The Pathogenesis of Ovarian Hyperstimulation Syndrome: A Continuing Enigma

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Purpose: Our purpose was to review the available literature concerning the pathogenesis of ovarian hyperstimulation syndrome and, in light of the most recent information, to attempt to provide further insight on this iatrogenic complication associated with the induction of ovulation.

Methods: Published studies related to this topic were identified through a computerized bibliographic search.

Conclusions: The exact mechanism for the development of ovarian hyperstimulation syndrome is still obscure. It is well established that the syndrome is associated with the process of ovulation induced by either luteinizing hormone or human chorionic gonadotropin. Following ovulation, one or more substances produced by the ovary are liberated in excess, increasing capillary permeability, resulting in the clinical features of the syndrome. It may well be that the syndrome is not triggered by a single mechanism but by the production and secretion of several substances acting in concert. These may include prostaglandins, cytokines, the ovarian renin-angiotensin system, vascular endothelial growth factor, and nitric oxide.

KEY WORDS: ovarian hyperstimulation syndrome; induction of ovulation.

INTRODUCTION

One of the most significant developments in the treatment of infertility during the second half of the 20th century has been the introduction of drugs for ovulation induction, e.g., clomiphene citrate and menotropins. Although these drugs were originally used in patients who do not ovulate regularly, the development of assisted reproductive technologies (ART) extended

their use to normally ovulating patients. The main goal in using these medications is the development of multiple follicles. However, ovarian stimulation may result in a supraphysiologic response leading to an iatrogenic complication known as the ovarian hyperstimulation syndrome (OHSS). This syndrome is potentially a lethal condition, the pathophysiologic hallmark of which is the accumulation of massive extravascular exudate combined with profound intravascular volume depletion and hemoconcentration.

Incidence

Before the introduction of ART, the incidence of OHSS with conventional induction of ovulation was reported by Schenker and Weinstein (1) to be 8–23% for mild OHSS, 0.005–7% for moderate OHSS, and 0.008–10% (less than 2% in most series) for the severe form of OHSS. With the introduction of ART and the extensive use of ovulation-inducing agents, one would have expected the incidence to increase; however, this did not occur for the moderate and severe forms of the syndrome. In patients undergoing ART, the incidence of moderate and severe OHSS was reported to range between 3 and 4 and between 0.1 and 0.2%, respectively (2). For mild OHSS it is difficult to estimate the exact incidence because most of the patients who are in a program of ART may experience this form of OHSS as a result of a more aggressive approach to induction of ovulation. The unchanged incidence of the moderate and severe forms in recent years can be attributed either to more advanced methods of monitoring of ovulation induction or to a better understanding of the syndrome and its prevention.

OHSS is much more frequent in conception cycles. Haning *et al.* (3) suggested that OHSS is four times more frequent in conception cycles than in nonconception ones. It is unclear whether the syndrome emanates from endogenous human chorionic gonadotropin (hCG) production when conception occurs or whether

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OHSS in itself represents a favorable preconception condition, with higher follicular and oocyte production, and a better hormonal milieu for both the oocytes and the developing endometrium. Whatever the relationship, the pregnancy rate among OHSS patients is significantly higher than in patients without OHSS and has been reported to range between 34.6 and 91% (4–12). In addition, the multiple pregnancy rate among these patients is also increased compared to that in non-OHSS cycles and ranges between 10 and 43% (4–12).

Induction of ovulation by clomiphene citrate (CC) is associated with a temporary increase in endogenous gonadotropins, and therefore, if OHSS develops, it is often as a milder form. Compared to exogenous human menopausal gonadotropin (hMG), treatment with CC exposes the ovaries to gonadotropins for a shorter time, resulting in the recruitment of fewer follicles. This may explain why OHSS is far less common after CC treatment and why the severe form is rare in comparison to its frequency following hMG treatment. Nevertheless, although rarely reported, OHSS in its severe form may still occur in patients with polycystic ovarian disease (PCOD) treated with CC (13,14).

PATHOGENESIS OF OHSS

Hormones and Vasoactive Mediators

The exact mechanism responsible for the development of OHSS remains obscure. However, it is well established that the syndrome involves enlargement of the ovaries with multiple ovarian cyst formation (theca lutein cysts) and an acute fluid shift from the intravascular to the extravascular space, resulting in ascites, hydrothorax, and generalized edema. Fluid shift contributes to the development of hypovolemia, a hypercoagulable state, and renal dysfunction. In fact, it is the main cause of morbidity and mortality associated with the syndrome.

The fluid shift seen in OHSS is caused by an increase in capillary permeability, which facilitates fluid escape into the third space. In animal experiments, Polishuk and Schenker demonstrated a direct and positive correlation among ovarian enlargement, extent of capillary permeability, severity of ascites and plural effusion, and dose of gonadotropins (1). It was suggested that the development of ascites was associated with an increased capillary permeability of the ovarian surface, peritoneum, omentum, or pleura. In addition, it is hypothesized that the increased capillary permeability, which also correlates with the hMG dose used, is medi-

ated by a vasoactive substance secreted by the stimulated ovaries.

Because estrogen levels are highly elevated in patients with OHSS, it has been suggested that high estrogen levels may be responsible for the increase in capillary permeability. This theory was supported by the experimental evidence in the rat and rabbit to show that excess estrogens may cause increased capillary permeability of the uterine and ovarian vessels (15). However, this hypothesis could not be substantiated by later studies, as OHSS could not be produced in animals exposed to high estrogen levels (16). In addition, OHSS was reported in patients with partial 17,20-desmolase deficiency (17) and late onset of adrenal hyperplasia (18), who underwent ovulation induction but had low serum estradiol concentrations. These observations question the role of estradiol in the pathogenesis of OHSS and led to the assumption that there must be another mediator of ovarian origin involved in the increased capillary permeability, rather than estrogen *per se*.

Several substances have been suggested as being involved in the pathogenesis of the syndrome. Histamine, with its well known effect on vascular permeability, was an obvious candidate for a role in the development of OHSS. This was, indeed, supported by several animal studies in which the blockade of histamine-1 (H1) receptors reduced the formation of ascites, with a partial decrease in ovarian size (19–21). However, Erlik *et al.* (22) did not demonstrate any difference in serum histamine levels between a group of OHSS rabbits and a control group, suggesting that histamine had no role in the pathogenesis of the syndrome.

Prostaglandins (PGs), originating from the enlarged stimulated ovaries, were proposed as a possible mediators in the development of increased capillary permeability. This was supported by the observation that indomethacin, an inhibitor of PG synthesis, was able to prevent the fluid shift responsible for the manifestations of OHSS (23). However, a later report by the same group, did not show any improvement in the clinical course of patients with severe OHSS treated by indomethacin (7). Therefore, the role of PGs in the pathogenesis of OHSS has not been proven.

OHSS is characterized by multiple theca lutein cysts and is accompanied by overproduction of other hormones including progesterone, testosterone, and prolactin (24). The high estrogen level associated with OHSS, through its effect on the pituitary gland, may be the cause of the elevated prolactin level. Prolactin has been shown to play a role in the water balance

of several systems. It affects the permeability of the amniotic membranes of guinea pigs as well as those of humans, influencing amniotic fluid production (25). It has also been shown to affect the production of aqueous humor and cerebrospinal fluid through its effect on membrane permeability (26). Thus, the hyperprolactinemia associated with the OHSS may play a role in some of the clinical features of the syndrome. This is supported by the study by Leung *et al.* (27), who showed that in a rabbit model, prolactin, although having no effect on ovarian size, may be involved in the pathogenesis of ascites formation. However, in other studies a significant effect on ascites formation in hyperstimulated rabbits treated with bromocriptine was not shown (16).

The Renin-Angiotensin System

The acute fluid accumulation in the peritoneal and pleural cavities results in hypovolemia and hemoconcentration, leading to increased osmolarity. Without correction, renal perfusion is impaired, resulting in oliguria, azotemia, hyperkalemia, and acidosis. In addition, thrombosis ensues from increased hemoconcentration and leads to the hypercoagulable state associated with the syndrome. Plasma levels of aldosterone and plasma renin activity (PRA) may be increased secondary to the elevated osmolarity associated with OHSS. Navot *et al.* (28) demonstrated elevated PRA in patients with OHSS and showed that the increased renin activity correlated positively with the severity of the syndrome. Furthermore, there is evidence to suggest that there is a local renin-angiotensin system in the ovary that plays a role in the development of OHSS. Thus, the high PRA may be a cause rather than a result of the syndrome. The existence of an ovarian renin-angiotensin system, which operates independently of the renal system, is well documented (29,30). Prorenin, an inactive precursor of renin and immunochemically identical to renal and plasma prorenin, has been detected in the follicular fluid aspirated during ovum pickup for IVF, at a concentration 12 times that of the concurrent plasma prorenin. This suggests a local production of prorenin in the ovary that can be transferred to the circulation, similar to other hormones produced by the ovaries. A sustained increase in prorenin has been found in stimulated cycles following hCG administration. Prorenin levels increase approximately 36 hr after hCG administration and peak 4–6 days after the injection. The concentration of prorenin following hCG administration in patients undergoing in vitro fertilization

(IVF) correlates well with the number of follicles at oocyte retrieval. The high prorenin level is a summation of the contribution of each follicle rather than an abnormal excessive production in an individual follicle. The corpus luteum probably secretes prorenin in response to hCG, and the increased level of prorenin found in early pregnancy supports this hypothesis. In patients who conceived after IVF, prorenin levels were decreased on days 3–5 after embryo transfer, but increased again on days 8 through 12, when endogenous hCG from the implanted embryo appeared in the circulation. In conception cycles resulting from oocyte donation, no such changes can be observed, indicating that the ovary rather than the placental tissue is responsible for the production of prorenin. Prorenin levels after hCG administration correlate with estradiol levels, leading to the assumption that estradiol or its precursors may play a role in ovarian prorenin synthesis (29). Angiotensin II, the active component of the ovarian renin angiotensin cascade, is luteinizing hormone (LH) and hCG dependent (31) and was found to be elevated in both the plasma and the ascitic fluid of patients with severe OHSS (32). Angiotensin II is an angiogenic factor (33), enhances steroidogenesis (34), mediates PG synthesis, and promotes small-vessel and large-vessel hyperpermeability (35,36). Taking all these observations together, one may speculate that the local active renin-angiotensin cascade plays a role in the pathogenesis of OHSS via new vessel formation in the ovary or other distant sites and that the increased vascular permeability might be mediated by PGs (28,32).

Despite compelling evidence for the role of the ovarian renin angiotensin system in the pathogenesis of OHSS, no therapeutic trials have been conducted or reported in humans. Recently, in a rabbit model, Morris *et al.* demonstrated that treatment with the angiotensin-converting enzyme (ACE) inhibitor (enalapril) inhibited the gonadotropin-stimulated rise in angiotensin II while increasing ovarian estradiol production (37). Furthermore, enalapril treatment was found to reduce some of the clinical and laboratory stigmata of severe OHSS such as weight gain and increased hematocrit, and it may also prevent the development of OHSS (38). These observations may suggest a clinical use for ACE inhibitors in cases of severe OHSS. However, because severe OHSS usually occurs with pregnancy, possible fetal effects must be considered. The use of both captopril and enalapril during the second and third trimesters has been associated with fetal anuria and renal failure, oligohydramnios, neonatal neurologic complications, and fetal death. The limited expe-

rience with these agents in early gestation precludes an accurate assessment of their teratogenic potential. No teratogenic effects have been observed in rats or rabbits at 330 and 50 times the recommended maximum human dose, respectively (39). However, one case report in humans raises the possibility that structural kidney anomalies may be induced by ACE inhibitors, so the use of these drugs in early gestation should be approached with considerable caution (40). Nevertheless, as more data become available, ACE inhibitors or angiotensin antagonists may prove to be effective, safe, and specific therapies for severe OHSS (41).

Cytokines

As indicated previously, hyperpermeability plays an important role in the pathogenesis of OHSS. Various immunologic factors have been shown to modulate permeability and therefore were investigated in association with OHSS.

Among potential novel intraovarian regulators, cytokines have been the focus of intense investigation. These agents are a group of factors that are released from cells and exert their function in an intracrine, an autocrine, a paracrine, and an endocrine fashion. The number of substances in the cytokine family is constantly increasing and includes interleukins (IL-1 to IL-13), colony stimulating factors (CSFs), tumor necrosis factor (TNF), and interferons. A characteristic feature of the cytokine family is that many of its members exhibit an overlapping set of effects in the target tissue.

Cytokines, mainly the interleukin group, have been recently considered to play a role in the regulation of ovarian function, modulating follicular growth, ovulation, and corpus luteum formation and demise (42). These polypeptides, originating mainly in activated immune and mesenchymal cells, have been shown to exert an effect on cells from origins other than the immune system (43).

Macrophages and monocytes have been identified in follicular aspirates of periovulatory follicles in significant numbers (44). This apparently acute inflammatory reaction is considered to be secondary to the increased periovulatory follicular neovascularization, with the release of follicular chemotactic factors (45,46). Following rupture of the follicle, capillaries and fibroblasts proliferate and penetrate the basal lamina. This rapid vascularization of the corpus luteum may be guided by angiogenic factors and associated with an increased vascular permeability (47).

The IL-1 Gene Family. The IL-1 gene family consists of two agonist molecules, termed IL-1 α and IL-1 β ,

and the high-affinity IL-1 receptor antagonist (IL-1RA). IL-1 α and IL-1 β are 26% homologous, bind the same receptor, and are both inhibited by IL-1RA. However, the β molecule appears to be more potent. When administered experimentally, IL-1 induces local and systemic effects, including prominent vascular leakage (48). IL-1 has been implicated in modulation and regulation of ovarian cell differentiation and follicular maturation. In addition, it has been shown to stimulate PG synthesis in bovine luteal cells (49). Hurwitz *et al.* (50) have shown that the intraovarian IL-1 system is highly compartmentalized and that the expression of IL-1 β is up-regulated in the murine ovary by gonadotropins, suggesting a role in the regulation of follicular development and ovulation. When evaluated in relation to OHSS, Revel *et al.* (51) have shown that IL-1 β concentrations are higher in ascitic fluid from severe OHSS patients compared to nonstimulated controls. Conversely, IL-1RA levels were higher in peritoneal fluid from controls compared to that of patients with severe OHSS. Although the differences in peritoneal levels of IL-1 and IL-1RA in both groups did not reach a statistical significance, the additive biological net effect of "free" IL-1 might be of importance. Similarly, Abramov *et al.* (52) have also reported a possible involvement of IL-1 in OHSS. IL-1 serum levels were found to be elevated in patients with OHSS at the time of their admission, and their concentration dropped significantly along with clinical improvement, reaching normal values after complete recovery.

IL-2. Orvietto and Ben-Rafael (53) reported that high intrafollicular IL-2 levels were found in the follicular fluid of seven patients with OHSS, and therefore suggested that IL-2 could predict the development of OHSS. Nevertheless, Revel *et al.* could not detect IL-2 in ascitic fluid from patients with OHSS (51). Thus, the actual role of IL-2 in the pathogenesis of OHSS has not yet been definitely established.

IL-6. Like IL-1 and TNF α , IL-6 mediates the acute phase response to injury—a systemic reaction characterized by leukocytosis, increased vascular permeability, and an increased level of acute-phase proteins synthesized by the liver. IL-1 and TNF α augment each other's production as well as the production of IL-6 in vivo and in vitro. The production of IL-6 mRNA during neovascularization or angiogenesis, which occurs in the development of ovarian follicles, suggests a role for this cytokine in normal ovarian function (54). The exact association between IL-6 and angiogenesis has not been totally elucidated. However, IL-6 and angiotensin II, an established angiogenic factor, were detected at high concentrations in the synovial

fluid of patients with rheumatic arthritis (55). Furthermore, IL-6 is produced by ovarian tumor cells and may play an important role in maintaining tumorigenicity, tumor vessel formation, and the development of ascites associated with ovarian cancer (56). In addition, IL-6 is reported to induce the expression of vascular endothelial growth factor (VEGF), which is itself a potent angiogenetic and vascular permeability factor (57). Thus, IL-6 may exert its effect on angiogenesis either directly or through another mediator such as VEGF.

Levels of IL-6 were found to be increased in the serum and ascitic fluid of patients with OHSS compared to controls (49,51,52,58). Moreover, IL-6 serum levels, which were elevated upon admission of patients with OHSS, dropped significantly along with clinical improvement, reaching normal levels after complete recovery (52).

Immunohistochemical localization of IL-6 revealed intense staining in corpora lutea and theca cells from large antral follicles and in luteinized granulosa cells in follicular aspirates after menotropin stimulation (58). These observations support the view that IL-6, which is secreted in excess by the ovaries of patients with OHSS, may serve as a mediator in the expression of the syndrome.

Although the mechanism through which IL-6 can mediate the development of OHSS is not totally understood, it may be related to its effect on angiogenesis, capillary permeability, and PG production. IL-6 has not been shown to cause vasodilatation or hypotension independent of IL-1 and TNF. Therefore, IL-1 and TNF may act in concert with IL-6 and be responsible for the development of the capillary leakage seen in OHSS.

In addition, IL-6 is a potential inhibitor of hepatic albumin production, switching the liver to the synthesis of acute phase reactants (59). Decreased albumin plasma levels, induced by IL-6, may contribute to the shift of fluid to the third space, which characterizes the OHSS.

In conclusion, IL-6 levels are markedly elevated in patients with OHSS compared to controls. High follicular fluid IL-6 levels suggest local secretion of this cytokine. Whether IL-6 is directly responsible for the clinical manifestations of this syndrome or acts together with other cytokines and VEGF is still a matter of further investigation.

TNF α . TNF α , previously known as cachectin, is a 17-kd polypeptide produced principally by stimulated macrophages. It is an extremely potent cytokine and is a known mediator of vascular hyperpermeability. In both animals and humans, TNF α has been shown to

stimulate ovarian cell growth, and to participate in the regulation of follicular development, and has been implicated as a promoter of epithelial ovarian cancer (60).

The ovary was shown to be a target as well as a source of TNF α . The production of TNF α is regulated by gonadotropins, and a high-affinity receptor for this cytokine has been detected in granulosa cells. Elevated levels of TNF α were found in the serum and ascitic fluid of patients with OHSS (51,52) and have been suggested to play a role in the pathogenesis of the syndrome (49). The exact role of TNF α in the development of OHSS has still to be investigated, as other reports have failed to demonstrate an increased level of this cytokine in patients with OHSS (61).

VEGF

Angiogenesis, the formation of new blood vessels, is induced by various growth factors and cytokines that act either directly or indirectly. VEGF, also known as vascular permeability factor (VPF), is a specific mitogen for vascular endothelial cells and, therefore, has a central role in physiological events of angiogenesis. Other cytokines, such as IL-6, may exert an indirect effect on angiogenesis by inducing VEGF expression (57).

VEGF is able to stimulate endothelial cell mitogenesis and render capillaries permeable to high molecular weight proteins. VEGF has been identified in rat and primates ovaries predominantly after the LH surge, suggesting its participation in angiogenesis within the developing corpus luteum. Using a VEGF radioimmunoassay, it was demonstrated that circulating and urinary VEGF values increase following gonadotropin stimulation, in parallel with the increased ovarian vascularity (62). Furthermore, VEGF production by granulosa cells has been shown to be mediated through hCG administration, substantiating the role of the latter hormone in the development and course of OHSS (63).

McClure *et al.* (64) suggested that VEGF plays an important role in the pathogenesis and development of OHSS. VEGF permeability activity was detected in the ascitic fluid of patients with OHSS but not in the ascitic fluid of patients with chronic liver failure. This activity decreased by 70% when rh VEGF antiserum was added to the OHSS ascitic fluid. Therefore, it has been suggested by these workers that the major capillary permeability agent in OHSS is VEGF secreted into the peritoneal cavity by the stimulated ovaries. Krasnow *et al.* (65) have shown that serum VEGF levels were significantly higher on day 14 after

hCG administration in a group who developed severe OHSS, compared with those who did not, corroborating the association between excess VEGF production and the development of OHSS. Similarly, Abramov *et al.* (66) demonstrated a high VEGF plasma levels in patients with severe OHSS. These levels dropped significantly with clinical improvement, reaching minimal values after complete resolution. In addition, a significant correlation was found between plasma VEGF levels and certain biologic characteristics of OHSS such as leukocytosis and an increased hematocrit, suggesting an association between VEGF and the pathogenesis of capillary leakage in OHSS.

In conclusion, there are data to suggest that excessive expression of VEGF from the ovary during gonadotropin-induced ovulation may contribute to the development of OHSS. The effect of VEGF is to support the development of the vascular net in the ovary after ovulation, on the one hand, and to increase capillary permeability, on the other.

Nitric Oxide (NO)

NO, previously known as endothelium-derived relaxing factor (EDRF), is a small messenger molecule with potent vasodilatory activity. NO production systems are widely distributed in body tissues, including the ovary. Van Voorhis *et al.* (67) have demonstrated the presence of NO synthase (NOS) in human ovarian follicular cells obtained from women undergoing IVF procedures. The ovarian NO system, among its multiple effects, seems to play a role in follicle rupture during ovulation. Shukovsky and Tsafiriri (68) have demonstrated the ability of NOS inhibitors to suppress hCG-induced ovulation in the rat *in vivo*. Recent studies suggest that endogenously generated NO may mediate the effects of cytokines in a variety of tissues including the ovary (69).

The presence of a NOS system in ovarian tissue and the interrelations of this system with cytokines, together along with the potential biologic effect of NO molecules on capillary blood vessels, raise the question about the possible role of this system in the production and maintenance of OHSS.

Preliminary observations by Revel *et al.* (51) suggest that NO may participate in the pathogenesis of OHSS. NO is a powerful vasodilator, and its inhibition modulates vascular permeability (70,71). NO has also been shown to be capable of inactivating superoxide anions, which are released regularly by mammalian cells as a result of oxygen consumption (72). These radicals cause lipid peroxidative damage to cell mem-

brane phospholipids, thus affecting membrane integrity and permeability. The free radical scavenging ability of NO may play a role in membrane stability and permeability. It may be postulated that low levels of NO could lead to increased membrane damage by peroxide, resulting in an increased permeability. Revel *et al.* (51) have measured nitrites, the main product of the unstable NO molecule, in the ascitic fluid of patients with OHSS. In comparison to those of a control group (i.e., peritoneal fluid achieved from normal diagnostic laparoscopies), barely detectable levels of nitrites were found in ascitic fluids of patients with OHSS. These findings support the view that decreased peritoneal NO levels may result in an increased capillary permeability and be involved in the pathogenesis of OHSS. Although the exact etiology of lowered NO levels in the ascitic fluid of patients with OHSS is currently under investigation, possible mechanisms include a dilution effect of the ascitic fluid, reduced NO production, or increased NOS inhibitory activity.

In summary, the exact mechanism for the development of OHSS is still obscure. It is well established that the syndrome is associated with the process of ovulation induced by either LH or hCG. Following ovulation, one or more substances produced by the ovary are liberated in excess, increasing capillary permeability, resulting in the clinical features of the syndrome. It may well be that the syndrome is not triggered by a single mechanism but by the production and secretion of several substances acting in concert.

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