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# Distribution and Determinants of Plasma Homocysteine Levels in a Preconception Population: A Retrospective Single-Center Study

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Data Interpretation D  
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**Background:** Raised plasma homocysteine (Hcy) levels have been associated with various diseases and pregnancy complications. Preconception is the primary prevention period to prevent birth defects. This retrospective study aimed to investigate the distribution of plasma Hcy levels among men and women at preconception and further evaluate the factors influencing plasma Hcy levels in a Southern China population.





**Material/Methods:** Sex, age, serum folate levels, plasma Hcy levels, and the time of Hcy and folate detection were obtained by medical records. Univariate analysis and multi-factor mixed virtual linear regression were used to explore the distribution and determinants of plasma Hcy levels.

**Results:** A total of 3031 participants (1091 men [35.99%] and 1940 women [64.01%]) were included. The average levels of Hcy and the rates of hyperhomocysteinemia (HHcy) in men were higher than those in women ( $P < 0.05$ ). Hcy levels were observed to be lowest during autumn and highest during winter ( $P < 0.05$ ). In the normal Hcy (NHcy) group, serum folate levels were higher than in the HHcy group ( $P < 0.05$ ). Regression analysis suggested that sex, season, and serum folate levels had an effect on Hcy levels, but age was not an influencing factor of Hcy level in the preconception population.

**Conclusions:** This retrospective study showed that Hcy levels are higher in men and in the winter season. Sex, season, and serum folate levels were the influencing factors of Hcy in the preconception population.

**Keywords:** **Folic Acid • Homocysteine • Hyperhomocysteinemia • Preconception Care • Seasons**

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## Background

The amino acid homocysteine (Hcy), which contains sulfur, is generally produced during methionine metabolism [1-3]. Several cross-sectional and prospective reports have presented the relationship between elevated plasma Hcy levels and more than 100 syndromes, diseases, and consequences, including ischemic stroke, bipolar disorder, cognitive decline, and cardiovascular disease [4-8]. Hyperhomocysteinemia (HHcy) is the term used to describe a condition when the Hcy level is  $>10 \mu\text{mol/L}$  [4,9,10]. Every  $5 \mu\text{mol/L}$  rise in Hcy levels resulted in a 33.6% increase in mortality risk [11].

Since pregnancy is a very complicated physiological process, for the pregnancy to become successful, the uterus and the embryo need to communicate with each other properly. Any adverse change occurring in a pregnant woman's physical state can complicate her pregnancy and lead to many adverse issues. Various studies have postulated that maternal Hcy-mediated placental thrombosis, inadequate placentation, apoptosis, inflammation, increased oxidative stress, and epigenetic alterations can lead to several pregnancy-linked issues [12-17], such as preeclampsia, placental abruption, early pregnancy loss, intrauterine growth restriction, and fetal death [18-20]. Normal Hcy values during pregnancy are 3.9 to 7.3 mmol/L before 16 gestational weeks, 3.5 to 5.3 mmol/L during gestational weeks 20 to 24, and 3.3 to 7.5 mmol/L after gestational week 36 [21]. Preconception care is provided for couples of reproductive age who are planning a pregnancy. Its aims are to identify pre-existing medical and social conditions that can affect pregnancy and the well-being of the mother and child. Preconception care also includes counseling for the parents.

Unhealthy physical conditions of the parents during the preconception period are linked to higher risks of morbidity and mortality in the child. Preconception care is advised by the World Health Organization as a means of enhancing the health and well-being of women and couples and improving the outcomes of subsequent pregnancies and child health [22].

A nationwide survey of residents  $\geq 40$  years of age from 31 provinces in the mainland of China showed that 25.9% of participants were defined as having HHcy [23]. However, there are very few studies focused on the Hcy status of couples at preconception. The goal of this study was to investigate the distribution of plasma Hcy levels in men and women at preconception and further evaluate the vital variables that impact Hcy levels. Results of this study will provide significant new information about the Hcy status among couples at preconception and provide a preliminary theoretical basis for the future study on Hcy and adverse pregnancy outcomes and complications.

## Material and Methods

### Participants

This retrospective study included couples who were seeking preconception care from January 2021 to May 2022 in the Department of Women's Health Care, Affiliated Foshan Maternal and Child Health Hospital, Southern Medical University, a large preconception care center. Hcy levels and serum folate levels were tested at the first visit. People who had chronic renal failure, hypothyroidism, malignant tumors, or incomplete medical records or who had Hcy and serum folate levels that were not measured at the same time were excluded from this study. Ethical approval was waived by the Ethics Committee of Affiliated Foshan Women and Children Hospital, Southern Medical University in view of the retrospective nature of the study and because all performed procedures were part of routine care. All data were extracted from medical records, and the consent to participate was unavailable due to the retrospective design of the study and difficulty in reconnection with participants.

### Sample Measurement and Collection

The Hcy and serum folate levels of the couples at preconception that were determined during their regular preconception care in the clinical laboratory were included in this report. After the participants fasted for 8 h, the nurses collected blood samples and immediately sent the samples for biochemical analyses in the hospital's laboratory. An automated biochemical analyzer (AU5800, Beckman Coulter Inc, Brea, CA, USA) was used for measuring Hcy levels, whereas an electrochemical luminescence automatic immune analyzer (Cobas e601, Roche Inc, Switzerland) was used for measuring serum folate levels. The time of measurement was also reviewed.

Hcy levels  $<10 \mu\text{mol/L}$  were defined as normal Hcy (NHcy), whereas Hcy levels  $\geq 10 \mu\text{mol/L}$  were defined as HHcy. According to the meteorological classification, the winter season includes the months of December, January, and February; spring season includes the months of March, April, and May; summer season includes the months of June, July, and August; and autumn season includes the months of September, October, and November. Data related to relevant factors, such as serum folate levels, sex, age, Hcy levels, and time of folate and Hcy detection, were obtained after examining the medical records of the participants.

### Statistical Analysis

Statistical analyses were conducted with SPSS Windows software (version 20.0; IBM Corp, Armonk, NY, USA). All data were tested for normality. The data that showed a normal distribution was represented as mean value  $\pm$  standard deviation (SD), whereas the non-normal distribution was described as median

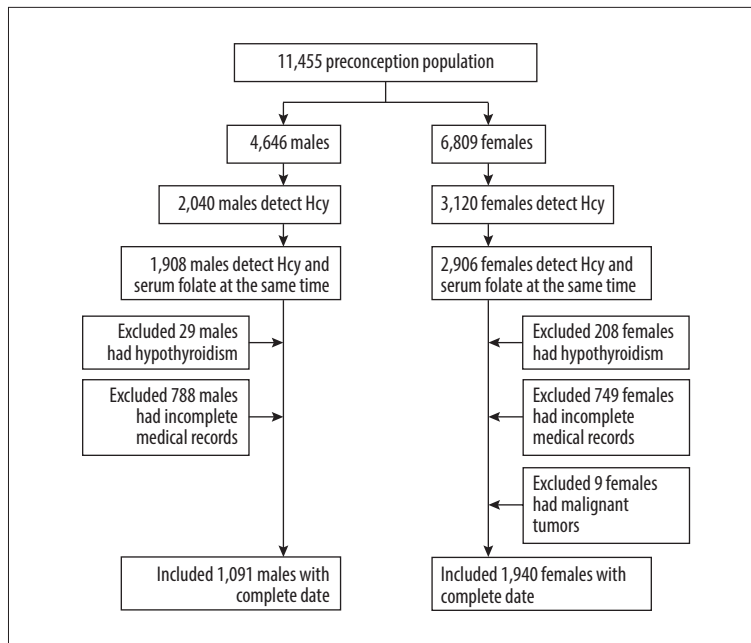


Figure 1. Flow chart of study participants.

Table 1. Comparison of age and homocysteine (Hcy) levels in the preconception population by sex.

Gender	N	Age (year)	Hcy (μmol/L)
Male	1091	31.17±4.65	10.9 (9.3, 12.9)
Female	1940	29.98±4.14	7.9 (6.8, 9.1)
t/Z value		7.279	31.49
P		0.000	0.000

and quartile (M [Q1, Q3]). Unpaired *t* tests were used for comparing 2 groups with normal distribution and homogeneity of variance, whereas the Mann-Whitney U test was used for comparing 2 groups with an unequal variance. The non-normal distribution between the data was also compared between 2 groups based on the nonparametric rank-sum test. The statistical data described the number of cases and percentage (N [%]), and the 2 groups were compared using the  $\chi^2$  test. The effects of the factors sex, age, season, Hcy levels, and serum folate levels were evaluated by multi-factor mixed virtual linear regression. A *P* value <0.05 was regarded as statistically significant.

## Results

A total of 3031 participants were included in this study, with 1091 men (35.99%) and 1940 women (64.01%) (Figure 1). The mean age of the men was 31.17±4.65 years, and the mean age of women, 29.98±4.14 years. The mean age of men was higher than that of women (*P*<0.05). The average Hcy level in men was 10.9 (9.3, 12.9) μmol/L and in women, 7.9 (6.8, 9.1)

Table 2. Normal homocysteine (NHcy) and hyperhomocysteinemia (HHcy) detection rates in the preconception population by sex.

Gender	N	NHcy n (%)	HHcy n (%)
Male	1091	366 (33.55)	725 (66.45)
Female	1940	1650 (85.05)	290 (14.95)
Total	3031	2016 (66.51)	1015 (33.49)

μmol/L. The results indicated that the average Hcy level in men was higher than that in women (*P*<0.05) (Table 1).

As shown in Table 2, the rates of NHcy and HHcy were 66.51% (2016/3031) and 33.91% (1015/3031) in all participants, while they were 33.55% (366/1091) and 66.45% (725/1091) in men, and 85.05% (1650/1940) and 14.95% (290/1940) in women, respectively. HHcy levels were significantly higher in the men than in women ( $\chi^2=831.656$ , *P*=0.000).

No significant difference was observed in Hcy levels in the male participants aged between 18 and 34 years and in the ≥35 years group (*P*>0.05). However, the female participants aged ≥35 years showed higher Hcy levels than those between 18 and 34 years, and this difference was statistically significant (*P*<0.05) (Table 3).

Hcy levels in the male and female groups were lowest in autumn and highest in winter (*P*<0.05). Further pairwise comparison showed no difference in Hcy levels between the spring and winter seasons (*P*>0.05), but a significant difference was noted in other seasons (*P*<0.05) (Table 4).

**Table 3.** Comparison of homocysteine (Hcy) levels in the preconception population by sex and age.

Age (year)	Male		Female	
	N	Hcy (μmol/L)	N	Hcy (μmol/L)
18-34	887	10.9 (9.3, 12.8)	1655	7.7 (6.6, 8.7)
≥35	204	10.9 (9.3, 12.9)	285	7.9 (6.9, 9.2)
Z value		31.49		2.182
P		0.873		0.029

**Table 4.** Comparison of homocysteine (Hcy) levels in the preconception population by sex and season.

Season	Male		Female	
	N	Hcy (μmol/L)	N	Hcy (μmol/L)
Winter	283	12.2 (7.8, 12.8)	469	8.2 (7.1, 9.6)
Spring	414	11 (9.5, 12.7)	733	8.0 (6.85, 9.1)
Summer	215	10.1 (9.9, 10.4)	384	7.9 (6.9, 9.1)
Autumn	179	9.5 (8.7, 15.4)	354	7.6 (6.6, 8.8)
Z value		58.88		19.238
P		0.000		0.000

**Table 5.** Comparison of serum folate levels in the preconception population by sex and homocysteine (Hcy) level.

Age (year)	Male		Female	
	N	Serum folate (nmol/L)	N	Serum folate (nmol/L)
NHcy	366	29.34±11.51	1650	36.32±12.69
HHcy	725	23.33±10.02	290	27.06±11.69
t value		8.898		11.583
P		0.00		0.000

**Table 6.** Regression analysis of the Hcy level with gender, age, serum folate level, and season.

Content	Unstandardized coefficients		Standardized coefficients	t-value	P
	B	SE	Bate		
Constant	14.318	0.834		17.173	0.000
Gender	-0.424	0.181	-0.060	-2.345	0.019
Age	0.041	0.026	0.033	1.588	0.112
Serum folate	-0.156	0.009	-0.381	-18.303	0.000
Season (spring)	0.160	0.356	0.012	0.448	0.655
Season (summer)	-1.417	0.386	-0.106	-3.670	0.000
Season (autumn)	-1.467	0.293	-0.133	-5.012	0.000

Higher serum folate levels were recorded in the NHcy group than in the HHcy group ( $P < 0.05$ ) among men and women (Table 5).

Regression analysis of Hcy levels with sex, age, season, and serum folate level suggested that sex, season, and serum folate levels affected Hcy levels, with serum folate having the greatest effect (standardized coefficient 0.381). Taking winter as a reference, Hcy levels in autumn, summer, and winter were significantly different ( $P < 0.05$ ). However, age was not the influencing factor of Hcy level in the preconception population ( $P > 0.05$ ), as shown in Table 6.

## Discussion

In this study, the results indicated that the average Hcy level in men was higher than that in women among the participants preparing for pregnancy ( $P > 0.05$ ). Interestingly, seasons affected Hcy levels, with the level of Hcy in both men and women lowest in autumn and highest in winter ( $P < 0.05$ ). However, difference in age was independent of Hcy levels in the preconception population.

In the present study, Hcy levels were higher in men than in women. A similar phenomenon has been reported in older adults and adolescents [24-27]. The present study not only confirmed that Hcy has a differential effect on different sexes during the preconception, but also showed that sex impacts the percentage of HHcy, similar to results of a recent Israeli study [28]. It is worth noting that the previous studies found that the sex differences even persisted during the subgroup analysis of participants aged over 55 years, when women experience perimenopause, with lower estrogen levels [24,28]. Therefore, in preconception population care, it is also important to focus on the assessment of men, not just women. However, the cause of the sex-induced Hcy difference is still controversial. Physical activity, endogenous estrogens, rates of homocysteine remethylation, and methylenetetrahydrofolate reductase (MTHFR) C677T genotypes are a few reasons [27,29-32].

Previous studies have suggested that age is a factor affecting Hcy and that Hcy levels increase each year, with the highest levels being found in elderly people (75 years or older) [33]. Also, age significantly affects Hcy metabolism dynamics in the pediatric population [34]. In the present study, age showed a significant difference in Hcy levels in women between 18 and 34 years old and  $\geq 35$  years old, before the confounders were adjusted. However, further multivariate regression analysis revealed that age was not a standalone risk factor for Hcy, which is different from the results indicated in earlier studies. The reason age was not associated with Hcy levels in the preconception population is puzzling. Xu et al [24] found that Hcy levels

in a Chinese population initially increase before decreasing, with the trend being generally constant from 30 to 50 years of age, and then levels significantly increase beyond 50 years of age. However, another study comparing White, Black, and Hispanic populations in the United States revealed that Hcy levels increase with increasing age throughout adulthood and differ by race-ethnicity [35]. Therefore, race may be the reason for the unrelated relationship between age and Hcy in this Chinese preconception population.

Surprisingly, Hcy levels varied with changing seasons. The levels of Hcy in men and women were lowest during the fall season and highest in winter. Recently, studies showed an inverse relationship between vitamin D levels and Hcy [36,37]. Also, Al-Bayyari et al [38] observed that vitamin D3 therapy remarkably lowered Hcy levels. According to several research reports, individuals have higher serum vitamin D concentrations in summer and fall than in winter and spring [37,39,40]. Therefore, we speculated that vitamin D was one of the reasons for the seasonal differences in Hcy levels. However, the relationship between Hcy and seasons is controversial. Similar to the present study, Clarke et al [41] found the median Hcy was 3% higher in summer than in winter. Meanwhile Checinska [42] found that cold-water swimmers have lower Hcy levels in winter, and Bates et al [43] observed that people aged 4 to 18 years and those aged 65 years and older often showed the lowest mean Hcy levels. In contrast, Michelle et al [44] showed there was no seasonal variation in plasma total Hcy levels. These inconsistent results may be due to differences in the geographic, racial, and social characteristics of participants. Therefore, further investigation needs to be conducted to validate the results of the present study. Nevertheless, the results of this study are still important for reminding preconception-care physicians to pay attention to the Hcy levels of couples preparing for pregnancy in winter.

Hcy is produced during methionine metabolism, and a proportion of Hcy binds to serine and creates cystathionine; however, most Hcy molecules are remethylated to produce methionine. This process requires plenty of reduced folate and a proper MTHFR effect [1]. Therefore, folate metabolism is closely related to Hcy metabolism. Many previous studies have suggested that folate deficiency is strongly associated with HHcy [45-47]. The results of the present study confirmed the earlier findings, specifically, whereby the HHcy group had a lower serum folate level. At present, there is a consensus that folic acid is one of the most common and effective supplements used to lower plasma Hcy levels [48,49]. Daily supplementation of 0.5 to 5.0 mg of folic acid typically lowers plasma Hcy levels by approximately 25% [50]. In one study, 56.41% of patients with HHcy reach the normal range (5-15 mmol/L) after 3 months of folic acid supplementation [51]. Therefore, it is important to take folic acid supplements 3 to 6 months before pregnancy.



This study had a few limitations. First, in this retrospective study, the preconception population who had received folic acid and vitamin D in advance were not excluded, which may have affected the levels of Hcy, leading to bias in the study. Second, this study lacked information about red blood cell folate, which, as the long-term indicator of folate status, is frequently used to assess folate status [52]. Third, Hcy levels are influenced by food groups to varying degrees [53], and Hcy levels are decreased under a low-protein diet and Mediterranean diet [54,55]; however, this study did not include an analysis of the participants' eating habits. Fourth, there was just one data center used in this investigation.

## Conclusions

In this retrospective study from a single center, we aimed to investigate the distribution of plasma Hcy levels in a

preconception population and further evaluate the factors influencing plasma Hcy levels in Southern China. The findings showed that Hcy levels were higher in men and in winter. Sex, season, and serum folate levels were the influencing factors of Hcy levels in this preconception population.

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## Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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