Age and menopausal status are important factors influencing the serum human epididymis secretory protein 4 level: a prospective cross-sectional study in healthy Chinese people

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Abstract

Background: Human epididymis secretory protein 4 (HE4) is a new ovarian cancer biomarker. The factors influencing HE4 levels are not clear, and the reference data in China are limited. Here, we aim to evaluate the effects of menopause and age on HE4 levels and to provide a possible reference value for HE4 in healthy Chinese people.

Methods: A total of 2493 healthy females aged 40 years or older were recruited from March 2013 to March 2017 with the cooperation of four medical institutions across Beijing, China. The serum levels of HE4 and cancer antigen 125 (CA125) were measured by enzyme-linked immunosorbent assay. The Wilcoxon rank-sum test of variance and a stratified analysis were used to analyze the relationships among age, menopausal status, and levels of HE4 or CA125. Confidence intervals (5%–95%) were determined for reference ranges in different populations.

Results: There was a statistically significant difference in median HE4 levels between the post-menopausal ($n = 2168$) and pre-menopausal groups ($n = 325$) (36.46 *vs.* 24.04 pmol/L, $Z = -14.41$, $P < 0.001$). HE4 increased significantly with age in the post-menopausal groups ($H = 408.18$, $P < 0.001$) but not in the pre-menopausal subjects ($Z = -0.43$, $P = 0.67$). The upper 95th percentile of HE4 levels were 44.63 pmol/L for pre-menopausal women, 78.17 pmol/L for post-menopausal women, and 73.3 pmol/L for all women. In the post-menopausal population, the HE4 reference ranges were 13.15 to 47.31, 14.31 to 58.04, 17.06 to 73.51, 24.50 to 115.25, and 35.71 to 212.37 pmol/L for different age groups from forty divided by decade. The CA125 level was affected mainly by menopausal status and not age.

Conclusions: Menopausal status and age were both important factors influencing the level of HE4, and age affected HE4 levels mainly in post-menopausal women. The HE4 level was higher in the post-menopausal population than in the pre-menopausal population and increased with age.

Keywords: Ovarian cancer; Biomarker; Human epididymis protein 4; Cancer antigen 125; Menopause status

Introduction

Ovarian cancer has the highest mortality of female reproductive tract cancers.[1] Due to the absence of detectable symptoms in the early stage of the disease and the lack of an effective screening method, more than 70% of patients are first diagnosed with advanced ovarian cancer. The 5-year relative survival rates for ovarian cancer patients are 89.3% and 65.5% for stage I and II, respectively, whereas the rates are only 33.5% and 17.9% for stage III and IV, respectively.[2] Therefore, early diagnosis is the key to improving the prognosis of ovarian cancer.

Cancer antigen 125 (CA125) is currently the most widely used clinical ovarian cancer marker. The sensitivity of CA125 for advanced ovarian cancer is approximately 90%, but for stage I ovarian cancer, the sensitivity is only approximately 50%, and the specificity is only 75%.[3] In addition, approximately 20% of epithelial ovarian cancer (EOC) does not express CA125, and CA125 can also be expressed in other benign gynecological diseases and some malignant systemic cancers. CA125 has certain limitations in terms of sensitivity and specificity. Hence, there is a critical need to identify an alternative tumor marker that has better sensitivity and specificity and is capable of detecting ovarian cancer at an early stage.

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HE4 is a recently identified ovarian cancer biomarker. A number of studies have found that HE4 is a specific serum marker of ovarian cancer.[4,5] The level of HE4 expression is high in EOCs, such as serous carcinoma, endometrial carcinoma, and clear cell carcinoma.[6,7] Compared with the traditional ovarian cancer marker CA125, HE4 has higher specificity and sensitivity for ovarian cancer, especially early ovarian cancer (stage I and II).[8-11]

Although HE4 has gradually increased in clinical use and has shown a good value for the diagnosis, prognosis, and follow-up of ovarian cancer,[12-15] there is still no common standard for reference values of the normal range, and the factors influencing the level of HE4 are not clear. The purpose of this study was to evaluate the factors, such as menopause status and age, influencing the levels of HE4 in Chinese people to provide possible HE4 reference values for healthy women.

Methods

Ethics approval

The study was conducted in accordance with the principles of the *Declaration of Helsinki* and was approved by the local ethics committee of the Peking University People's Hospital (No. 2012-19). Informed written consent was obtained from all patients before their enrollment in this study.

Recruitment of study population

Women who were 40 years or older were enrolled in this study, except those who had undergone bilateral ovary resection or with malignant tumors.

From March 2013 to March 2017, volunteers were recruited with the cooperation of Peking University People's Hospital, Peking University First Hospital, and Beijing Cancer Hospital. Demographic data, including age, menopausal status, pregnancy, fertility, and lactation parameters; and personal and familial disease histories, were all obtained. Post-menopausal women were defined as those with an absence of menses for more than 1 year (physiological menopause).

Serum collection and detection process

All blood samples were acquired according to a standard collection protocol. Approximately 5 mL of venous blood was collected in yellow blood collecting vials containing inert separation gel and coagulant, the tube was centrifuged at $1200 \times g$ for 20 min, and the supernatant was then collected and frozen at −80°C until testing. The serum levels of HE4 and CA125 were detected by enzyme-linked immunosorbent assay (ELISA) kits (Fujirebio Diagnostics, Inc., Sweden).

Statistical analysis of data

A cross-sectional study was conducted by analyzing the results of a single blood sample collection. According to the reference,[16,17] PASS V14 software was used to estimate the sample size. At the expected accuracy of 1.5 pmol/L within the 95% confidence intervals, 198 and 698 participants were needed in the pre-menopausal and post-menopausal groups, and 919 and 352 participants were needed in the post-menopausal group older than and younger than 60 years, respectively.

The statistical analyses in this study were performed using SPSS (IBM SPSS version 20.0, Chicago, IL, USA). According to age and menopausal status, the Wilcoxon rank-sum test of variance was conducted for the HE4 and CA125 levels. Considering the interaction between age and menopausal status, a stratified analysis was applied to compare the levels of HE4 and CA125 in different groups. Reference ranges of HE4 levels were evaluated using the 5% to 95% cut-offs in different age groups as well as in pre- and post-menopausal subjects. Log2-transformed scatter plots were generated for HE4 levels by decadal age groups and menopausal status. The percentages of individuals with abnormal HE4 and CA125 levels were also calculated in different age groups and menopausal statuses.

Results

Characteristics of the study population

A total of 2493 subjects were enrolled in this study, 325 for pre-menopausal group and 2168 for post-menopausal group. The mean age of all the women was 58 ± 9 years, with a minimum age of 40 and a maximum age of 91 years. The population was divided into five age groups by decade: 40 to 49 ($n = 378$), 50 to 59 ($n = 1187$), 60 to 69 ($n = 665$), 70 to 79 ($n = 196$), and over 80 ($n = 67$) years.

Factors affecting HE4 and CA125 levels

From the results of the Wilcoxon rank-sum test of variance, we observed significant differences in median HE4 levels among groups with different ages (24.93, 31.95, 39.35, 52.24, and 70.27 pmol/L, $H = 562.86$, $P < 0.001$) and menopausal statuses (post-menopausal *vs.* pre-menopausal, 36.46 *vs.* 24.04 pmol/L, $Z = -14.41$, $P < 0.001$). There were significant differences in median CA125 levels according to menopausal status (post-menopausal *vs.* pre-menopausal, 4.72 *vs.* 7.78 U/mL, $Z = -13.05$, $P < 0.001$) and age group (7.49, 4.86, 4.38, 4.65, and 5.00 U/mL, $H = 196.68$, $P < 0.001$). Both age and menopausal status were factors influencing HE4 and CA125 levels [Table 1].

To investigate the interaction between age and menopausal status, a stratified analysis was further performed to determine whether, age, menopausal status, or both, affected HE4 levels. There was a statistically significant difference in the median HE4 levels between the post-menopausal and pre-menopausal groups, regardless of whether the subjects were in the 40- to 49-year-old (27.18 *vs.* 24.15 pmol/L, $Z = -3.22$, $P = 0.001$) or 50- to 59-year-old (32.38 *vs.* 23.88 pmol/L, $Z = -4.43$, $P < 0.001$) age group. In the post-menopausal population, the median HE4 level differed significantly among age groups and increased with age (27.18, 32.38, 39.35, 52.24, and 70.27 pmol/L, $H = 408.18$, $P < 0.001$), while in the pre-menopausal

Table 1: Effects of age and menopausal status on levels of HE4 or CA125 by Wilcoxon rank-sum tests of variance analysis.

	HE4						CA125				
			Reference range					Reference Range			
Items	Case number	Median (IQR) (pmol/L)	5%	95%	*H*/*Z* value	*P* value	Median (IQR) (U/mL)	5%	95%	*H*/*Z* value	*P* value
Age groups (years)					562.86	<0.001				196.68	<0.001
40–49	378	24.93 (18.37, 32.82)	11.40	44.79			7.49 (5.33, 11.45)	2.64	25.76		
50–59	1187	31.95 (24.03, 40.86)	14.07	57.68			4.86 (3.25, 7.19)	1.50	14.21		
60–69	665	39.35 (30.69, 48.43)	17.06	73.51			4.38 (2.86, 6.22)	1.49	11.47		
70–79	196	52.24 (41.28, 68.90)	24.50	115.25			4.65 (2.92, 6.56)	1.52	11.40		
≥80	67	70.27 (51.31, 94.06)	35.71	212.37			5.00 (3.38, 7.36)	2.12	23.25		
Total	2493	34.93 (24.94, 45.17)	14.31	73.30			5.00 (3.30, 7.48)	1.55	15.12		
Menopausal Status					−14.41	<0.001				−13.05	<0.001
Post-menopausal	2168	36.46 (26.99, 46.75)	15.33	78.17			4.72 (3.18, 6.90)	1.52	12.58		
Pre-menopausal	325	24.04 (17.63, 31.66)	10.89	44.63			7.78 (5.34, 12.54)	2.50	29.33		
Total	2493	34.93 (24.94, 45.17)	14.31	73.30			5.00 (3.30, 7.48)	1.55	15.12		

HE4: Human epididymis secretory protein 4; CA125: Cancer antigen 125; IQR: Interquartile range.

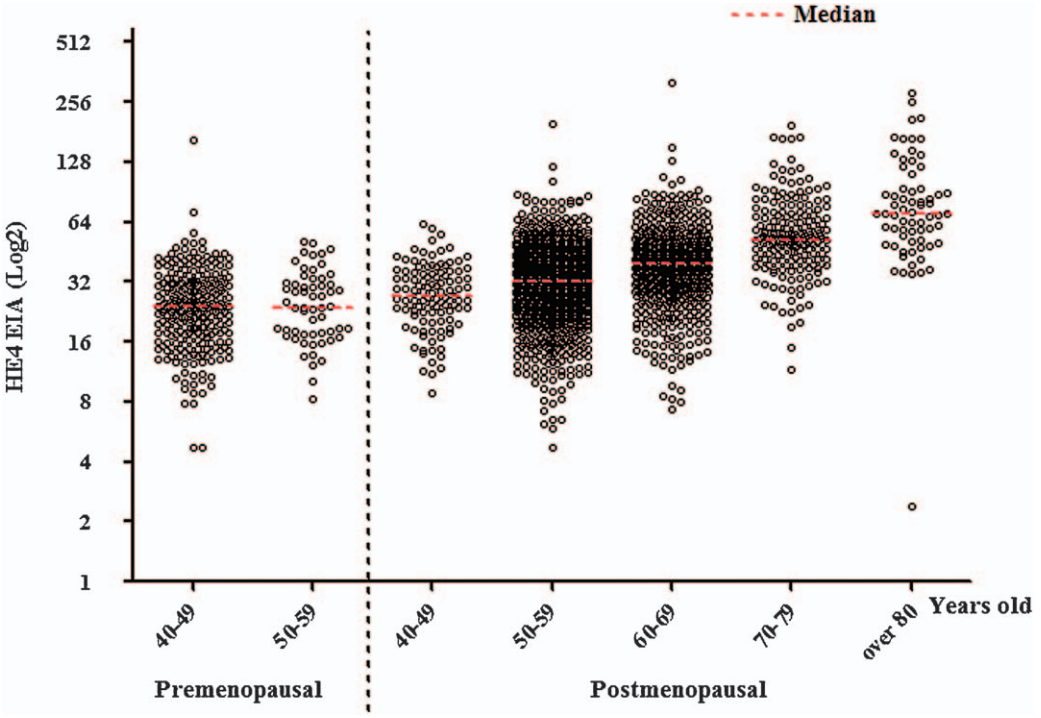

Figure 1: Scatter plot of the serum HE4 levels for pre-menopausal or post-menopausal women stratified by age group. HE4: Human epididymis secretory protein 4.

population, there was no difference in median HE4 levels between the 40- to 49-year-old and 50- to 59-year-old groups (24.15 *vs.* 23.88 pmol/L, $Z = -0.43$, $P = 0.67$) [Figure 1 and Table 2]. Thus, menopausal status and age were both important factors influencing HE4, and age affected HE4 levels mainly in post-menopausal women.

Regarding CA125, there was also a statistically significant difference in the median CA125 levels between the post-menopausal group and the pre-menopausal group, regardless of whether the subjects were in the 40- to 49-year old (6.75 *vs.* 7.93 U/mL, $Z = -3.34$, $P = 0.001$) or 50- to 59-year-old (4.79 *vs.* 6.33 U/mL, $Z = -3.87$, $P < 0.001$) age group. In the pre-menopausal population, there was no statistically significant difference in the median CA125 level between the different age groups (7.93 and 6.33 U/mL in the 40- to 49-year old and 50- to 59-year-old age groups, respectively, $Z = -1.73$, $P = 0.08$). Although the median CA125 level in the post-menopausal population appeared to vary significantly among age groups (6.75, 4.79, 4.38, 4.65, and 5.00 U/mL, $H = 46.34$, $P < 0.001$), in fact, only the 40-to 49-year-old age group (6.75 U/mL) had levels that were significantly different from those in other groups; this difference might have been affected by the peri-menopause period [Table 2]. Thus, the CA125 level seems to be affected mainly by menopausal status and not age.

HE4 normal reference ranges in different populations

The detailed reference ranges for HE4 were evaluated using the 5% to 95% cut-offs in different age groups as well as in pre- and post-menopausal subjects; these ranges are listed in Table 2. The 95th percentile is often used as the upper limit of normal in biomarker analysis. Using

Table 2: Effects of age and menopausal status on levels of HE4 or CA125 by stratified analysis.

	Post-menopausal				Pre-menopausal group					
			Reference range				Reference range			
Items	Number	Median (IQR)	5%	95%	Number	Median (IQR)	5%	95%	*Z* value	*P* value
HE4										
Age groups (years)										
40–49	111	27.18 (22.44, 35.95)	13.15	47.31	267	24.15 (17.65, 31.67)	10.73	43.75	−3.22	0.001
50–59	1129	32.38 (24.56, 41.13)	14.31	58.04	58	23.88 (17.50, 32.05)	12.04	46.93	−4.43	<0.001
60–69	665	39.35 (30.69, 48.43)	17.06	73.51	–	–	–	–	–	–
70–79	196	52.24 (41.28, 68.90)	24.50	115.25	–	–	–	–	–	–
Over 80	67	70.27 (51.31, 94.06)	35.71	212.37	–	–	–	–	–	–
Total	2168	36.46 (26.99, 46.75)	15.33	78.17	325	24.04 (17.63, 31.66)	10.89	44.63	−14.41	<0.001
H/*Z* value		408.18				−0.43				
P value		<0.001				0.67				
CA125										
Age groups (years)										
40–49	111	6.75 (4.39, 9.20)	2.66	18.19	267	7.93 (5.54, 12.74)	2.58	28.94	−3.34	0.001
50–59	1129	4.79 (3.22, 7.03)	1.50	13.63	58	6.33 (4.43, 11.14)	1.95	42.67	−3.87	<0.001
60–69	665	4.38 (2.86, 6.22)	1.49	11.47	–	–	–	–	–	–
70–79	196	4.65 (2.92. 6.56)	1.52	11.40	–	–	–	–	–	–
Over 80	67	5.00 (3.38, 7.36)	2.12	23.25	–	–	–	–	–	–
Total	2168	4.72 (3.18, 6.90)	1.52	12.58	325	7.78 (5.34, 12.54)	2.50	29.33	−13.05	<0.001
H/*Z* value		46.34				−1.73				
P value		<0.001				0.083				

HE4: Human epididymis secretory protein 4; CA125: Cancer antigen 125; IQR: Interquartile range.

Table 3: Age and menopausal status distribution of individuals with abnormal HE4 and CA125 levels.

	HE4				CA125			
	Post-menopausal group		Pre-menopausal group		Post-menopausal group		Pre-menopausal group	
Age group (years)	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
40–49	0	0	1	5.26	0	0	9	50.00
50–59	2	10.53	0		3	16.67	3	16.67
60–69	3	15.79	0		1	5.56	0	0
70–79	5	26.32	0		0	0	0	0
Over 80	8	42.11	0		2	11.11	0	0
Total	18	94.75	1	5.26	6	33.34	12	66.67

HE4: Human epididymis secretory protein 4; CA125: Cancer antigen 125.

the 95th percentile as the upper limit of normal cut-off point, the upper 95th percentile for the HE4 level was 44.63 pmol/L for pre-menopausal women, 78.17 pmol/L for post-menopausal women, and 73.3 pmol/L for all women. In the post-menopausal population, the reference ranges were 13.15 to 47.31, 14.31 to 58.04, 17.06 to 73.51, 24.50 to 115.25, and 35.71 to 212.37 pmol/L for different age groups from forty divided by decade.

Individuals with abnormal serum levels of HE4 or CA125

In total, according to the 150 pmol/L threshold in the package insert for the HE4 ELISA assay and the threshold of 35 U/mL for the CA125 ELISA assay, 19 individuals with abnormal serum levels of HE4 and 18 individuals with abnormal serum levels of CA125 were identified in this study. As shown in Table 3, 18/19 of the individuals with abnormal serum levels of HE4 were in the post-menopausal group. The number of individuals with abnormal HE4 increased with increasing age in the post-menopausal group (0/18, 2/18, 3/18, 5/18, and 8/18 in the age groups of 40–49, 50–59, 60–69, 70–79 and over 80 years old, respectively). It was consistent with the previous results showing that the serum HE4 level was affected by menopausal status and age, was higher in the post-menopausal population and increased with age. For CA125, the pre-menopausal group had more individuals with an abnormal level (12/18) than the post-menopausal group (6/18), which was also consistent with the previous results showing that the CA125 level was more affected by menopausal status than by age and was higher in the pre-menopausal group than the post-menopausal group.

In addition, of the women with abnormal serum HE4 levels, 5/19 had kidney disease, all of whom were in the post-menopausal group. Five individuals found to have uterine fibroids and adenomyosis were all in the pre-menopausal group and accounted for 5/18 of the women with abnormal serum CA125 levels (data not shown).

Discussion

HE4 was approved in 2009 by the United States Food and Drug Administration (U.S. FDA) as a new serological biomarker for the monitoring of women diagnosed with EOC. It is the only tumor marker approved for clinical use in the past 25 years.[18] A large number of studies have shown that HE4 is of great value for the diagnosis, treatment evaluation, prognostic assessment, and follow-up monitoring of ovarian cancer, especially in combination with CA125.[19-21] However, there have been few large trials examining serum HE4 levels in healthy women,[16,17,22] and the factors influencing the HE4 level are still not clear. The detection method, race, age, menopausal status, pregnancy, and other physical states might affect the HE4 level.[23]

The reference data in the Chinese population were limited.[16,24] At present, as recommended by the U.S. FDA, the upper limit of the 95% confidence interval for the normal threshold of HE4 ELISA kits (Fujirebio Diagnostics Inc, Malvern, PA, USA) is 150 pmol/L. This value does not take into consideration of patient age, menopausal status or actual normal levels in healthy women. It is very important to clarify the factors influencing HE4 levels and provide reference values in healthy women.

In this study, by stratification analysis to exclude the mutual interference of age and menopause factors, we confirmed that both age and menopause were important influencing factors of HE4 level in healthy women. The median level of HE4 in post-menopausal group was higher than that in pre-menopausal group (36.46 *vs*. 24.04, $P < 0.001$). Age mainly affected the level of HE4 in the menopausal population. In the post-menopausal population, the median HE4 levels differed significantly among different age groups divided by decade, and increased with age, while there were no differences among age groups in the pre-menopausal population.

Similar to our results, in 2012, Moore *et al*[17] showed that the HE4 concentration increased with age in healthy women over 40 years old, and there was a significant difference in the median serum HE4 levels between pre- and post-menopausal women (46.6 *vs*. 57.6 pmol/L, $P < 0.001$). However, because the median HE4 levels for pre-menopausal women age 40 years and older (50.5 pmol/L) and post-menopausal women younger than 60 years (50.7 pmol/L) were not significantly difference, Moore thought differences in HE4 levels might not be related to menopausal status but rather to age, which is different from the results of our study. In our study, there was a statistically significant difference in the median serum levels of HE4 between the post-menopausal and pre-menopausal groups for subjects in the age groups of 40 to 49 years (27.18 *vs*. 24.15 pmol/L, $P = 0.001$) and 50 to 59 years (32.38 *vs*. 23.88 pmol/L, $P < 0.001$), indicating that differences in HE4 levels might be related to menopausal status.

One explanation for the differences between the study by Moore and the present study could be a difference in the definition of menopausal status. Women aged 55 years or older were considered post-menopausal, while women aged 45 years or younger were defined as pre-menopausal by Moore, and no samples were obtained between the ages of 46 to 54 years in one study center. In contrast, the menopausal status of subjects in our study was strictly determined by consultation. The post-menopausal women were defined as those with an absence of menses for more than 1 year (physiological menopause), and the enrolled population included samples of all ages. These differences might affect the statistics in the peri-menopause group.

In addition, the median serum HE4 level and the upper limit of the 95% confidence interval that we identified were lower than the values in Moore's study. In pre-menopausal people, the median serum HE4 level in our study was 24.04, while it was 46.6 pmol/L in the study by Moore, and the upper limit of the 95% confidence interval was 44.63 in our study and 89 pmol/L in the study by Moore. In the present study and that performed by Moore, in post-menopausal people, the median serum HE4 levels were 36.46 and 57.6 pmol/L, and the upper limits of the 95% confidence interval were 78.17 and 128 pmol/L, respectively. The values were larger in the study by Moore than in our study. In addition to the possible influence of the different definitions of menopausal status, the racial differences between the Chinese and American populations might account for the differences in values.

A study in multiple Asian ethnicities suggested that age, as well as ethnicity, was associated with HE4 levels.[24] The concentration of HE4 increased with increasing age, especially in women who were more than 50 years old. The HE4 levels were significantly different between Malays and Indians but were not significantly different between Malays and Chinese. In addition, similar to the results in our study, the authors also found that the upper reference limit they proposed was lower than the value found by Moore[17] and the level given on the insert by the manufacturer of the Abbott Architect HE4 kit (58.4 *vs*. 70 pmol/L in the pre-menopausal group and 69.0 *vs*. 140 pmol/L in the post-menopausal group). The differences were presumably related to genetics or body mass index.

Different studies have reached different conclusions about the effects of age and menopausal status on HE4 levels. Tian *et al*,[16] in a study with 618 healthy Chinese people, reported that although there were significant differences in HE4 levels among groups with different ages (older > younger) and menopausal statuses (post-menopausal > pre-menopausal), multivariate analysis showed that menopause but not age was independently associated with HE4 levels. Age was not an independently associated factor. This conclusion was opposite to the conclusion drawn in the study by Moore. Although it was not entirely consistent with our results, it supported our finding that

the HE4 level was affected by menopausal status, which was the difference between our conclusion and Moore's.

Although Tian conducted a detailed study on the HE4 level of healthy population (618 cases) and patients with benign tumor (767 cases) or malignant tumor (951 cases), the sample size of healthy population was smaller than ours (2493 cases). Furthermore, a multivariate analysis was used in Tian's study while our study used a stratified analysis that could exclude the interferences between age and menopausal factors. These might be the reasons to the differences in conclusions.

In Tian's study, the reference values ranged from 29.30 to 68.79 pmol/L in pre-menopausal healthy women and from 35.96 to 114.43 pmol/L in post-menopausal women. Although the Chinese population was evaluated in both Tian's study and the present study, Tian used a chemiluminescence reagent kit from Roche rather than an ELISA kit from Fujirebio Diagnostics Inc. Thus, the normal reference values could not be compared because of the different methods used.

Yu *et al*[25] performed a study in 1809 healthy Korean women and found that HE4 levels increased in women over 50 years old and were influenced by age rather than menopausal status, which also illustrated the debate about age and menopause. This finding also showed that the reference limit of HE4 differed according to racial and regional differences.

In conclusion, almost all the above studies showed that HE4 levels changed with age and menopausal status, while it was still controversial whether it was age or menopausal status, or both affected the HE4 levels because age and menopausal status interfered with each other. Our study suggested that HE4 levels were influenced both by menopausal status and age in Chinese females. Menopausal status was an important influential factor for HE4, and HE4 levels were higher in post-menopausal women than in pre-menopausal women. Age affected HE4 levels mainly in post-menopausal women. The HE4 level in post-menopausal women increased with age. While the precise reason for these increases remains uncertain, they are likely secondary to age-related declines in renal function or perhaps an increased prevalence of comorbid conditions.[17] In addition, our research also confirmed that CA125 was influenced mainly by menopausal status and not age. Uterine fibroids and adenomyosis, which are common in pre-menopausal populations, might be associated with high levels of CA125.

Our study supplemented data on the influence factors and normal reference values of HE4 in the Chinese population. However, the population in this study was limited to the Beijing area, and large multi-center and large-sample studies need to be carried out in more areas of China to determine and verify the reference value of the HE4 levels in different populations. In addition, the influences of multiple factors on HE4 levels need to be determined in future studies, as previous studies have shown that age, menopausal status, fertility status, smoking, renal function, ethnicity, detection method, and other factors may affect serum HE4 levels. The normal value range of HE4 levels for clinical tests should be adjusted to enable accurate diagnoses.

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Conflicts of interest

None.

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