Retrospective Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
48(8) 1–9

DOI: 10.1177/0300060520945551
journals.sagepub.com/home/imr
SAGE

Prediction of severe ovarian hyperstimulation syndrome in women undergoing *in vitro* fertilization using estradiol levels, collected ova, and number of follicles

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Abstract

Objective: Our objective was to determine whether estradiol (E2) levels (Day 3 and fold change to Day 10), antral follicle count (AFC), and number of ova collected could predict ovarian hyperstimulation syndrome (OHSS) and culdocentesis intervention.

Methods: We conducted a retrospective review of patient charts between January 2008 and December 2017. OHSS was defined using American Society for Reproductive Medicine criteria. Predictability was evaluated by measuring the area under the receiver operating characteristic curve (AUC).

Results: The cohort included 319 women (166 controls, 153 OHSS, of whom 54 had severe OHSS). The OHSS group had higher $E2_{Day\,3}$ (249 ± 177 vs. 150 ± 230 ng/L), $E2_{FoldChange}$ (32.2 ± 29.1 vs. 20.1 ± 23.8), AFC (18.2 ± 9.1 vs. 11.6 ± 8.3), and number of ova collected (21.1 ± 9.0 vs. 10.1 ± 6.5). $E2_{Day\,3}$ (AUC = 0.76, 95%CI: 0.71–0.82), $E2_{FoldChange}$ (AUC = 0.71, 95%CI: 0.65–0.77), AFC (AUC = 0.75, 95%CI: 0.70–0.81), and number of ova collected (AUC = 0.85, 95% CI: 0.81–0.89) were predictive for OHSS. All variables were predictive for culdocentesis

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intervention ($E2_{Day\,3}$: AUC = 0.63, 95%CI: 0.55–0.70; $E2_{FoldChange}$: AUC = 0.63, 95%CI: 0.55–0.71; AFC: AUC = 0.74, 95%CI: 0.68–0.80; number of ova collected: AUC = 0.80, 95%CI: 0.75–0.85).

Conclusions: Day 3 E2 levels and number of ova collected predict patients who could develop OHSS and may require culdocentesis.

Keywords

Culdocentesis, assisted reproduction, antral follicle count, ovarian hyperstimulation syndrome, in vitro fertilization, estradiol

Date received: 5 December 2019; accepted: 6 July 2020

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of ovarian stimulation, usually occurring during the luteal phase or the early part of pregnancy.[1] The use of human chorionic gonadotropin (hCG) as an ovulatory trigger is associated with the development of OHSS, and hCG is associated with increased production of vascular endothelial growth factor (VEGF).[2,3] VEGF causes angiogenesis and increased vascular permeability. Moreover, VEGF and VEGF receptor levels are elevated during the gonadotropin stimulation phase that precedes the hCG injection, and are further stimulated by hCG administration.[4] Before stimulation, VEGF receptors are found in the corpus luteum vessels, but after hCG stimulation, these receptors are found throughout the corpus luteum.[5] Similarly, the severity of OHSS has been directly linked to VEGF levels.[6,7] Elevated levels of pro-inflammatory immune cytokines [i.e., interleukin (IL)-1β, IL- 6, IL-8, tumor necrosis factor-α, and VEGF] are characteristic of OHSS and are associated with increased capillary permeability,[6] which can result in abdominal distention or discomfort, ovarian rupture or hemorrhage, ovarian torsion, ascites, and abdominal compartmental syndrome.[8]

OHSS, potentially life-threatening, has been shown to decrease pregnancy potential[9] and can lead to massive fluid shifts from the intravascular space, leading to accumulation in the pouch of Douglas.[10–12] The fluid can be removed by conservative management, but for the few patients who suffer from severe OHSS (sOHSS), a corrective procedure—culdocentesis—is required.[13] Culdocentesis is a minor procedure with minimal complications.

To date, there are few indicators or risk factors for OHSS or for patients in whom culdocentesis would be required. One biomarker, anti-Müllerian hormone (AMH), has been studied and shows promise as a predictor of OHSS;[14–16] however, serum AMH levels vary significantly with age, body mass index (BMI), and presence of hormonal disorders.[15,17] Moreover, AMH levels are significantly affected by ethnicity, with Hispanics having lower ranges than Caucasians or other populations.[18] As indicated by Dewailly and colleagues, AMH is a possible biomarker for OHSS, but additional studies are required.[15] Because of inter-laboratory and kit variation and the lack of a standard cutoff for AMH, there is a need to examine other potential markers of OHSS.[15] Studies have shown that age, BMI, antral follicle count (AFC), the number of ova collected, and serum estradiol (E2) levels during stimulation are predictive of patients who could

develop OHSS;[1,19–21] however, early measurements of serum E2 levels and its rate of change have not been assessed as predictors of OHSS. Moreover, to our knowledge, no studies have been performed to determine the predictive capabilities of these factors for culdocentesis. Therefore, this study aimed to assess serum E2 levels on Day 3, fold change in E2 by Day 10, and AFC to predict not only OHSS but also the need for culdocentesis intervention.

Patients and methods

Selection of patients

Patient chart review was performed between January 2008 and December 2017 at Ingenes in Mexico City for this retrospective study. To be included in this study, patients had to fulfill the following criteria: abdominal distention, ultrasonographic evidence of ascites, severe abdominal pain, severe dyspnea, oliguria/anuria, nausea, or vomiting. For controls, we included patients who were seen at our facilities during the same period who did not present with the inclusion criteria and were also matched (one to one) for age, BMI, and ovarian stimulation protocol. Patients were excluded from the analysis for the presence of diabetes or other chronic disorders that could promote sOHSS, or if culdocentesis was performed at a location other than Ingenes. OHSS and sOHSS were defined using the American Society for Reproductive Medicine criteria.[22] Only data from patients who agreed to participate and signed consent in accordance with the Declaration of Helsinki were considered. This study was approved by the Ethics Committee of the Ingenes Institute (approval number: ISF150108).

Ovarian stimulation

All patients were subjected to controlled ovarian stimulation with the antagonist protocol. Antagonist administration was initiated according to one of the following patient-specific criteria: (1) at least one follicle measuring >14 mm or (2) estradiol levels >400 pg/mL until hCG application, usually 4 to 5 days. The gonadotropin-releasing hormone (GnRH) long agonist protocol was started by administering 0.1 mg of a GnRH agonist (triptorelin) on cycle Day 21 of the previous cycle, followed by gonadotropins at a dose per the physician's recommendation that were started on cycle Day 2. The agonist dosage was reduced to 0.05 mg on the day that gonadotropin was started. The gonadotropin dose was adjusted based on follicular development. Administration of the GnRH agonist and gonadotropin continued until the start of the hCG injections, which was approximately 14 days after the GnRH agonist regimen or when follicles reached 16 to 18 mm.

Ovarian response was assessed by measuring serum E2 levels, and follicular development was evaluated by ultrasound examination. Oocyte retrieval was conducted under ultrasound guidance 36 hours after administration of β-hCG. An embryologist monitored and recorded all information about BMI, ovarian volume, AFC, the number of ova collected, and serum E2 levels during the stimulation. AFC was defined as the total number of follicles measuring between 2 and 10 mm in diameter that were observed during the early follicular phase by transvaginal ultrasound.

Culdocentesis

Culdocentesis was performed when clinical assessment of the patient indicated features such as nausea, vomiting, oral intolerance, or ascites identified by endovaginal ultrasound and abdominal ultrasound (renal and hepatic areas with visible ascites) that did not respond to conservative management. The patient was placed in a lithotomy position, with an empty bladder, using the

same equipment used for transvaginal ultrasound-guided oocyte recovery. Under general anesthesia with propofol 1% (Diprivan, Aspen Pharma, Dublin, Ireland) and fentanyl 5% (Fenodid, Ethypharm, Saint-Cloud, France), vaginal asepsis was performed using 11% povidone-iodine solution (Germisin, Prodinsa, Madrid, Spain) followed by irrigation with distilled water. An echo-tipped needle (size 17 Cook Medical Ovum aspiration needle, William A. Cook Australia, Brisbane, Australia) was inserted through the pouch of Douglas and the fluid was aspirated. Because of the risk of hemodynamic compensation, no more than 2 L of fluid was removed. The needle was then extracted and hemostasis verified.

Statistical analysis

The normality of the data was assessed by the Shapiro–Wilk test. Differences between groups were determined by either Student's *t*-test (parametric) or by the Mann U test (nonparametric). Predictability was evaluated by measuring the area under the receiver operating characteristic (ROC) curve (AUC). Using the sensitivity and specificity, Youden's index (sensitivity + specificity – 1) was calculated, and the highest Youden's index score was considered to be the optimal cutoff value to predict OHSS or the need for culdocentesis. All analyses were carried out using SPSS version 22.0 (IBM Corp., Armonk, NY, USA); *p*-values <0.05 (two-tailed) were considered significant.

Results

Predictability of IVF parameters for OHSS

Three hundred nineteen women agreed to participate. The characteristics of the cohort, separated by the presence of OHSS, are shown in Table 1. Patients without and with OHSS differed considerably in serum E2 levels on Day 3 (1.67-fold change in patients with OHSS), which was similar to the fold change between Day 3 and Day 10 (1.60-fold change). As expected, the AFC was significantly higher in OHSS patients (1.57-fold change), as was the number of ova

Table 1. Characteristics of the cohort.

		OHSS			sOHSS (culdocentesis)		
Category	Total	Negative	Positive	*p*[a]	Negative	Positive	*p*[a]
Sample	319	166	153		265	54	
Age (years)	34.2 ± 4.9	35.4 ± 4.9	32.9 ± 4.5	<0.001*	34.7 ± 4.8	31.9 ± 4.5	<0.001*
BMI (kg/m^2)	25.3 ± 4.4	25.3 ± 4.7	25.4 ± 4.1	0.832	25.4 ± 4.5	24.8 ± 3.8	0.367
E2 (ng/mL)							
Day 3	198 ± 212	150 ± 230	249 ± 177	<0.001*	191 ± 221	232 ± 158	0.191
Day 10	3623 ± 2695	1728 ± 787	5678 ± 2519	<0.001*	3205 ± 2389	5671 ± 3160	<0.001*
Fold change	25.9 ± 27.2	20.1 ± 23.8	32.2 ± 29.1	<0.001*	24.5 ± 26.6	32.9 ± 28.8	0.038*
AFC (n)	14.7 ± 9.3	11.6 ± 8.3	18.2 ± 9.1	<0.001*	13.7 ± 9.0	19.8 ± 8.9	<0.001*
Ova collected (n)	15.3 ± 9.5	10.1 ± 6.5	21.1 ± 9.0	<0.001*	13.7 ± 8.9	23.3 ± 8.1	<0.001*

Values are means ± standard deviations.

AFC, antral follicle count; BMI, body mass index; E2, serum estradiol levels; OHSS, ovarian hyperstimulation syndrome; sOHSS, severe ovarian hyperstimulation syndrome.

[a]Differences between groups were determined by either Student's *t*-test (parametric) or by the Mann U test (nonparametric).

*indicates a significant difference between the two groups ($p < 0.05$, two-tailed).

collected (2.01-fold change). From the IVF parameters assessed, serum E2 levels on Day 3 (AUC = 0.76, 95%CI: 0.71–0.82), fold change between serum E2 levels on Day 3 and Day 10 (AUC = 0.71, 95%CI: 0.65–0.77), and AFC (AUC = 0.75, 95% CI: 0.70–0.81) were all moderate predictors of OHSS, with the number of ova collected (AUC = 0.85, 95%CI: 0.81–0.89) being a strong predictor (Figure 1a). Using ROC curve analysis, cutoff values were calculated (Table 2). The best predictor was the number of ova collected (accuracy = 76.5%, sensitivity = 79.5%, and specificity = 73.5%), followed by serum E2 levels on Day 3 (accuracy = 71.5%, sensitivity = 83.4%, and specificity = 60.8%), as determined by test accuracies.

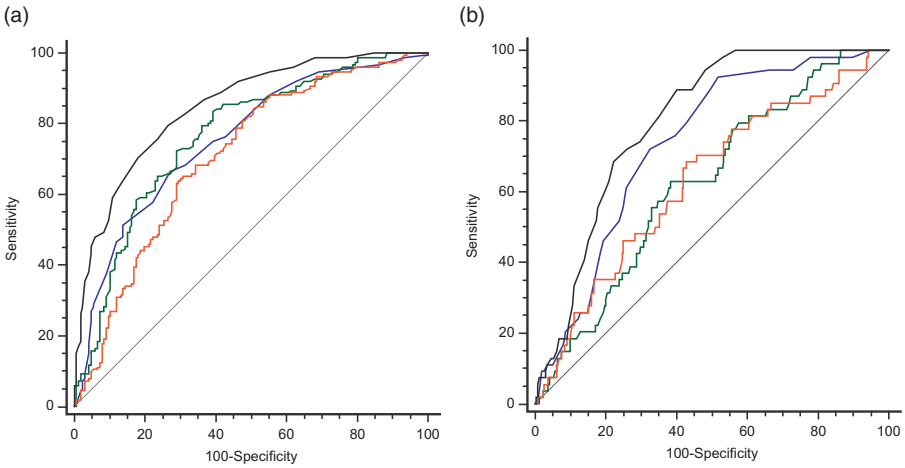

Figure 1. Receiver operating characteristic curve for serum estradiol levels on Day 3 (green line), fold change between Day 3 and Day 10 serum estradiol levels (orange line), number of ova collected (black line), and antral follicle count (blue line) for detecting ovarian hyperstimulation syndrome (A) and the requirement for culdocentesis (B). The diagonal line is the reference line of area under the receiver operating characteristic curve (AUC).

Table 2. Proposed cutoff values for IVF parameters to predict OHSS and culdocentesis.

Category	Cutoff	Sensitivity	Specificity	Youden	Accuracy	PPV	NPV
OHSS							
E2 Day 3 (ng/mL)	≥126.5	83.4%	60.8%	0.443	71.5%	66.1%	79.5%
E2 fold change	≥19.25	65.6%	69.3%	0.348	67.4%	66.2%	68.5%
AFC (n)	≥13.50	66.2%	72.9%	0.391	69.5%	69.2%	69.9%
Ova collected (n)	≥13.50	79.5%	73.5%	0.530	76.5%	73.5%	79.7%
Culdocentesis							
E2 Day 3 (ng/mL)	≥169.5	63.0%	62.0%	0.249	62.1%	25.2%	89.1%
E2 fold change	≥19.25	68.5%	57.0%	0.256	58.9%	24.5%	89.9%
AFC (n)	≥10.50	92.6%	48.3%	0.409	55.5%	26.6%	96.9%
Ova collected (n)	≥14.50	88.9%	60.1%	0.490	65.0%	31.4%	96.3%

AFC, antral follicle count; E2, serum estradiol levels; NPV, negative predictive values; OHSS, ovarian hyperstimulation syndrome; PPV, positive predictive values.

Predictability of IVF parameters for culdocentesis

Of the 153 patients with OHSS, 35.3% were diagnosed as having sOHSS (16.9% of the total cohort). Nevertheless, when the sOHSS patients were compared with the rest of the cohort, we observed no difference in serum E2 levels on Day 3 (Table 1). However, the fold change in serum E2 levels from Day 3 to Day 10 was significantly greater in sOHSS patients (1.34-fold change, $p = 0.038$), AFC (1.45-fold change, $p < 0.001$), and the number of ova collected (1.70-fold change, $p < 0.001$). Interestingly, each parameter assessed was predictive of patients requiring culdocentesis; however, serum E2 levels on Day 3 (AUC = 0.63, 95%CI: 0.55–0.70, $p = 0.003$) and E2 fold change (AUC = 0.63, 95%CI: 0.55–0.71, $p = 0.003$) were weakly predictive for culdocentesis, whereas AFC (AUC = 0.74, 95%CI: 0.68-0.80, $p < 0.001$) and the number of ova collected (AUC = 0.80, 95%CI: 0.75-0.85, $p < 0.001$) were moderately to strongly predictive (Figure 1b). The best predictors determined (Table 2) were the number of ova collected (accuracy = 65.0%, sensitivity = 88.9%, and specificity = 60.1%) and serum E2 levels on Day 3 (accuracy = 62.1%, sensitivity = 63.0%, and specificity = 62.0%).

Discussion

OHSS presents a potential problem for women undergoing IVF. Here, we examined whether serum E2 levels could predict OHSS. Indeed, serum E2 levels on Day 3 and their fold increase on Day 10 could predict OHSS; moreover, AFC and the number of ova collected were predictive for OHSS. As expected, women who developed OHSS produced more ova per cycle. Our results are in agreement with the systematic review by Nastri et al.[23] However, serum E2 levels on Day 3 >126 ng/mL were associated with an increased risk of OHSS. It is worth noting here that our results were based on serum E2 levels on Day 3, a measurement that may not be standard between IVF centers due to its variability and the frequency of measurements. Serum E2 levels on subsequent days would be augmented and more analysis is needed to determine the predictive ability of serum E2 levels measured on a different or later day.

We also assessed the change in serum E2 levels between Day 3 and Day 10. This analysis gives a rate-of-change measurement, which is independent of the initial serum E2 level. Even though the parameter was fairly predictive for OHSS, it presented lower accuracy but was not clinically inferior to serum E2 level on Day 3. Nevertheless, this result suggested that if the E2 concentration increased by more than 19 times, then the patient is likely to develop OHSS. As mentioned above, these results are based on serum E2 levels on Day 3, and measuring and comparing E2 levels over a different interval may show different results.

In this study, serum E2 levels were considered over the more postulated AMH biomarker. Even though AMH levels have been shown to correlate with follicular development, where early antral and antral follicles release the majority of AMH,[15] AMH does not promote VEGF expression as well as E2;[24] moreover, E2 negatively regulates AMH expression. Therefore, we posited that E2 levels would correlate strongly with OHSS development and the need for culdocentesis. Indeed, serum E2 levels did correlate. In Mexico, most IVF facilities do not use AMH measurements because of the large variability between external laboratories and the cost associated with the measurement. Therefore, using serum E2 levels, which are routinely collected during the IVF protocol, to monitor for OHSS makes sense because it will lead to no additional costs or procedures. As noted by Zheng and colleagues, many risk factors for OHSS, such as low BMI, high serum levels of AMH or

E2, or a large number of oocytes, among others, have yet to be confirmed as independent predictors of OHSS.[25] Thus, additional studies are required.

The rate of moderate OHSS to sOHSS for IVF cycles ranges from 3% to 10%.[26,27] Here, our rate for sOHSS was 16.9%. We believe that our rate was high because of the study design and the selection criteria, which could skew for a higher prevalence. However, it has been shown that, the rate can reach 20% for high-risk women.[26,27] Moreover, according to a review by Delvigne and Rozenberg, moderate OHSS has an incidence rate between 3% and 6% of all OHSS cases, whereas that of sOHSS is between 0.1% and 2%.[28] This suggests that in a cohort of patients with moderate OHSS and sOHSS, between 3% and 30% of the cohort would be in the sOHSS category, which in our study would be consistent with an incidence rate of 35.3%.

When fluid builds up in the pouch of Douglas, the preferred remedy in Mexico is culdocentesis. Even though this procedure is minimally invasive, the recovery time can delay embryo transfer in the next cycle. Therefore, an early predicting parameter would be beneficial in women in whom OHSS is suspected or in patients with a history of OHSS. Of the four parameters assessed here, only serum E2 levels on Day 3 and the number of ova collected were predictive. These two parameters showed cutoff values for culdocentesis greater than the cutoff values for OHSS, which would correspond to the degree of pathology. Nevertheless, the poor test accuracy (62.0% and 60.1%, respectively) suggests that these parameters should not be utilized individually.

This study has a few limitations. First, this was a retrospective study and any conclusions postulated here should be further investigated. A retrospective study can only generate future hypothesis to be tested. Second, the women in our cohort were from Mexico and any ethnic effects should be examined by studying other populations. Third, these parameters were studied individually and interactions were not considered. This is a preliminary report and future studies should be performed using a prospective study design. Furthermore, because the mechanism underlying OHSS development is multifactorial and may not follow one singular pathway, additional studies are required to determine the conditions (ovarian stimulation procedure and patient characteristics) that correlate best with these parameters.

In conclusion, we demonstrated that the number of ova collected and the fold increase in serum E2 from Day 3 to Day 10 could predict development of OHSS. Moreover, AFC and serum E2 levels on Day 3 could predict OHSS and the possibility of a patient undergoing culdocentesis. This research proposes cutoff values that can be easily considered during ovarian stimulation protocols.

Acknowledgements

We express our gratitude to the participants of this study, as well the IVF and medical staff at Ingenes.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received funding from Consejo Nacional de Ciencia y Tecnología (Conacyt grant number: 23179).

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