

Research Article

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GPER was associated with hypertension in postmenopausal women

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Abstract: Objective: To explore the relationship between G protein-coupled estrogen receptor (GPER) and hypertension in postmenopausal women.

Methods: Using a matched case-control design, clinical and laboratory data were collected. Conditional logistic regression with stratified analysis was conducted to identify the association between GPER and hypertension.

Results: The GPER level was significantly lower in the case group than in the control group (126.3 ± 21.6 vs. 133.6 ± 27.3 , $P=0.000$). The GPER levels of the hypertension cases with and those without menopause were significant (120.5 ± 11.8 and 127.2 ± 12.1 , $P=0.000$). No significant difference in the GPER level between the controls with and those without menopause was observed ($P=0.241$). Logistic regression revealed that the GPER quartile was related to hypertension (odds ratio [OR]: 0.63, 95% confidence interval [CI]: 0.13–0.93, $P=0.018$) after adjusting for potential confounding factors. Stratified analysis revealed that the GPER quartile was not associated with hypertension in premenopausal women, and the fourth GPER quartile showed a predictive association with hypertension (OR: 0.43, 95% CI: 0.29–0.90) in menopausal women.

Conclusions: GPER level is associated with hypertension and is a protective factor for hypertension in menopausal women but not premenopausal women. Further research is required due to study limitations.

Keywords: Hypertension; G protein-coupled estrogen receptor; Menopause; Estrogen receptor; Case-control

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1 Introduction

Previous studies have reported an obvious elevation in blood pressure in women after menopause [1]. In women, hormonal changes and the use of exogenous hormones result in crucial differences in blood pressure rhythm [2]. Estrogen plays an important regulatory role not only in the reproductive system but also in the cardiovascular, immune, and nervous systems [3]. It was recently reported that estrogen exerts its biological effects on hypertension by binding to the estrogen receptor. Estrogen receptor- α and - β are typical estrogen receptors. Both receptors reduce estrogen's regulatory function via specific transcription of target genes [4]. G protein-coupled estrogen receptor (GPER) is a transmembrane protein [5]. It has been suggested that GPER mediates the rapid non-genetic response of estrogen, acting as an estrogen receptor. It has been confirmed both *in vitro* and *in vivo* that GPER is widely expressed in the cardiovascular system and is associated with estrogen specificity. It was also found that selective activated GPER can expand blood vessels, lower blood pressure, and inhibit vascular smooth muscle cell differentiation, making GPER an indispensable material for maintaining normal blood pressure [6,7]. However, these results are inconsistent with those from clinical studies. In humans, blood pressure is affected by a variety of factors. However, it is uncertain whether GPER is associated with hypertension and whether it is an independent risk factor in hypertensive patients, especially females. This study explored the relationship between the GPER level and hypertension in females after adjusting for potential confounding factors, with the aims of identifying new indicators and therapeutic targets for hypertension.

2 Methods and materials

2.1 Study population

The study population included women who visited the physical examination center of our hospital between May

2016 and October 2016. The subject age ranged from 35 to 80 years old. Hypertension was diagnosed as follows: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, a history of hypertension, or receiving a drug treatment during the past year [8]. We only included newly diagnosed hypertensive patients to exclude drug interference. The normal population (without hypertension) was treated as a control group during the same period. One patient was matched by age (± 2 years). The following were excluded: those with coronary heart disease, kidney disease, cancer, or stroke or those who used estrogen. A total of 400 hypertensive patients and 400 healthy controls were included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Data collection

A standard questionnaire was used to collect the following information: general demographic data, family history, smoking and drinking habits, menopause status, and history of drug usage. The research was performed by trained investigators. Height, weight, and waist circumference were measured using standard physical examination methods. Body mass index (BMI) was categorized as follows: obese, >28 ; overweight, 24–28; normal, 18.5–24; and underweight, <18.5 [9]. Experienced doctors and nurses adopted an internationally standardized method for blood pressure measurements using the mercury blood pressure gauge. Blood pressure was measured three times after 5 min of rest with at least 15 s between measurements, with the mean taken as the final value. If a difference of > 5 mm Hg among measurements for a subject was found, blood pressure was measured again.

For biochemical data, 5 mL of venous blood were extracted from each subject after fasting for 12h. Laboratory examinations, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood glucose, and uric acid levels, were performed by an automatic biochemical analyzer (Hitachi 7020, Tokyo, Japan). A level of TC ≥ 6.22 mmol/L, LDL-C ≥ 4.14 mmol/L, HDL-C

≤ 1.04 mmol/L, or TG ≥ 2.26 mmol was defined as abnormal[10]. Blood glucose levels were categorized as follows: normal, < 6.1 mmol/L; high, ≥ 6.1 mmol/L. A uric acid level ≥ 357 was considered high [11]. The GPER level was measured using a double antibody enzyme-linked immunosorbent assay kit (Shanghai Xinyu Biological Technology Co., Ltd., Shanghai, China) with a detection range of 18–600 ng/L. The detection process was completed by the same researchers under the same conditions.

2.3 Statistical analysis

Continuous data are expressed as means \pm standard error or as medians (quartile: Q25–Q75) according to the Kolmogorov–Smirnov test. Either Student's t-test or the Mann–Whitney U test was used to determine differences between two groups. Categorical data are expressed as percentages, and differences between two groups were assessed using the Chi-square test. The GPER level was divided into four categories according to quartiles 1–4. Conditional logistic regression analysis was conducted to identify an association between the GPER level and hypertension after adjusting for age, family history, menopause status, overweight status, and high uric acid, high blood lipid, and high blood glucose levels. The relative odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. Stratified analysis was performed by stratifying by menopause status. All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA), and P values < 0.05 were considered to indicate statistical significance.

3 Results

3.1 General characteristics of the study population

The present study included 400 hypertension cases and 400 normal controls. In the case group, the mean age was 53.8 ± 9.0 years, and 87% of subjects were in menopause. No hypertensive patients were on antihypertensive drug treatments. The mean age of the control group was 54.3 ± 8.2 years, and 77.7% of subjects were in menopause. The GPER level was significantly lower in the case group than in the control group (126.3 ± 21.6 vs. 133.6 ± 27.3 ng/L, respectively, $P=0.000$). The GPER levels of the hypertensive cases with and those without menopause were 120.5 ± 11.8 and 127.2 ± 12.1 , respectively, and this difference was

significant ($P=0.000$). The GPER levels of the controls with and those without menopause were 132.5 ± 12.1 and 134.2 ± 11.8 , respectively; no significant difference was observed ($P=0.241$). Compared with the control group, the case group tended to have a greater waist circumference ($P=0.000$), BMI ($P=0.000$), systolic blood pressure ($P=0.000$), diastolic blood pressure ($P=0.000$), and uric acid ($P=0.000$), TG ($P=0.000$), and blood glucose ($P=0.000$) levels and a family history of hypertension ($P=0.000$). There were no significant differences in age, smoking or drinking habits, or total cholesterol, HDL-C, or LDL-C levels between the case and control groups ($P>0.05$). Table 1 presents the detailed results.

3.2 Univariate and multivariate logistic regression analyses

We conducted conditional logistics regression analyses with and without adjusting for potential confounding factors. The results indicated that the GPER quartile (OR: 0.67, 95% CI: 0.31–0.81, $P=0.000$), age, overweight status, family history, menopause status, high uric acid level, dyslipidemia, and high blood glucose level were associ-

ated with hypertension before adjusting for potential confounding factors. The GPER quartile was still related to hypertension (OR: 0.63, 95% CI: 0.13–0.93, $P=0.018$) after adjusting for potential confounding factors. Other factors included age (OR: 2.89, 95% CI: 1.22–6.80, $P=0.015$), family history (OR: 1.37, 95% CI: 1.17–1.61, $P=0.000$), menopause status (OR: 2.88, 95% CI: 1.42–7.80, $P=0.002$), high uric acid level (OR: 1.69, 95% CI: 1.17–2.45, $P=0.005$), and high blood glucose level (OR: 1.30, 95% CI: 1.12–2.65, $P=0.026$). Table 2 presents the detailed results.

3.3 Stratified analysis

The study population was stratified by menopause status. The GPER level was divided into four categories according to quartile. We conducted conditional logistic regression using the first quartile as the reference. In premenopausal women, the GPER level was not associated with hypertension before adjusting for potential confounding factors, including age, smoking and drinking habits, family history, overweight status, high uric acid level, dyslipidemia, and high blood glucose level. Compared with the first quartile, the ORs for the second, third, and

Table 1: Comparisons of general characteristics between case and control group

Parameters	Control group (n=400)	Case group (n=400)	t/ χ^2	P
Age (year)	54.3±8.2	53.8±9.0	0.821	0.412
Waist (cm)	74.6±8.1	77.5±8.3	5.001	0.000
BMI (kg/m ²)	22.8±3.2	23.6±2.8	3.763	0.000
SBP (mmHg)	122.6±11.7	147.9±12.8	29.178	0.000
DBP (mmHg)	74.7±8.6	85.6±9.2	22.075	0.000
History of family	183 (45.8%)	240 (60.0%)	16.299	0.000
Smoking (n, %)	12 (3.0%)	16 (4.0%)	0.592	0.442
Drinking (n, %)	8 (2.0%)	6 (1.5%)	0.291	0.589
Menopause (n, %)	311 (77.7%)	348 (87.0%)	6.783	0.009
Uric acid (μmol/L)	261.2±21.8	277.8±23.5	10.357	0.000
Total cholesterol (mmol/L)	5.1±2.1	5.2±2.8	0.571	0.568
Triglyceride (mmol/L)	1.1±0.8	1.4±0.8	5.303	0.000
HDL-C (mmol/L)	1.3±0.2	1.3±0.1	0.000	0.999
LDL-C (mmol/L)	3.1±	3.0±	1.661	0.097
Blood glucose (mmol/L)	4.7±2.5	5.1±3.5	2.789	0.001
GPER(ng/L)	133.6±27.3	126.3±21.6	4.194	0.000

BMI: body mass index, SBP: systolic blood pressure, diastolic blood pressure: DBP, HDL-C: high-density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, GPER: G protein-coupled estrogen receptor

Table 2: Logistic regression analysis results for female hypertensive patients

Factors	Univariate analysis			Multivariate analysis		
	B	OR(95%CI)	P value	B	OR(95%CI)	P value
Age	0.248	1.28(1.06-1.54)	0.009	1.060	2.89(1.22-6.80)	0.015
Overweight	1.131	3.11(1.21-7.99)	0.018	0.047	1.05(0.98-1.12)	0.196
History of family	0.512	1.67(1.38-2.02)	0.000	0.314	1.37(1.17-1.61)	0.000
Smoking	0.016	1.02(0.79-1.31)	0.902	0.034	1.03(0.81-1.21)	0.476
Drinking	-0.237	0.79(0.62-1.76)	0.602	-0.321	0.73(0.76-1.67)	0.512
Menopause	1.095	2.99(1.11-8.05)	0.000	1.058	2.88(1.42-7.80)	0.002
High uric acid	0.450	1.57(1.19-2.07)	0.001	0.526	1.69(1.17-2.45)	0.005
Dyslipidemia	0.570	1.77(1.23-2.55)	0.012	-0.296	0.74(0.55-1.21)	0.061
High blood glucose	1.142	3.13(2.45-4.01)	0.000	0.265	1.30(1.12-2.65)	0.026
GPER quartile	-0.397	0.67(0.31-0.81)	0.000	-0.465	0.63(0.13-0.93)	0.018

Table 3: Logistic regression analysis results for hypertensive in female with and without menopause

GPER	premenopausal		menopause	
	Unadjusted	Adjusted	Unadjusted	Adjusted
The first quartile	1.00	1.00	1.00	1.00
The second quartile	1.05(0.73-1.50)	1.03(0.64-1.72)	1.08(0.91-1.43)	1.05(0.28-1.19)
The third quartile	0.41(0.41-1.97)	0.62(0.56-2.01)	0.81(0.63-1.49)	0.61(0.51-1.76)
The fourth quartile	0.26(0.30-3.40)	0.49(0.26-1.42)	0.56(0.38-0.71)	0.43(0.29-0.90)

*adjusted for age, smoking, drinking, history of family, overweight, high uric acid, dyslipidemia, high blood glucose

fourth quartiles were 1.05 (95% CI: 0.73–1.50), 0.41 (95% CI: 0.41–1.97), and 0.26 (95% CI: 0.30–3.40), respectively. After adjusting for potential confounding factors, the GPER quartile was still not associated with hypertension in premenopausal women, and the ORs were 1.03 (95% CI: 0.64–1.72), 0.62 (95% CI: 0.56–2.01), and 0.49 (95% CI: 0.26–1.42), respectively. In menopausal women, the results indicated that the second and third GPER quartiles were not associated with hypertension before adjusting for potential confounding factors. Compared with the first quartile, the ORs for the second and third quartiles were 1.08 (95% CI: 0.91–1.43) and 0.81 (95% CI: 0.63–1.49), respectively. The fourth quartile was associated with hypertension (OR: 0.56, 95% CI: 0.38–0.71). After adjusting for potential confounding factors, the second and third quartiles were still not associated with hypertension in premenopausal women. However, the fourth quartile showed a predictive association with hypertension (OR: 0.43, 95% CI: 0.29–0.90).

4 Discussion

Our study found (1) lower serum GPER levels in the hypertension group than in the normal healthy control group, (2) an independent association between the serum GPER level and hypertension in women after adjusting for potential confounding factors and a decreased risk of hypertension with elevated GPER levels, and (3) an association between the serum GPER level and hypertension in menopausal but not premenopausal women.

This epidemiologic investigation showed that the prevalence of hypertension is significantly lower in premenopausal women than in males [12]. The function of the ovary decreases gradually with the arrival of menopause, and the level of secreted estrogen is also gradually reduced [13]. The prevalence of hypertension increased and even outnumbered the male counterparts. It has been acknowledged that estrogen exerts its regulatory function via the estrogen receptor. Estrogen- α and - β belong to a

nuclear receptor family that is widely distributed in many tissues, including those of the reproductive and cardiovascular systems [5]. It is believed that it takes several hours or even several days for estrogen to exert effects on gene transcription via its nuclear receptor. However, it takes only several seconds or minutes for GPER and its membrane receptor to exert such effects. Most importantly, this process cannot be blocked by protein and RNA production [14]. The biomedical function of GPER differs from that of estrogen- α and - β , and there may be synergistic interactions or antagonistic or independent effects [15,16]. The current research on the relationship between GPER levels and blood regulation has been restricted to *in vitro* and animal experiments. Animal studies have revealed that the risk of hypertension increases when the GPER gene is mutated [17]. GPER agonists cause blood vessel expansion in rodents and humans. The binding of GPER to its ligands exerts a cardiovascular-protective function via many mechanisms, such as improving local blood circulation, lowering blood pressure, exerting an anti-inflammation effect, and regulating glucose and lipid metabolism [18]. Activated GPER generates a vasodilation effect by promoting the release of nitric oxide from endothelial cells, generating an endothelium-dependent hyperpolarization factor, inhibiting Ca^+ channels, and opening K^+ channels [19]. Moreover, GPER also can inhibit vasoconstriction by downregulating angiotensin II type 1 receptor expression and reducing angiotensin-converting enzyme activity [20]. Our results confirm the protective effects of GPER on hypertension in menopausal women.

Although the current study is based on a case-control design, it is limited in that it did not evaluate the causal relationship between the GPER level and hypertension. The current results support the use of estrogen replacement therapy and hypertension prevention in menopausal women. Estrogen replacement therapy, which reduces the risk of cardiovascular disease in menopausal women, is practiced in the clinic. However, a previous multi-center randomized controlled study with a large sample size suggested that estrogen replacement therapy does not reduce the risk of cardiovascular disease in those diagnosed with hypertension. In contrast, estrogen replacement therapy may increase the risks of stroke and deep vein thrombosis for reasons that remain unclear [21]. The *in vitro* and *in vivo* factors, including receptors, that may affect GPER-mediated signaling pathways are complicated and may vary. The serum estrogen level is associated with hypertension and regulates blood pressure via different mechanisms, but the specific mechanism has not yet been elucidated. Therefore, further study is required. Our study evaluated the serum GPER level only, and it should be confirmed

whether this factor reflects the physiological or pathological status of estrogen.

In conclusion, our study found that the GPER level is associated with hypertension in menopausal women and is a protective factor for hypertension. This relationship remained after adjusting for potential confounding factors. Further research is required due to the limitations of the present study.

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Conflict of interests: The authors declare that they have no competing or any commercial or proprietary interest in any drug, device, or equipment mentioned in the submitted article.

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