Value of serum and follicular fluid cytokine profile in the prediction of moderate to severe ovarian hyperstimulation syndrome

Chin-Der Chen, Hsin-Fu Chen, Hsin-Fen Lu, Shee-Uan Chen, Hong-Nerng Ho and Yu-Shih Yang

Department of Obstetrics and Gynecology, National Taiwan University College of Medicine and Hospital, 7 Chung-Shan South Road, Taipei, Taiwan

© European Society of Human Reproduction and Embryology 1037

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a relatively common complication of ovarian stimulation and can be life threatening. The underlying mechanism responsible for the clinical manifestations of OHSS appears to be an increase in capillary permeability of the mesothelial surface with acute fluid shift out of the intravascular space (Polishuk and Schenker, 1969). Several recent reports suggest a pathophysiological increase in follicular fluid, ascitic fluid, and even serum concentrations of vasoactive mediators including interleukin (IL)-6, IL-8, tumour necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) (McClure et al., 1994; Abramov et al., 1997; Agrawal et al., 1999). Cytokines appear to participate in OHSS and may potentially serve as valuable predictive tools.

Materials and methods

Subjects and study design

From September 1998 to March 1999, 156 consecutive in-vitro fertilization (IVF) patients were recruited. All patients were followed for the development of OHSS. According to the classification proposed by Golan et al. (1989), seven (4.5%) had moderate OHSS and five (3.2%) had severe OHSS.

The study group was comprised of 12 patients who developed early-form moderate (n = 7) or severe (n = 5) OHSS requiring hospitalization. The two control groups were comprised of
randomized selection of 12 high-risk women and 12 low-risk women in whom OHSS did not develop.

To define patients at high risk for the development of OHSS, the records of the women in the study group who subsequently developed moderate/severe OHSS were reviewed. Because it was revealed that the lowest serum oestradiol concentration on the day of HCG administration in these cases was 1665 pg/ml (conversion factor to SI units, 3.671), this concentration was chosen as the threshold value for selection of control patients. Serum oestradiol concentration was chosen to define high-risk patients because this is the most commonly accepted risk factor for the development of OHSS (Delvigne et al., 1993).

Ovarian stimulation protocols

The ovarian stimulation protocols were performed as previously described (Chen et al., 1999). Briefly, pituitary desensitization was initiated using buserelin (Supremon®, Hoechst, Frankfurt, Germany) by a long protocol, which was administered by intranasal spray from the mid-luteal phase of the previous cycle at a dose of 200 µg, four times a day, until the day of menses. In the short protocol, buserelin was started on the second day of the treatment cycle and continued at a dose of 200 µg, given four times a day until the day before transvaginal aspiration of oocytes. Follicle stimulating hormone (FSH, Metrodin®, Serono, Rome, Italy) (150 IU/day) and human menopausal gonadotrophin (HMG, Pergonal®; Serono) (150 IU/day) were injected from cycle days 3–6 in the long protocol, while FSH and HMG were administered on cycle days 5 and 6 in the short protocol. Individualized injections of HMG were then continued until the administration of HCG (10 000 IU, Profasi®, Serono) when two or more leading follicles had reached a diameter of 18 mm. Transvaginal oocyte retrieval was performed 34–36 h later.

In all patients, peripheral venous blood was drawn in the morning on the days of HCG administration, oocyte retrieval, and embryo transfer. Transvaginal oocyte retrieval was performed 34–36 h after HCG administration. All embryo transfers were performed 2 days after oocyte retrieval. During oocyte retrieval, clear follicular fluid from individual follicles of ≥18 mm was collected and pooled in each patient. The follicular fluid was centrifuged immediately (1000 g), and the supernatant was stored, together with the serum samples, at –70°C before assay for cytokines and steroids.

Cytokine and hormone assays

Concentrations of IL-6, IL-8 and TNF-α in serum and follicular fluid were measured with a solid-phase chemiluminescent enzyme immunoassay system (Immulite®; Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of these cytokine assays was 1 pg/ml for IL-6, 2 pg/ml for IL-8, and 1.7 pg/ml for TNF-α. Serum and follicular VEGF concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) (Quantikine®; R&D System, Inc., Minneapolis, MN, USA) according to the manufacturer’s instructions. The sensitivity of the assay was 5 pg/ml. All samples were assayed at the same time to avoid interassay variations.

Serum and follicular fluid oestradiol and progesterone concentrations were assayed using a chemiluminescent immunoassay (Immulite®; Diagnostic Products Corporation). The intra-assay and interassay coefficients of variation for oestradiol at a concentration of 480 pg/ml were 6.3 and 6.4% respectively, and 6.3 and 5.8% for progesterone at a concentration of 7.2 ng/ml.

Statistical analysis

Statistical calculations were done using the Statistical Package for the Social Sciences (version 9.0, SPSS Inc., Chicago, IL, USA). For data that were not normally distributed, the Mann–Whitney U test was used if only two groups were being compared; the Kruskal–Wallis one-way analysis of variance was used if more than two groups were being compared. For normally distributed data, analysis of variance was used. Spearman rank correlation was used to determine if there were correlations between variables. Results are expressed as means ± SD and means with 95% confidence intervals as appropriate. Statistical significance was defined as a value of $P < 0.05$.

Results

The clinical and laboratory data of patients in the study and the two control groups are presented in Table I. There were no significant differences in any studied parameter between the study group and the high-risk control group. However, significant differences were observed between the OHSS group and the low-risk group in number of patients with polycystic ovary syndrome, serum oestradiol concentrations on the day of HCG administration, number of oocytes retrieved, and number of embryos transferred ($P < 0.05$ for all variables).

Follicular fluid concentrations of IL-6 as well as progesterone (Table II) were significantly higher in the OHSS group than in the two control groups ($P = 0.026$ and $P = 0.043$ respectively). Follicular fluid concentrations of IL-8, TNF-α, VEGF, and oestradiol showed no statistically significant difference among the three groups.

Table III shows changes in the serum concentrations of various cytokines and steroids on the days of HCG administration, oocyte retrieval, and embryo transfer. Serum IL-6 concentrations on the day of HCG administration were significantly higher in patients at risk for OHSS (OHSS and high-risk control groups) than that in the low-risk control group ($P = 0.015$, Mann–Whitney U test); however, no significant difference was observed between the OHSS group and the high-risk control group. Significantly elevated concentrations of IL-8 on the day of embryo transfer were observed in the OHSS group compared to that in the two control groups ($P = 0.017$, Kruskal–Wallis test). Levels of TNF-α and VEGF showed no statistically significant difference between the OHSS group and the two control groups at any studied time point. Serum oestradiol and progesterone concentrations were significantly different ($P < 0.05$) among the three groups at each studied time-point.

Table IV shows the correlation coefficients of cytokine concentrations with steroid concentrations in serum and follicular fluid. Follicular fluid cytokine (IL-6, IL-8, and TNF-α) concentrations were not correlated with oestradiol or progesterone concentrations. Follicular fluid VEGF concentrations were positively correlated with progesterone concentrations ($r = 0.49, P < 0.01$) and with IL-8 concentrations ($r = 0.397, P < 0.05$). Serum oestradiol concentrations were positively correlated with serum IL-6 concentrations ($r = 0.379, P < 0.01$) and with serum IL-8 concentrations ($r = 0.280, P < 0.01$), but were negatively correlated with serum TNF-α concentrations ($r = -0.281, P < 0.01$).

Discussion

The unique feature of this study is that multiple relevant cytokines were assessed in serum and follicular fluid
Predicting ovarian hyperstimulation

Table I. Clinical and laboratory data of patients in the study and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group OHSS (n = 12)</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High risk (n = 12)</td>
<td>Low risk (n = 12)</td>
<td></td>
</tr>
<tr>
<td>No. of patients with PCOS (%)</td>
<td>7 (58.3)</td>
<td>1 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.9 ± 4.8</td>
<td>33.7 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>No. of HMG ampoules</td>
<td>23.5 ± 9.6</td>
<td>27.05 ± 757</td>
<td></td>
</tr>
<tr>
<td>Oestradiol concentration on day of HCG administration (pg/ml)</td>
<td>3607 ± 2121</td>
<td>1117 ± 375oga</td>
<td></td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>23.4 ± 9.1</td>
<td>16.5 ± 8.8</td>
<td></td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>5.3 ± 1.1</td>
<td>4.8 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>8 (75)</td>
<td>4 (25)</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancies (%)</td>
<td>3/8 (37.5)</td>
<td>2/4 (50)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise indicated. HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin; OHSS = ovarian hyperstimulation syndrome; PCOS = polycystic ovarian syndrome.
aga P < 0.05 (versus study group).
oga No. of patients who became pregnant (%).

Table II. Follicular fluid concentrations of various cytokines and steroid hormones in the study and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group OHSS (n = 12)</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High risk (n = 12)</td>
<td>Low risk (n = 12)</td>
<td></td>
</tr>
<tr>
<td>Cytokine (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>42.7 (0–106.6)</td>
<td>6.1 (3.7–8.6)</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>357.0 (0–716.2)</td>
<td>160.5 (91.5–229.6)</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>21.7 (10.8–32.6)</td>
<td>10.4 (7.8–12.9)</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>2989 (2094–3884)</td>
<td>2492 (1288–3696)</td>
<td></td>
</tr>
<tr>
<td>Oestradiol (ng/ml)</td>
<td>255.3 (139.7–350.9)</td>
<td>198.7 (70.4–327.0)</td>
<td></td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>6962 (4585–9339)</td>
<td>4275 (3140–5409)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (95% confidence intervals). IL = interleukin; NS = not significant; OHSS = ovarian hyperstimulation syndrome; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor.
aga Determined by Kruskal–Wallis test.

Simultaneously in patients who subsequently developed OHSS and controls who did not. The possible role of serial measurement of these mediators was also examined from the day of HCG administration up to the day of embryo transfer in the prediction of OHSS. It was found that follicular fluid IL-6 concentrations at the time of oocyte retrieval and serum IL-8 concentrations on the day of embryo transfer may serve as early predictors for this syndrome.

A previous study has shown that IL-6 ribonucleic acid is produced during follicular neovascularization (Motro et al., 1990). The potential role of IL-6 in ovulation could be to enhance vascular permeability. Elevated concentrations of IL-6 in ascites and serum of women with OHSS were found (Friedlander et al., 1993), suggesting its role as a marker of OHSS. In addition, it was found that elevated concentrations of IL-6 in the follicular fluid at the time of oocyte retrieval may predict the development of early-form OHSS in high responders (oestradiol concentrations >3000 pg/ml and/or 20 oocytes recovered) (Geva et al., 1997). However, no comparison was made with normal responders in whom OHSS did develop. The results of the current study confirm the observation that increased follicular fluid IL-6 concentrations at the time of oocyte retrieval may indicate the early stages of OHSS in IVF patients. The study further expanded on the assay of circulating IL-6 concentration by showing that serum IL-6 concentrations differed significantly on the days of HCG administration in the OHSS group compared to the low risk group. There was no difference between the OHSS and high-risk group without OHSS. Serum concentrations of IL-6 were also correlated to oestradiol concentrations. It is of note that the statistical results for serum IL-6 concentrations on the days of HCG administration were on the lower detection concentrations for the assay and probably not clinically significant. This suggests the ovary may be the main resource of mediators that work as the initiators of the changes and lead to the full appearance of OHSS.

The findings of this study agree with previous studies (Krasnow et al., 1996; Geva et al., 1999), where no difference in follicular fluid VEGF concentrations was detected between OHSS and controls. However, lower concentrations of VEGF were found in the follicular fluid of patients who were at risk for OHSS (Pellicer et al., 1999). This finding is difficult to
understand because excessive ovarian angiogenesis is a key factor involved in the syndrome. The authors speculated that the ovary might not be involved initially in the pathogenesis of OHSS. Rather, a systemic response to HCG seems plausible. Whether these opposite patterns of VEGF found in follicular fluid are representative of regulation of the cytokine by other cytokines or steroid hormones requires further study.

Although some studies (Abramov et al., 1997; Artini et al., 1998; Pellicer et al., 1999; Agrawal et al., 1999) have established correlations between the development of OHSS and detectable circulating VEGF concentrations, the correlation is somewhat inconsistent. Pellicer et al. (1999) showed that there was a significant increase in serum VEGF concentrations after HCG administration in patients who were at risk for OHSS compared with those who were not at risk. Agrawal et al. (1999) reported that the increase in the VEGF concentration that occurred between the day of HCG administration and the day of oocyte retrieval (the ‘VEGF rise’) was an important non-steroidal marker of OHSS. The VEGF rise predicted 40.3% of cases of moderate and severe OHSS, with no false-negative results. However, the serum VEGF concentrations as a single marker during the early follicular phase, on the days of HCG administration, oocyte retrieval and embryo transfer did not predict the development of OHSS. Ludwig et al. (1999) described a correlation between the values of free VEGF on the day of HCG and the occurrence of OHSS, but could not show this effect for the total VEGF. The divergent results may be explained by different patient groups with inclusion of even
lower degree of OHSS and the small sample sizes in these studies. Another factor may be the kit used to measure VEGF concentration in serum or plasma (Ludwig et al., 1999).

Moreover, Lee et al. (1997) recently reported that serum VEGF concentrations at the time of oocyte retrieval are not useful in predicting which patients undergoing IVF are at increased risk for the development of OHSS. Furthermore, Krasnow et al. (1996) did not find any significant difference in pre-ovulatory and on day 7 after the ovulatory dose of HCG plasma VEGF concentrations between women who subsequently developed OHSS and matched controls who did not. Thus, based on the work presented here and previous reports (Krasnow et al., 1996; Lee et al., 1997; Ludwig et al., 1998), circulating VEGF concentrations from the day of HCG administration up to the days of oocyte retrieval or embryo transfer do not appear to be a good marker or predictor of OHSS. The presence of other factors that interact with VEGF may be required for the development of severe OHSS (Geva et al., 1999). Another possible explanation is that the VEGF may be involved, but it is not necessarily implicated in the early events of the syndrome when its action would be clinically relevant.

Interleukin-8, a cytokine with neutrophil chemotactic activity, is a potent angiogenic factor (Koch et al., 1992). Recently, it was found that IL-8 concentrations were significantly higher in peritoneal fluid in 12 patients with severe OHSS compared with 20 controls (Revel et al., 1996), but no statistically significant difference was observed in the serum concentrations of patients and controls. The potential value of IL-8 measurements for predicting OHSS has not been fully investigated. The present results showed that a significantly higher serum concentration of IL-8 on the day of embryo transfer were observed in the OHSS group compared to the two control groups. Serum IL-8 concentrations may be a good parameter to predict an OHSS, even if it is only possible on the day of embryo transfer. However, it is of note that serum concentrations of IL-8 were also correlated strongly to serum oestradiol concentrations in this study.

Of interest are the relationships between the individual cytokines and steroid hormones in serum and follicular fluid. It is important to state that in many of its functions IL-6 probably acts in concert with other cytokines (Jones, 1994). Interleukin-6 also has been shown to induce the expression of VEGF in several cell lines (Cohen et al., 1996). The data of the current study showed that serum IL-6 and IL-8 concentrations tend to increase with an increase in serum oestradiol concentration, and there is no correlation between serum VEGF and oestradiol concentrations. This indicates that a complex pattern of cytokines exists in serum and follicular fluid from patients with OHSS, suggesting the importance of 'profiling' cytokines rather than monitoring the concentration of only a single type. It is speculated that the presence of other factors that interact with IL-6 and IL-8 may be required for the development of severe OHSS. The interrelations between the individual cytokines remain to be established in further studies, as does the feasibility of performing measurements in serum and follicular fluid in clinical practice.

This study had limitations. The present study design did not answer the question of whether serum IL-6 and IL-8 are independent risk factors, or just an epiphenomenon of high oestradiol values. A more reasonable study design would have been to obtain another control group without OHSS including patients who are matched to the group of patients with OHSS according to both age at oocyte retrieval and oestradiol values on day of HCG administration. Furthermore, we have no prospective experience with measurements of serum and follicular fluid, pro-inflammatory cytokines and VEGF in a different series of patients. Therefore, a prospective study is necessary to evaluate the proposed predictors of OHSS.

Acknowledgements

This study was supported by the National Health Research Institute (NHRI-96-ET98841L), the National Science Council of the Republic of China (NSC88-2314-B-002-385), and National Taiwan University Hospital (NTUH88A-008).

References


Received on November 16, 1999; accepted on February 4, 2000