ORIGINAL ARTICLE

Relationship of self-reported exercise tolerance with inflammatory markers in women with stable ischemic heart disease

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Abstract

OBJECTIVE: Ischemic heart disease (IHD) is associated with decreased exercise tolerance and it is subjectively reported as angina pectoris or dyspnea. Inflammation and pro- inflammatory cytokines are related to progression of IHD, but their level is seldom analyzed in association with self reported exercise tolerance.

METHODS: Women aged 35–75 years with stable IHD from Homocysteine Slovakia study (N=175) were analyzed for monocyte chemoatractant protein-1 (MCP-1), interleukin 6 (IL-6), transforming growth factor β1 (TGF β1), Mannan binding lectin (MBL), heat shock proteins 60 (HSP60), carbonyl protein (CP), high sensitivity C-reactive protein (hsCRP) and oxidized glutathione (GSSG) in relation to exercise induced dyspnea or angina pectoris (AP) (≤200 m).

RESULTS: Patients with dyspnea had higher HSP60 (77.3±107.2 vs 43.7±48.9 ng/ml; p=0.014) and IL-6 (2.9±1.3 vs 1.9±0.6 pg/ml; p=0.04) levels. IL-6 and HSP60 demonstrated direct correlation with dyspnea (rho=0.39; p=0.02 resp. rho=0.22; p=0.01). AP≤200 m patients showed only decreased protein carbonyl a marker of protein oxidation and increased oxidative stress (CP 61.7±27.3 vs. 72.1±23.1 pg/ml; p=0.001). CP indirectly correlates with AP≤200 m (rho=–0.25; p=0.001).

CONCLUSIONS: We have found associations of pro-inflammatory cytokines and inflammation markers with dyspnea or angina pectoris, but the relationship was not consistent in our patients with stable ischemic heart disease.

Abbreviations:

AP	- angina pectoris
BMI	- body mass index
CP	- carbonyl protein
GSSG	- oxidized glutathione
hsCRP	- high sensitivity C-reactive protein
HSP60	- heat shock proteins 60
IHD	- Ischemic heart disease
IL-6	- interleukin 6
MBL	- Mannan binding lectin
MCP-1	- monocyte chemoatractant protein-1
MI	- myocardial infarction
TGF β1	- transforming growth factor β1

INTRODUCTION

Atherosclerosis is still the leading health-related problem worldwide. Both lipid accumulation and inflammation have been recognized as key players in atherogenesis. Advances in understanding the pathobiology of atherosclerosis have implicated several pro-inflammatory and inflammatory factors in the cardiovascular disease prediction and identification (Zakynthinos & Pappa, 2009; Vohnout *et al.* 2011). These inflammatory markers and mediators, released at different stages in the pathobiology of atherothrombosis, can enter the circulation, where they can be easily measured in a peripheral vein (Packard & Libby, 2008).

Ischemic heart disease (IHD) is associated with decreased exercise tolerance and it is subjectively reported as angina pectoris or dyspnea. Inflammation and pro-inflammatory cytokines are related to progression of IHD (Zakynthinos & Pappa, 2009), but their level is seldom analysed in association with self reported exercise tolerance.

Aim of our study was to analyse relationship between markers of inflammation released at different stages in the pathobiology of atherothrombosis and self reported exercise level in women with self-reported exercise induced angina pectoris or dyspnea. We focused on women due to general lack of information for female populations in the cardiovascular field.

PATIENTS AND METHODS

The Homocysteine Slovakia study and the selection of subjects and methods have been described in detail previously (Lietava *et al.* 2012). Briefly, one hundred seventy five female patients aged between 35–75 years with stable IHD were recruited from two cardiological registers in Bratislava and Nové Zámky. Stable IHD was defined as an absence of history of prior acute coronary syndrome, heart failure and hypertensive crisis in last three month before the study. IHD was defined as a hospitalization for myocardial infarction (MI) and/or typical angina pectoris (AP) or proven and treated silent IHD documented by medical report and ECG signs of ischemia. Tolerance of physical load was defined as self reported estimated distance in meters tolerated without angina. Tolerance ≤200 m was considered as positive for angina pectoris. Self reported distance in meters tolerated without dyspnea was also evaluated. Tolerance ≤200 m was considered as positive.

The study was approved by the Ethics Committee of the Medical School of Comenius University in Bratislava and all subjects signed informed consent.

Venous blood samples were collected in EDTA-Potassium tubes to obtain plasma and in standard tubes to obtain serum after overnight fasting without cubital compression. Blood for plasma was immediately centrifuged in cooled centrifuge (4 °C) for 30 minutes, blood for serum was allowed to clot for 15 minutes at room temperature between 22–25 °C and underwent the same centrifugation procedure. Full methodology for measurement of basic biochemistry was described previously (Lietava *et al.* 2012). The serum level of heat shock proteins 60 (HSP60) was determined by original HSP60 ELISA kit (Stressgen, USA; Microtitrate plate reader MRX II – Dynex technologies, USA) according to the instructions of manufacturer. Blood for oxidized glutathione (GSSG) measurement was deproteinized by 10% sulfosalicylic acid (SSA) (400 µl 10% SSA, 750 µl blood), centrifuged at 4 °C at 10 000 rpm for 12 minutes and the supernatant was stored in cryovials at –70 °C until spectrophotometrical analysis at 410 nm using microplate reader (TECAN Spectra Fluor, Austria) in kinetic-type reaction (Atalay & Sen 1999). Protein carbonyls (CP) were measured using modified ELISA method according to Buss *et al.* (1997). ELISA method was used also for measurement of Monocyte chemoatractant protein-1 (MCP-1) (ELISA, Bender Medsystems, Germany), high sensitivity C-reactive protein (hsCRP) (Bender Medsystems, Germany), Transforming growth factor β1 (TGF β1) (ELISA, Bender Medsystems, Germany) and Mannan binding lectin (MBL) (MBL Oligomerkit, AntibodyShop, UK) according to the instructions of manufacturers. Interleukin 6 (IL-6) levels were measured luminometrically (Athena Multi-Lyte™, Luminex, USA).

All data from questionnaires and results of laboratory tests were input into databases and underwent three step control. Data were analyzed with SPSS 10.0 for Windows. Normal distributions of parametric variables were examined by using one-sample Kolmogorov-Smirnova test. Homogenously distributed data were compared using independent 2-samples Student t-test. Heterogeneously distributed data were compared using non-parametrical Mann-Whitney U test for two independent groups using. Correlation between the exercise tolerance and individual clinical or laboratory parameters were tested using two-tailed bivariate correlation according to the Pearson or Spearman, considering type of analysed data – categorical, interval or binomial variables. Relationships between exercise induced angina pectoris or dyspnea and inflammatory markers parameters were tested by hierarchic logistic regression for angina or dyspnea given as binomial parameter.

Risk factors as age, body mass index (BMI), uric acid, leucocytes and trombocytes were included into analysis as controls for possible bias. Statistical significance was considered at the level of $p<0.05$ with power=0.80.

RESULTS

Main characteristics of the populations are shown in the Table 1. Exercise induced dyspnea with tolerance ≤200 metres was reported by 75.9% and exercise induced angina by 78.9% of females.

Patients with angina pectoris had significantly decreased carbonyl proteins (CP 61.7±27.3 vs. 72.1±23.1 pg/ml; p=0.001) (see Table 2). Carbonyl proteins indirectly correlated with AP≤200 metres (rho=–0.25; p=0.001, Table 3). Any of analysed parameters was identified as independent predictor of angina using hierarchic logistic regression analysis with angina pectoris as a dependent value (data not shown).

Patients with dyspnea had higher HSP60 (77.3±107.2 vs 43.7±48.9 ng/ml; p=0.014) and IL-6 (2.85±1.28 vs 1.90±0.55 pg/ml; p=0.040) (Table 2). IL-6 and HSP60 demonstrated direct correlation with dyspnea (rho=0.39; p=0.021 resp. rho=0.22; p=0.013) and indirect correlation with GSSG (rho=–0.16; p=0.036; Table 3). No one of analysed parameters was identified as independent predictor of dyspnea using hierarchic logistic regression analysis with angina pectoris as dependent value (data not shown).

Significant associations of CP with AP and HSP60, IL-6 and GSSG with dyspnea remained statistically significant even after adjustment of data on age, BMI, metabolic syndrome and/or diabetes mellitus.

DISCUSSION

Levels of inflammatory cytokines are seldom analysed in association with self reported exercise tolerance. In the present study we found that patients with exercise induced dyspnea had higher HSP60 and IL-6 levels, moreover IL-6 and HSP60 demonstrated direct and GSSG indirect correlation with dyspnea. To our knowledge, this is the first study to show relation between inflammatory biomarkers and exercise induced dyspnea in patients with IHD. Inflammation is related to progression of atherosclerosis (Mizuno *et al.* 2011), we can therefore hypothesis that presence of the dyspnea or angina with increased inflammatory status reflects more severe atherosclerosis in the patients. In agreement with the hypothesis, the elevated pro-inflam-

Tab. 1. Main characteristics of the patients.

	All	Dyspnea	No dyspnea	*p*-value	Angina	No angina	*p*-value
N	175	137	38		136	39	
Age (years)	63.5±7.8	62.5±9.0	63.3±7.8	0.58	63.5±8.2	61.6±7.8	0.19
Systolic blood pressure (mmHg)	158±24	151±21	160±25	0.04	151±19	160±25	0.20
Diastolic blood pressure (mmHg)	94±11	93±10	95±12	0.47	95±11	93±10	0.51
Waist >80 cm (%)	91.9	94.6	83.3	0.05	90.4	97.3	0.31
Obesity (%)	54.9	58.2	46.8	0.24	54.6	57.5	0.86
Myocardial infarction (%)	22.9	25.4	12.8	0.10	19.1	32.5	0.09
Hypertension (%)	91.4	91.8	80.9	0.07	88.7	87.5	0.78
Diabetes (%)	26.3	27.6	23.4	0.70	27.0	25.0	0.80
Metabolic syndrome (%)	57.1	57.6	51.1	0.61	57.3	57.0	0.99
Glucose (mmol/L)	6.7 (3.8–17.6)	6.6 (3.8–17.6)	6.7 (4.8–16.6)	0.10	6.8 (3.8–17.6)	6.3 (4.7–16.2)	0.262
Cholesterol (mmol/L)	6.2±1.2	6.0±1.2	6.3±1.1	0.15	6.3±1.2	6.0±1.0	0.21
Triglycerides (mmol/L)	1.6 (0.5–6.0)	1.63 (0.5–6.0)	1.57 (0.5–4.1)	0.38	1.6 (0.5–4.8)	1.6 (0.7–6.0)	0.89
HDL (mmol/L)	1.4±0.3	1.3±0.4	1.4±0.3	0.38	1.4 (0.8–1.4)	1.4 (1.0–2.2)	0.29
LDL (mmol/L)	4.2±1.0	4.0±1.1	4.2±1.0	0.16	4.2 (1.8–7.6)	4.0 (2.1–6.6)	0.73
Bilirubin (μmol/l)	12.8±4.2	12.8±4.3	12.7±4.0	0.93	12.4 (5–29)	14.1 (8–24)	0.02
Uric acid (μmol/l)	315.8±87.2	303±78	321±90	0.24	313±88	327±83	0.36
Angina ≤200 m (%)	78.9	77.7	79.7	NA	77.9	22.1	NA
Dyspnoe (%)	75.9	74.0	26.0	NA	74.8	76.0	NA
BMI (kg/m^2)	30.9±5.2	29.5±5.9	31.4±5.2	0.03	30.8±5.4	31.4±4.5	0.53

Data with Gaussian distribution are presented as mean ± SD, heterogeneously distributed data are presented as median (minimum–maximum). NA – Non-applicable.

Tab. 2. Comparison of inflammatory markers dichotomized according to the exercise induced angina pectoris or dyspnea.

	Angina ≤200 m (n=136)	Angina >200 m (n=39)	*p*-value	Dyspnea (n=137)	No dyspnea (n=38)	*p*-value
MBL [ng/ml]	1350±1124	1136±1117	*p*=0.40	1275±1116	1402±1153	*p*=0.60
TGF β1 [ng/ml]	34.2±28.8	31.3±29.3	*p*=0.68	34.4±26.9	29.7±34.4	*p*=0.47
HSP60 [ng/ml]	71.5±107.0	60.3±44.5	*p*=0.17	77.3±107.2	43.7±48.9	*p*=0.01
CP [pg/ml]	61.7±27.3	72.1±23.1	*p*=0.001	62.2±18.3	69.0±43.7	*p*=0.66
hsCRP [mg/l]	2.8±2.9	2.6±2.8	*p*=0.60	3.0±3.0	2.2±2.5	*p*=0.21
MCP1 [pg/ml]	216.0±59.1	233.1±86.3	*p*=0.44	214.2±57.1	229.8±78.1	*p*=0.39
GSSG [μmol/l]	22.2±18.5	23.0±23.5	*p*=0.84	21.7±20.8	24.5±15.4	*p*=0.42
IL-6 [pg/ml]	2.83±1.44	2.27±0.65	*p*=0.13	2.85±1.28	1.90±0.55	*p*=0.04

means±SD, Abbreviations: CP-Carbonyle proteins, TGF β1 – Transforming growth factor β1, HSP60 – Heat shock proteins 60, hsCRP – high sensitivity C-reactive proteine, MCP1 – Monocyte chemoatractant protein-1, GSSG – oxidized glutathione, IL-6 – Interleukin 6

matory cytokines and inflammation markers showed associations with dyspnea, but the relationship was not consistent for all markers. We however cannot exclude participation of pulmonary impairment on both the inflammatory status and presence of dyspnea in our patients. Inflammation that originates in the lung and extends beyond the pulmonary system is thought to contribute to the cardiovascular consequences of lung disease (Sin & Man 2005). In patients with angina, we have found decreased levels of carbonyl proteins and no significant difference in other inflammatory markers. Moreover only several parameters demonstrated significant association, and no one was identified as independent predictor of angina or dyspnea. Lack of tight association may be explained by exclusion of unstable ischemic heart disease, which is known with more pronounce inflammatory reaction (Zakynthinos & Pappa 2009; Rogers *et al.* 2010).

Our finding correspond with recent study Schlager *et al.* (2012) who did not observe lasting changes of markers of inflammation in patients undergoing supervised exercise training. Intervened group of peripheral arterial disease patients with intermittent claudication despite of increase of walking capacity did not improve inflammatory markers (Schlager *et al.* 2012). On the other hand, other studies on exercise training in patients with coronary artery disease and congestive heart failure were able to demonstrate reduction in inflammatory biomarkers, such as hsCRP, TNF-alpha, and IL-6 (Adamopoulos *et al.* 2002; Milani *et al.* 2004). Similarly to these studies, our female IHD patients also demonstrated general tendency to increased IL-6 in subgroups with angina or dyspnea, however only dyspnea analysis demonstrated significant differences.

At least partially, the beneficial effect of physical activity on inflammatory status is related to reduction of body weight (Calder *et al.* 2011) which is difficult to evaluate in our study due to its cross-sectional nature. BMI was however considered in our regression analyses. We also cannot exclude, that particular analyte may participate clearly in a pathogenic pathway, albeit with minor role but not serve as an effective biomarker.

Tab. 3. Correlation between inflammatory markers and exercise induced angina or dyspnea.

	Angina [rhó]	*p*-value	Dyspnea [rhó]	*p*-value
MBL	0.10	0.28	–0.06	0.53
TGF β1	0.05	0.64	0.15	0.15
HSP60	–0.12	0.17	0.22	0.01
CP	–0.25	0.001	–0.03	0.66
hsCRP	0.04	0.60	0.10	0.21
MCP1	0.07	0.44	0.14	0.12
GSSG	0.01	0.93	–0.16	0.04
IL-6	0.14	0.42	0.39	0.02

Abbreviations: CP-Carbonyle proteins, TGF β1 – Transforming growth factor β1, HSP60 – Heat shock proteins 60, hsCRP – high sensitivity C-reactive proteine, MCP1 – Monocyte chemoatractant protein-1, GSSG – oxidized glutathione, IL-6 – Interleukin 6

We are aware of some limitations of our study. The cross-sectional nature of our study does not enable determination of causality. Both dyspnea and exercise tolerance were self-reported and such subjective evaluation is prone to bias. Another source of statistical underestimation of the differences between subgroups may be asymmetrical occurrence of angina and dyspnea: the both pathologies were present in prevalence cca 75:25, what significantly increase necessity for contrasts between analyzed subgroups.

In conclusions we have found associations of pro-inflammatory cytokines and inflammation markers with dyspnea or angina pectoris, but the relationship was not consistent in our patients with stable ischemic heart disease.

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