CLINICAL ASSISTED REPRODUCTION

Features of the Renin–Angiotensin System in Ascites and Pleural Effusion During Severe Ovarian Hyperstimulation Syndrome

ANNE DELBAERE,^{1,3} PIERRE J. M. BERGMANN,^{1,2} and YVON ENGLERT¹

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Purpose: The purpose of this work was to investigate the ovarian renin–angiotensin system (RAS) during severe ovarian hyperstimulation syndrome (OHSS) with ascites and pleural effusion.

Methods: Two patients who developed severe OHSS after ovarian stimulation for in vitro fertilization were investigated. Both patients presented ascites and pleural effusion. Blood, ascites, and pleural fluid were simultaneously sampled during therapeutic paracentesis and thoracocentesis. Renin activity, active renin, prorenin, and angiotensin II immunoreactivity (Ang II-ir) were measured simultaneously in plasma, ascites, and pleural fluid.

Results: Prorenin, renin activity, active renin, and Ang II-ir levels were much higher than normal plasmatic laboratory norms in the three compartments. Prorenin and Ang II-ir levels were the highest in the ascites, while they were in the same range in the pleural fluid and in the plasma.

Conclusions: The present findings provide additional evidence for the ovarian origin of the prorenin and Ang II-ir in the ascites of severe OHSS.

KEY WORDS: angiotensin II; ascites; ovarian hyperstimulation syndrome; pleural effusion; prorenin; renin; ovarian renin–angiotensin system.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) remains the most important complication of ovulation induction. It is clinically characterized by a massive ovarian enlargement associated with an acute third-space fluid shift responsible for the development of ascites and, sometimes, pleural and/or pericardial effusion (1). The pathogenesis of the vascular fluid leakage is unknown but is thought to be related to an increase in vascular permeability of mesothelial surfaces, caused by one or several mediators secreted by the hyperstimulated ovaries (2). Various observations have suggested that the ovarian renin–angiotensin system (RAS) could be involved in the development of the third-space fluid shift, through the synthesis of angiotensin II (Ang II), the bioactive peptide of the system, able to promote angiogenesis (3) and to increase vascular permeability (4). A direct correlation between plasma renin activity and the severity of OHSS has been demonstrated previously (5). Very elevated levels of Ang II immunoreactivity (Ang II-ir) (6) and of prorenin (7) have been found in the ascites from severe OHSS. The present investigation reports two cases of severe OHSS with ascites and pleural effusion. To characterize further the components of the RAS in each compartment, renin activity, active renin, prorenin, and Ang II-ir were measured in plasma, ascites, and pleural fluid.

MATERIALS AND METHODS

Case Reports

The first patient was a 25-year-old woman with 3 years of primary infertility. Her husband presented

¹ Fertility Clinic, Department of Gynecology and Obstetrics, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

² Radioimmunology and Experimental Medicine Laboratory, Brugmann Hospital, Université Libre de Bruxelles, Brussels, Belgium.

³ To whom correspondence should be addressed at Fertility Clinic, Department of Gynecology and Obstetrics, Erasme Hospital, route de Lennik 808, 1070 Brussels, Belgium.

severe oligoasthenoteratospermia. A first cycle of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) was started. Pituitary desensitization was carried out by intranasal administration of buserelin acetate (Suprefact; Hoechst Inc., Frankfurt, Germany). Ovarian stimulation required daily im administration of 150 IU of human menopausal gonadotropins (hMG; Humegon; Organon Inc., Oss, The Netherlands) for 7 days. The dose was then modified according to response, and she received 187.5 IU for 2 days, 225 IU for 3 days, and 242.5 IU for 7 days. Ovulation induction was performed using 10,000 IU of human chorionic gonadotropins (hCG; Pregnyl; Organon Inc.). At that time, serum E₂ levels had reached 2100 pg/ml, and 22 follicles were observed at vaginal ultrasonography. The oocyte pickup (OPU) was performed 36 hr later transvaginally, under ultrasonographic control. Nineteen oocytes were obtained, of which 17 were in metaphase II and microinjected. Consequently, 13 oocytes were fertilized and 3 embryos were transferred 48 hr after the OPU. The patient was given a daily intramuscular injection of 100 mg of progesterone in oil, for luteal supplementation. Two weeks after the oocyte retrieval, the patient complained of abdominal distension, nausea, vomiting, and dyspnea. Her abdomen was distended, with clinical signs of ascites, and she was hospitalized for severe OHSS. Physical examination revealed decreased breath sounds at the lung bases, and a bilateral hydrothorax, predominant on the right side; was confirmed by a chest X-ray film. An abdominal ultrasound scan revealed bilateral ovarian enlargement (right, 130 × 80 × 80 mm; left, 80 × 70 × 60 mm) and confirmed the presence of massive ascites. On admission, the blood pressure was 130/80 mm Hg and the pulse was 120/min. The hematocrit was 47%. A pregnancy test was positive. Treatment first consisted in bed rest and intravenous hydration with crystalloids and colloids solutions. Two days after admission, a transabdominal paracentesis under ultrasound control was performed and 1500 ml of ascitic fluid (protein count of 4.3 g/dl) was removed. A therapeutic thoracocentesis performed the same day drained 1200 ml of fluid noted to be an exudate (protein count of 4.0 g/dl). Subsequently, the patient's condition improved dramatically and remained satisfactory despite a partial reaccumulation of both pleural and abdominal effusions. The patient was discharged on hospital day 7, as ascites, pleural effusion, and ovarian enlargement began resolving. A pelvic ultrasound performed 4 days later revealed a triplet pregnancy which is ongoing uneventfully.

The second patient was a 30-year old woman with a 1.5-year history of primary infertility. Her husband presented extreme oligoasthenoteratospermia. She had irregular cycles and presented biological and ultrasonographical characteristics of polycystic ovary syndrome. A chromolaparoscopy revealed the presence of mild endometriosis in the peritoneal cavity. IVF with intracytoplasmic sperm injection was attempted. Pituitary desensitization was achieved by intranasal administration of buserelin acetate. Ovarian stimulation required im administration of 150 IU of follicle-stimulating hormone (FSH; Metrodin; Serono Inc., Aubonne, Switzerland) for 5 days, 225 IU of FSH for 2 days, and 150 IU for the next 4 days. The serum E₂ levels reached 4800 pg/ml. To avoid canceling the cycle, and to prevent as much as possible the development of OHSS, the "prolonged coasting" method (8) was applied to the stimulation protocol by stopping the gonadotropin therapy for 2 days, while continuing the administration of buserelin. Ovulation induction was performed using 10,000 IU hCG when serum E₂ levels had fallen to 3200 pg/ml. Thirty-seven follicles were punctured 36 hr later, and 23 oocytes were obtained, of which 20 were microinjected. Eleven oocytes were fertilized and 3 embryos were transferred. Luteal phase was supplemented with progesterone. Three days after OPU, the patient complained of abdominal discomfort, nausea, and dyspnea. Her abdomen was distended, with clinical signs of ascites, and she was hospitalized for severe OHSS. A right-sided hydrothorax was diagnosed clinically and confirmed by a chest X-ray film. An abdominal ultrasound scan showed bilateral ovarian enlargement (right, 130 × 110 × 80 mm; left, 70 × 60 × 40 mm), with multiple cysts, and confirmed the presence of moderate amounts of ascites. On admission, the blood pressure was 130/80 mm Hg and the pulse was 120/min. The hematocrit was 46%. Treatment consisted in bed rest and iv administration of crystalloids and colloids solutions. Two days after the admission, the dyspnea worsened, and the chest X-ray film showed an enlargement of the hydrothorax. A thoracocentesis drained 200 ml of fluid (protein count of 4.3 g/dl). A transabdominal paracentesis under ultrasound control was also performed on the same day. Although the total volume of ascites estimated by ultrasonography was at least of 1000 ml, only 100 ml of ascitic fluid (protein count of 4.5 g/dl) was removed for technical reasons. The patient was discharged on hospital day 5. No pregnancy ensued.

Samples

Samples of peripheral venous blood, ascites, and pleural fluid intended for Ang II-ir assay were collected in the presence of inhibitors of angiotensin converting enzyme and angiotensinases as described previously (6). Blood, ascitic, and pleural samples intended for RA, active renin, and total renin assays were collected in EDTA tubes and centrifuged at room temperature. Supernatants were frozen at -20°C until assayed.

Assays

Ang II-ir was measured in the plasma, ascites, and pleural fluid as described previously (6). Briefly, each sample was extracted using Dowex H^{+} ion-exchange resin (AG 50 W-X2; Bio-Rad Laboratories, Eke, Belgium). The extracts were dried and redissolved in RIA buffer and assayed for RIA using ^{125}I -labeled Ang II and a rabbit anti-Ang II antiserum. The intraassay and interassay variations did not exceed 17%.

RA was measured in the plasma, ascites, and pleural effusion by the generation rate of angiotensin I (Ang I) at 37°C and pH 6 for 60 min, as described previously (6). Angiotensin I generated was quantified by direct RIA using a rabbit anti-Ang I antiserum and ^{125}I -labeled Ang I. Intra- and interassay coefficients of variation were less than 11%.

Active renin and total renin were measured by commercially available immunoradiometric assays (Active Renin and Total Renin kits; Nichols Institute Diagnostics B.V., Wychen, The Netherlands) according to the manufacturer's instructions. The prorenin concentration was obtained by subtraction of the active renin concentration from the total renin concentration. Intra and interassay coefficients of variation for active renin and total renin were less than 10%.

RESULTS

Levels of RA, active renin, prorenin, and Ang II-ir, in the plasma, ascites, and pleural fluid of the two patients studied are shown in Table I. All the investigated parameters of the RAS were much higher in the three compartments than the plasmatic laboratory normal values (Table I). Levels of Ang II-ir and of prorenin were in the same range of magnitude in the plasma and in the pleural fluid, while they were much higher in the ascites. RA (measured by enzymatic assay) was in the same range of magnitude in the three compartments, while active renin (measured by

immunoradiometric assay) was slightly higher in the ascites.

DISCUSSION

This work describes for the first time the features of the RAS in ascites and pleural fluid from two patients presenting severe OHSS. We have shown previously levels of Ang II-ir hundred times higher in the ascites of OHSS than in control ascites (6). Moreover, the levels of Ang II-ir in the ascites of OHSS were much higher than in the plasma (6). Others have demonstrated very high levels of prorenin in the ascites of OHSS compared to control ascites (7). The present work confirms those previous findings and shows, moreover, that prorenin levels in the ascites of OHSS were, as for Ang II-ir, much higher than in the plasma. Besides, levels of Ang II-ir and of prorenin were comparable in the plasma and in the pleural liquid, while they were clearly higher in the ascites, even though ascites and pleural fluid displayed a similar total protein count. Our present observation provides additional support for the ovarian origin of prorenin and Ang II-ir in the ascites of OHSS, given the existence of an ovarian RAS able to produce prorenin locally (9) and Ang II (10) and the stimulatory role of gonadotropins on this local system (11). In patient 2, levels of prorenin and Ang II-ir were slightly higher in the pleural fluid than in the plasma. This could be due to preferential ways between ascites and pleural fluid through lymphatic channels. In this regard, the correction of abdominal ascites by a chest tube placed to drain pleural effusion has been reported recently in a patient with severe OHSS (12). Plasma levels of renin activity, active renin, prorenin, and Ang II-ir were clearly higher than those observed in patients stimulated with gonadotropins for IVF on the day of oocyte retrieval (9–11, 13), suggesting peripheral stimulation of the RAS during OHSS. Active renin was measured by an immunoradiometric assay. However, as ovarian Ang I could be generated from angiotensinogen by proteolytic enzymes other than renin (13), renin activity was also measured. With both methods, active renin did not display large variations among the three compartments, suggesting that renin is the main enzyme responsible for Ang I generation in the ovary. The slight discrepancies observed between RA and active renin values could be due, at least partially, to the fact that in the enzymatic assay, the rate of the renin reaction is also determined by the concentration of angiotensinogen, which could vary among the three compartments. The

Table I. Levels of Renin Activity (RA), Active Renin, Prorenin, and Angiotensin II Immunoreactivity (Ang II-ir), in the Plasma, Ascites, and Pleural Fluid of the Two Investigated Patients^a

	Patient 1			Patient 2		
	Plasma	Pleural fluid	Ascites	Plasma	Pleural fluid	Ascites
RA (ng ANGI/ml/hr)	16	17	14	37	22	29
Active renin (mU/L)	575	526	873	664	316	690
Prorenin (mU/L)	3260	3250	8210	1310	1597	3660
Ang II-ir (pg/ml)	386	371	613	124	208	800

^a Plasma laboratory norms: RA, 0.3 to 3.6 ng Ang I/ml/hr; Ang II, 3 to 35 pg/ml; active renin, 12 to 45 mU/L; prorenin, 100 to 360 mU/L.

differences observed in the expression of prorenin and of active renin in the three compartments are consistent with the reported observation that ovarian renin is secreted largely in its inactive form prorenin, whether in follicular fluid (9) or in blood (14).

In summary, the present investigation shows levels of prorenin and of Ang II-ir higher in the ascites of severe OHSS than in the pleural fluid and in the plasma, providing additional support for their ovarian origin in the ascites during the syndrome. The precise roles of prorenin and Ang II in the pathogenesis of the OHSS, whether by themselves or in synergy with other factors, remain to be clarified. However, the huge amounts of Ang II in the ascites together with its known vasoactive properties support that this mediator constitutes an important piece in the puzzle of OHSS pathophysiology.

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