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# Associations of proteins relevant to MAPK signaling pathway (p38MAPK-1, HIF-1 and HO-1) with coronary lesion characteristics and prognosis of peri-menopausal women

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## Abstract

**Background:** The present study was intended to explore whether three proteins within MAPK signaling pathway (i.e. p38MAPK-1, HIF-1 and HO-1) were correlated with peri-menopausal women's coronary lesion features and prognosis.

**Methods:** Altogether 1449 peri-menopausal women were divided into non-coronary artery disease (CAD) group ( $n = 860$ ) and CAD group ( $n = 589$ ), including 167 pre-menopausal CAD populations and 422 post-menopausal CAD populations. General information about CAD risk parameters were gathered, including age, family history of CAD or hypertension or diabetes mellitus, bilirubin, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) and so on. Coronary angiography results were judged, and CAD score was calculated with application of Genisin scoring method. Besides, detection of MAPK-1 levels was implemented with Strept Avidin-Biotin Complex (SABC) method, while HIF-1 and HO-1 expressions in the serum were determined utilizing ELISA detection kit. Correlations among protein expressions, characteristics of coronary lesions and prognosis of CAD populations were finally evaluated.

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**Results:** Hypertension, hyperlipidemia, diabetes and smoking history were more prevalent among postmenopausal CAD women than premenopausal CAD women ( $P < 0.05$ ). Furthermore, postmenopausal women seemed to be significantly associated with multiple (i.e. double and triple) vessel lesions and severe lesion types (type B and C), when compared with premenopausal CAD group ( $P < 0.05$ ). Similarly, remarkably elevated expressions of p38MAPK-1, HIF-1 and HO-1 were found within postmenopausal CAD populations in comparison to premenopausal ones ( $P < 0.05$ ). The internal CysC, hs-CRP, TG and LDL-C concentrations all accorded with the following tendency: postmenopausal CAD women > premenopausal CAD women > non-CAD women. Moreover, p38MAPK-1, HIF-1 and HO-1 expressions were up-regulated with increasing number of vessel lesions and severity of coronary lesions among peri-menopausal women. Besides, among both pre-menopausal and post-menopausal CAD groups, positive correlations could be observed between MAPK-1 and TG ( $r_s = 0.271$ ;  $r_s = 0.476$ ), between HIF-1 $\alpha$  and LDL-C ( $r_s = 0.077$ ;  $r_s = 0.470$ ), as well as between HO-1 and CysC ( $r_s = 0.492$ ;  $r_s = 0.190$ ) or hs-CRP ( $r_s = 0.569$ ;  $r_s = 0.542$ ) (all  $P < 0.05$ ). MAPK-1, HIF-1 $\alpha$  and HO-1 were also, respectively, positively correlated with CysC ( $r_s = 0.415$ ), hs-CRP ( $r_s = 0.137$ ), and TG ( $r_s = 0.142$ ), regarding post-menopausal CAD women (all  $P < 0.05$ ). Finally, only SBP and TG were regarded as independent risk factors for CAD prognosis (i.e. high Genisin score) among premenopausal women (OR = 1.02, 95%CI: 1.01–1.18,  $P = 0.043$ ; OR = 1.82, 95%CI: 1.01–3.33,  $P = 0.047$ ).

**Conclusions:** Expressions of p38MAPK-1, HIF-1 and HO-1 could serve as predictive roles for coronary lesions among peri-menopausal women.

**Keywords:** Perimenopausal CAD, p38MAPK-1, HIF-1, HO-1, Coronary lesion characteristics, Prognosis

## Background

Cardiovascular disorders, especially coronary artery disease (CAD), always stand high in their causal mortality and morbidity [1]. It has been stressed that CAD was more prevalently found in men than in women for that estrogen can, to some extent, safeguard women from risk of CAD [2]. Nonetheless, emerging documentaries suggested that women who were below 50 years old appeared to be associated with 2 folds of mortality rate when compared with men of matched ages [3]. The potential account for this distinct contrast could be hypothesized as hypoestrogenaemia, apart from certain well-established classical risk elements, including smoking history, hypertension, hyperlipidemia, diabetes and so on [4]. Epidemiological data has also confirmed a remarkable rise of CAD prevalence among postmenopausal populations, despite a lower susceptibility to CAD among premenopausal women than that within men [5]. However, due to the under-estimation of CAD among women, systematic evaluations targeting peri-menopausal women and relevant underlying mechanisms still remained lacking [6].

Diverse proteins have been speculated as reliable serum biomarkers for susceptibility to CAD, such as adipocyte fatty acid-binding protein (A-FABP), retinol-binding protein-4 (RBP4), hypoxia inducible factor-1 (HIF-1), heme oxygenase-1 (HO-1) and so on [7–10]. Notably, HIF-1 was subject to regulation of estrogen via correlated Akt and p38-mitogen activated protein kinase (p38MAPK) pathways, implying that HIF-1 might emerge as the featured biomarker between premenopausal and postmenopausal populations [11]. In addition, since

HIF-1/HO-1 signaling pathway was involved with the molecular mechanism that contributed to development of myocardial ischemia, HO-1 was also incorporated as a candidate protein that could be modified by estrogen [12, 13]. Besides, p38MAPK appeared to be commonly investigated in the area of myocardial inflammation, rather than CAD, yet it functioned as the upstream molecule of HIF-1 [14]. As myocardial inflammation was highly correlated with CAD [15], p38MAPK could also count much in differentiating CAD from non-CAD, even in marking the discrepancies between premenopausal and postmenopausal CAD.

Above all, multiple factors related with CAD for women were distinct from that for men, regardless of clinical manifestations, pathogenesis, diagnosis, drug metabolism and prevention strategies. The present study was intended to explore characteristic proteins that could be tightly linked with peri-menopausal CAD risk and coronary artery lesions, providing solid evidence for further exploration of treatment targets for CAD.

## Methods

### Subjects

Altogether 1449 peri-menopausal women suspected with CAD were checked with coronary angiography in Cangzhou central hospital from January 2007 to June 2010. For retrospective analysis, the subjects were divided into non-CAD group ( $n = 860$ ) and CAD group ( $n = 589$ ), which was then sub-grouped into premenopausal CAD group ( $n = 167$ ) and post-menopausal CAD group ( $n = 422$ ). The baseline characteristics of the participants were recorded, including history of diabetes,

hypertension, hyperlipidemia and myocardial infarction. We also gathered information relevant to medications and treatment strategies managed for the subjects, as well as the results of coronary angiography. The participants were included into the CAD group when at least one of their vascular lumens within left main (LM), left anterior descending (LAD), left circumflex artery (LCX) and right coronary artery (RCA) showed a diameter stenosis of more than 50 %. Besides, patients satisfying the following criterion would be excluded from our study: (1) they have incomplete medical records; (2) they simultaneously suffered from other myocardial disorders, such as dilated cardiomyopathy and rheumatic heart disease; (3) they concurrently have other severe diseases related with liver, kidney, blood, tumor, endocrine, immunity system and so on; (4) they suffered from amenorrhoea due to diverse causes or underwent estrogen replacement treatment (ERT); (5) they were diagnosed as psychosis; (6) they had history of operation or trauma within 1 month. All participants have signed informed consents, and the present study was approved by the ethics committee of Cangzhou central hospital, Hebei province.

#### Detection of biochemical indicators

Early next morning after hospitalization, fasting venous food were drawn from patients, and fully automatic dry biochemical analyzer (model: OLYMPUS-AU-5400) was applied to detect certain biochemical indicators, including levels of bilirubin, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Besides, blood routine indicators, which mainly included leucocyte count, neutrocyte proportion, red blood cell count and haemoglobin level, were determined by blood analyzer (model: LH 750), and fibrinogen levels were checked with coagulation analyzer (model: ACL TOP).

#### Coronary arteriography

Right radial artery or femoral artery was pierced with Seldinger method, and they were projected with Judkin method in multiple perspectives and multi-positions. Left coronary artery was projected with  $\geq 4$  positions, while right coronary artery was projected with  $\geq 2$  positions. The arteriography results were reviewed and judged by experienced cardiologists and interventional physicians. Based on the count of stenosed coronary vessel, CAD lesions were categorized into single-vessel lesions, double-vessel lesions and triple-vessel lesions. Diagonal branch lesions were regarded as LAD, while blunt circular lesions were deemed as LCX. Posterior descending lesions and left ventricular lesions were incorporated into RCA, and LM was calculated as a single lesion. Moreover, considering the complexity of

the CAD lesions, they were classified as type A, type B and type C in accordance with the guidelines of American College of Cardiology (ACC)/American Heart Association (AHA).

#### Genisin scoring

The degree of coronary artery stenosis was quantitatively assessed by Genisin scoring: (1) 0 score when proportion of stenosis = 0; (2) 1 score when proportion of stenosis  $\leq 25$  %; (3) 2 scores when proportion of stenosis achieved 26 % ~ 50 %; (4) 4 scores when proportion of stenosis achieved 51 % ~ 75 %; (5) 8 scores when proportion of stenosis achieved 76 % ~ 90 %; (6) 16 scores when proportion of stenosis achieved 91 % ~ 99 %; (7) 32 scores when proportion of stenosis achieved 100 %. Different stenosis segments of coronary arteries were based on Genisin scores multiplied by corresponding coefficients: (1)  $\times 5$  for LM; (2)  $\times 2.5$  for proximal ends of LCX and LAD; (3)  $\times 1.5$  for medium-ends of LCX and LAD; (4)  $\times 1$  for far-ends of RCA/LAD/LCX, first diagonal branch, second diagonal branch, posterior descending branch, posterior collateral branch and obtuse marginal branch. The stenosis score of coronary arteries for each patient was the sum score of all branches. Finally, five groups were drawn based on Genisin scores: (1) group 1 ( $0 < \text{sum} \leq 10$ ); (2) group 2 ( $10 < \text{sum} \leq 20$ ); (3) group 3 ( $20 < \text{sum} \leq 34$ ); (4) group 4 ( $34 < \text{sum} \leq 64$ ); (5) group 5 ( $\text{sum} > 64$ ).

#### Definition of risk indicators

The participants would be diagnosed with hypertension if their systolic pressure was  $\geq 140$  mmHg and/or diastolic pressure was  $\geq 90$  mmHg for three times in different days without taking anti-hypertensive drugs. Diabetes was defined as the station that blood sugar level was  $\geq 11.1$  mmol/L at any time, or fasting blood-glucose (FDG) was  $\geq 7.0$  mmol/L, or blood sugar level remained  $\geq 11.1$  mmol/L 2 h after loading [16]. The diagnosis of hypertension accorded with the criteria that the sitting blood pressure of participants were  $\geq 130/85$  mmHg. Smoking history was confirmed if subjects had habits of regular smoking previously, or they smoked  $\geq 1$  cigarette/day for  $\geq 1$  year.

#### Detection of MAPK-1 levels with Strept Avidin-Biotin Complex (SABC) method

Paraffin sections of samples were routinely placed in xylene for transparent dewaxing and gradient alcohol for hydration. After rinsing with distilled water for 3 times (5 min for each time), the sections were immersed in 3 % H<sub>2</sub>O<sub>2</sub> at room temperature for 10 min to inactivate endogenous enzymes. Then the sections were immersed in 0.01 M citrate buffer solution (pH6.0) and simultaneously heated to boiling. When the boiled solution was

cooled, it was cleansed with phosphate buffer saline (PBS) (pH7.2-7.6) for 1–2 times. The bovine serum albumin (BSA) sealing fluid was then dipped at room temperature for 20 min. The primary antibody for p-MAPK (Stancruz corporation, USA) was firstly dipped to incubate tissues at 37 °C for two hours, and then biotin goat-anti-mouse secondary antibody was dipped at 37 °C for 20 min. Subsequently, the reagent of SABC was dipped at the temperature ranging from 20 to 37 °C for 20 min. Finally, DAB color developing reagent kit was applied to color the tissues, and hematoxylin was employed to redye the tissues. The sections were observed under the microscope and corresponding integrated optical density (IOD) was processed with application of Image pro plus software.

#### Evaluations of HIF-1 and HO-1 levels

The concentrations of HIF-1 and HO-1 in the serum were detected with usage of ELISA detection kit (Shanghai langdun biotech corporation, China). The sensitivities of HIF-1 and HO-1 were 2 ng/L and 50 ng/L, respectively. The experimental procedures were strictly in accordance with the instructions of the kit. Specifically, HIF-1 and HO-1 were added to the enzyme labeled pores that were pre-packaged with antibodies of HIF-

1 and HO-1, and were then incubated. After cleansing, HIF-1 and HO-1 antibodies that were marked with horseradish peroxidase (HRP) were added, and then substrates of A and B were supplemented to generate blue, which was finally converted to yellow under the influence of acid. The depth of colour was positively correlated with concentrations of HIF-1 and HO-1 in samples.

#### Statistics

All the statistical analyses were conducted on the basis of SPSS 18.0 software. Measurement data (mean  $\pm$  SD) were compared with student's *t* test, while enumeration data in the form of percentages (%) were compared with chi-square test. Data of each group were examined to confirm whether they conformed to normal distribution and homogeneity of variance. Multi-group comparisons were conducted with application of analysis of variance (ANOVA). Correlation analysis was performed with Pearson analysis. The regression model was established to explore independent risk factors for premenopausal and postmenopausal CAD. It was considered statistically significant when *P* values were found to be less than 0.05.

**Table 1** Comparison of baseline clinical characteristics among groups of non-CAD, premenopausal CAD and postmenopausal CAD

Indicators	Non-CAD group	Premenopausal-CAD group	Postmenopausal-CAD group	F/ $\chi^2$	<i>P</i> value
Size	860	167	422		
BMI (kg/m <sup>2</sup> )	24.1 $\pm$ 2.4	24.3 $\pm$ 3.1	24.0 $\pm$ 2.1	1.0	0.36
Age (years old)	50.7 $\pm$ 2.7	50.8 $\pm$ 2.9	50.7 $\pm$ 1.2	2.4	0.09
Hypertension					
yes	291 (33.8 %)	70 (41.9 %)	229 (54.3 %)	49.1	<0.05
no	569 (66.2 %)	97 (58.1 %)	193 (45.7 %)		
Hyperlipoidemia					
yes	204 (23.7 %)	37 (22.2 %)	141 (33.4 %)	15.4	<0.05
no	656 (76.3 %)	130 (77.8 %)	281 (66.6 %)		
Diabetes					
yes	108 (12.6 %)	29 (17.4 %)	124 (29.4 %)	54.3	<0.05
no	752 (87.4 %)	138 (82.6 %)	298 (70.6 %)		
Family history					
yes	140 (16.3 %)	64 (38.3 %)	75 (17.8 %)	44.6	<0.05
no	720 (83.7 %)	103 (61.7 %)	347 (82.2 %)		
Smoking history					
yes	151 (17.6 %)	55 (32.9 %)	96 (22.7 %)	21.4	<0.05
no	709 (82.4 %)	112 (67.1 %)	326 (77.3 %)		
Obesity					
yes	192 (22.3 %)	43 (25.7)	116 (27.5%)	4.4	0.11
no	668 (77.7 %)	124 (74.3 %)	306 (72.5 %)		

**Table 2** Comparison of coronary angiography indicators between groups of premenopausal CAD and postmenopausal CAD

Indicators	Premenopausal-CAD group	Postmenopausal-CAD group	$\chi^2$	P value
Lesion vessels				
Left main coronary artery	6 (3.6 %)	18 (4.3 %)	1.1	0.78
Anterior descending artery	97 (58.1 %)	279 (66.1 %)		
Circumflex coronary artery	92 (55.1 %)	269 (63.7 %)		
Right coronary artery	72 (43.1 %)	246 (58.3 %)		
Number of stenosed coronary vessel				
Single vessel lesion	103 (61.7 %)	175 (41.5 %)	19.7	<0.05
Double vessel lesion	28 (16.8 %)	104 (24.6 %)		
Triple vessel lesion	36 (21.5 %)	143 (33.9 %)		
Lesion type				
Type A	59 (35.3 %)	53 (12.6 %)	40.6	<0.05
Type B	71 (42.5 %)	230 (54.5 %)		
Type C	37 (22.2 %)	139 (32.9 %)		

## Result

### Baseline characteristics of subjects

Participants in non-CAD group, pre-menopausal CAD group and post-menopausal CAD group were well matched in terms of BMI and age without statistical significance ( $P > 0.05$ ) (Table 1). The occurrence of either CAD or menopause seemed to be associated with distinct incidences of hypertension, hyperlipidemia, diabetes ( $P < 0.05$ ). Family history and smoking history might also render development of CAD to be significantly distinct ( $P < 0.05$ ), yet the prevalence of obesity was similar in this population ( $P > 0.05$ ).

### Comparison of coronary angiography indicators

Whether menopause occurred or not might not be associated with the location of lesion vessels, since that no significant difference was observed between the two groups regarding lesion vessels within left main coronary artery, anterior descending artery, circumflex coronary artery and right coronary artery ( $P > 0.05$ ) (Table 2). However, post-menopause could be correlated with larger proportions of double/triple vessel lesions and higher degrees of lesion type (type A and type B) than pre-menopause ( $P < 0.05$ ).

### Comparison of protein expressions relevant to MAPK signaling pathway

Expressions of MAPK-1, HIF-1 $\alpha$  and HO-1 in the pre-menopausal group were found to over-number those in the non-CAD group ( $P < 0.05$ ), and the three proteins in the post-menopausal group expressed even higher than those in the pre-menopausal group ( $P < 0.05$ ) (Table 3).

Additionally, significant correlations were found in the non-CAD group, respectively, between HO-1 and HIF-1 ( $r_s = 0.25$ ,  $P < 0.05$ ), p38MAPK and HIF-1 ( $r_s = 0.20$ ,  $P <$

$0.05$ ), p38MAPK and HO-1 ( $r_s = 0.12$ ,  $P < 0.05$ ) (Fig. 1). Pre-menopausal group and post-menopausal group also exhibited the similar tendencies of the three relations as non-CAD group, which were specifically displayed as: HO-1 and HIF-1 ( $r_s = 0.52$ ,  $P < 0.05$ ;  $r_s = 0.34$ ,  $P < 0.05$ ), p38MAPK and HIF-1 ( $r_s = 0.28$ ,  $P < 0.05$ ;  $r_s = 0.20$ ,  $P < 0.05$ ), p38MAPK and HO-1 ( $r_s = 0.38$ ,  $P < 0.05$ ;  $r_s = 0.23$ ,  $P < 0.05$ ) (Figs. 2 and 3).

### Comparison of CAD-related indicators

Among the thirteen indicators, the measured values of around five parameters (i.e. SBP, CysC, hs-CRP, TG and LDL-C) all conformed to the following tendency with statistical significance: post-menopausal group > pre-menopausal group > non-CAD group ( $P < 0.05$ ) (Table 4). Nonetheless, the remaining indicators were approximate in their detection values among groups of non-CAD, pre-menopausal CAD and post-menopausal CAD ( $P > 0.05$ ).

Among premenopausal women, MAPK-1 was indicated to be positively correlated with TG ( $r_s = 0.271$ ,  $P < 0.001$ ), while HO-1 expressions increased with elevated CysC ( $r_s = 0.492$ ,  $P < 0.001$ ) and hs-CRP ( $r_s = 0.569$ ,  $P < 0.001$ ).

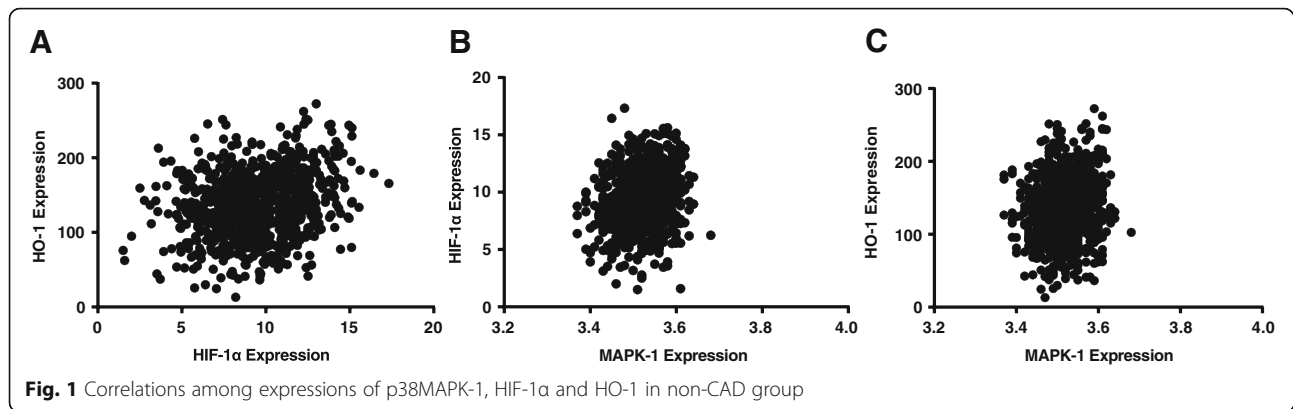
**Table 3** Comparison of protein expressions (MAPK-1, HIF-1 $\alpha$  and HO-1) among groups of non-CAD, premenopausal CAD and postmenopausal CAD

Proteins	Non-CAD group	Premenopausal CAD group	Postmenopausal CAD group
MAPK-1 <sup>a</sup>	3.5 $\pm$ 0.1	4.5 $\pm$ 0.1 <sup>b</sup>	4.6 $\pm$ 0.1 <sup>b,c</sup>
HIF-1 $\alpha$ (ng/L)	9.4 $\pm$ 2.5	20.7 $\pm$ 6.1 <sup>b</sup>	24.4 $\pm$ 7.6 <sup>b,c</sup>
HO-1 (ng/L)	136.6 $\pm$ 52.8	382.1 $\pm$ 135.4 <sup>b</sup>	413.7 $\pm$ 112.9 <sup>b,c</sup>

<sup>a</sup>integrated optical density (IOD) values

<sup>b</sup>compared with non-CAD group

<sup>c</sup>compared with pre-menopausal CAD group



(Table 5). Slightly different from premenopausal populations, postmenopausal ones revealed positive associations of MAPK-1 with CysC ( $r_s = 0.415, P < 0.001$ ) and TG ( $r_s = 0.476, P < 0.001$ ). The positive correlations between HIF-1 and either hs-CRP ( $r_s = 0.137, P = 0.005$ ) or LDL-C ( $r_s = 0.470, P < 0.001$ ) could also be found. Additionally, HO-1 exhibited positive linkages with CysC ( $r_s = 0.190, P < 0.001$ ), hs-CRP ( $r_s = 0.542, P < 0.001$ ) and TG ( $r_s = 0.142, P = 0.004$ ) as well.

**Correlation between protein expressions relevant to MAPK signaling pathway and coronary angiography indicators**

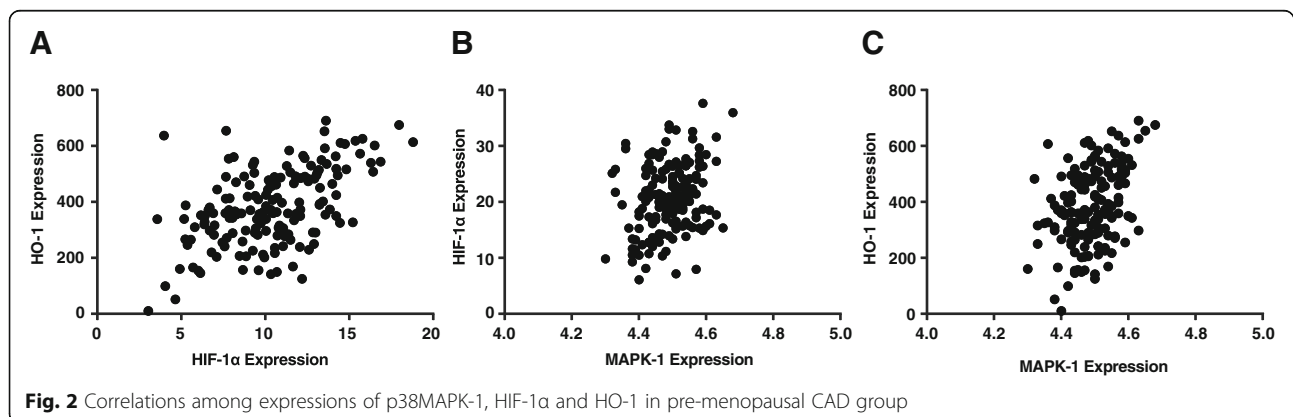
Interestingly, despite groups of non-CAD, pre-menopausal CAD and post-menopausal CAD, expressions of HIF-1α and HO-1 increased significantly with changes of lesion vessels (left main coronary artery < anterior descending artery < circumflex coronary artery < right coronary artery), number of stenosed coronary vessel (single vessel lesion < double vessel lesion < triple vessel lesion) and lesion type (type A < type B < type C) ( $P < 0.05$ ) (Table 6). However, p38MAPK expressions stayed stable irrespective of the place of lesion vessels ( $P < 0.05$ ), though its expressions also elevated with rise in number of stenosed coronary vessel and lesion type ( $P < 0.05$ ).

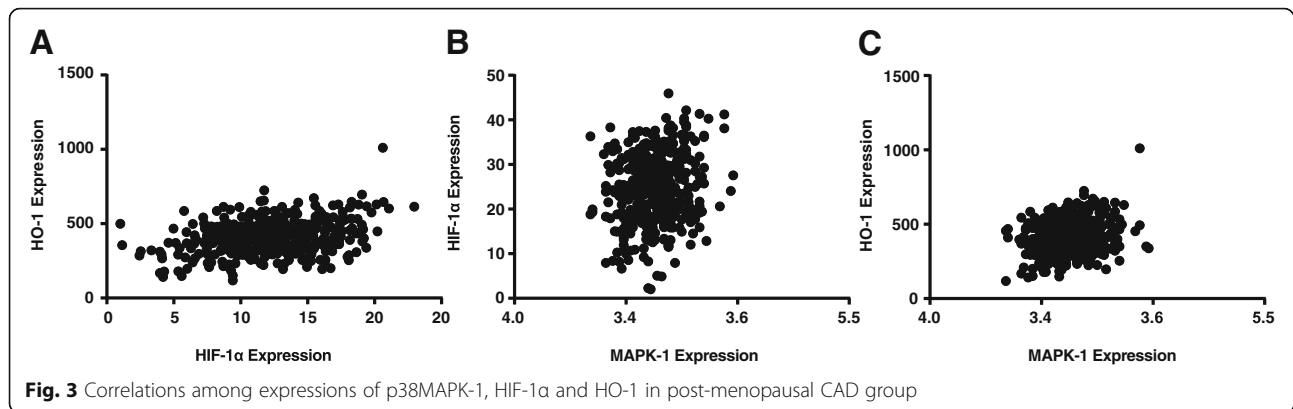
**Correlation between CAD-relevant indicators and Genesis score**

It was observed that merely SBP and TG appeared as independently hazardous factors for prediction of CAD prognosis, with ORs of 1.02 (95 % CI = 1.01–1.18,  $P < 0.05$ ) and 1.82 (95 % CI = 1.01–3.33,  $P < 0.05$ ), respectively (Table 7). Nevertheless, none of any other available associations were found between CAD-indicators and Genesis score among both premenopausal and postmenopausal populations (all  $P > 0.05$ ).

**Discussion**

Among the classical factors, hypertension was demonstrated to accelerate progression of atherosclerosis through inducing continuous or repeated mechanical injuries of vascular endothelial cell layers [17]. Diabetes was always accompanied by numerous metabolic disorders, and it can even counteract the protective effects of estrogen [18]. Moreover, the role of dyslipidemia, such as high triglyceride (TG) in the plasm, in CAD development also appeared to be crucial since that TG enabled patients' blood to be in a hypercoagulative state and thereby elevated CAD risk [19]. C-reactive protein (CRP) stimulated endothelial cells of aorta to generate high levels of plasminogen activator inhibitor-1 (PAI-1), which induce injuries of endarterium and instability of





plaques [20]. Increased heart rate (HR) reduced oxygen supply and raised oxygen consumption, which spurred production of arteriosclerosis and augmented incidence of thrombus [21].

Besides, the onset of CAD seemed to be accompanied with myocardial hypoxia [22], and inhibited p38MAPK has been documented to worsen cellular injury under exposure to hypoxia [23, 24]. In fact, p38MAPK could be deemed as a candidate biomarker for CHD, owing to the facts that it mediated development of cardiac failure and that cardiac failure existed as a most severe disorder of CHD [25]. It was also documented that suppression of tumorigenicity 2 (ST2) could bind interleukin-33 (IL-33) on inflammatory membranes, thereby restraining and facilitating development of cardiovascular disorders [26, 27]. The *in vivo/in vitro* experiments conducted by Sanda et al. also demonstrated that administration of IL-33 could serve to lower

p38MAPK expressions and Jun N-terminal kinase phosphorylation aroused by angiotensin II, which caused cardiac dysfunctions through generation of certain reactive oxygen species (ROS) [28]. All in all, the effects of p38MAPK on cardiovascular functions appeared to be regulated by ST2/IL-33 signaling, though the inherent pathogenesis needed to be further identified.

In addition, p38MAPK also remarkably up-regulated HIF-1α expressions in the hypoxic myocardium [29, 30]. The elevated HIF-1α expressions in acute ischemic myocardial tissue facilitated increase of capillary density within the damaged myocardium areas [31], where micro-circulation was effectively constructed [32]. Accumulating evidence also confirmed that the hypoxic preconditioning (HPC) mechanism mobilized by activated HIF-1α not only eased arrhythmia after ischemia-reperfusion via improving intracellular ion disorder, but also ameliorated blood supply by way of releasing endothelium-derived relaxing factor (EDRF) [33, 34]. Moreover, restrained HIF-1α expressions could antagonize the formation of new blood cells by repressing VEGF expressions, accordingly alleviating the incidence of in-stent restenosis when inhibitors of HIF-1α (e.g. mTOR and pacitaxel) were painted on the bracket [35].

As an endogenous protective factor, HO-1 was subject to regulation of HIF-1α [36]. Under the stimulation of ischemia and hypoxia, HO-1 functioned to resist atherosclerosis, ischemia reperfusion injury and myocardial hypertrophy mainly through catalysing degradation of haem into bilirubin, CO and ferroprotein [37, 38]. Bilirubin has been documented to be associated with less susceptibility to CHD for its crucial roles in inhibition of lipid oxidation, clearance of oxyradicals and suppression of complement response during inflammation development [39–41]. In addition, CO could potentially block the inflammatory process through depressing generation of inflammatory factors that were relevant to NO and cGMP, whereas ferroprotein prevented cells from damage with its anti-oxidative capability [38, 42, 43].

**Table 4** Comparison of CAD-related indicators among groups of non-CAD, premenopausal CAD and postmenopausal CAD

Indicators	Non-CAD group	Premenopausal CAD group	Postmenopausal CAD group
SBP (mm Hg)	123.0 ± 14.7	134.9 ± 16.3 <sup>a</sup>	140.5 ± 18.1 <sup>a,b</sup>
DBP (mm Hg)	80.5 ± 9.2	83.8 ± 10.5	82.6 ± 9.7
Heart rate (/min)	86 ± 10	88 ± 17	88 ± 14
FBG (mmol/L)	7.6 ± 1.5	9.3 ± 0.8	10.4 ± 1.3
2 h PG (mmol/L)	12.3 ± 1.9	15.2 ± 2.3	15.9 ± 2.1
BUN (mmol/L)	6.9 ± 1.6	7.7 ± 2.7	8.2 ± 3.5
Cr (μmol/L)	76.7 ± 16.5	84.1 ± 24.1	88.8 ± 21.9
CysC (mg/dL)	1.1 ± 0.2	1.4 ± 0.3 <sup>a</sup>	1.8 ± 0.2 <sup>a,b</sup>
hs-CRP (mg/L)	0.7 ± 0.2	12.8 ± 8.3 <sup>a</sup>	16.5 ± 7.9 <sup>a,b</sup>
TC (mmol/L)	4.4 ± 0.7	4.7 ± 1.1	4.6 ± 0.8
TG (mmol/L)	1.7 ± 0.1	1.8 ± 0.7 <sup>a</sup>	2.1 ± 0.6 <sup>a,b</sup>
HDL-C (mmol/L)	1.4 ± 0.3	1.2 ± 0.1	1.4 ± 0.3
LDL-C (mmol/L)	2.4 ± 0.9	3.2 ± 0.6 <sup>a</sup>	3.9 ± 0.7 <sup>a,b</sup>

<sup>a</sup>compared with non-CAD group

<sup>b</sup>compared with pre-menopausal CAD group

**Table 5** Correlations between protein expressions (MAPK-1, HIF-1 $\alpha$  and HO-1) and CAD-related indicators among groups of premenopausal and postmenopausal women

Indicators	Premenopausal-CAD group			Postmenopausal-CAD group		
	MAPK-1	HIF-1 $\alpha$	HO-1	MAPK-1	HIF-1 $\alpha$	HO-1
BMI (kg/m <sup>2</sup> )	-0.07 (0.39)	-0.02 (0.83)	0.05 (0.55)	-0.06 (0.23)	-0.02 (0.74)	-0.01 (0.84)
SBP (mm Hg)	-0.01 (0.93)	0.08 (0.32)	-0.09 (0.26)	0.05 (0.36)	-0.01 (0.83)	0.08 (0.12)
DBP (mm Hg)	0.11 (0.15)	0.02 (0.78)	0.09 (0.23)	-0.05 (0.3)	-0.02 (0.63)	0.07 (0.15)
Heart rate (/min)	-0.04 (0.59)	0.04 (0.65)	0.01 (0.91)	0.02 (0.66)	0.01 (0.88)	-0.06 (0.26)
FBG (mmol/L)	0.06 (0.41)	0.09 (0.23)	-0.1 (0.22)	0.01 (0.81)	0.04 (0.42)	0.04 (0.42)
2 h PG (mmol/L)	0.14 (0.08)	-0.06 (0.43)	-0.03 (0.7)	0.08 (0.1)	-0.01 (0.78)	0.02 (0.72)
BUN (mmol/L)	-0.13 (0.09)	0.04 (0.62)	0.06 (0.42)	-0.03 (0.59)	0.06 (0.24)	-0.01 (0.78)
Cr ( $\mu$ mol/L)	0.06 (0.43)	0.02 (0.78)	0.04 (0.65)	0.05 (0.35)	-0.01 (0.93)	0.00 (0.95)
CysC (mg/dL)	0.08 (0.31)	0.05 (0.52)	0.49 (<0.05)	0.42 (<0.05)	0.00 (0.98)	0.19 (<0.05)
hs-CRP (mg/L)	0.08 (0.29)	0.03 (0.75)	0.57 (<0.05)	0.08 (0.11)	0.14 (0.01)	0.54 (<0.05)
TC (mmol/L)	-0.15 (0.05)	0.08 (0.31)	0.01 (0.88)	0.01 (0.91)	0.00 (0.99)	0.06 (0.24)
TG (mmol/L)	0.27 (<0.05)	0.06 (0.43)	0.03 (0.72)	0.48 (<0.05)	0.04 (0.45)	0.14 (<0.05)
HDL-C (mmol/L)	0.01 (0.87)	0.07 (0.39)	-0.12 (0.13)	-0.02 (0.72)	0.01 (0.82)	0.03 (0.53)
LDL-C (mmol/L)	-0.15 (0.05)	0.08 (0.32)	-0.08 (0.33)	0.01 (0.92)	0.47 (<0.05)	0.01 (0.88)

<sup>a</sup>The results were presented as rs and P value

In addition, postmenopause made female CAD patients more susceptible to hazard factors of cardiovascular diseases owing to shortage of estrogen. Estrogen not merely regulated blood lipid levels and reduced the chance of atherosclerosis, but also ameliorated a series of vascular endothelial functions, which were specifically indicated as dilation of coronary vessels, increase of coronary flow and reduction of coronary artery spasm. Furthermore, estrogen also inhibited migration of

monocytes to subendothelial, and thereby restrained their transformation to foam cells after swallowing lipids [44]. Estrogen was also advantageous in restraining proliferation of vascular smooth muscle cells and antagonizing aggregation of platelets. In spite of the above merits, estrogen could aggravate premenopausal CAD when diabetes was combined, which might be explained by that estrogen can stimulate RAGE expressions within endothelial cells and potentiate advanced glycation end products (AGE)-

**Table 6** Correlations between protein expressions (MAPK-1, HIF-1 $\alpha$  and HO-1) and coronary angiography indicators among groups of premenopausal and postmenopausal women

Indicators	Premenopausal-CAD group			Postmenopausal-CAD group		
	MAPK-1	HIF-1 $\alpha$ (ng/L)	HO-1 (ng/L)	MAPK-1	HIF-1 $\alpha$ (ng/L)	HO-1 (ng/L)
Lesion vessels						
Left main coronary artery	4.4 $\pm$ 0.1	13.8 $\pm$ 4.3	230.5 $\pm$ 50.2	4.7 $\pm$ 0.2	15.8 $\pm$ 4.2	264.3 $\pm$ 89.8
Anterior descending artery	4.5 $\pm$ 0.1	17.2 $\pm$ 7.3 <sup>*</sup>	374.4 $\pm$ 195.3 <sup>*</sup>	4.7 $\pm$ 0.12	21.4 $\pm$ 10.1 <sup>*</sup>	397.7 $\pm$ 159.4 <sup>*</sup>
Circumflex coronary artery	4.5 $\pm$ 0.1	20.3 $\pm$ 11.3 <sup>®</sup>	419.6 $\pm$ 210.1 <sup>®</sup>	4.8 $\pm$ 0.1	29.9 $\pm$ 12.1 <sup>®</sup>	482.8 $\pm$ 278.4 <sup>®</sup>
Right coronary artery	4.6 $\pm$ 0.2	24.1 $\pm$ 14.2 <sup>&amp;</sup>	527.1 $\pm$ 273.1 <sup>&amp;</sup>	4.8 $\pm$ 0.1	35.9 $\pm$ 15.0 <sup>&amp;</sup>	598.1 $\pm$ 262.1 <sup>&amp;</sup>
Number of stenosed coronary vessel						
Single vessel lesion	4.5 $\pm$ 0.2	16.0 $\pm$ 3.5	283.6 $\pm$ 82.0	4.7 $\pm$ 0.2	16.1 $\pm$ 3.7	278.4 $\pm$ 91.7
Double vessel lesion	4.5 $\pm$ 0.1 <sup>a</sup>	19.3 $\pm$ 9.9 <sup>a</sup>	409.7 $\pm$ 175.3 <sup>a</sup>	4.7 $\pm$ 0.1 <sup>a</sup>	23.6 $\pm$ 10.1 <sup>a</sup>	349.8 $\pm$ 183.2 <sup>a</sup>
Triple vessel lesion	4.5 $\pm$ 0.2 <sup>b</sup>	29.1 $\pm$ 13.2 <sup>b</sup>	561.6 $\pm$ 280.6 <sup>b</sup>	4.8 $\pm$ 0.1 <sup>b</sup>	39.6 $\pm$ 13.2 <sup>b</sup>	637.9 $\pm$ 257.6 <sup>b</sup>
Lesion type						
Type A	4.4 $\pm$ 0.2	14.7 $\pm$ 2.9	236.9 $\pm$ 90.2	4.7 $\pm$ 0.1	18.7 $\pm$ 4.5	293.5 $\pm$ 85.4
Type B	4.5 $\pm$ 0.1 <sup>c</sup>	17.6 $\pm$ 8.1 <sup>c</sup>	418.7 $\pm$ 161.3 <sup>c</sup>	4.7 $\pm$ 0.1 <sup>c</sup>	25.1 $\pm$ 7.9 <sup>c</sup>	375.8 $\pm$ 155.5 <sup>c</sup>
Type C	4.6 $\pm$ 0.2 <sup>d</sup>	30.7 $\pm$ 11.4 <sup>d</sup>	520.6 $\pm$ 224.5 <sup>d</sup>	4.8 $\pm$ 0.2 <sup>d</sup>	34.7 $\pm$ 10.8 <sup>d</sup>	614.5 $\pm$ 249.5 <sup>d</sup>

<sup>\*</sup>: compared with left main coronary artery; <sup>®</sup>: compared with anterior descending artery; <sup>&</sup>: compared with circumflex coronary artery; <sup>a</sup>: compared with single vessel lesion; <sup>b</sup>: compared with double vessel; <sup>c</sup>: compared with type A; <sup>d</sup>: compared with type B



**Table 7** Correlations between CAD-related indicators and Genisin scores

Items	Premenopausal-CAD group		Postmenopausal-CAD group	
	OR (95 % CI)	P	OR (95 % CI)	P
Hypertension	0.63 (0.29, 1.35)	0.232	0.91 (0.61, 1.36)	0.64
Hyperlipoidemia	2.63 (0.91, 7.62)	0.075	0.70 (0.45, 1.08)	0.11
Diabetes	0.95 (0.35, 2.56)	0.915	0.84 (0.54, 1.30)	0.43
Family History	0.95 (0.44, 2.06)	0.905	1.39 (0.83, 2.34)	0.21
Smoking History	1.38 (0.60, 3.16)	0.444	1.29 (0.80, 2.06)	0.29
MAPK-1	0.13 (0.00, 42.64)	0.488	0.41 (0.05, 3.48)	0.41
HIF-1 $\alpha$	1.02 (0.96, 1.08)	0.586	0.99 (0.96, 1.02)	0.52
HO-1	1.00 (1.00, 1.00)	0.966	1.00 (1.00, 1.00)	0.54
SBP (mm Hg)	1.02 (1.01, 1.18)	0.043	1.00 (0.99, 1.01)	0.98
CysC (mg/dL)	1.66 (0.47, 5.90)	0.433	0.95 (0.33, 2.75)	0.92
hs-CRP (mg/L)	0.99 (0.93, 1.05)	0.620	1.00 (0.97, 1.04)	0.88
TG (mmol/L)	1.82 (1.01, 3.33)	0.047	1.13 (0.72, 1.78)	0.59
LDL-C (mmol/L)	1.24 (0.63, 2.45)	0.537	1.10 (0.80, 1.51)	0.56

RAGE interactions, finally exacerbating vascular inflammation [45].

Intriguingly, estrogen was also suggested to modulate p38 MAPK activity and HO-1 expressions [46]. Particularly, administration of 17 beta-estradiol (E(2)) for trauma-hemorrhage patients would normalized p38 MAPK, yet not HO-1 expressions, whereas addition of p38MAPK inhibitor abolished the increase of HO-1 expressions due to trauma haemorrhage [46]. It was also indicated that MAPK may phosphorylate certain targets (e.g. p27) that resulted in anti-estrogen resistance [47]. For another, E2 imposed effects primarily through binding to the estrogen receptors (ER $\alpha$  and ERbeta) or interacting with elements related with estrogen response [48]. Lower ER $\alpha$  expressions were determined in HIF-1 $\alpha$  positive breast cancers than in HIF-1 $\alpha$  ones [49]. Simultaneously, down-regulated ER $\alpha$  and inhibited influence of anti-estrogen could also be observed in the oxygen-lacking state [50], suggesting the inter-correlation between hypoxia, ER $\alpha$  and HIF-1 $\alpha$ .

## Conclusions

Thus, it was reasonable to consider that p38MAPK-1/HIF-1 $\alpha$ /HO-1 were highly associated with coronary lesion characteristics and prognosis for peri-menopausal women, providing clues that p38MAPK-1/HIF-1 $\alpha$ /HO-1 could act as biomarkers for diagnosis and prognosis of peri-menopausal women suffering from CAD.

## Abbreviations

A-FABP: Adipocyte fatty acid-binding protein; BSA: Bovine serum albumin; CAD: Coronary artery disease; CRP: C-reactive protein; EDRF: Endothelium-derived relaxing factor; FDG: Fasting blood-glucose; HDL-C: High-density lipoprotein cholesterol; HR: Heart rate; IOD: Integrated optical density; LDL-C: Low-density lipoprotein cholesterol; p38MAPK: p38-mitogen activated protein kinase; PAI-1: Plasminogen activator inhibitor-1; PBS: Phosphate buffer saline; RBP4: Retinol-binding protein-4

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## Availability of data and materials

All data generated or analyzed during this study are included in this article.

## Authors' contributions

LY, XC and SZ: conceived and designed the experiments. ZL and JW: performed the experiments. FL: analyzed the data. YW and YL: drafted the manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Consent for publication

All participants have signed informed consents for publication.

## Ethics approval and consent to participate

All participants have signed informed consents, and the present study was approved by the ethics committee of Cangzhou central hospital, Hebei province.

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