Increased erythrocyte aggregation in ovarian hyperstimulation syndrome: a possible contributing factor in the pathophysiology of this disease

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BACKGROUND: Many theories regarding the pathophysiology leading to ovarian hyperstimulation syndrome (OHSS) have been proposed and tested. Increased erythrocyte aggregation is associated with capillary slow flow and tissue hypoxaemia. We performed this study in order to assess the degree of erythrocyte aggregation in the peripheral blood of individuals with OHSS and undergoing controlled ovarian stimulation (COH).

METHODS: Twenty women with severe OHSS, 20 women undergoing COH under IVF protocol, and 20 healthy matched controls were recruited for this prospective study. Blood samples were drawn for determination of erythrocyte aggregation as well as haematological indices. The percentage of slide covered by the cells (`erythrocyte percentage': EP) was determined using a simple slide test and image analysis. Lower EP values correspond to higher degrees of aggregation.

RESULTS: The respective measures of EP were 59.2 ± 66.3, 42.0 ± 66.3 and 35.0 ± 2.4% for the controls, women with COH and OHSS (P < 0.01 between controls and the two stimulation groups).

CONCLUSIONS: The degree of erythrocyte aggregation is enhanced in the peripheral venous blood of patients with both COH and OHSS. This finding, known to cause capillary leak, may contribute to the pathophysiology of the OHSS.

Key words: controlled ovarian stimulation/erythrocyte aggregation/ovarian hyperstimulation syndrome

Introduction

Ovarian hyperstimulation syndrome (OHSS) and controlled ovarian stimulation (COH) are associated with several haemostatic changes brought about by gonadotrophin administration. The typical haemostatic changes in OHSS include an increase in the haematocrit, leukocyte and platelet counts with the concomitant elaboration of vasoactive substances that are dependent on plasma volume such as renin activity, aldosterone, norepinephrine and anti diuretic hormone (ADH) (Delvigne and Rozenberg, 2003). In its severe form, OHSS can be life-threatening due to severe haemoconcentration, thromboembolic phenomena, hypovolaemia, electrolyte imbalances and respiratory failure due to fluid shift to third space (Whelan and Vlahos, 2000). The pathophysiological processes leading to the syndrome are not entirely clear.

Many theories have evolved over the years regarding the aetiology of OHSS. These theories, at best, can only partly explain the full cascade of events leading to this clinical entity. Hyperoestrogenaemia as an example cannot be solely responsible for mediating OHSS, as patients with partial 17,20-desmolase deficiency with low estradiol levels were reported to have severe OHSS (Pellicer et al., 1991). Prostaglandins in high levels can only partly lead to OHSS, as indomethacin did not prevent the occurrence of OHSS (Pride et al., 1986). Vascular endothelial growth factor (VEGF) has been assigned a significant role in mediating OHSS by its ability to induce vessel permeability (Levin et al., 1998). VEGF receptor inhibitors demonstrated the capability of preventing the increase in vascular permeability brought about by the elevated levels of VEGF (Gomez et al., 2002). Secretion of interleukin-6 by human lung microvascular endothelial cells was demonstrated to be increased following administration of hCG, causing an increase in vessel permeability (Albert et al., 2002).

The common denominator of most theories has been endothelial activation and an increase in capillary permeability leading to fluid shifting to third spaces (Delvigne and Rozenberg, 2002).

An increase in erythrocyte aggregation is associated with unfavourable haemorrhological effects in terms of microcirculatory slow flow, tissue hypoxaemia and microcirculatory occlusions (Tateishi et al., 2001, 2002). These changes alter the peripheral resistance, reduce capillary perfusion and oxygen transfer to tissues and bring about a degeneration of the vascular wall leading to capillary leak (Rainer et al., 1987;
Soutani et al., 1995; Cabel et al., 1997; Pfafferott et al., 1999; Bishop et al., 2001).

Increased capillary leak brought about by the elevation of erythrocyte aggregation could be a newly discovered mediator in the pathophysiology of OHSS. In our present study we undertook to determine if women with COH and OHSS have increased red cell aggregation in their peripheral blood.

**Materials and methods**

**Patients and controls**

This was a prospective case–control study. All participating women gave their consent for participation in the study that was approved by the local ethics committee. Twenty successive women who were admitted to the Gynecology Department at the Lis Maternity Hospital from February 2001 to December 2001 and who met the criteria for moderate to severe OHSS were recruited for the study (group 1). The criteria were according to Golan et al. (1989) and included clinical evidence of nausea and abdominal distention, sonographic evidence of ascites and in severe cases clinical evidence of ascites and difficulty in breathing. The indications for IVF were male factor in six (30.0%) couples, mechanical factor in three (15.0%) women, unexplained in 11 (55.0%) women and lack of ovulation in two (10.0%) women. All women had previous clomiphene or gonadotrophin treatment; however, an unstimulated natural cycle was a prerequisite before treatment.

Twenty successive women from our IVF unit, undergoing COH from December 2001 to January 2002, were recruited for the study (group 2). The indications for IVF were male factor in five (25.0%) couples, mechanical factor in three (15.0%) women, unexplained in 11 (55.0%) women and lack of ovulation in one (5.0%) individual. COS in groups 1 and 2 was performed using down-regulation with GnRH analogues from day 1 of the cycle followed by gonadotrophin administration from cycle day 3 until the administration of hCG.

Twenty healthy control women (group 3) matched for age and body mass index were recruited for the study. Women from all groups were healthy and had no chronic disease. Coagulation disorders or past events of thrombosis were excluded.

Blood samples were drawn and prepared using the technique described below. Blood was collected from the hospitalized women (group 1) upon admission. Blood was drawn from the COH women (group 2) on the morning of day 12–13 of the treatment cycle, after hCG administration.

**Laboratory tests**

Blood samples were analysed for cell count as well as the erythrocyte sedimentation rate (ESR) and fibrinogen concentration by the method of Clauss (1957).

**Erythrocyte aggregation**

The Erythrosense™ biomarker is based on the previously described erythrocyte adhesiveness/aggregation test (Rogowski et al., 2000). Venous blood from the antecubital vein was obtained between 08:00 and 11:00. Blood was drawn into a syringe containing sodium citrate (one volume of 3.8% sodium citrate and three volumes of whole blood). One drop of the citrated whole blood was trickled onto a slide inclined at an angle of 30° and allowed to run down by gravity, leaving a fine film. The slides were left to dry in that position, at room temperature. A technician who was blinded to the clinical and laboratory results of the patients scanned the slides by using an image analysis system (Inflamet™; Inflamet Ltd, Tel Aviv, Israel), as previously described by Fusman et al. (2002).

The variable that was used to describe the state of erythrocyte aggregation was the erythrocyte percentage (EP). This is the slide area covered by the erythrocytes. When there is no aggregation, 100% of the slide area is covered with erythrocytes; during aggregation this percentage is reduced due to the appearance of clear areas between the groups of aggregated cells.

**Statistical analysis**

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 Fisher’s exact test, one-way analysis of variance, or the Kruskal–Wallis test where appropriate. The non-paired t-test or post-hoc Bonferroni analyses were used to perform pair-wise comparisons between group means. A two-tailed Pearson’s correlation was performed for the correlation coefficient and significance between EP and fibrinogen levels. According to power analysis, the minimum sample size required in each sub-group was 20 subjects (alpha of 5% and power of 80%). P < 0.05 was considered statistically significant. Calculations were performed using the SPSS software package (SPSS Inc., USA).

**Results**

We have presently included 20 women with COH, 20 with OHSS as well as 20 matched controls. The mean ± S.E. age, body mass index and the percentage of smokers are presented in Table II. No significant changes were found regarding these variables in the two study groups and the controls. Relevant haematological data are reported in Table II. The concentration of fibrinogen as well as the ESR was not significantly changed from patients and controls, however, women with OHSS had significantly elevated haematocrit and concentration of leukocytes and platelets.

A significant elevation in the degree of erythrocyte aggregation was noted in the two groups of COS and OHSS (Figure 1). In fact, the mean ± SE (range) percentages of slide area covered by red blood cells (EP values) were 59.2 ± 3.0 (35–82) in the controls and 42.0 ± 3.0 (16–73) and 35.0 ± 2.4 (6–57) in the COH and OHSS groups respectively. Lower EP values correspond to higher degrees of aggregation and vice versa. The difference between the controls and the two stimulation groups was significant at P < 0.01. Representative photos of slides are shown in Figure 2. The EP and levels of fibrinogen were significantly correlated, as shown in Figure 3, with a correlation coefficient of r = −0.387, and a significance of P = 0.012. The negative correlation coefficient describes the fact that EP decreases, i.e. erythrocyte aggregation increases with an increase in fibrinogen concentration.

**Discussion**

The administration of gonadotrophins for ovulation induction is a common practice worldwide. This practice, however, is not without risks with an incidence of 0.5–5% of severe ovarian hyperstimulation in patients undergoing COH (Delvigne and Rozenberg, 2002). This iatrogenic complication can be mild and cause only a little discomfort, but it can evolve into a life-threatening disease. The grave complications of OHSS are
electrolyte and fluid imbalances, thrombotic complications and respiratory difficulty. These complications are a result of fluid shifting to third spaces and haemoconcentration due to increased permeability of membranes (Myrianthefs et al., 2000).

The present study clearly showed that enhanced erythrocyte aggregation as expressed by using the Erythrosense® can be detected in the peripheral blood of women following gonadotrophin administration. This phenomenon of erythrocyte aggregability can have a deleterious effect on the microcirculatory flow (Weng et al., 1998, 1999; Froom, 2000; Bishop et al., 2001). In fact, pathological red cell aggregation characterized by large aggregates with strong intercellular links is involved in microcirculatory sludging and stagnation, which alter the peripheral vascular resistance, reduce capillary perfusion and oxygen transfer to tissues and cause ischaemia, local metabolic acidosis as well as degeneration of the vascular wall and capillary leak (Rainer et al., 1987; Soutani et al., 1995; Cabel et al., 1997; Pfafferott et al., 1999; Bishop et al., 2001; Tateishi et al., 2001, 2002; Suckfull, 2002). Moreover, in a recent study by Baskurt et al. (2004) it was shown that enhanced red blood cell aggregation results in suppressed expression of NO synthesizing mechanisms. In an animal model of enhanced erythrocyte aggregation, they showed that this suppression causes an alteration in vasomotor tone. The mechanisms involved most likely relate to decreased wall shear stresses due to decreased blood flow and/or increased axial accumulation of red blood cells.

We believe that increased erythrocyte aggregation in patients with COH and OHSS after the administration of gonadotrophins is brought about by hyperfibrinogenaemia. It has been shown that fibrinogen has a major role in the induction and/or maintenance of increased erythrocyte aggregation in the peripheral blood (Weng et al., 1996). The enhanced synthesis of this macromolecule is probably a result of the presence of enhanced IL-6 concentrations in the peripheral blood (Gabay et al., 1999). In fact, enhanced concentrations of IL-6 have been shown in OHSS (Aboulghar et al., 1999). In the present study, the fibrinogen concentration was significantly correlated with erythrocyte aggregation (Figure 3). The findings of the present study are therefore in accordance with previous observations related to the role of this macromolecule in the tendency of red blood cells to aggregate during hyperfibrinogenaemic conditions (Schechner et al., 2003).

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<tr>
<th>Table I. Demographic and clinical characteristics for normal controls, women with controlled ovarian stimulation (COS) and women with ovarian hyperstimulation syndrome (OHSS)</th>
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<td>Variable</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<td>Rate of smoking (%)</td>
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Values are mean ± SE (range). No values are significantly different by analysis of variance or Fisher’s exact test.

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<th>Table II. Haematological values for normal controls, women with controlled ovarian stimulation (COS) and women with ovarian hyperstimulation syndrome (OHSS)</th>
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<tr>
<td>Variable</td>
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<tr>
<td>Haematocrit (%)</td>
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<td>White blood cells (×10³/mm³)</td>
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<td>Platelets (×10³/mm³)</td>
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<td>Fibrinogen (mg/dl)</td>
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<td>ESR (mm/h)</td>
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Values are mean ± SE (range). *Significantly different (P < 0.05) from the corresponding value in the two other sub-groups (OHSS versus controls and COH). **Significantly different (P < 0.05) between the specified values (OHSS versus controls). ESR = erythrocyte sedimentation rate.

Figure 1. Mean ± SE of erythrocyte percentage (EP) in the three study groups. Lower EP values correspond to higher degrees of aggregation and vice versa. The difference between the controls and the two stimulation groups was significant at P < 0.01.
Patients with OHSS demonstrated erythrocyte aggregation to a larger extent than patients undergoing COH. This further increase in aggregation may be the necessary step in the evolution from a silent state to a clinical entity.

Finally, we would like to comment on the potential relationship, if any, between the degree of erythrocyte aggregation and the day of the menstrual cycle. In an ongoing study, we have evaluated 418 apparently healthy women and found no clear evidence that the degree of erythrocyte aggregation changes significantly according to the day of the menstrual cycle.

We conclude that following gonadotrophin administration for ovarian stimulation, enhanced red blood cell aggregation can be detected in the peripheral blood. These findings may have pathophysiological relevance in terms of capillary slow flow, tissue hypoxia, changes in vasomotor tone, reduced NO synthase expression and capillary leak. These observations are important in order to define therapeutic strategies that might be applied to those women who develop life-threatening side effects during ovarian hyperstimulation.

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References


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