Hyperhomocysteinemia and metabolic syndrome show differences in ovulation, conception, miscarriage, and live birth in women with PCOS: a secondary analysis of PCOSAct

Abstract:

Objective: To determine the association between hyperhomocysteinemia (HHCY), metabolic syndrome (MS), and reproductive outcome among the women enrolled in the Acupuncture and Clomiphene in Polycystic Ovary Syndrome Trial (PCOSAct).

Design: Secondary analysis of the data from a randomized clinical trial.

Setting: 21 sites (25 hospitals) in mainland China.

Population: A total of 1,000 women diagnosed with polycystic ovary syndrome (PCOS) according to the modified Rotterdam criteria were included in the PCOSAct, and 936 of these had baseline measurements of homocysteine (HCY) levels and metabolic parameters and were included in the present study.

Methods: Baseline serum HCY levels were compared according to tertile levels, and patients' reproductive outcomes, clinical parameters, and biochemical results were recorded.

Main outcome measures: Live birth, ovulation, conception, and miscarriage rates.

Results:

(1) The higher HCY tertile was associated with higher BMI and free testosterone levels and with lower follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), and fasting glucose levels compared with the median and lower tertile groups. (2) The higher HCY tertile was associated with a lower ovulation rate among all women ($p = 0.003$), but not per ovulaion induction cycle ($p = 0.20$), a higher rate of late miscarriage in the second and third trimesters ($p = 0.05$), and comparable rates of conception, pregnancy, and live birth compared to the median and lower tertile groups. After adjusting for acupuncture and clomiphene treatment in the PCOSAct data, the higher tertile HCY group lost the significant association with late miscarriage rate. (3) There were comparable levels of BMI, fasting glucose/insulin, HOMA-IR, and hirsutism

scores in the HCY and control groups, and these were all significantly lower than the values in patients with MS. Luteinizing hormone (LH), LH/FSH ratio, and SHBG in the HHCY group (>12.14 μmol/L HCY) were similar to the control group and were significantly higher than in the MS group. Furthermore, the HHCY group had comparable waist-to-hip ratios and estradiol and free testosterone levels as the MS group, and these were significantly different from controls. (4) There were comparable rates of ovulation and conception among PCOS patients in the HHCY group and controls, and these were significantly higher than in the MS group, indicating relatively normal oocyte fertilization under conditions of HHCY and impaired oocyte fertilization with concurrent MS in women with PCOS undergoing infertility treatment. A novel finding is that there were significantly lower rates of pregnancy and live birth in the MS group and similarly higher rates of total and late pregnancy loss in both the HHCY and MS groups ($p < 0.001$ and $p = 0.05$, respectively) compared with controls. After adjustment for treatment in PCOSAct, the higher rate of late pregnancy loss lost significance, but the HHCY group retained significantly lower ovulation rates per subject and higher miscarriage rates per conception (similar to the MS group) compared with the control group.

Conclusions: In PCOS patients, HHCY contributes to increased miscarriage and reduced ovulation, and MS contributes to defects in conception, pregnancy, ovulation, and live birth, indicating that the two conditions lead to defects at different reproductive stages.

Key words: Hyperhomocysteinemia, metabolic syndrome, outcomes, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder in women of reproductive age (1), with a worldwide prevalence ranging from 5% to 20% (2). It is considered to be the most common cause of female infertility, and it is characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovaries (2).

Homocysteine (HCY) is an amino acid with biological functions in methionine metabolism, and it is derived from the essential amino acid methionine. Hyperhomocysteinemia (HHCY), defined as the presence of abnormally elevated concentrations of plasma or serum total HCY (more than 12.14 μmol/L) (4), has been regarded as a strong independent risk factor for cardiovascular disease and stroke in humans (5,**Error! Reference source not found.**). Previous studies also established that there was a possible link between HHCY, dyslipidemia, and atherosclerosis. A growing body of evidence suggests that HCY metabolism is disrupted in women with PCOS, and previous studies have demonstrated higher circulating blood HCY levels in PCOS patients compared to healthy controls (**Error! Reference source not found.**,6). However, others have suggested that HCY levels do not differ between PCOS patients and controls (7). HCY is associated with increased insulin resistance, increased blood pressure, and increased low-density lipoprotein cholesterol, which are three of the five characteristics of metabolic syndrome (MS) (1,9). However, It has been pointed out that high HCY levels in women with PCOS are not related to the degree of obesity, insulin resistance, or androgen levels (9). The negative effects of HHCY on reproductive processes have been documented in numerous studies, including poor oocyte quality; male infertility due to abnormal sperm morphology, low sperm counts, and loss of motility; congenital malformation; miscarriage; hypertension; and low birth weight (12-14).

However, the association between HCY levels and pregnancy outcome in PCOS patients has not been previously evaluated. The purpose of this study was to determine the association and

contribution of increased concentrations of HCY on ovulation, conception, pregnancy, miscarriage, and live birth in infertile women with PCOS. We aimed to determine whether higher HCY levels are predictive of a rapid decline in ovulation function and an increase in pregnancy loss among PCOS patients.

Methods

Design and target population

This is a post hoc analysis of metabolic parameter and reproductive outcome data from the Acupuncture and Clomiphene in Polycystic Ovary Syndrome Trial (PCOSAct), a large-sample, multicenter, randomized controlled clinical trial conducted from 2012 to 2015 in mainland China. All 1,000 subjects were diagnosed with PCOS according to the modified Rotterdam criteria (1,15). In brief, 936 women with PCOS who had baseline measurements of HCY were enrolled in the present study, and reproductive outcomes, including ovulation, conception, pregnancy, miscarriage, and live birth were recorded at the end of study. The trial was registered on ClinicalTrials.gov (No. NCT01573858). All participants and their partners provided written informed consent before participation in the study. The study design, methods, and inclusion and exclusion criteria have been described in detail elsewhere (3). Women aged from 20 to 40 years with oligomenorrhea (defined as a menstrual interval ≥35 days and/or ≤8 menses in the past year) or amenorrhea combined with clinical hyperandrogenism (modified Ferriman-Gallwey score ≥5 (16, 17)) and/or polycystic ovaries (defined as the presence of >12 antral follicles (≤9mm) and ovarian volume >10 ml by ultrasound) were considered to have PCOS (1,15).

Subjects with any three of the following five factors were considered to have MS: 1) fasting glucose level of 110–126 mg/dL, 2) fasting triglycerides greater than 150 mg/dL, 3) fasting high-density lipoprotein cholesterol level lower than 50 mg/dL, 4) waist circumference more than 88 cm, and 5) systolic blood pressure greater than 130 mm Hg or diastolic blood pressure greater than 85 mm Hg (1). Documentation for patency of at least one tube and a normal uterine cavity

was required by hysterosalpingogram, HyCosi, or diagnostic laparoscopy. Male partners were required to have a sperm count of at least 14 million per milliliter. Exclusion criteria included patients with hyperprolactinemia, FSH levels >15 mIU/mL, uncorrected thyroid disease, poorly controlled Type I or Type II diabetes, taking antidiabetes medications, and suspected Cushing's syndrome as well as the use of hormones or other medications, pregnancy, abortion, postpartum, or breastfeeding within the past 6 weeks.

Data Collection

Baseline laboratory, anthropometric, degree of hirsutism, acne, and ultrasonography measurements were performed after an overnight fast, and all biochemical assays were performed in a core laboratory. Fasting blood collected at baseline was used for all hormone assays (18). Fasting glucose levels were determined on a glucose analyzer using the glucose oxidase method, with all intra-assay and inter-assay coefficients of variation less than 1.5% (18). Total testosterone was measured by radioimmunoassay (Siemens Diagnostics, Deerfield, Illinois), with intra-assay and inter-assay coefficients of variation less than 7.1%. HCY levels were available for 954 of the 1,000 women enrolled in the trial, and these were measured in samples stored at –80°C using an ELISA assay with no pre-dilution. BMI was calculated as weight in kilograms divided by height in meters squared. Insulin resistance index determined by HOMA-IR was computed according to the following formula (19): HOMA-IR = fasting plasma insulin (mIU/L) x fasting plasma glucose (mmol/L) / 22.5.

Statistical Analysis

Descriptive statistics were used to compare characteristics between different groups. Continuous variables were reported as means with standard deviations, and categorical variables were summarized in frequencies and percentages. Analysis of variance/Kruskal–Wallis test and Fisher's exact test were used to determine differences among three groups, and Student's t-test or Fisher's exact test were used for pairwise comparisons between the groups. Logistic regression was performed to analyze the differences in reproductive outcomes in the three

groups after adjusting for acupuncture and clomiphene treatment in the PCOSAct. All analyses were performed in SAS version 9.3 software (SAS Institute Inc), and p-values <0.05 were considered statistically significant.

Results

All participants were divided into three HCY tertiles (lower tertile HCY ≤6.09 µmol/L, median tertile HCY 6.10 -9.33 µmol/L, and higher tertile HCY ≥9.34 µmol/L), and their demographic characteristics of are shown in Table 1. Women in the higher tertile of HCY tended to have higher BMI and free testosterone levels and lower FSH, SHBG, and fasting glucose levels compared with the median and lower tertile groups. There were no major differences in ovary morphology parameters, WHR, fasting insulin, HOMA-IR, or MS prevalence according to HCY tertile (Table 1).

Higher HCY tertile levels were associated with lower ovulation rate among all women (p = 0.003) but were not associated with ovulation per ovulation induction cycle (p = 0.20). Higher rates of late miscarriage in the second and third trimesters were observed in the higher HCY tertile group (p = 0.05), but after adjusting for treatment the late miscarriage rate in the higher tertile HCY group was no longer significant (p = 0.5032). There were comparable rates of conception, pregnancy, and live birth for the three HCY tertiles (Table 2).

The 908 participants were then divided into three groups – HHCY (defined as a serum total serum concentrations of HCY >12.14 µmol/L (4)), MS, and controls. We also observed that there were comparable levels of BMI, fasting glucose, insulin, HOMA-IR, and hirsutism scores in the HHCY and control groups, and these were significantly lower than in the MS group. LH, LH/FSH, and SHBG were similar in the HHCY and control groups and were significantly higher than the MS group. The HHCY group had comparable levels of WHR, estradiol, and free testosterone as the MS group, and these were significantly different from the control group (Table 3).

There were comparable rates of ovulation and conception among all women between the HHCY and control groups, and these were significantly higher than in the MS group, indicating relatively normal oocyte fertilization under conditions of HHCY and impaired oocytes with concurrent MS in women with PCOS undergoing infertility treatment (Table 4).

A novel finding is that there were significantly lower rates of pregnancy and live birth in the MS group, and there were comparably higher rates of total and late pregnancy loss in both the HHCY and MS groups (p < 0.001 and p = 0.05, respectively) compared with controls. After adjusting for treatment, the higher rate of pregnancy loss lost significance in the MS group, while

the HHCY group maintained significantly lower ovulation rates among all women and higher miscarriage rates per conception compared with the control group.

Thus, HHCY contributes to defects in ovulation and pregnancy among all women, and MS contributes to defects in ovulation, conception, pregnancy, and live birth, indicating that the two conditions lead to defects at different reproductive stages.

Table 1. Baseline characteristics of the subjects.

Characteristic	Quartile 1	Quartile 2	Quartile 3	Overall	p-value
HCY(μmol/L)	HCY≥9.34	6.09<HCY<9.34	HCY≤6.09	8.36 ± 4.83 (936)	<0.0001
Biometric features					
Age, years	27.9 ± 3.3 (311)	27.9 ± 3.5 (312)	27.9 ± 3.2 (312)	27.9 ± 3.3 (935)	0.96
BMI, kg/m^2	24.9 ± 4.3 (310)**	24.0 ± 4.3 (312)	23.5 ± 4.0 (312)	24.1 ± 4.2 (934)	<0.001
Obesity (%)					
Waist-to-hip ratio (WHR)	0.9 ± 0.1 (311)	0.9 ± 0.1 (312)	0.9 ± 0.1 (312)	0.9 ± 0.1 (935)	0.10
Modified Ferriman-Gallwey score	3.1 ± 2.7 (311)	3.1 ± 3.0 (312)	3.0 ± 2.8 (312)	3.0 ± 2.8 (935)	0.67
Acne score	0.4 ± 0.7 (311)	0.4 ± 0.7 (312)	0.5 ± 0.8 (312)	0.4 ± 0.8 (935)	0.88
Ultrasonographic findings					
Polycystic ovary morphology of any ovary	255/289[b] (88.2)	260/293 (88.7)	258/298 (86.6)	773/880 (87.8)	0.70
Any ovary volume ≥ 10 cm^3	95/132 (72.0)	110/153 (71.9)	106/163 (65.0)	311/448 (69.4)	0.31
Polycystic ovaries according to the Rotterdam criteria,	276/299 (92.3)	275/299 (92.0)	274/304 (90.1)	825/902 (91.5)	0.59
Fasting serum levels					
LH (mIU/mL)	10.3 ± 5.2 (310)	10.6 ± 6.5 (310)	10.7 ± 5.9 (307)	10.5 ± 5.9 (927)	0.78
FSH (mIU/mL)	5.9 ± 1.5 (310)	6.0 ± 1.6 (310)	6.3 ± 1.9 (308)**	6.1 ± 1.7 (928)	0.005
LH to FSH ratio	1.8 ± 0.9 (310)	1.8 ± 1.0 (310)	1.8 ± 1.4 (307)	1.8 ± 1.1 (927)	0.41
Progesterone (ng/ml)	2.5 ± 5.2 (308)	2.3 ± 2.9 (310)	2.9 ± 6.5 (308)	2.6 ± 5.1 (926)	0.41
Estradiol (pg/mL)	76.0 ± 101.9 (309)	67.3 ± 63.7 (310)	75.7 ± 85.4 (309)	73.0 ± 85.1 (928)	0.47
Total testosterone (ng/dL)	48.9 ± 18.1 (310)	47.8 ± 17.3 (310)	47.1 ± 20.0 (309)	47.9 ± 18.5 (929)	0.24
Sex hormone-binding globulin (nmol/L)	42.2 ± 32.7 (307)	41.0 ± 29.9 (307)	44.9 ± 28.5 (306)**	42.7 ± 30.4 (920)	0.003
Free testosterone (pg/ml)	2.5 ± 0.9 (311)**	2.3 ± 0.8 (307)	2.1 ± 0.8 (312)	2.3 ± 0.8 (930)	<0.001

Characteristic	Quartile 1	Quartile 2	Quartile 3	Overall	p-value
Fasting glucose (mg/dL)	87.3 ± 18.4 (309)	91.2 ± 15.5 (309)	96.2 ± 17.6 (308)**	91.6 ± 17.6 (926)	<0.001
Fasting insulin (μIU/mL)	13.5 ± 11.0 (310)	14.1 ± 12.0 (310)	13.6 ± 13.7 (307)	13.7 ± 12.3 (927)	0.55
Metabolic Parameter					
HOMA-IR	3.0 ± 2.7(308)	3.3 ± 3.4 (307)	3.4 ± 4.2 (306)	3.2 ± 3.5 (921)	0.62
Metabolic syndrome	66/312 (21.2)	67/312 (21.5)	54/312 (17.3)	187/936 (20.0)	0.35

** $p < 0.05$, compared with others.

Table 2. Outcomes with regard to live birth, ovulation, pregnancy, and pregnancy loss in the subjects with different HCY tertiles.

Outcome	Quartile 1	Quartile 2	Quartile 3	p-value	p-value Higher vs. Median	p-value Higher vs. Lower	p-value Median vs. Lower	p-value after adjusting for treatment
HCY(μmol/L)	≥ 9.34	6.09 - 9.34	≤ 6.09		.	.	.	
Primary outcome								
Live births/All women[a]	56/312 (17.9)	64/312 (20.5)	72/312 (23.1)	0.28	0.4164479	0.1126869	0.4379165	0.3706
Secondary outcomes					.	.	.	
Conceptions/All women	98/312 (31.4)	94/312 (30.1)	109/312 (34.9)	0.41	0.7286331	0.349655	0.1999395	0.6251
Pregnancies/All women	62/312 (19.9)	65/312 (20.8)	76/312 (24.4)	0.36	0.7654846	0.1768875	0.2923705	0.4840
Twin pregnancies/All pregnancies	4/62 (6.5)	5/65 (7.7)	6/76 (7.9)	1.00	1	1	0.9643653	0.9755
Pregnancy losses/All women who conceived	40/98 (40.8)	29/94 (30.9)	35/109 (32.1)	0.28	0.1502647	0.1932103	0.8473393	0.2499
Losses in first trimester/ All women who conceived	35/98 (35.7)	29/94 (30.9)	33/109 (30.3)	0.68	0.4748627	0.4054632	0.9292239	0.6054
Losses in second or third trimester/All women who conceived	5/98 (5.1)	0/94 (0.0)	2/109 (1.8)	0.05	0.0264838	0.2594171	0.5002683	0.5032
Ovulations/All women	224/312 (71.8)*	253/312 (81.1)	255/312 (81.7)	0.003	0.0062243	0.0032997	0.8369412	0.0040**
Ovulations/Total number of treatment cycles	489/1045 (46.8)	515/1083 (47.6)	524/1037 (50.5)	0.20	0.7259226	0.0881282	0.1704403	0.5547

[a] All women who had HCY measurements taken.

** $p < 0.05$, compared with others.

* $p < 0.05$, compared with

Table 3. Baseline characteristics of the subjects with higher HCY, concurrent MS, and normal controls

Characteristic	Control	HHCY	MS	Overall	p-value
Number of women	628	121	159	908	
HCY(µmol/L)	6.73 ± 2.77 (628)	16.57 ± 5.37 (121)**	7.11 ± 2.68 (159)	8.10 ± 4.63 (908)	<0.0001
Biometric features					
Age (years)	27.8 ± 3.3 (628)	27.7 ± 3.1 (120)	28.5 ± 3.7 (159)	27.9 ± 3.3 (907)	0.07
BMI (kg/m^2)	22.9 ± 3.7 (628)	24.4 ± 3.6 (120)	27.9 ± 3.6 (158)*	24.0 ± 4.1 (906)	<0.001
Waist-to-hip ratio	0.8 ± 0.1 (628)	0.9 ± 0.1 (120)*	0.9 ± 0.1 (159)*	0.9 ± 0.1 (907)	<0.001
Modified Ferriman-Gallwey score	2.9 ± 2.8 (628)	3.0 ± 2.3 (120)	3.6 ± 3.0 (159)*	3.0 ± 2.8 (907)	0.02
Acne score	0.5 ± 0.8 (628)	0.4 ± 0.7 (120)	0.4 ± 0.8 (159)	0.4 ± 0.8 (907)	0.72
Ultrasonographic findings					
Polycystic ovary morphology of any ovary (%)	516/589[a] (87.6)	98/112 (87.5)	135/153 (88.2)	749/854 (87.7)	0.98
Any ovary volume ≥10 cm^3 (%)	215/321 (67.0)	37/46 (80.4)	53/71 (74.6)	305/438 (69.6)	0.11
Polycystic ovaries according to the Rotterdam criteria(%)	551/605 (91.1)	107/114 (93.9)	142/156 (91.0)	800/875 (91.4)	0.61
Fasting serum levels					
LH (mIU/mL)	11.1 ± 6.2 (621)	10.5 ± 5.3 (120)	8.6 ± 4.8 (158)*	10.6 ± 6.0 (899)	<0.001
FSH (mIU/mL)	6.2 ± 1.7 (621)	6.1 ± 1.5 (120)	5.9 ± 1.5 (159)*	6.1 ± 1.7 (900)	0.06
LH to FSH ratio	1.9 ± 1.3 (621)	1.7 ± 0.9 (120)	1.5 ± 0.8 (158)*	1.8 ± 1.1 (899)	<0.001
Progesterone (ng/ml)	2.8 ± 6.0 (620)	2.0 ± 1.2 (119)	2.1 ± 2.5 (159)	2.6 ± 5.1 (898)	0.40
Estradiol (pg/mL)	77.7 ± 98.6 (621)	63.7 ± 29.9 (120)*	62.0 ± 53.7 (159)*	73.1 ± 85.9 (900)	0.04
Total testosterone (ng/dL)	47.5 ± 18.9 (622)	48.8 ± 16.0 (120)	48.1 ± 19.5 (159)	47.8 ± 18.6 (901)	0.50

Characteristic	Control	HHCY	MS	Overall	p-value
Sex hormone-binding globulin (nmol/L)	46.8 ± 29.9 (615)	47.1 ± 39.7 (119)	26.1 ± 17.6 (158)*	43.2 ± 30.7 (892)	<0.001
Free testosterone (pg/ml)	2.2 ± 0.8 (623)	2.5 ± 0.8 (120)*	2.4 ± 0.8 (159)*	2.3 ± 0.8 (902)	<0.001
Fasting glucose (mg/dL)	90.4 ± 14.0 (620)	84.2 ± 20.4 (120)	101.6 ± 22.5 (159)*	91.5 ± 17.5 (899)	<0.001
Fasting insulin (µIU/mL)	11.3 ± 10.1 (621)	11.6 ± 7.5 (120)	23.0 ± 16.6 (158)*	13.4 ± 12.1 (899)	<0.001
HOMA-IR	2.6 ± 2.6 (617)	2.5 ± 1.9 (119)	6.0 ± 5.5 (158)*	3.2 ± 3.5 (894)	<0.001

[a] The first number is the number of women, and the second number is the total number of women with ultrasonographic findings in the PCOSAct.

Table 4. Outcomes with regard to live birth, ovulation, pregnancy, and pregnancy loss in the subjects with higher HCY and concurrent MS.

Outcome	Control group a	HHCY group b	MS Group c	p-value	p-value between group b and group a	p-value between group c and group a	p-value between group b and group c	p-value after adjusting for treatment
Number of women	**628**	**121**	**159**		.	.	.	
Primary outcome								
Live births/All women	151/628 (24.0)	21/121 (17.4)	18/159 (11.3)	0.001	0.1091769	0.0004827	0.1485408	0.0027
Secondary outcomes					.	.	.	
Conceptions/All women	217/628 (34.6)	40/121 (33.1)	36/159 (22.6)**	0.02	0.7508997	0.0040654	0.0521835	0.0303
Pregnancies/All women	155/628 (24.7)	24/121 (19.8)	21/159 (13.2)**	0.006	0.2523176	0.001924	0.1347199	0.0126
Twin pregnancies/All pregnancies	12/155 (7.7)	2/24 (8.3)	1/21 (4.8)	1.00	1	1	1	0.8400
Pregnancy losses/All women who conceived	64/217 (29.5)**	18/40 (45.0)	16/36 (44.4)	0.05	0.0531847 = 0.05	0.0739845 = 0.07	0.96121	0.0494 <0.05
Losses in first trimester/All women who conceived	64/217 (29.5)	15/40 (37.5)	13/36 (36.1)	0.49	0.3132233	0.4241611	0.9002621	0.4514
Losses in second or third trimester/All women who conceived	0/217 (0.0)**	3/40 (7.5)	3/36 (8.3)	<0.001	0.0035334	0.002677	1	0.9531
Ovulations/All women	513/628 (81.7)	88/121 (72.7) *	113/159 (71.1) *	0.003	0.0234119	0.003026	0.7600627	0.0093
Ovulations/Total number of treatment cycles	1085/2119 (51.2)	190/410 (46.3)	216/542 (39.9)*	<0.001	0.0714917	2.3874E-6	0.0450033	0.0001

Discussion

The most important finding of our study was that there were significantly lower rates of live birth, pregnancy, conception, and ovulation (both among all women and per treatment cycle) in infertile women with PCOS and MS and that there were lower ovulation rates among all women and higher miscarriage rates in infertile women with PCOS and HHCY. This suggests that HHCY contributes to reduced ovulation and miscarriage among all women and that MS contributes to defects in ovulation, conception, pregnancy, and live birth, which indicates that HHCY and MS lead to defects at different reproductive stages. Another interesting finding was that the ovulation rate among all women was significantly lower in the group with the higher baseline HCY level. Moreover, patients with PCOS in the higher HCY tertile tended to have higher BMI and free testosterone and lower FSH and fasting glucose than those in the median and lower tertiles. Women with MS had higher BMI, WHR, free testosterone, fasting glucose, fasting insulin, and HOMA-IR and lower LH, FSH, LH/FSH, estradiol, and SHBG compared to controls.

This study found that HCY is related to fasting glucose and that HHCY is associated with lower fasting glucose but not with insulin and HOMA-IR, and that MS is associated with fasting glucose, fasting insulin, and HOMA-IR. These results are consistent with a series of investigations that also showed no association between HCY and insulin resistance, insulin levels, degree of obesity, or diabetes status (20, 21).

However, accumulating evidence suggests that there is a positive correlation between HCY levels and fasting insulin level and HOMA-IR and impaired glucose tolerance (22-25). Recent studies have shown that insulin resistance is an independent risk factor for the progression of abnormal glucose metabolism (26), and HHCY might be correlated with impaired glucose tolerance in Chinese adults (27). HCY is mainly obtained from the diet, and thus HCY levels are influenced by many nonenzymatic factors, including age, gender, sex-steroid environment, smoking, chronic inflammation, nutrition, coffee consumption, and physical activity. A previous study has reported that there is a positive correlation between HCY and serum insulin independent of BMI (28). The mechanisms linking HHCY and glucose and fasting insulin or insulin resistance might be caused by the insulin-mediated reduction in the activity of key enzymes that govern the demethylation pathway or control the transsulfuration pathway (29,30). We found that HCY was related to BMI and that HHCY subjects tended to have higher WHR, and these findings were consistent with a previous study showing that HCY level is positively correlated

with BMI (31). Elevated plasma HCY levels in PCOS patients are independent of obesity (32), and an increase in body weight is not an independent risk factor for an increase in plasma HCY levels in women with PCOS (33).

Serum HCY level was found to be correlated with free testosterone and estradiol in this study, but no associations were found between serum HCY level and total testosterone and LH. This finding was contradictory with some studies that found HCY level to be correlated with total testosterone (34). Women with HHCY tended to have lower estradiol levels, and one possible reason is that higher follicular fluid HCY levels might suppress estradiol production and interfere with the development of dominant follicles, oocyte maturation, and fertilization in women with PCOS undergoing assisted reproduction treatment. However, recent research has suggested that high HCY levels in women with PCOS are not related to androgen levels or estradiol levels (35,36).

Our present study showed that HHCY subjects had significantly lower ovulation among all women and higher pregnancy loss, while MS subjects had significantly lower rates of conception, pregnancy, ovulation, and live birth after adjusting for acupuncture and clomiphene citrate treatment. These findings suggest that high HCY concentrations in early pregnancy might adversely influence the success of pregnancy and birth outcomes.

HHCY during pregnancy is suggested to play a significant role in pregnancy loss, and endothelial cell dysfunction is a central theme. Our findings are consistent with a series of investigations showing similar relations between higher HCY concentrations and miscarriage, and some authors report that HHCY leads to as much as a three-fold increase in the risk for early pregnancy loss (37). Other studies have confirmed that mild HHCY is associated with vascular-related pregnancy complications, such as recurrent miscarriages and intrauterine growth restriction (38,39). The increased maternal serum HCY levels in PCOS patients might inhibit the enzymatic activity of methyltransferase, thus increasing the magnitude and frequency of uterine smooth muscle contractions, which in turn might induce abortion in the early stage of pregnancy and increase the risk of pregnancy-related complications (40,41). Experimental studies have shown that moderately elevated HCY concentrations (16–24 μmol/l) might induce cytotoxic and oxidative stress leading to endothelial cell impairment (42). As far as we know, there is little research about plasma HCY levels and reproductive outcome, and most of the studies have focused on HCY levels in follicular fluid.

Previous studies showed that high HCY levels in the follicular fluid cause decreased cell division and high fragmentation in embryo cultures, thus leading to a decrease in oocyte and embryo quality (43, 44), and others have reported associations between mild to moderate HHCY and detrimental effects on reproductive outcome (45). However, other contradictory studies have reported no differences in follicular parameters or clinical pregnancy rates between PCOS patients with HHCY and controls after in vitro fertilization (46). A recent study reported that HHCY could increase the incidence of obstetric complications and that maternal serum HCY levels were negatively correlated with the birth weight of the neonate (47), but no such correlation was observed in our study.

Strengths and Limitations

Strengths of this study include measurement of HCY in a large cohort of 936 women, making this the largest study of its kind to evaluate the association between HCY and reproductive outcomes in infertile women with PCOS. To our knowledge, this is the first study to examine the correlation between levels of HCY in pregnant women with adverse pregnancy outcomes. One limitation of this study is that this was a secondary analysis of data from a randomized clinical trial, and it is hard to confirm causality. Another limitation is that HCY levels were only available at baseline and not during the whole pregnancy.

Interpretation

Because elevated HCY levels are treatable, early preventive interventions could be important. If low cost and effective interventions to reduce plasma HCY, such as folic acid supplements, can also reduce the risk of undesirable reproductive outcomes in these patients, then the benefits for public health could be substantial (in addition to explaining the link between these diseases).

Conclusion

In summary, elevated maternal serum levels of HCY might be a sensitive and independent biomarker for the early prediction of abnormal glucose and lipid metabolism and adverse pregnancy outcomes, such as ovulation defects, in infertile patients with PCOS. This is a novel objective parameter for the prevention and treatment of adverse pregnancy outcomes.

Disclosure of conflicts of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Contribution to authorship

Dr. XK Wu had full access to all of the data in the study (including statistical reports and tables) and thus takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Dr. XK Wu, H Chang, LZ Xie, and Dr. CC Wang. Drafting of the manuscript: H Chang. Data collection and data interpretation: H Chang and LZ Xie. Statistical analysis: LZ Xie, Q Wu, and Dr. CC Wang. Supervision: Dr. XK Wu. All authors critically reviewed the article and approved it for submission.

Details of ethics approval

The study was approved by the regional ethics committee at The First Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, Harbin, China.

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References

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.

2. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS,et al. Polycystic ovary syndrome. Nat Rev Dis Primers 2016;2:16057.

3. Li R, Zhang Q, Yang D, Li S, Lu S, Wu X,et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. Hum Reprod.2013;28(9):2562-9.

4. Lin YH, Huang SY, Hsu MI, Chang YC, Cheng CY, Hsu CS, Tzeng CR. Hyperhomocysteinaemia is associated with biochemical hyperandrogenaemia in women with reproductive age. European Journal of Obstetrics & Gynecology and Reproductive Biology 2013;171:314-8.

5. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA.1995;274(13):1049-1057.

6. Kwon HM, Lee YS, Bae HJ, Kang DW. Homocysteine as a predictor of early neurological deterioration in acute is chemic stroke. Stroke 2014;45(3): 871-3.

7. Toulis KA, Goulis DG, Mintziori G, Kintiraki E, Eukarpidis E, Mouratoglou SA, et al. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Human reproduction update 2011;17(6):741–60.

8. Soares GM, Vieira CS, Martins WP, Franceschini SA, dos Reis RM, Silva de Sá MF, et al. Increased arterial stiffness in nonobese women with polycystic ovary syndrome (PCOS) without comorbidities: one more characteristic inherent to the syndrome. Clin Endocrinol (Oxf). 2009;71: 406-11.

9. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF.Circulating markers of oxidative stress and polycystic ovary syndrome (pcos): A systematic review and meta-analysis. Human reproduction update 2013;19(3):268-88.

10. Wang X, Ye P, Cao R, Yang X, Xiao W, Zhang Y,et al. The association of homocysteine with metabolic syndrome in a community-dwelling population: homocysteine might be concomitant with metabolic syndrome. PLoS One 2014;9 (11):e113148.

11. Meng Y, Chen X, Peng Z, Liu X, Sun Y, Dai S. Association between High Serum Homocysteine Levels and Biochemical Characteristics in Women with Polycystic Ovarian Syndrome: A Systematic Review and Meta-Analysis.PLoS One 2016;11(6):e0157389.

12. Jia L, Li J, He B, Jia Y, Niu Y, Wang C,et al. Abnormally activated one-carbon metabolic pathway is associated with mtDNA hypermethylation and mitochondrial malfunction in the oocytes of polycystic gilt ovaries.Sci Rep 2016, 6: 19436.

13. Van den Boogaard E, Hermens RP, Franssen AM, Doornbos JP, Kremer JA, van der Veen F,et al. Recurrent miscarriage: do professionals adhere to their guidelines. Hum. Reprod 2013;28(11): 2898-904.

14. Yajnik CS, Chandak GR, Joglekar C, Katre P, Bhat DS, Singh SN,et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis.Int J Epidemiol 2014; 43(5): 1487-97.

15. Chen Z, Zhang Y, Liu J. Diagnosis of polycystic ovary syndrome: standard and guideline of Ministry of Health of People's Republic of China. Zhong Hua Fu Chan Ke Za Zhi 2012;47(1):74-5.

16. Li R, Qiao J, Yang D, Li S, Lu S, Wu X, et al. Epidemiology of hirsutism among women of reproductive age in the community: a simplified scoring system.Eur J Obstet Gynecol Reprod Biol 2012;163(2):165-9.

17. Zhao X, Ni R, Li L, Mo Y, Huang J, Huang M,et al. Defining hirsutism in Chinese women: a cross-sectional study. Fertil Steril 2011;96(3):792-6.

18. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Alvero R, et al. The Pregnancy in Polycystic Ovary Syndrome II study: baseline characteristics and effects of obesity from a multicenter randomized clinical trial. Fertility and sterility 2014;101(1):258-69.

19. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care. 2004;27(6):1487-95.

20. Tessari P, Vettore M, Puricelli L, Cecchet D, Coracina A, Kivanuka E. Decreased homocysteine trans-sulfuration in hypertension with hyperhomocysteinemia: relationship with insulin resistance. J Clin Endocrinol Metab 2017-01076

21. Li Y, Jiang C, Xu G, Wang N, Zhu Y, Tang C, et al. Homocysteine upregulates resistin production from adipocytes in vivo and in vitro. Diabetes 2008;57(4):817-27.

22. Grodnitskaya EE, Kurtser MA. Homocysteine metabolism in polycystic ovary syndrome. Gynecol Endocrinol 2012;28(3):186-9.

23. Pang Y, Li Y, Lv Y, Sun L, Zhang S, Li Y, et al. Intermedin Restores Hyperhomocysteinemia-induced Macrophage Polarization and Improves Insulin Resistance in Mice. J Biol Chem 2016;291(23):12336-45.

24. Qi X, Zhang B, Zhao Y, Li R, Chang HM, Pang Y,et al.Hyperhomocysteinemia Promotes Insulin Resistance and Adipose Tissue Inflammation in PCOS Mice Through Modulating M2 Macrophage Polarization via Estrogen Suppression. Endocrinology 2017;158(5):1181-1193.

25. Feng X, Xu Y.Hyperhomocysteinemia as a Metabolic Risk Factor for Glucose Intolerance Among High-Risk Groups of Chinese Adults. Med Sci Monit 2017;23:2775-2781.

26. Festa A, Williams K, D'Agostino R Jr, Wagenknecht LE, Haffner SM.The natural course of beta-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study. Diabetes 2006;55(4):1114-20.

27. Feng X, Xu Y.Hyperhomocysteinemia as a Metabolic Risk Factor for Glucose Intolerance Among High-Risk Groups of Chinese Adults. Med Sci Monit 2017;23,2775-81.

28. Altuğ Şen T, Köken R, Narcı A, Yılmazer M.Homocysteine and ghrelin link with polcystic ovary syndrome in relation to obesity., J Pediatr Adolesc Gynecol 2011;24(4): 211-7.

29. Dicker-Brown A, Fonseca VA, Fink LM, Kern PA.The effect of glucose and insulin on the activity of methylene tetrahydrofolate reductase and cystathionine-beta-synthase: studies in hepatocytes.Atherosclerosis 2001;158(2): 297-301.

30. Fonseca V, Keebler M, Dicker-Brown A, Desouza C, Poirier LA, Murthy SN. The effect of troglitazone on plasma homocysteine, hepatic and red blood cell S-adenosyl methionine, and S-adenosyl homocysteine and enzymes in homocysteine metabolism in Zucker rats. Metabolism 2002;51(6):783-6.

31. He Y, Liu S, Zhang Z, Liao C, Lin F, Yao W,et al. Imbalance of Endogenous Hydrogen Sulfide and Homocysteine in Chronic Obstructive Pulmonary Disease Combined with Cardiovascular Disease. Front Pharmacol 2017;12;8:624.

32. Altug Sen T, Koken R, Narci A, Yilmazer M. Homocysteine and ghrelin link with polycystic ovary syndrome in relation to obesity. J Pediatr Adolesc Gynecol 2011;24(4):211-7.

33. I Al-Gareeb A,Abd Al-Amieer WS, M Alkuraishy H, J Al-Mayahi T.Effect of body weight on serum homocysteine level in patients with polycystic ovarian syndrome: A case control study. Int J Reprod Biomed (Yazd) 2016;14(2):81-8.

34. Lin YH, Huang SY, Hsu MI, Chang YC, Cheng CY, Hsu CS,et al. Hyperhomocysteinaemia is associated with biochemical hyperandrogenaemia in women with reproductive age.Eur J Obstet Gynecol Reprod Biol 2013;171(2):314-8.

35. Meng Y, Chen X, Peng Z, Liu X, Sun Y, Dai S. Association between High Serum Homocysteine Levels and Biochemical Characteristics in Women with Polycystic Ovarian Syndrome: A Systematic Review and Meta-Analysis.PLoS ONE 2016;11(6):e0157389.

36. Eskandari Z, Sadrkhanlou RA, Nejati V, Tizro G. PCOS women show significantly higher homocysteine level, independent to glucose and E2 level. Int J Reprod Biomed (Yazd) 2016;14(8): 495-500.

37. Chakraborty P, Goswami SK, Rajani S, Sharma S, Kabir SN, Chakravarty B,et al. Recurrent pregnancy loss in polycystic ovary syndrome: role of hyperhomocysteinemia and insulin resistance. PLoS ONE 2013;8(5): e64446.

38. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr 2000;71(4):962-8.

39. Steegers-Theunissen RP, Boers GH, Blom HJ, Trijbels FJ, Eskes TK. Hyperhomocysteinaemia and recurrent spontaneous abortion or abruption placentae. Lancet 1992;339(8801):1122-3.

40. Qiu X,Gao F, Qiu Y, Bao J, Gu X, Long Y. Association of maternal serum homocysteine concentration levels in late stage of pregnancy with preterm births: a nested case-control study. J Matern Fetal Neonatal Med 2017;11:1-5.

41. Kawamura M, Fukuda N, Suzuki A, Kobayashi Y, Matsuda M, Kanda R,et al.Use of fibrin monomer complex for screening for venous thrombo-embolism in the late pregnancy and post-partum period. J Obstet Gynaecol Res 2014;40(3):700-4.

42. Mohamed R, Sharma I,Ibrahim AS, Saleh H, Elsherbiny NM, Fulzele S, et al. Hyperhomocysteinemia Alters Retinal Endothelial Cells Barrier Function and Angiogenic Potential via Activation of Oxidative Stress. Sci Rep 2017;7(1):11952.

43. Berker B, Kaya C, Aytac R, Satiroglu H. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. Hum Reprod 2009;24(9):2293-302.

44. Ocal Pelin,Ersoylu Bilge,Cepni Ismail, Guralp O, Atakul N, Irez T,et al. The association between homocysteine in the follicular fluid with embryo quality and pregnancy rate in assisted reproductive techniques. J Assist Reprod Genet 2012;29(4):299-304.

45. Boxmeer JC, Steegers-Theunissen RP, Lindemans J, Wildhagen MF, Martini E, Steegers EA, et al. Homocysteine metabolism in the preovulatory follicle during ovarian stimulation. Hum Reprod 2008;23(11):292-930.

46. Nafiye Y, Sevtap K, Muammer D, Emre O, Senol K, Leyla M. The effect of serum and intrafollicular insulin resistance parameters and homocysteine levels of nonobese, nonhyperandrogenemic polycystic ovary syndrome patients on in vitro fertilization outcome. Fertil Steril 2010;93(6):1864-69.

47. Dhobale M, Chavan P, Kulkarni A, Mehendale S, Pisal H, Joshi S. Reduced folate, increased vitamin B(12) and homocysteine concentrations in women delivering preterm. Ann Nutr Metab 2012;61(1):7-14.