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Gender difference in C-reactive protein concentrations in individuals with atherothrombotic risk factors and apparently healthy ones

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Recent studies have shown that C-reactive proteins have a pathogenetic role in atherothrombosis and concentrations of these substances could be used as a marker for future vascular events. The objective of this study was to determine gender differences in highly sensitive C-reactive protein (hs-CRP) in individuals with atherothrombotic risk factors and apparently healthy ones. We have presently matched 469 females and 469 males having the same age and body mass index (BMI). Of these, 210 men and 210 women had no atherothrombotic risk factors. In this group the hs-CRP concentrations were 1.6 ± 3.4 mg l^{-1} in women and 1.0 ± 2.7 mg l^{-1} in men ($p < 0.0005$). These values were 2.1 ± 3.4 mg l^{-1} and 1.5 ± 2.8 mg l^{-1}, respectively, in the entire cohort ($p < 0.0005$), which included also individuals with atherothrombotic risk factors. We conclude that significant gender differences exist in hs-CRP concentrations despite perfect matching for age and BMI. These differences should be reflected in guidelines that suggest hs-CRP cut-off points for the stratification of vascular risk.

Keywords: biomarker, highly sensitive C-reactive protein, gender differences, risk factors.

Introduction

Atherothrombosis is a leading cause of morbidity and mortality in the Western world. It is accompanied by a low grade, subclinical, smoldering internal inflammatory response that can be detected by the determination of highly sensitive C-reactive protein (hs-CRP) concentrations (Ridker 2001). In addition to being a prognostic biomarker (Ridker *et al.* 2002), this protein may have a pathogenetic role in the atherothrombotic process (Ballou and Lozanski 1992, Cermak *et al.* 2000, Pasceri *et al.* 2000, 2001, Torzewski *et al.* 2000, Labarrere *et al.* 2002, Verma *et al.* 2002a Verma *et al.* 2002b, Venugopal *et al.* 2002, Devaraj *et al.* 2003), and the hs-CRP level may have extensive clinical implications.

In a recent publication (Yeh and Willerson 2003), it was suggested that individuals should be divided into risk categories based on cut-off points of 1 and 3 mg l^{-1}. Individuals with hs-CRP concentrations of up to 1 mg l^{-1} are considered to be at low risk, those with a level between 1 and 3 mg l^{-1} to be at intermediate

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risk, while those who present with higher concentrations are at a relatively high risk. However, this categorical stratification did not take into account the significant gender differences that exist with regard to the expression of hs-CRP concentrations in individuals with atherothrombotic risk factors and in apparently healthy individuals.

In the present study, we analysed the differences in hs-CRP between females and males based on a head-to-head comparison following age and body mass index (BMI) matching. In addition, we took into account possible confounding factors, including the number of atherothrombotic risk factors and the intake of cardiovascular medications. The results of this study are relevant for the application of hs-CRP measurements in clinical practice.

Materials and methods

Patients and apparently healthy volunteers

We performed a cross-sectional study to which we recruited apparently healthy employees of a tertiary hospital and a municipality as well as individuals with atherothrombotic risk factors who were being followed up in the outpatient clinics of the Medical Center, Tel Aviv, Israel. Patients attending the Tel Aviv Sourasky Medical Center for a routine health examination between September 2002 and June 2003 were asked to participate in the Tel Aviv Medical Center Inflammation Survey (TAMCIS) (Cohen *et al.* 2003). Recruitment was based on local announcements and advertisements in the monthly pay bill of medical personnel as well as personal appeals to patients in the various outpatient clinics to participate in the inflammation survey.

Any individuals with an underlying inflammatory disease (arthritis, inflammatory bowel disease, etc.) as well as those with any infection or other inflammatory condition, including infarction, surgery or angiography during the 6 months prior to their recruitment into the present study, were excluded. We also excluded any individual being treated with steroids or non-steroidal anti-inflammatory agents, and those who had a past history of a vascular event (myocardial infraction, stroke, etc.).

Study design

From a cohort of 2114 recruited individuals, women and men who had the same age and BMI were matched. Women receiving hormone replacement therapy were excluded due its effect on hs-CRP concentrations. The matching was performed in a systematic way, regardless of the hs-CRP concentration. The only individuals who were not included were those for whom a perfect age and BMI match could not be found.

Definition of atherothrombotic risk factors

Diabetes mellitus was defined as a fasting blood glucose of $\geq 126 \text{mg dl}^{-1}$ or a random blood glucose $> 200 \text{ mg dl}^{-1}$ on two separate occasions, or treatment with insulin or oral hypoglycaemic agents. Hypertension was defined as a blood pressure of $\geq 140/90$ mmHg or treatment with antihypertensive drugs, while hyperlipidaemia was defined as a low density lipoprotein (LDL) cholesterol of $\geq 130 \text{ mg dl}^{-1}$ or triglyceride concentrations of $\geq 160 \text{ mg dl}^{-1}$, or treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or fibrates.

Laboratory variables

hs-CRP levels were measured using the Behring BN II nephelometer (Dade Boering, Marburg, Germany) and the method of Rifai (1999). The lower limit of hs-CRP detection by the Dade Behring nephelometer is 0.17 mg l^{-1}. Samples were tested in a random sequence.

Statistical analysis

The continuous variable data was summarized and reported as the mean $\pm$ SD for each gender. The categorical data was summarized and reported as the number and percentage in each group. The hs-CRP concentration values had a non-normal distribution; thus a logarithmic transformation was used, and the hs-CRP results given are back-transformed geometric means $\pm$ SD. For the continuous variables, a comparison between the genders was performed using the Student's t-test for paired samples. For the categorical variables, the paired McNemar test was used to test possible differences between the groups.

To check the possible relationship of the CRP differences between the genders and age, the pairs were divided to quartiles according to their age and a one-way analysis of variance (ANOVA) was performed to compare the mean level of difference in hs-CRP between those groups. Bonferroni's multiple comparison technique was used for pairwise comparison between the age categories. All analyses were carried out using SPSS software (SPSS Inc., Chicago, Illinois, USA).

Results

We have to date matched 469 women and 469 men with the same age and BMI out of a cohort of 2114 individuals (1003 women and 1111 men) who participated in the study. The remaining 1176 individuals (534 women and 642 men) could not be matched on the basis of age and BMI and were excluded.

Of the matched individuals, 210 men and 210 women had no atherothrombotic risk factors and were not receiving any medication. They were perfectly matched for age and BMI (table 1), but the men were somewhat more obese. The mean $\pm$ SD hs-CRP, cholesterol and triglyceride levels in these subjects are given in table 2. There is a significant difference in hs-CRP concentrations between women and men, with men having clearly lower values.

We also added 48 pairs of obese individuals who do not have any other atherothrombotic risk factors and who were not taking any cardiovascular medications to the previous 210 pairs. The mean age and BMI as well as the hs-CRP and lipid levels in these 258 pairs is reported in table 3. Again, a highly significant difference in hs-CRP concentrations is seen between women and men. In fact, the hs-CRP concentrations in women were almost double those observed in men, despite the fact that the men were somewhat more obese and had higher concentrations of cholesterol and triglycerides.

We also analysed the results in the 469 pairs of women and men matched for age and BMI (table 4). These subjects included those with atherothrombotic risk factors (table 5) and those who were taking medications (table 6). Several findings should be noted. There were no significant differences in the intake of

Table 1. Basic characteristics in the healthy volunteers.

	Women ($n = 210$)	Men ($n = 210$)	p value (paired t-test)
Age (years)	45.8 $\pm$ 11.4	45.9 $\pm$ 11.4	NS
BMI (kg m^{-2})	24.6 $\pm$ 2.8	24.8 $\pm$ 2.6	0.01

BMI, body mass index; NS, not significant.

Table 2. Highly sensitive C-reactive protein (hs-CRP) concentration and lipid profile in healthy volunteers.

	Women ($n = 210$)	Men ($n = 210$)	p value (paired t-test)
hs-CRP (mg l^{-1})	1.6 $\pm$ 3.4 (1.5)	1.0 $\pm$ 2.7 (1.0)	< 0.0005
LDL cholesterol (mg dl^{-1})	131 $\pm$ 31	139 $\pm$ 30	0.005
HDL cholesterol (mg dl^{-1})	64 $\pm$ 14	53 $\pm$ 12	< 0.0005
Triglycerides (mg dl^{-1})	106 $\pm$ 60	116 $\pm$ 62	NS

Values are the mean $\pm$ SD, with the median in parentheses.
LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

Table 3. Basic characteristics, highly sensitive C-reactive protein (hs-CRP) concentration and lipid profile in healthy volunteers, including obese subjects.

	Women ($n = 258$)	Men ($n = 258$)	p value (paired t-test)
Age (years)	46.0 ± 11.2	46.0 ± 11.3	NS
BMI (kg m^{-2})	26.0 ± 4.0	26.2 ± 3.8	0.006
hs-CRP (mg l^{-1})	2.0 ± 3.6 (1.9)	1.2 ± 2.7 (1.2)	< 0.0005
LDL cholesterol (mg dl^{-1})	131 ± 30	137 ± 30	0.017
HDL cholesterol (mg dl^{-1})	62 ± 14	52 ± 12	< 0.0005
Triglycerides (mg dl^{-1})	111 ± 62	129 ± 71	0.002

Values are the mean ± SD, with the median in parentheses.

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

Table 4. Basic characteristics, highly sensitive C-reactive protein (hs-CRP) concentration and lipid profile in all volunteers, including those with atherothrombotic risk factors.

	Women ($n = 469$)	Men ($n = 469$)	p value (paired t-test)
Age (years)	45.8 ± 10.6	45.9 ± 10.6	NS
BMI (kg m^{-2})	26.8 ± 4.4	26.9 ± 4.4	0.001
hs-CRP (mg l^{-1})	2.1 ± 3.4 (2.1)	1.5 ± 2.8 (1.4)	< 0.0005
LD cholesterol (mg dl^{-1})	134 ± 34	140 ± 33	0.014
HDL cholesterol (mg dl^{-1})	61 ± 15	50 ± 11	< 0.0005
Triglycerides (mg dl^{-1})	117 ± 64	134 ± 72	< 0.0005

Values are the mean ± SD, with the median in parentheses.

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

cardiovascular medications between women and men (table 6), and the percentage of smokers was higher in men (table 5). The BMI was somewhat higher in men (table 4), as were the cholesterol and triglyceride concentrations. Despite these differences in favour of higher CRP concentrations in men, the hs-CRP concentration was clearly and significantly higher in women (table 4).

hs-CRP concentrations in the 210 healthy individuals (reported in table 1 and table 2) were further analysed in age quartiles; the results are shown in figure 1. It can be seen that age *per se* cannot explain the results of the present study.

The distribution of the hs-CRP results in the 210 pairs of women and men is shown in figure 2. It can be seen that women tend to have higher hs-CRP concentrations; more men are represented in the left (low hs-CRP) than in the right (high hs-CRP) part of the graph.

Table 5. Number and percentage of different risk factors in the two gender groups.

	Women ($n = 469$)		Men ($n = 469$)		
	No.	%	No.	%	p value (paired McNemar test)
Smoker	167	35.6%	180	38.4%	0.026
Diabetes mellitus	4	0.9%	4	0.9%	NS
Hyperlipidaemia	99	21.1%	99	21.1%	NS
Hypertension	29	6.2%	29	6.2%	NS

NS, not significant.

Table 6. Number and percentage of different medications used by the two gender groups.

	Women ($n = 469$)		Men ($n = 469$)		
	No.	%	No.	%	p value (paired McNemar test)
Nitrates	0	0.0%	0	0.0%	NS
Amiodarone	0	0.0%	0	0.0%	NS
Alpha blockers	2	0.4%	6	1.3%	NS
Calcium channel blockers	6	1.3%	7	1.5%	NS
ACE inhibitors	5	1.1%	6	1.3%	NS
Angiotensin II receptor blockers	3	0.6%	2	0.4%	NS

ACE, angiotensin-converting enzyme; NS, not significant.

Discussion

hs-CRP is a useful biomarker for the detection of low grade inflammation in apparently healthy individuals as well as those with atherothrombotic risk factors. Since hs-CRP has a non-normal distribution, most studies divide their cohorts into quartiles or quintiles and have come to the conclusion that the quintile distributions of hs-CRP for men and for women are remarkably similar. Various cut-off points for this risk stratification have been suggested (Ridker 2003), but it should be mentioned that most studies were carried separately in women or men (Ridker *et al.* 1997, 2000, 2002, Pradham *et al.* 2002).

Potential differences in hs-CRP concentrations between women and men were noted by several researchers. Wener *et al.* (2000) evaluated the differences in the upper normal limit of CRP (represented by the 95th percentile in the overall population) in women and men and concluded that demographic factors, including age, sex and race, should be used to adjust the upper reference limit for CRP. Wang *et al.* (2002) studied the relationship between CRP and carotid atherosclerosis.

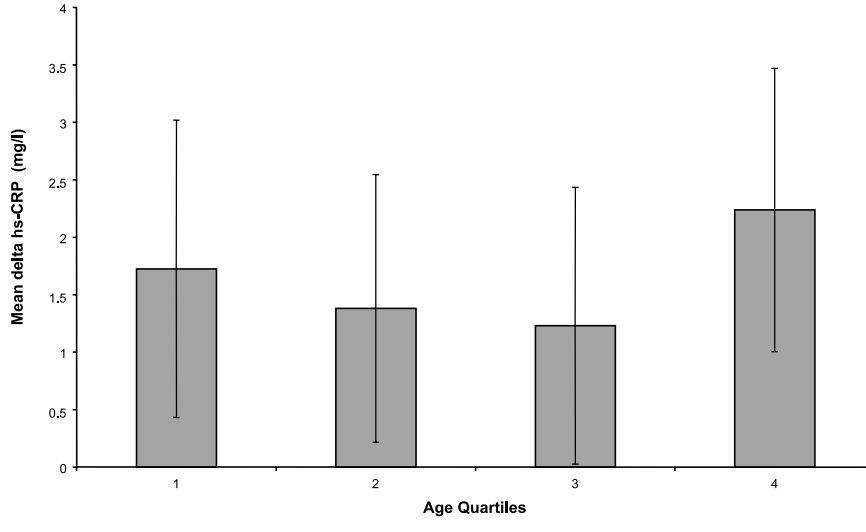

Figure 1. Mean ± SE delta highly sensitive C-reactive protein (hs-CRP) values between women and men in the quartiles of age among healthy non-obese individuals.

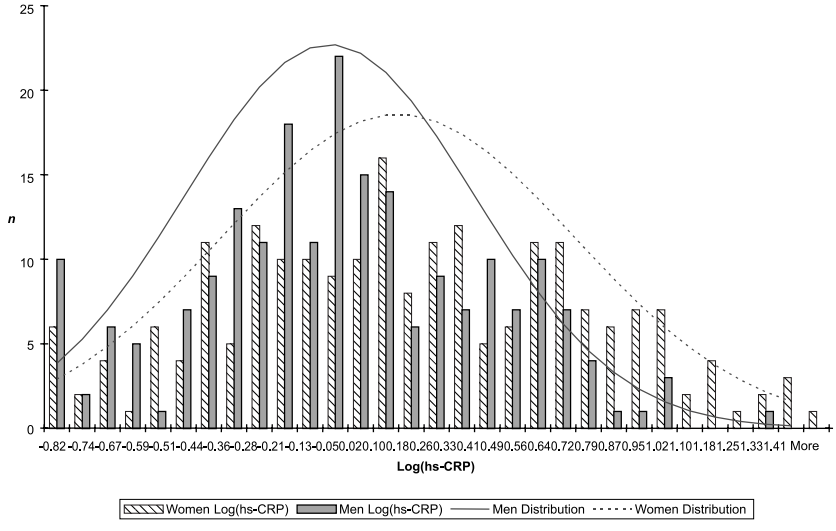

Figure 2. Distribution of highly sensitive C-reactive protein (hs-CRP) levels in women and men and their normal distribution curves among healthy non-obese individuals.

They divided their cohort based on gender and quartiles of CRP, and concluded that there is a significant difference in CRP levels between the genders and recommended a sex-specific analyses in future studies involving CRP. Roeters van Lennep *et al.* (2002), in a detailed review, summarized the differences between women and men in regard to different risk factors for coronary heart disease (CHD). They found that many factors such as diabetes and high density lipoprotein and triglyceride levels have a greater impact on CHD risk in women compared with men. There are indications that risk factors such as smoking, family history and inflammation characterized as CRP have a more negative influence on CHD in women than in men. The authors concluded that for optimal treatment and prevention of CHD it is necessary to acknowledge that it is not self-evident that women and men share a similar response to risk factors or to treatment, and it is essential that studies present results according to gender in order to comprehend to what extent CHD prevention measures are similar for men and women. McConnell *et al.* (2002) looked at the differences in CRP between the genders using two sensitive methods of measurement. They found that the gender difference was independent of age differences, and concluded that it had important implications for the establishment of cut-off points for cardiovascular risk stratification. Finally, Wong *et al.* (2001) studied a large cohort of 4472 men and 5212 women aged 30–74 years, and calculated the 10 year risk of CHD for women and men separately for CRP groups of ≤ 0.21, > 0.21 to < 0.5, ≥ 0.5 to < 1.0, and ≥ 1.0 mg l^{-1}. They found higher absolute mean levels of CRP among women, a higher percentage of women with levels ≥ 1.0 mg l^{-1}, and a higher percentage of the 10 year risk of CHD among men for the same CRP group; these findings imply that women have higher levels of CRP but these do not necessarily contribute to a higher risk for CHD. All these studies point towards the importance in differentiating between men and women with regard to CRP levels and CHD risk.

In the present study we evaluated the differences in hs-CRP concentrations between women and men, based on a direct head-to-head comparative analysis between pairs of women and men matched for age and BMI. The main finding was that, even when matched for age and BMI, hs-CRP concentrations were significantly higher in women. This is especially remarkable since the men had higher concentrations of lipids and had a higher rate of smoking. Individuals were matched for age and BMI because CRP concentrations tend to be higher in the elderly as well as in obese individuals.

A possible practical outcome of these findings is the movement of a given individual from one risk category to another on the basis of gender. With the cut-off points suggested by Ridker (2003) of low risk up to 1 mg l^{-1}, intermediate risk between 1 and 3 mg l^{-1} and high risk for ≥ 3 mg l^{-1}, if absolute CRP levels are used and gender is ignored, more women than men might be classified in a higher risk group due to the gender differences and not necessarily due to specific atherothrombotic risk factors. For example, a man with an hs-CRP concentration of 0.8 mg l^{-1} would be considered to be at low risk according to the above categorization. However, a woman with the same age, BMI and atherothrombotic risk factors could have, for example, an hs-CRP concentration of 1.3 mg l^{-1} only because of the gender difference. According to the above classification, she would be considered to be at intermediate risk.

A major deficiency in this study is the lack of follow-up to confirm that higher CRP levels in females have an influence on atherothrombotic risk. Thus, we currently have no evidence that the noted genetic differences in hs-CRP concentrations have any relevance in term of future vascular events. A prospective evaluation of vascular events in subgroups by gender is required to clarify this issue.

We conclude that women have higher CRP concentrations than men with a comparable atherothrombotic risk profile. Not taking into consideration gender differences in hs-CRP concentrations distribution could have an effect on the clinical application of the recently suggested hs-CRP cut-off values.

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