were collected, including age, serum calcium, body mass index (BMI), years since menopause (YSM), serum phosphorus, and the BMD of femoral neck and lumbar spine that was assessed utilizing DXA (Discovery W; Hologic, Waltham, MA, USA). Menopause was defined as the permanent cessation of menstruation (in the absence of pregnancy) due to the failure of ovarian function. The menopause status was ascertained as the cessation of menstruation over the past 12 months since the last menstrual period in women over 40 years old based on their self-report, medical records, or both. A 6 mL fasting venous blood sample was collected from the elbow of each eligible subject on admission and divided into two equally parts. One portion was kept in collection tubes without anticoagulant and left to stand for 30 min at room temperature for spontaneous agglutination. Subsequently, the blood sample was centrifuged at 2000 r/min for 20 min, with the serum obtained and stored at -80°C. The other portion was preserved in anticoagulant-containing collection tubes. Then, the blood sample was mixed with the anticoagulant and the mixture was allowed to stand for 1 h. After centrifugation, the supernatant (plasma) was collected and stored at -80°C.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of procollagen type I N-propeptide (PINP) and β-C-terminal telopeptide of type 1 collagen (β-CTX) were examined by ELISA using an FK-SY96S multi-functional ELISA analyzer (Fangke instruments Co., Ltd., Weifang, Shandong, China). The kits were obtained from Mlbio (Shanghai, China). Human Trp (Jianglaibio, Shanghai, China), 5-HIAA, 5-HT and 5-HTP ELISA kits (Camilo, Nanjing, Jiangsu, China) were applied for assessing the plasma Trp, 5-HIAA, 5-HT and 5-HTP levels according to the manufacturer’s instructions.

Ethical statement

This study was approved by the Ethics Committee of The Tenth Affiliated Hospital, Southern Medical University (Dongguan People's Hospital) and conducted in accordance with principles of the *Declaration of Helsinki*. The study involved the use of existing data from medical records, where informed consent had been exempted during the original data collection. As per the ethical guidelines and the approval from the Institutional Review Board (IRB) at The Tenth Affiliated Hospital, Southern Medical University (Dongguan People's Hospital), informed consent for the current study was waived.

Statistical analysis

Statistical analyses and graphing of data were conducted using SPSS 27.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.5 software (GraphPad Software, San Diego, CA, USA). Normally

distributed measurement data tested by the Kolmogorov-Smirnov test were expressed as mean ± standard deviation. One-way ANOVA with a Tukey post-hoc analysis was used for comparisons involving multiple groups. Pearson's analysis test was applied for analyzing the correlations of 5-HTP, Trp, 5-HIAA and 5-HT with PINP, β-CTX, femoral neck BMD and lumbar spine BMD in PMOP patients. A multivariate logistic regression model was established to determine the independent risk factors of PMOP. Receiver operating characteristic (ROC) curves were plotted to evaluate the predictive value of 5-HTP, 5-HT and 5-HIAA, alone or in combination for PMOP. Diagnostic accuracy analysis was performed using MedCalc statistical software (20.0.15, MedCalc Software Ltd., Ostend, Belgium) with a DeLong test used to compare the differences in areas under the curve (AUCs) in ROC analysis. The effects of the expression differences of 5-HT precursors and metabolites on the risk of PMOP occurrence were analyzed by Kapan-Meier curves. A two-tailed test with $P < 0.05$ indicated a significant difference.

RESULTS

Comparisons of the baseline characteristics of patients in the normal, OP and osteopenia groups

The enrolled PM women were grouped into the normal, OP and osteopenia groups based on the results of BMD measurements. In terms of BMI, age, and serum calcium and serum phosphorus levels, the three groups represented no statistically noticeable variations (all $P > 0.05$); by contrast, YSM, and PINP and β-CTX levels were higher while femoral neck BMD, and lumbar spine BMD were lower in the OP group than in the normal and osteopenia groups (all $P < 0.01$) (Table 1).

Trp, 5-HT, 5-HTP and 5-HIAA levels were augmented in the plasma of PMOP patients, and prominently correlated with bone turnover markers (BTMs)

We then compared the plasma levels of 5-HTP, Trp, 5-HT and 5-HIAA among the three groups and unveiled enhancements in the levels of these indicators in the OP group relative to the osteopenia and normal groups, with the former showing higher levels than the latter (all $P < 0.05$, Figure 1A-D). Further, Pearson's correlation analysis implied that plasma 5-HT, Trp, 5-HTP and 5-HIAA had positive correlations with PINP and β-CTX in patients with PMOP, and negative correlations with femoral neck BMD and lumbar spine BMD (all $P < 0.05$, Table 2).

Elevated levels of 5-HT precursors and metabolites were independent risk factors for the occurrence of OP in PM women

To precisely appraise the impacts of 5-HT precursor and metabolite levels on the occurrence of OP in PM women, we performed multivariate logistic regression analyses by including the PMOP occurrence (0 = no, 1 = yes) as the dependent variable, and YSM, 5-HIAA, 5-HTP, Trp, 5-HT, PINP, β-CTX, femoral neck BMD and lumbar spine BMD as the independent variables. Results showed that increased YSM and elevated levels of β-CTX, PINP, 5-HIAA, YSM, 5-HTP and 5-HT were

independent risk factors for the occurrence of PMOP, whilst high lumbar spine BMD and femoral neck BMD were protective factors (Table 3).

5-HT precursors and metabolites provided diagnostic value for PMOP

Subsequently, we further delved into the predictive value of 5-HT precursor and metabolite levels for diagnosing PMOP. ROC curve analysis demonstrated that the AUCs of 5-HTP, 5-HT and 5-HIAA in diagnosing PMOP were 0.800 (95% CI: 0.749~0.845), 0.864 (95% CI: 0.819~0.902) and 0.827 (95% CI: 0.778~0.869), with the cut-off values of 76.02, 73.03 and 26.68, sensitivity of 89.57%, 86.09% and 90.43%, and specificity of 55.81%, 72.67% and 63.37%, respectively (Table 4, Figure 2A-C). Moreover, the AUC of the combined analysis of 5-HT precursors and metabolites for predicting PMOP was superior to that of each indicator alone (all $P < 0.05$, Table 4, Figure 2D-E). The above results support the evidence that 5-HT precursors and metabolites have high diagnostic value for PMOP. In addition, 5-HT precursors combined with the metabolites provided added diagnostic value above the 5-HT precursors and metabolites alone for the PMOP occurrence.

High levels of 5-HT precursors and metabolites increased the risk of PMOP

Finally, we categorized patients into the YSM ≥ 12 years group (n = 168) and the YSM < 12 years group (n = 119) using the median value of YSM as the cut-off value, and then compared their plasma levels of 5-HTP, 5-HT and 5-HIAA. The results showed higher plasma levels of 5-HTP, 5-HT and 5-HIAA in the YSM ≥ 12 years group than in the YSM < 12 years group (all $p < 0.05$, Table 5). In addition, patients were divided into the high and low expression groups based on the ROC cut-off values of 5-HT precursors and metabolites (76.02 ng/mL, 73.03 ng/mL, or ≥ 26.68 ng/mL). The Kapan-Meier curve was employed to analyze the risk of PMOP occurrence in patients with different expression patterns of 5-HT precursors and metabolites. The patients with plasma 5-HTP ≥ 76.02 ng/mL, 5-HT ≥ 73.03 ng/mL or 5-HIAA ≥ 26.68 ng/mL had curves that shifted to the left relative to those with low levels of 5-HTP, 5-HT and 5-HIAA (all $P < 0.001$, Figure 3A-C). This suggested that the levels of 5-HTP, 5-HT and 5-HIAA were elevated as the YSM increased and their elevated levels (5-HTP: HR = 2.898; 5-HT: HR = 3.349; 5-HIAA: HR = 3.195) noticeably shortened the YSM for PMOP and amplified the risk of PMOP occurrence. Overall, these results provide a potential target for prolonging the postmenopausal time for PMOP occurrence.

DISCUSSION

PMOP places tremendous financial and physical burdens on aging females, with an estimation of 1/3 of females over 50 years old having OP-triggered fractures [24]. Emerging evidence supports the contribution of 5-HT to the onset and progression of OP by modulating bone mass [25, 26]. Herein, our study highlighted that changes in the plasma levels of 5-HT precursors and metabolites aided in the diagnosis of OP.

BMD is defined as the amount of mineral per unit of bone and is used as an important determinant of bone strength and a primary indicator of osteopenia or OP [27]. Patients with OP have reduced BMD and bone strength, which is associated with an increased fracture risk under low-energy injuries [28]. Besides, biochemical BTMs, including β-CTX and PINP, are augmented in OP women with fracture [29]. Specifically, serum β-CTX is a well-established marker of bone loss and bone resorption, while serum PINP and BALP are markers of bone formation and osteoblast viability [30, 31]. OP patients have been found to exhibit elevated levels of PINP and β-CTX, suggesting a high turnover in bone metabolism [32]. In this study, our observations demonstrated that PMOP patients had higher levels of β-CTX, YSM and PINP, and lower femoral neck BMD and lumbar spine BMD than those with normal bone mass and osteopenia. However, there was no statistically significant difference in terms of BMI, age, or serum calcium and serum phosphorus levels among the normal, OP and osteopenia groups. PMOP is characterized by reduced bone mass and mineral density that are attributable to estrogen deficiency, genetics, smoking, diet, exercise, alcohol consumption, advanced age, weight loss, and calcium absorption [2, 3], as well as alterations in immune status [4]. However, BMD is not directly related to BMI, age, and serum calcium and serum phosphorus levels [33]. In fact, changes in the bone mass in postmenopausal women are mainly affected by estrogen levels, osteogenic differentiation, osteoclast activity and other factors [2, 34, 35]. In our study, there was no statistically significant difference in terms of BMI, age, or serum calcium and serum phosphorus levels among the normal, OP and osteopenia groups. The reason may be that these indicators are not factors directly influencing osteopenia or OP. On the other hand, our study was a retrospective study conducted in a single center with a relatively small sample size, which might also affect the analysis results. Additionally, in the present study, we discovered higher levels of 5-HTP, Trp, 5-HIAA and 5-HT in the plasma of PMOP patients, than those in the PM subjects with osteopenia and normal bone mass. To the best of our knowledge, this is the first study to reveal the significant alterations in the levels of 5-HT precursors and metabolites in PMOP.

BTMs change sensitively and reflect bone formation and bone resorption; an increase of which is often associated with an increased risk of fracture [36, 37]. 5-HT is a key regulator of bone turnover [38]. There is also evidence suggesting that PMOP patients with high 5-HT, 5-HIAA and 5-HTP levels are more likely to have augmented PINP and β-CTX levels; in addition, the 5-HT, 5-HIAA and 5-HTP are negatively correlated with BMD and positively correlated with PINP and β-CTX [39]. Furthermore, selective inhibition of 5-HT is a major contributing factor to BTM reductions and the reduced facture risk [39-44]. These studies directly support our findings that the levels of 5-HIAA, 5-HT, and 5-HTP had a positive association with β-CTX and PINP levels, and negative

association with lumbar spine BMD and femoral neck BMD in the plasma of PMOP patients. Altogether, the findings of the current study taken together with those of previous studies add values to the use of plasms 5-HIAA, 5-HT, and 5-HTP in the determination of occurrence of PMOP. Our multivariate regression analysis further disclosed that increased YSM and elevated levels of 5-HT precursors and metabolites, as well as β-CTX and PINP were independent risk factors for PMOP. By contrast, high lumbar spine BMD and femoral neck BMD were protective. Notably, elevated plasma levels of 5-HT precursors and metabolites led to shortened YSM for PMOP and increased the risk of OP in postmenopausal women. It has been reported that PM subjects with OP, osteopenia and normal bone mass have different YSM [45]. YSM demonstrates close association with the osteoporotic fractures in PM women [46]. In addition, low total hip and lumbar spine BMD are confirmed to be risk factors for recurrent and primary fractures in women with PMOP [47]. For men and women with OP, PINP and β-CTX levels in serum are proven to be independent risk factors [48]. There have been relatively fewer reports regarding the predictive value of 5-HT precursors and metabolites for diagnosing PMOP, and we for the first time uncovered that 5-HT precursors and metabolites could serve as independent risk factors that were associated with PMOP occurrence. With future study, these markers in combination may be helpful to further gauge PMOP risk.

CONCLUSION

This study uncovered that 5-HT precursors and metabolites were significantly correlated to bone turnover indicators and BMD. They were independent risk factors for the occurrence of PMOP. Elevated plasma levels of 5-HT precursors and metabolites strikingly shortened the YSM for PMOP and increased the risk of OP in postmenopausal women. These observations highlight the clinical potential of 5-HT precursors and metabolites as plasma biomarkers for the diagnosis and treatment for PMOP. Meanwhile, this study provides potential targets for delaying the onset of OP in postmenopausal women in terms of YSM. Nonetheless, there are several limitations in the current study. First, this study was a retrospective study conducted in a single center with a relatively small sample size. Hence, subsequent studies involving multiple centers and a larger sample size should form objectives in future studies. Second, YSM is one of the potential influencing factors for PMOP but we failed to conduct relevant research on the effect of menopause duration on PMOP occurrence because of the limited time and work arrangement. Furthermore, due to time and funding constraints, the mechanisms of 5-HT precursors and metabolites at the cellular and molecular levels have not been characterized, which will be explored in future studies.

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Data availability statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Comparisons of baseline characteristics of patients in the normal, osteopenia and OP groups

Clinical data	Normal group (n = 79)	Osteopenia group (n = 93)	OP group (n = 115)	*P*
Age (years)	61.80 ± 4.38	62.09 ± 5.09	62.43 ± 6.04	0.715
BMI (kg/m^2)	24.09 ± 2.53	23.68 ± 2.47	23.54 ± 2.96	0.372
YSM (years)	10.58 ± 3.07	11.00 (2.00, 23.00)	14.09 ± 3.56^{aabb}	< 0.001
Lumbar spine BMD (g/cm^2)	1.03 ± 0.15	0.87 ± 0.14^{aa}	0.80 ± 0.10^{aabb}	< 0.001
Femoral neck BMD (g/cm^2)	0.95 ± 0.11	0.86 ± 0.11^{aa}	0.78 ± 0.09^{aabb}	< 0.001
Serum phosphorus (mmol/L)	1.05 ± 0.12	1.04 ± 0.14	1.02 ± 0.11	0.262
Serum calcium (mmol/L)	2.35 ± 0.16	2.33 ± 0.11	2.32 ± 0.14	0.329
β-CTX (ng/mL)	0.34 ± 0.11	0.37 (0.06, 0.59)	0.46 ± 0.13^{aabb}	< 0.001
PINP (ng/mL)	33.25 ± 5.36	36.89 ± 6.45^{aa}	47.51 ± 8.05^{aabb}	< 0.001

BMI, body mass index; BMD, bone mineral density; YSM, years since menopause; β-CTX, β-C-terminal telopeptide of type 1 collagen; PINP, procollagen type I N-propeptide. Measurement data conforming to normal distribution were depicted as mean ± standard deviation. One-way ANOVA was employed for analyzing inter-group comparisons, followed by Tukey's multiple comparison test. Measurement data that did not display normal distribution were presented as median (minimum value-maximum value) and compared by the Kruskal-Wallis with Dunn's multiple comparison test. a $p < 0.05$, aa $p < 0.01$, *vs.* the normal group. b $p < 0.05$, bb $p < 0.01$, *vs.* the osteopenia group.

Table 2. Analyses on the correlations of plasma 5-HT precursors and metabolites with BTMs and BMD in patients with PMOP

Indicators	Trp		5-HTP		5-HT		5-HIAA	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value
Lumbar spine BMD	- 0.418	< 0.001	- 0.429	< 0.001	- 0.533	< 0.001	- 0.477	< 0.001
Femoral neck BMD	- 0.208	0.026	- 0.422	< 0.001	- 0.323	< 0.001	- 0.398	< 0.001
β-CTX	0.477	< 0.001	0.343	< 0.001	0.565	< 0.001	0.422	< 0.001
PINP	0.499	< 0.001	0.562	< 0.001	0.766	< 0.001	0.631	< 0.001

5-HT, serotonin; BMD, bone mineral density; PINP, procollagen type I N-propeptide; β-CTX, β-C-terminal telopeptide of type 1 collagen; Trp, tryptophan; 5-HTP, 5-hydroxytryptophane; 5-HIAA, 5-hydroxyindoleacetic acid.

Table 3. Multivariate logistic regression analyses on the OP occurrence in PM women

Independent variable	*P* value	OR value	95%CI

YSM	< 0.001	1.426	1.242~1.637
Trp	0.795	1.012	0.926~1.105
5-HTP	0.046	1.057	1.001~1.116
5-HT	0.039	1.046	1.002~1.092
5-HIAA	0.029	1.049	1.005~1.095
β-CTX	0.045	25.441	1.074~602.398
PINP	0.001	1.132	1.049~1.222
Lumbar spine BMD	0.043	0.026	0.001~0.894
Femoral neck BMD	0.041	0.014	0.000~0.839

YSM, years since menopause; BMD, bone mineral density; PINP, procollagen type I N-propeptide; β-CTX, β-C-terminal telopeptide of type 1 collagen; Trp, tryptophan; 5-HTP, 5-hydroxytryptophane; 5-HIAA, 5-hydroxyindoleacetic acid.

Table 4. Comparative analyses on AUCs of the 5-HT precursors and metabolites alone and joint detection for predicting PMOP

Items	AUC	95%CI	Sensitivity	Specificity
5-HTP	0.800	0.749~0.845	89.57	55.81
5-HT	0.864	0.819~0.902	86.09	72.67
5-HIAA	0.827	0.778~0.869	90.43	63.37
combination	0.903	0.862~0.934	66.96	94.19
5-HTP *vs.* combination	$P < 0.001$			
5-HT *vs.* combination	$P < 0.001$			
5-HIAA *vs.* combination	$P < 0.001$			

The differences of AUC of multiple ROC curves were compared using the Delong test in MEDCALC 20.0.15 software (MedCalc software, Ostend, Belgium). Combination represented the combined analysis of of 5-HTP, 5-HT and 5-HIAA.

Table 5. Comparison of 5-HTP, 5-HT and 5-HIAA levels in the YSM ≥ 12 years group and the YSM < 12 years group

Item	YSM ≥ 12 years group (n = 168)	YSM < 12 years group (n = 119)	*P* value
5-HTP (ng/mL)	81.02 ± 10.08	77.30 ± 10.93	0.0031
5-HT (ng/mL)	75.74 ± 13.12	72.41 ± 12.65	0.0325
5-HIAA (ng/mL)	31.48 ± 10.30	21.79 (2.56, 52.23)	< 0.0001

Measurement data conforming to normal distribution were expressed as mean ± standard deviation. Comparison between two groups was performed using unpaired *t* test. Measurement data that do not display normal distribution are presented as median (minimum value-maximum value) and compared by Mann-Whitney test.

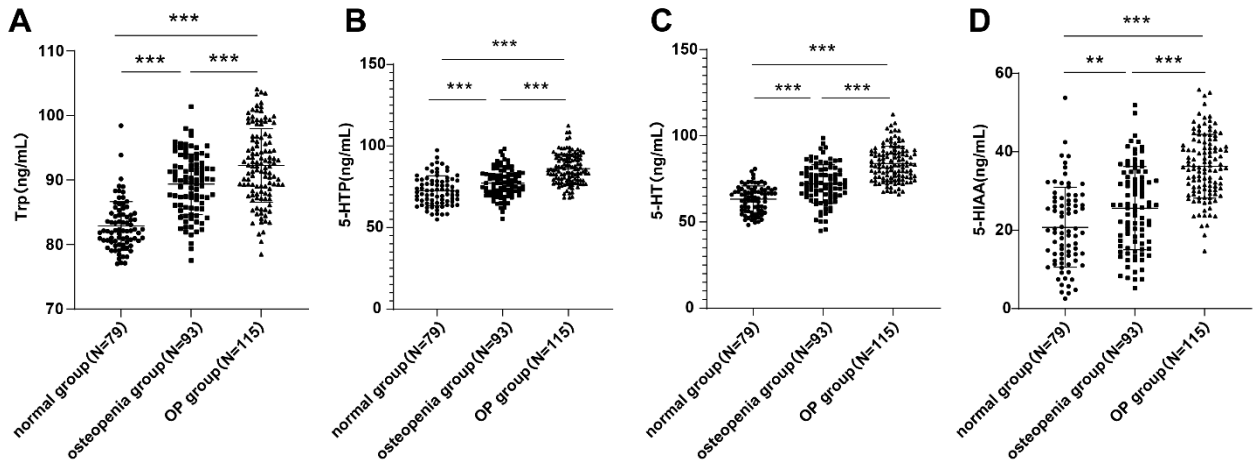

Figure 1. Plasma levels of 5-HT, Trp, 5-HTP and 5-HIAA in PM women among the normal, osteopenia and OP groups. Levels of Trp, 5-HT, 5-HTP and 5-HIAA were assessed by ELISA and the differences in plasma levels of Trp (A), 5-HTP (B), 5-HT (C) and 5-HIAA (D) were compared among the normal, osteopenia and OP groups. Data were presented as mean ± standard deviation, and compared by one-way ANOVA with Tukey's post hoc test. *** $P < 0.001$.

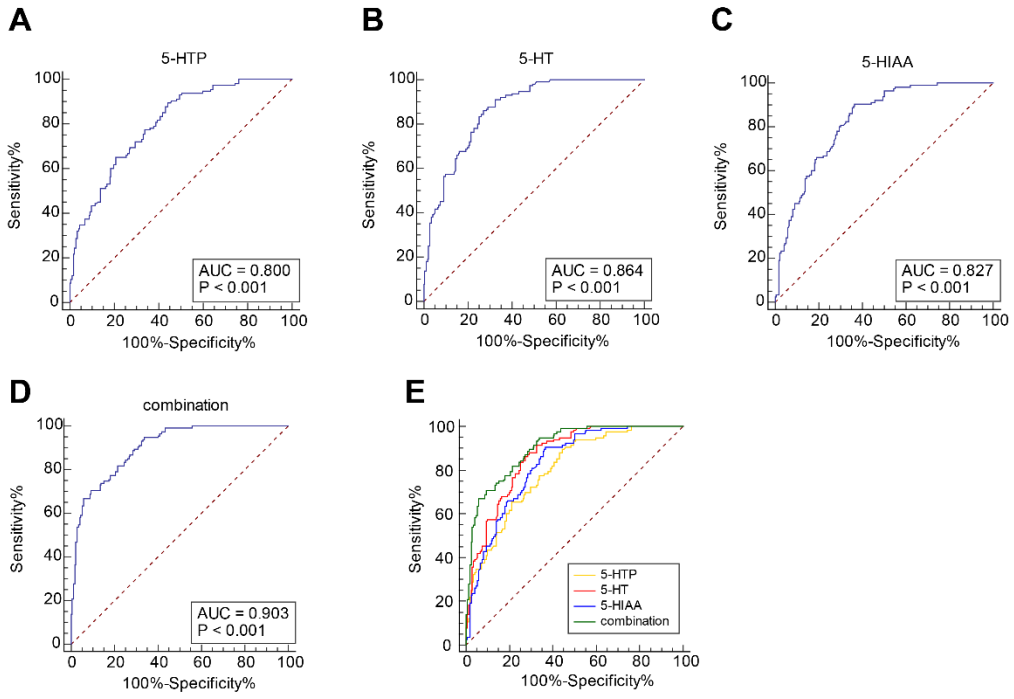

Figure 2. Predictive values of 5-HT precursors and metabolites alone or in combination for PMOP

occurrence. ROC curves were plotted for analyzing the predictive value of plasma 5-HTP (A), 5-HT (B), 5-HIAA (C) levels, and the combined assay (D) for PMOP. The AUCs of these indicators (E) were compared.

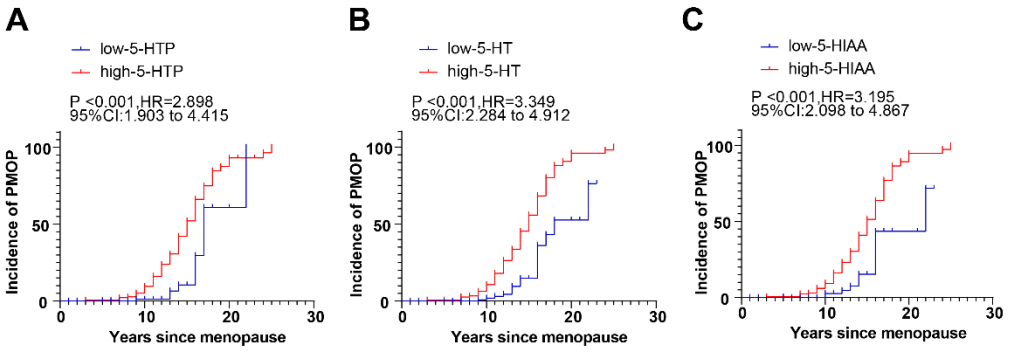

Figure 3. Influence of plasma levels of 5-HT precursors and metabolites on the occurrence of PMOP analyzed by Kapan-Meier curves. The influence of plasma 5-HTP (A), 5-HT (B) and 5-HIAA (C) levels on the risk of PMOP occurrence analyzed by Kapan-Meier curves.

SUPPLEMENTAL DATA

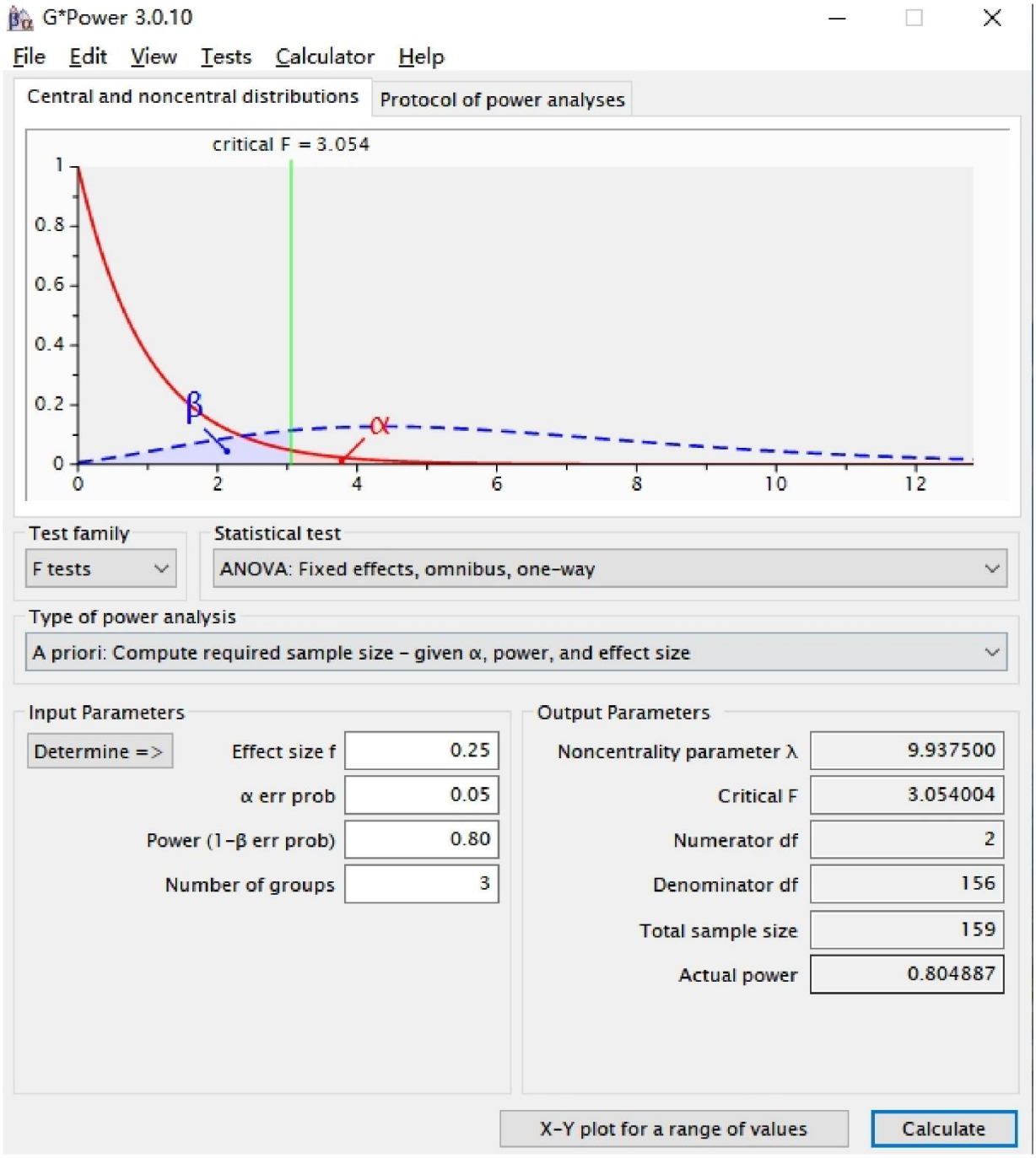

Figure S1. Sample size estimation by G Power.