A Rise of the Serum Level of von Willebrand Factor Occurs Before Clinical Manifestation of the Severe Form of Ovarian Hyperstimulation Syndrome

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Submitted: May 30, 2000

Accepted: July 25, 2000

Background: Ovarian hyperstimulation syndrome (OHSS) appears to be caused by increased capillary permeability in the vascular endothelial cells. Such cells secrete excess amounts of von Willebrand factor (vWF), a large adhesive glycoprotein.

Methods: We retrospectively evaluated the circulating levels of vWF and of vascular endothelial growth factor (VEGF) twice, on the days of oocyte retrieval and embryo transfer, in 46 women who developed early-onset OHSS.

Results: Nineteen, 14, and 13 women developed mild, moderate, and severe OHSS, respectively. Inconsistent changes were observed in the VEGF during oocyte retrieval and embryo transfer. However, the net increase in serum vWF during that period showed an increase in absolute value at the time of embryo transfer that paralleled an increase in the severity of OHSS. That is, in mild OHSS, the serum vWF increased from 140 ± 44 to $164 \pm 28\%$; in moderate OHSS, it increased from 113 ± 47 to $186 \pm 22\%$; and in severe OHSS, it increased from 120 ± 35 to $274 \pm 63\%$. All 9 women with a vWF level $>230\%$ at embryo transfer developed severe OHSS, while 9 of 13 women with severe OHSS exhibited a vWF $>230\%$ at embryo transfer.

Conclusion: The results suggest that a rise of the serum level of vWF occurs prior to clinical manifestation of OHSS in patients with severe OHSS but not in patients with mild OHSS.

KEY WORDS: Capillary permeability; IVF-ET; ovarian hyperstimulation syndrome; prediction; von Willebrand factor.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS), which is often caused by artificial stimulation of the ovaries, can result in serious, and even fatal, complications. The incidence of mild, moderate, and severe cases ranges from 23 to 33, 3 to 6, and 0.3 to 5%, respectively (1,2). Because the available treatments for OHSS have only limited efficacy, methods for preventing the severe syndrome are sought. Risk factors for OHSS include the patients' age (<35 years), the presence of polycystic ovary syndrome (PCOS), a high serum level of estradiol (>2500 pg/ml), a large number of developed follicles, and ultrasonographic evidence of the ovarian necklace sign (3). The presence of these factors can be used to estimate the risk of subsequent occurrences of OHSS. In addition, the avoidance of human chorionic gonadotropin (hCG) administration after oocyte retrieval and the cryopreservation of all embryos during the ovulation-induction cycle have been proposed (2,4).

The underlying cause of OHSS appears to involve an increased capillary permeability of the ovarian tissues and of other mesothelial surfaces (5–8). The secretion of vasoactive substances by the ovary seems to be increased by hCG (8,9). Changes in serum levels of substances such as renin, angiotensin, interleukins, vascular endothelial growth factor (VEGF), tumor necrosis factor α , and endothelin-1 have appeared to coincide with the onset of OHSS (10–16). Because OHSS is heralded by the accumulation of ascites, with a concomitant shrinkage of the circulating plasma volume, increased permeability of vascular vessels may occur prior to the clinical onset of OHSS, suggesting that dysfunction of the endothelial cells may be

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an early step in pathogenesis. von Willebrand factor (vWF) is reportedly released by vascular endothelial cells that have been injured or stimulated by certain bioactive substances (17,18). The serum level of vWF gradually rises in stimulated cycles, then declines in nonhyperstimulated patients (19). However, the serum level of vWF continues to rise in patients with OHSS (19). These observations suggest that the level of vWF around the time of oocyte retrieval reflects a dysfunction of the endothelial cells. The present study retrospectively evaluated changes in serum levels of vWF during oocyte retrieval and embryo transfer to determine whether they reflected the severity of OHSS that occurred subsequently.

MATERIALS OF METHODS

Patients

Between January 1996 and December 1998, approximately 1200 Japanese women underwent controlled ovarian hyperstimulation related to the use of assisted reproductive technologies at the Jichi Medical School Hospital and the Central Clinic, and approximately 120 women developed OHSS. Of these, 46 women were included in the present analysis because blood samples were available at the scheduled time, the patients developed early-onset OHSS, and they gave their informed consent for determining serum levels of VEGF and vWF (Table I).

Controlled Ovarian Hyperstimulation and Luteal Support

The same ovulation induction regimens were used in all cases. Each case received 300 μ g of busereline

acetate (Hoechst, Tokyo) as a nasal spray three times daily beginning in the midluteal phase of the preceding menstrual cycle until the day of hCG administration. A daily intramuscular injection of 300 IU pure FSH (Serono, Tokyo) was administered from the third day of the menstrual cycle up to the time at which the diameter of the leading follicles reached 8–12 mm. Then each subject received a combination of 150 IU pure FSH and 150 IU hMG (Nihon Organon, Tokyo) administered daily by intramuscular injection. After the follicles had reached a diameter of 14–16 mm, treatment consisted of 300 IU hMG. Then, when several prominent follicles had developed to a diameter of 18–20 mm, 10,000 IU of hCG (Mochida Seiyaku, Tokyo) was administered intramuscularly. Oocyte retrieval was performed 34–36 hr after the administration of hCG.

Collection of Blood and Assay of VEGF and vWF

Peripheral blood samples were collected twice, once on the day of oocyte retrieval and again at the time of embryo transfer, which was performed approximately 48 hr after oocyte retrieval in cases of IVF-ET. In cases of gamete intrafallopian transfer (GIFT), the second blood sample was obtained 48 hr after oocyte retrieval. Blood samples were collected for evaluation of vWF into tubes containing 1 part 3.13% sodium citrate and 9 parts blood. For evaluation of VEGF an aliquot of 2 ml blood was collected into tubes that contained 2 mg ethylenediaminetetraacetic acid. Plasma was stored frozen at -20°C after being centrifuged at 4°C and 3000g for 15 min. Levels of vWF were determined by quantitative ELISA using the VIDAS immunoanalyzer (BioMerieux, Marcy l'Etoile, France) (20) according

Table I. Clinical Features of 46 Patients with OHSS^a

	Mild OHSS	Moderate OHSS	Severe OHSS
No. of patients	19	14	13
GIFT	7	2	3
Age (years)	32.1 $\pm$ 4.3	31.5 $\pm$ 4.1	31.0 $\pm$ 2.7
No. <35 years old	14	10	11
Positive history of OHSS	2	5	5
Polycystic ovary syndrome	0	1	4
Dose of hMG (IU)	2661 $\pm$ 291	2503 $\pm$ 281	2567 $\pm$ 197
No. of oocytes retrieved	15 $\pm$ 6.1	23 $\pm$ 6.2*	29 $\pm$ 10*
Embryo transfer canceled	0	2	5
Hospital stay (days)	2.6 $\pm$ 1.7	6.9 $\pm$ 1.3*	10.3 $\pm$ 2.4*,†
Days of albumin supplementation	0.0 $\pm$ 0.0	3.2 $\pm$ 1.1*	5.7 $\pm$ 1.3*,†

^a GIFT, gamete intrafallopian transfer; hMG, human menopausal gonadotropin.

* $P < 0.01$ vs moderate OHSS.

† $P < 0.01$ vs severe OHSS.

to the manufacturer's instructions. Levels of VEGF were measured by solid-phase ELISA (R&D Systems Inc., Minneapolis, MN). The minimum detectable concentration of vWF was 1.0%, and that of VEGF was 15.6 pg/ml. The intra- and interassay coefficients of variation for each variable were <8.0 and <12%, respectively. We also determined the white blood cell count (WBC), hematocrit (Ht), and serum levels of total protein and albumin.

Grading of OHSS

At the time of the second blood sampling on the day of embryo transfer, none of the 46 women showed signs of OHSS such as ascites, hemoconcentration, oliguria, or enhanced coagulability. However, all 46 women developed early-onset OHSS, described by Orvieto and Ben-Rafael (1998). Patients were divided into three groups according to the severity of OHSS. Patients with mild OHSS exhibited a maximum Ht of <45% and a minimum serum level of total protein of >6.5 g/dl in the presence of ascites. Patients with moderate OHSS exhibited a maximum Ht of $\geq 45\%$ and/or a minimum serum level of total protein of ≤ 6.5 g/dl in the presence of ascites without evidence of increased blood coagulability. Of the patients with moderate OHSS, some exhibited a urine volume of <300 ml/day and/or evidence of increased blood coagulability [serum levels of fibrin degradation products (FDP) of ≥ 80 μ g/ml and antithrombin III activity of <70% normal] during their clinical course. These patients were classified as having severe OHSS. In patients with moderate or severe OHSS, a commercially available human albumin solution was used. A total of 37.5 g human albumin in 350 ml [100 ml 25% human albumin (Alpha Therapeutic Corporation, Los Angeles, CA) and 250 ml 5% human albu-

min (Baxter Health Care, Glendale, CA)] was given in 1 hr by intravenous drip infusion once a day.

Data are reported as the mean $\pm$ SD. The paired Student *t* test, the unpaired Student *t* test, the Mann-Whitney *U* test, and the chi-square test with Yates' correction were used for statistical analysis as appropriate. A level of *P* < 0.05 was regarded as statistically significant.

RESULTS

On the day of embryo transfer, none of the 46 women showed signs of OHSS such as ascites, hemoconcentration, oliguria, or enhanced coagulability. However, embryo transfer was canceled in seven women because more than 25 oocytes had been retrieved and/or these patients had a history of severe OHSS. All 46 women developed early-onset OHSS, described by Orvieto and Ben-Rafael (1998). Ultimately, 19 patients developed mild, 14 patients developed moderate, and 13 patients developed severe OHSS (Table I). The three groups did not differ as to age or the total dose of hMG. The number of oocytes retrieved was significantly larger in patients with moderate or severe OHSS than in patients with mild OHSS. The length of hospital stay and the amount of albumin supplementation increased significantly with increased severity of OHSS.

The WBC count in the three groups did not differ significantly on the day of oocyte retrieval (Table II). The WBC count tended to be decreased at the time of embryo transfer compared with that at oocyte retrieval in the groups with mild and moderate OHSS, whereas the WBC count showed a significant increase in the severe OHSS group at that time. The Ht did not differ significantly at the time of oocyte retrieval

Table II. Differences in Laboratory Data Between the Time of Oocyte Retrieval and the Time of Embryo Transfer^{a,*}

	Mild OHSS (<i>n</i> = 19)		Moderate OHSS (<i>n</i> = 14)		Severe OHSS (<i>n</i> = 13)	
	Oocyte retrieval	Embryo transfer	Oocyte retrieval	Embryo transfer	Oocyte retrieval	Embryo transfer
WBC count (per mm ³)	8453 $\pm$ 2595	8173 $\pm$ 1931	8471 $\pm$ 2466	8113 $\pm$ 3054	8162 $\pm$ 1541	11062 $\pm$ 3123 ^{a,e}
Ht (%)	37.7 $\pm$ 3.0	40.0 $\pm$ 2.6	39.7 $\pm$ 3.4	42.3 $\pm$ 1.8 ^a	38.9 $\pm$ 3.2	43.2 $\pm$ 3.2 ^{b,e}
Total protein (g/dl)	6.6 $\pm$ 0.5	7.1 $\pm$ 0.2 ^d	6.3 $\pm$ 0.3	6.6 $\pm$ 0.4	6.2 $\pm$ 0.3 ^a	6.5 $\pm$ 0.6
Albumin (g/dl)	3.9 $\pm$ 0.5	3.9 $\pm$ 0.1	3.9 $\pm$ 0.3	3.9 $\pm$ 0.1	3.7 $\pm$ 0.4	4.0 $\pm$ 0.7
VEGF (pg/ml)	28 $\pm$ 13	20 $\pm$ 5.2	25 $\pm$ 12	19 $\pm$ 7.6	26 $\pm$ 14	30 $\pm$ 21
vWF (%)	140 $\pm$ 44	164 $\pm$ 28 ^d	113 $\pm$ 47	186 $\pm$ 22 ^{a,f}	120 $\pm$ 35	274 $\pm$ 63 ^{b,c,f}

^a WBC, white blood cell; Ht, hematocrit; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

* ^a*P* < 0.05 and ^b*P* < 0.01 vs the value for the group with moderate OHSS at the corresponding time. ^c*P* < 0.01 vs the value for the group with severe OHSS at the corresponding time. ^d*P* < 0.05, ^e*P* < 0.01, and ^f*P* < 0.001 vs the value at oocyte retrieval for the same group.

among the three groups. The Ht tended to show an increase on the day of embryo transfer in all three groups, with the value being significantly higher in the patients with moderate or severe OHSS vs those with mild OHSS at that time. The total protein concentration increased by 0.3 to 0.5 g/dl at embryo transfer compared with that at the time of oocyte retrieval in all three groups. The serum albumin level did not differ among the three groups at the time of oocyte retrieval or at embryo transfer.

The serum level of VEGF in the three groups did not differ significantly at the time of oocyte retrieval or at embryo transfer. Inconsistent changes in VEGF were observed between groups during oocyte retrieval and at embryo transfer; it tended to decrease in the groups with mild and moderate OHSS, whereas it tended to increase in the group with severe OHSS.

Interestingly, in all three groups, the mean vWF was higher at the time of embryo transfer than at oocyte retrieval; the magnitude of the net increase between these periods became greater with increased severity of OHSS: mild, $25 \pm 49\%$; moderate, $73 \pm 36\%$; and severe, $154 \pm 75\%$. The mean absolute serum level of vWF on the day of embryo transfer showed a significant increase with an increase in the severity of

OHSS (Table II). Fourteen of the 46 women were at high risk to develop OHSS based on a positive history of OHSS, the presence of PCOS (Table I), and/or an increased number of oocytes retrieved, ≥ 25 . Of the 14 women, 2, 5, and 7 developed mild, moderate, and severe OHSS, respectively. The mean vWF at oocyte retrieval did not differ between the seven women who developed severe OHSS and the seven women who did not develop severe OHSS (111 ± 47 vs $128 \pm 41\%$, respectively). However, the mean vWF at the time of embryo transfer was significantly higher in the seven women who developed severe OHSS than in the seven women who did not develop severe OHSS (304 ± 56 vs $181 \pm 26\%$, respectively; $P < 0.001$).

Individual changes in vWF levels are shown in Fig. 1. An apparent ($>20\%$) increase in the serum level of vWF was seen in 6 of 19 patients (32%) with mild OHSS, in 13 of 14 patients (93%) with moderate OHSS, and in all 13 patients (100%) with severe OHSS. Nine of the thirteen patients with severe OHSS and none of the 33 patients with mild or moderate OHSS exhibited a vWF level $>230\%$ on the day of embryo transfer. In terms of predicting the severity of OHSS, no level of vWF at the time of embryo transfer was able to differentiate patients with moderate vs

Fig. 1. Change in the serum level of vWF in three groups of patients with OHSS: (A) mild ($n = 19$); (B) moderate ($n = 14$); (C) severe ($n = 13$). The dotted line indicates the cutoff value of 230%.

severe OHSS. However, a cutoff vWF value of 230% was able to differentiate patients with severe OHSS from those with mild to moderate OHSS. All 9 women with a vWF level >230% at the time of embryo transfer developed severe OHSS, while 9 of 13 with severe OHSS exhibited a vWF level >230% at the time of embryo transfer. The cutoff value of 230% vWF on the day of embryo transfer had a positive predictive value of 100% and a sensitivity of 69%.

DISCUSSION

The present study demonstrated that the serum level of vWF exhibited a significant rise before the development of clinical manifestations of moderate to severe OHSS. The magnitude of the rise was associated with the clinical severity of OHSS. Twenty-six (96%) of the 27 patients with moderate to severe OHSS exhibited a significant elevation of vWF (>20%) during this period, whereas only 6 (32%) of the 19 patients with mild OHSS did. A cutoff value of 230% vWF differentiated the patients who subsequently developed severe OHSS from those who developed a milder form of the syndrome.

The vWF is a large adhesive plasma glycoprotein that is produced mainly by the vascular endothelial cells and is released into the circulation as a multimer (21). vWF has gained attention as a marker for the activation of endothelial cells by virtue of its interaction with the platelets and the vessel walls (22). Increased levels of vWF have been found in clinical conditions such as preeclampsia (17,18), atherosclerosis, thrombotic thrombocytopenic purpura, myeloproliferative disorders, hemolytic uremic syndrome (23), and pregnancy (24), which are characterized by endothelial cell dysfunction and stimulation. Our finding that the vWF level rose prior to the onset of clinical manifestations of OHSS suggests that a dysfunction of vascular endothelial cells preceded the clinical occurrence of OHSS as has been suspected. Patients who developed OHSS in the present study showed a very low level of vWF in the follicular fluid (less than 10%) (data not shown). This finding suggests that a high level of circulating vWF did not originate in the ovaries. The release of vWF from the vascular endothelial cells may have been stimulated in response to the ovarian release of vasoactive substances in patients with OHSS (8,9).

An acute shift of intravascular fluid into a third space such as the peritoneal cavity occurs in patients with OHSS. This phenomenon is related to the

increased permeability of the small blood vessels. Various factors seem to be responsible for the increased capillary permeability of the ovarian vessels and the other mesothelial surfaces (25–27). VEGF is now found to be one of the most important causal factors in OHSS. Its production by luteinized human granulosa cells has been demonstrated in vitro (28). High concentrations of circulating VEGF lead to the extravasation of plasma, followed by pronounced hemoconcentration (29). Capillary permeability is suppressed by the administration to rats of an antiserum to recombinant human VEGF (25). VEGF may play an important role in inducing OHSS. However, in the present study, the serum level of VEGF did not rise prior to the onset of clinical manifestations of OHSS.

In the present study, the number of oocytes retrieved and the Ht value on the day of embryo transfer were associated with the severity of OHSS. However, from the clinical viewpoint of predicting the development of moderate vs severe OHSS, the number of oocytes retrieved or the Ht value did not clearly differentiate such cases compared with the vWF level (data not shown). Whether trauma to the capillaries and bleeding associated with oocyte retrieval may be responsible for the increased level of vWF on the day of embryo transfer remains to be determined. This seems unlikely, because the level of vWF begins to rise before oocyte retrieval and declines thereafter in non-hyperstimulated patients (19). The higher Ht value in the moderate and severe OHSS patients than in the mild OHSS patients on the day of embryo transfer may reflect the severity of their vascular endothelial dysfunction.

In conclusion, the serum level of vWF on the day of embryo transfer was markedly elevated before clinical manifestation of OHSS in patients who exhibited extreme oliguria (<300 ml/day) and/or increased blood coagulability compared with patients who did not exhibit such abnormalities. Although a large prospective study is needed to confirm the present findings, it is possible that determination of serum vWF on the day of embryo transfer may be useful in predicting the severe form of OHSS.

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