Elevated levels of CRP in ovarian hyperstimulation syndrome: an unrecognised potential hazard?

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Objective Elevated levels of C-reactive protein (CRP) were found recently to be a crucial marker for cardiovascular disease. This protein might have a role in endothelial cell activation, vascular damage and a thrombotic tendency. We sought to determine whether concentrations of CRP are altered in women with controlled ovarian hyperstimulation (COH) or hyperstimulation syndrome (OHSS).

Design A prospective cohort study.

Setting The gynecology department and IVF unit of the Lis maternity hospital.

Population Twenty women with OHSS, 20 women undergoing COH and 20 women who participated as controls were included in the study.

Methods Venous blood was withdrawn for analysis of high sensitive C-reactive protein (hs-CRP) using the Boering BN-II nephelometer.

Main outcome measures hs-CRP levels were determined.

Results There was a significant ($P < 0.05$) difference in the hs-CRP concentrations in women with OHSS compared with women with COH and controls. The respective values for hs-CRP were 19.0 [4.0], 7.0 [1.7] and 4.6 [1.1] mg/L.

Conclusions Women with OHSS have elevated concentrations of hs-CRP. This finding, previously believed to merely represent an acute phase response, may actually represent a yet unrecognised pathophysiological factor of this syndrome and pave the way for new investigational directions of this potentially hazardous condition.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening disease due to the severe haemoconcentration, hypovolemia, electrolyte imbalances and respiratory failure brought about by fluid shifting to third spaces.1 The pathophysiological processes leading to the syndrome are not entirely clear, and many theories about them share the common denominator of endothelial activation and an increase in capillary permeability.2 C-reactive protein (CRP), previously believed to merely represent an acute phase response, was found recently to have a role in endothelial cell activation and a thrombotic tendency and is now known to be a crucial marker for cardiovascular disease.3–10

We recently conducted a study to define the degree of erythrocyte aggregation in women with OHSS and women with controlled ovarian hyperstimulation (COH).11 In this study, we used an assay for the determination of high sensitivity C-reactive protein (hs-CRP) concentrations in order to determine whether patients with OHSS demonstrate increased levels of this protein in their peripheral blood.

METHODS

In all the study, women gave their consent to participate in this investigation that was approved by the local ethics committee. Twenty consecutive women who were admitted to the Department of Obstetrics and Gynecology at the Lis Maternity Hospital from February 2001 to December 2001 and who met the criteria for severe OHSS were recruited for the study (group 1). These criteria were selected according to Golan et al.,12 and included sonographic evidence of ovaries enlarged to $>5$ cm in diameter and clinical evidence of nausea, abdominal distention and, in the severe cases, difficulty in breathing and ascites. The indications for IVF in women admitted with OHSS were male factor in six (30.0%), mechanical factor in three (15.0%) women, unexplained in nine women (45.0%) and lack of ovulation in two (10.0%) women.

Twenty consecutive women from our IVF unit who were undergoing COH from December 2001 to January 2002...
were also recruited for the study (group 2). The indications for IVF were male factor in 5 couples (25.0%), mechanical factor in 3 women (15.0%), unexplained in 11 women (55.0%) and lack of ovulation in 1 woman (5.0%).

Twenty women matched for age and at different stages of the menstrual cycle were also recruited for the study (group 3, controls). The women in all three groups were apparently healthy and had no chronic disease. Coagulation disorders or past thrombotic events were ruled out.

Venous blood was collected from the hospitalised women (group 1) upon admission, while blood samples were drawn from the COH women (group 2) on the morning of days 12–13 after human chorionic gonadotrophin (hCG) administration.

Additional information about the expected hs-CRP concentrations in a group of healthy women aged 18–39 years was obtained from the database of the Tel-Aviv Medical Center’s Inflammation Survey.13 Age groups, body mass indexes (BMIs) and smoking status, all known to affect CRP concentrations, were selected to match the data of the study group, and 145 suitable women selected from ~3000 apparently healthy individuals undergoing their annual routine health screening programme were enrolled.

The hs-CRP test was performed by using the Boering BN-II (DADE Boering, Marburg, Germany) nephelometer and a method according to Rifai et al.14 The lower limit of hs-CRP detection by the Dade Boering nephelometer hs-CRP is 0.17 mg/L.

All the variables were analysed for the normality of their distribution by the one-sample Kolmogorov–Smirnov test procedure. Differences between parameters in different patient groups were evaluated using the Fisher exact test, one-way ANOVA or Kruskal–Wallis where appropriate. The non-paired t test or post hoc Bonferroni analysis was used to perform pairwise comparisons between group means. According to power analysis, 20 subjects represented the minimum sample size required in each subgroup (alpha of 5% and power of 80%). A $P$ value of $<0.05$ was considered statistically significant. Calculations were performed using the SPSS software package (SPSS Inc., Chicago, IL).

Table 1. Demographic and clinical characteristics for normal controls, women with COH and women with OHSS. Values are given as mean [SE] and range.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (natural cycle), $n = 20$</th>
<th>COH, $n = 20$</th>
<th>OHSS, $n = 20$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30 [1.0]</td>
<td>29 [0.7]</td>
<td>31 [1.1]</td>
</tr>
<tr>
<td></td>
<td>22–39</td>
<td>18–39</td>
<td>18–39</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 [1.5]</td>
<td>23 [0.7]</td>
<td>24 [0.9]</td>
</tr>
<tr>
<td></td>
<td>18–34</td>
<td>18–32</td>
<td>17–32</td>
</tr>
<tr>
<td>Rate of smoking (%)</td>
<td>15</td>
<td>20</td>
<td>15</td>
</tr>
</tbody>
</table>

All values are not significantly different by ANOVA or Fisher exact test.

RESULTS

The 60 study women were equally divided into three groups, group 1 who had OHSS, group 2 who had COH and group 3 who were healthy controls. The mean [SE] age, BMI and the percent of smokers are presented in Table 1; there were no significant demographic differences between the three groups.

The results of the hs-CRP concentration in the three study groups are displayed in Fig. 1; there was a significant difference ($P < 0.05$) between the mean hs-CRP concentration in the OHSS group compared with the other two groups, with the values being 19.0 [4.0] mg/L for group 1, 7.0 [1.7] mg/L for group 2 and 4.6 [1.1] mg/L for group 3. No thrombotic events were recorded in our study groups. Five patients in the OHSS group and four patients in the COH were pregnant.

The expected hs-CRP concentrations in 145 apparently healthy age-matched women were obtained from the database of the Tel-Aviv Medical Center Inflammation Survey (Fig. 2). Average hs-CRP value in this patient group was

vascular permeability. Recent studies have suggested that CRP might not be an innocent bystander but that it may actually participate in endothelial cell activation and a prothrombotic tendency. In this preliminary study, we sought to determine whether OHSS is associated with an increase in CRP levels.

The possibility that CRP might be augmented or even underline the pathophysiology in OHSS has not been considered in the past. This protein is a non-specific acute phase reactant, the concentration of which can be increased following oestrogen administration. It has been suggested that it is synthesised following direct stimulation of receptors in the hepatocytes, although it is not clear whether this exacerbated synthesis is a sign of the presence of a generalised inflammatory response. Recent studies have suggested that CRP might not be an innocent bystander but that it may actually participate in cell activation, in inflammation intensification and as a prothrombotic protein. In fact, it has been shown that CRP can directly activate endothelial cells, induce the synthesis of tissue factor by monocytes, induce inflammatory cytokine release from human monocytes, increase plasminogen activator inhibitor-1 expression, activate the complement cascade and promote tissue necrosis in a model of myocardial infarction. The pro-inflammatory activities of CRP were recently summarised by Ridker et al. It would stand to reason, then, that CRP might have some pathologic role during endothelial cell activation, inflammation and thrombotic conditions.

Based on these previous reports, we believe that there is an increase in CRP levels in a step-up fashion in patients undergoing COH and with OHSS. The elevation of CRP demonstrated in COH is silent clinically but a further increase of this protein may promote vascular leak and set the background for the full blown syndrome of OHSS.

Expected levels of CRP in 145 healthy matched patients were also included in order to exclude the possibility that our present findings in our control group (group 3) were coincidental. This group of patients was retrieved from a large database (13) of apparently healthy individuals who were recruited during their routine annual health screening programme. From the data presented in Fig. 2, it could be deduced that the chance of finding women with a mean concentration of hs-CRP of ≥19 mg/L in this random population is relatively low. In fact, only four out of 145 (2.8%) women had hs-CRP concentrations >19.0 mg/L. This further supports the contention that the high hs-CRP concentrations reported in the group of women with OHSS represent an effect of the treatment and not a coincidental observation.

We conclude that the concentrations of hs-CRP are significantly elevated in women with OHSS. This finding may represent a heretofore unrecognized pathophysiological factor in the syndrome and set the background for further investigation. In a large scale prospective study, we are currently evaluating the profile of CRP and other pro-inflammatory markers in women undergoing IVF treatment. We plan to assess whether these markers are correlated with IVF results and pregnancy.

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Accepted 26 October 2004