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Listening to the heart-brain talk: persistent depressive symptoms are associated with hsCRP in apparently healthy individuals at high risk for coronary artery disease

Rima Azar[1], Robert P Nolan[2] and Donna E Stewart[3]

Abstract

Background: This study examined whether mild-to-moderate depressive symptoms are associated with increased high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL-6) levels in apparently healthy individuals at high risk for coronary artery disease. We investigated in individuals whether: (1) current depressive symptoms were associated with increased hsCRP and IL-6 levels; (2) persistent depressive symptoms at two time points 6 months apart were associated with hsCRP and IL-6; and (3), sex-based differences in inflammation were a function of depressive symptoms.

Methods: We measured depressive symptoms (twice), hsCRP, and IL-6 (follow-up time point) in 84 apparently healthy individuals (52% women) at high cardiac risk.

Results: Patients with persistent depressive symptoms had higher hsCRP, compared to participants without persistent symptoms (5.55 vs. 1.70 mg/l, $p < 0.05$, 95% CI 0.11 to 1.09, $d = 0.67$). Participants with current depressive symptoms had higher hsCRP (3.99 vs. 1.70 mg/l, $p = 0.059$) than those without symptoms. Findings remained unchanged after controlling for covariates. Women had higher adjusted hsCRP than men (2.91 vs. 1.87 mg/l, $p < 0.001$). When we entered depressive symptoms, the model remained significant, with a significant interaction between sex and symptoms: women with depressive symptoms had higher hsCRP than men with depressive symptoms and than women without symptoms (6.75 vs. 1.11 mg/l). The hypothesized differences were not observed with respect to IL-6, after controlling for body mass index (95% CI−0.77 to 0.73).

Conclusions: Before a first ischaemic coronary event, persistent mild-to-moderate depressive symptoms were associated with increased hsCRP. Women with depressive symptoms had higher hsCRP than men with symptoms.

Keywords

Coronary artery disease, C-reactive protein, depressive symptoms, high risk, inflammation, interleukin 6

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Introduction

The development of coronary artery disease (CAD) in apparently healthy individuals is multi-factorial. Inflammation has emerged over the past few years as a suspected risk factor.[1–5] Among the best-established CAD psychosocial risk factors are depressive symptoms. A burgeoning literature shows that, depression is independently associated with cardiac morbidity (e.g., hospitalizations) or mortality in healthy individuals.[4] Inflammatory processes are an important pathophysiological pathway that could link depressive symptoms to CAD,[5] in addition to behavioural pathways such as poor diet or smoking.[6] One argument favouring a depression–inflammation relationship is that the hypothalamic–pituitary–adrenal axis hormones are consistently dysregulated in adults with

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major depression. Indeed, depressive symptoms may disrupt the anti-inflammatory effect of glucocorticoids (e.g., excess cortisol disrupts the negative feedback loop).[7] This study investigated whether persistent mild-to-moderate depressive symptoms are associated with increased pro-inflammatory markers.

Depressive symptoms are associated with higher acute-phase proteins and pro-inflammatory cytokines.[8–12] Independent of CAD, recent studies showed that clinical depression is associated with higher high-sensitivity C-reactive protein (CRP) and interleukin (IL) 6 levels.[13,14] Kop et al.[12] found depressive symptoms to be correlated with higher CRP levels. Lespérance et al.[15] showed that depressed patients, not taking statins, had markedly higher CRP levels. In a recent meta-analysis, Howren et al.[16] found that: (1) depression, CRP, and pro-inflammatory cytokines (e.g., IL-6, IL-1) are associated in clinical and community samples; and (2) there seems to be a dose–response relationship between depression and inflammatory markers but this association was the strongest in clinically depressed samples. Although Miller et al.[17] also found a graded relationship between CRP levels and depressive symptoms, they did not find evidence of a link between clinical depression diagnosis and CRP.

The findings by Empana et al.[18] also support an association between depressive mood and inflammation. However, cross-sectional studies using the Whitehall II epidemiological cohort[19] failed to show an association between depressive mood and inflammatory markers. The episodic nature of depressive symptoms may have accounted for the lack of statistically significant associations. Examining these associations in apparently healthy individuals, at two time points, before an acute coronary syndrome, can help tease apart causal ordering of variables more than in some previous studies.[15,17] Therefore, we evaluated symptoms of depression over a 6-month interval in order to establish the stability of depressed mood among apparently healthy women and men at high risk for CAD. We subsequently examined the relation between depressive symptoms, hsCRP and IL-6. To our knowledge, only two studies[5,18] have examined the relationship between depressive mood and inflammation in initially healthy patients. In addition, there is evidence that the relative risk for CAD linked to CRP is higher for women than men.[19] Regardless of depression, CRP levels have been found to be higher in women than men.[19] Other investigations[20] did not find any sex differences in CRP. There is abundant epidemiological data showing that prevalence and morbidity risk of depression are higher in females from mid-puberty throughout adult life.[21] Clearly, we should investigate potential sex-based differences in immune parameters in at-risk individuals. We hypothesized that: (1) individuals with current depressive symptoms would have increased hsCRP and IL-6 levels; (2) individuals with persistent depressive symptoms at two time points 6 months apart would have increased hsCRP and IL-6 levels; and (3) women with depressive symptoms would have higher hsCRP and IL-6 than men with depressive symptoms.

Materials and methods

Participants

The sample was comprised of 84 (out of potentially 86) apparently healthy participants with high cardiac risk factors recruited from a tertiary care cardiac centre. Participants were at $\geq$15% on the Framingham 10-year absolute risk index (age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes), free of ischaemic heart event (arrhythmia or unstable angina), and free of a diagnosis of congenital cardiac condition, based on medical records. Exclusion criteria were: taking anti-inflammatory drugs (i.e. aspirin, steroids) known to suppress IL-6,[22] antidepressants given that some reduce inflammation,[23] and being on estrogen therapy. The latter is linked to increased hsCRP levels.[24] From our potential sample of 86 participants, one woman and one man refused to participate because of a busy schedule. The final sample ($n = 84$) consisted of 44 women (52%) and 40 men (48%). Table 1 shows women's and men's characteristics. This study was approved by the local research ethics board. Before entering the study, each patient gave written informed consent.

Measures

Depressive symptoms. We measured depressive symptoms with the Beck Depression Inventory, second edition (BDI-II), a 21-item self-report scale, with excellent reliability (average coefficient alpha of 0.91) and validity.[25] The BDI-II is one of the most widely used instruments to measure severity of depression components (i.e. affective, cognitive, psychomotor) over the 2 previous weeks. The items rate symptom intensity on a 0–3 scale. The BDI-II was completed twice: at entry to study (T1) and 6 months later (T2). To compare women and men with and without mild-to-moderate depressive symptoms, we used a cut-off of 10 to assign participants to either a group with current symptoms (BDI-II >10, $n = 18$) or to a comparison group (BDI-II ≤ 10, $n = 66$). The BDI and BDI-II have frequently been used with post-myocardial infarction patients[26] and a score ≤ 10 is considered normal.[27]

Inflammatory markers. We measured serum hsCRP and IL-6 at follow-up (T2). hsCRP levels less than

Table 1. Demographic and clinical characteristics of participants ($n = 84$)

Characteristic	Women ($n = 44$, 52%)	Men ($n = 40$, 48%)	p-value
Age (years)	55.8 ± 8.6 [a]	56.9 ± 6.9	0.50
Medication use			
Statin	1 (2.3)	0 (0)	–
Beta-blocker	1 (2.3)	2 (5)	–
Other[b]	11 (25)	6 (15)	–
Body mass index (kg/m^2)	29.5 ± 5.5	27.4 ± 3.6	<0.05
Smoking status	0 (0)	5 (12.5)	–
Family history of heart disease or stroke	11 (25)	7 (17.5)	–
Total cholesterol (mmol/l)[c]	6.1 ± 1.3	5.0 ± 0.9	<0.01
LDL (mmol/l)[c]	3.7 ± 1.1	3.0 ± 0.8	<0.01
HDL (mmol/l)[c]	1.5 ± 0.4	1.2 ± 0.2	<0.01
Ratio of total to HDL cholesterol	4.3 ± 1.4	4.2 ± 1.0	0.65
Triglyceride (nmol/l)	1.8 ± 1.1	1.8 ± 1.2	0.92
Fasting plasma glucose (mmol/l)	6.7 ± 2.9	6.2 ± 1.5	0.32
Systolic blood pressure (mmHg)	134.7 ± 18.1	135.6 ± 15.9	0.76
Diastolic blood pressure (mmHg)	80.9 ± 10.8	83.7 ± 10.2	0.76

Values are mean $\pm$ standard deviation or n (%). [a]Among women under 51 years of age (mean age for menopause in North America), none reported using a birth control pill. [b]Angiotensin-converting enzyme inhibitor, diuretic, osteoporosis, and insomnia drugs. [c]No significant correlation with either hsCRP or IL-6 levels. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

1 mg/l indicate a low overall cardiac risk. Levels between 1 and 3 mg/l reflect a moderate risk while levels higher than 3 mg/l suggest an elevated risk (about 2–3 times higher than low levels).[28] Circulating IL-6 is found in normal individuals in the 1 pg/ml range, with slight elevations during menstrual cycle.[29] Only 12 women were under the average age of menopause (51 years) but their menstrual status was not assessed.

Collection of samples. There is no diurnal variation of hsCRP concentrations.[30] To avoid any potential diurnal variation in IL-6, we measured all serum samples in the morning (0830 and 1230 hours at T2 (BDI-II of T2 completed). We instructed participants to have a light breakfast and avoid drinking coffee or smoking. They completed a brief questionnaire, asking about medication, what they ate at their last meal, whether they smoked or had a recent infection/injury. The serum was immediately stored at -80 C until assay.

hsCRP was assessed using a high sensitivity immunoturbidimetric assay on an ABX Pentra 400, a clinical chemistry analyser (HORIBA ABX, Chicksands, Shefford, UK). For the hsCRP assay, the analytic measurement range was 2–380 µl with automatic dilution from 1/2 to 1/22,500. The coefficient of variation values of the ABX Pentra 400 are less than 7.04% (conventional range). The analyser shows good reliability with low sample volume.[31]

Serum IL-6 was measured with a Quantikine HS enzyme linked immunosorbent assay (ELISA) for human IL-6 kit (R & D Systems, Minneapolis, MN, USA). This solid-phase ELISA is designed to measure human IL-6 in serum, plasma, and urine. The assay has a sensitivity <0.03 pg/ml, an intra-assay variation $<8\%$, and inter-assay variation $<10\%$.

Data analysis

We performed analyses with SPSS version 12.0 (SPSS, Chicago, IL, USA). Significance was defined as $p < 0.05$. Tests were two-tailed.

Because of positively skewed distributions, we log transformed the data for the inflammatory markers hsCRP and IL6. The transformations resulted in normal distributions.

Based on previous literature, we specified a priori the following potential covariates: infection within the past 2–3 weeks, medication use such as statins and beta-blockers, age, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, diabetes mellitus, and BMI. Four participants (two women, two men) reported using statins and beta-blockers. Given their potential to reduce inflammation,[32] we controlled for the latter in the analyses.

Statistics

To test hypothesis 1, we ran a bivariate Pearson-product-moment correlation coefficient for the entire sample. We then ran two t-tests comparing hsCRP and IL-6 levels respectively in the two groups of individuals with mild-to-moderate depressive symptoms and in individuals without depressive symptoms. To test hypothesis 2, we performed a t-test on hsCRP and IL-6 levels in participants whose depressive symptoms were persistent (BDI-II scores >10 at the two time points) and those without persistent scores (BDI-II >10 at one time or no symptoms at all). To test hypotheses 3 and 4, we investigated hsCRP and IL-6 levels respectively as a function of sex and/or depressive symptoms, using analysis of covariance (ANCOVA) to control for potentially confounding variables.

Results

Descriptive findings

Actual mean hsCRP levels were 2.32 mg/l (standard deviation, SD 7.8, median 1.00 mg/l), with half of the sample with levels raised above the conventional clinical cut-off point: 50% of participants had hsCRP levels <1 mg/l, 25% 1−3 mg/l, and 25% >3 mg/l. Actual mean IL-6 levels were 1.93 (SD 1.44, median 1.6). hsCRP and IL-6 were positively correlated ($r = 0.5$, $p < 0.001$). Mean current BDI-II score (T2) was 6.43 (SD 5.89, range 0–22). Mean T1 BDI-II score was 6.82 (SD 5.75, range 0–22). Eight women and ten men had current mild-to-moderate depressive symptoms (BDI-II >10). Of these, 12 had persistent symptoms, with BDI-II scores higher than 10 at T1 and T2. Women had BDI-II scores slightly higher than men but this was not statistically significant (see Table 2).

Potentially confounding variables

In the ANCOVA, we adjusted for BMI, blood glucose, and triglycerides (potential mediators for the relation between sex and hsCRP). No participant had a current known infection and 12 participants had upper respiratory infection symptoms more than 2 weeks earlier. However, there was no difference in hsCRP and IL-6 in participants with/without past upper respiratory infection and those taking/not taking statins, beta-blockers, and other medications ($p = 0.5$). Neither hsCRP nor IL-6 differed as a function of age ($p = 0.2$).

Are current and persistent mild-to-moderate depressive symptoms associated with hsCRP and IL-6 levels?

In the whole sample, depression scores and hsCRP levels were significantly correlated at the two time points ($r = 0.22$ and $r = 0.25$ respectively). Participants with current depressive symptoms had higher hsCRP levels (actual means 3.99 vs. 1.70 mg/l) than participants without depressive symptoms but this did not reach significance [t-test(78, 22.60) = 1.92, $p = 0.059$]. Participants with persistent mild-to-moderate depressive symptoms (BDI-II >10 at two time points) had higher hsCRP levels than participants without persistent symptoms: past depressive symptoms were associated with increased hsCRP [4.62 vs. 1.61, t-test(60, 17.87) = 2.41, $p < 0.05$]; concurrent depression treated as continuous was not associated with hsCRP ($F = 0.18$, $p > 0.05$), actual means 5.55 vs. 1.70 mg/l, t-test(61, 11.55) = 2.44, 95% CI on transformed scale 0.11 to 1.09, $p = 0.018$, with a substantial effect size ($d = 0.67$). Although in the hypothesized direction, IL-6 levels were not associated with depressive symptoms ($p = 0.4$). The 95% confidence interval of the mean differences in IL-6 levels were –0.77 and 0.73. These findings remained unchanged when analyses were repeated, controlling for covariates.

Do women have higher hsCRP and IL-6 levels?

A first examination of the distribution of women and men in the three hsCRP clinical groups yielded no

Table 2. Past and current mean depressive scores (BDI-II), hsCRP, and IL-6 levels in women and men in the sample

	Women ($n = 44$)	Men ($n = 40$)	p-value
Past depressive scores (T1)	7.3 (0−22)	6.4 (0−22)	0.76
Current depressive scores (T2)	6.6 (0−22)	6.2 (0–20)	0.56
Median hsCRP (mg/l)	0.7 (0.0–26.8)[a]	1.0 (0.0–11.6)[a]	0.30
hsCRP <1 mg/l (%)	21	19	0.90
hsCRP >3 mg/l (%)	11	9	0.90
Median IL-6 (pg/ml)[l]	1.6 (0.2–8.5)	1.6 (0.1−6.3)	0.30

Values are mean (range) unless otherwise stated. hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6. [a]Patients with hsCRP levels ≥20 are considered to have the very highest risk levels of future vascular events.

significant results (Pearson chi-squared = 0.45, df = 2, $p = 0.7$). However, an ANCOVA with sex as an independent variable, adjusting for BMI, blood glucose, and triglycerides levels, indicated that women (without depression) had higher hsCRP levels than men [adjusted means 2.91 vs. 1.87 mg/l, F(2, 53) = 7.69, $p < 0.01$]. When we entered current depressive symptoms in the ANCOVA (BDI-II >10 and comparison group) as fixed factor, the model remained significant [whole model $R^2 = 0.40$, F(6, 52) = 7.59, $p < 0.01$]. There was an interaction between sex and symptoms [F(3, 52) = 2.75, $p = 0.05$]: women had higher adjusted hsCRP levels than men. Women with depressive symptoms had higher hsCRP than men with depressive symptoms or women without symptoms (adjusted actual means 6.75 vs. 1.11 mg/l). IL-6 levels did not differ by sex ($p = 0.2$).

Discussion

Our study investigated whether depressive symptoms can add 'fuel to the fire' of the inflammatory processes involved in early atherosclerosis. Given the finding by Miller et al.[17] in early heart syndromes, we investigated whether the severity of depressive mood, as measured by persistent symptoms at two time points 6 months apart, can lead to higher hsCRP and IL-6 in individuals at high risk for CAD. We found that persistent mild-to-moderate depressive symptoms were associated with higher hsCRP levels and the effect size was medium-to-large.[33] Our findings corroborate those of others with patients who had recent[15] or earlier acute coronary syndromes.[17] Even if our underlying hypothesis was that depressive symptoms 'initiate' inflammation, inflammation can also precede depression. Indeed, depressive symptoms, if persistent or strong enough, might aggravate inflammation, and the latter might further induce depressive mood.

Women had higher adjusted hsCRP levels than men. Despite the very small and non-significant gender difference in BDI-II scores, there was an interaction between sex and current symptoms: women with depressive symptoms had higher hsCRP levels than men. It is not possible to ascertain the reason for the small gender differences in depressive scores in our study. The interaction between sex and depressive symptoms reached statistical significance, despite small gender differences in BDI-II scores and the small number of women with depressive symptoms. Our finding differs from the results of Matthews et al.[24] of a 5-year longitudinal study of women during transition to menopause in which depressive symptoms were not associated with hsCRP but rather with increased fibrinogen, which facilitates platelet aggregation.[3]

hsCRP, depressive symptoms, and CAD risk?

Newer hsCRP assays detect subtle variations in persistent low-grade inflammation. Longitudinal epidemiological studies with women and men show that hsCRP (which has a heritability of 35–40%) is a strong independent risk factor for the likelihood of rupture of an atherosclerotic plaque; not only in cardiac patients or in patients with multiple risk factors but also, interestingly, in (apparently) healthy individuals.[2] CAD and hsCRP might be related through chronic infection, obesity, or the pre-existing inflammatory atherosclerotic lesion. Inflammation can be related to both depression and atherosclerosis. Depressive symptoms persisting over 6 months might have had more time to instigate an inflammatory response. If so, it is possible that low-to-moderate depressive symptoms, which do not reach clinical significance for a diagnosis, are more likely to be related to hsCRP levels when they are chronic. hsCRP may add prognostic information at all levels of the Framingham risk. Although hsCRP confers risk in both sexes, prospective data show that, in healthy postmenopausal women, CRP and IL-6 independently provide a two-fold increase in CAD risk.[34] The clinical significance of increased adjusted hsCRP in women with and without depressive symptoms is unclear.

Role of IL-6 in CAD risk?

IL-6 induces hsCRP production. Although hsCRP and IL-6 were highly correlated, which corroborates other findings,[15] IL-6 was not associated with depressive symptoms.

Strengths and limitations

The data should be interpreted cautiously, as sample was small and there was likely a restricted range in the depression and inflammation scores in this apparently healthy sample. It would have been preferable to measure hsCRP at the beginning of the study as this might have provided an initial look at the possible direction of the relationship between depression and hsCRP. This would have allowed us to better assess the trajectories and test meditational models.[16] Finally, possible bi-directional associations between depressive symptoms and CRP could have limited the interpretability of our findings.

Conclusion

In apparently healthy individuals with cardiac risk factors, mild-to-moderate depressive symptoms persistent over 6 months were associated with increased hsCRP.

As women with depressive symptoms had higher hsCRP levels than men with symptoms, we suggest that more clinical attention should be given to women at high cardiac risk when they experience depressive mood.

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Conflict of interest

None.

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