Calcium gluconate infusion is as effective as the vascular endothelial growth factor antagonist cabergoline for the prevention of ovarian hyperstimulation syndrome

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ABSTRACT

BACKGROUND: Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic and potentially life-threatening disease process, which may occur in healthy young women undergoing controlled ovarian hyperstimulation for assisted reproduction. As the treatment is largely empirical, prevention forms the mainstay of management. **OBJECTIVE:** The present study was aimed to evaluate the effectiveness of intravenous (IV) calcium gluconate infusion in comparison to the dopamine agonist cabergoline (Cb2) in preventing OHSS in high risk patients undergoing assisted reproductive technique cycles. **MATERIALS AND METHODS:** It was a comparative study wherein the 202 high risk patients undergoing *in vitro*-fertilization over a period of 18 months after meeting the strict inclusion and the exclusion criteria, were randomly divided into two groups (98 subjects in Group I and 104 in Group II). Women in Group I were administered IV calcium gluconate while the remaining 104 received the dopamine agonist Cb2. The 104 patients belonging to Group II were started Cb2 0.5 mg/day from the day of ovulation trigger and continued until the next 8 days while the 98 high risk patients from Group I were infused with 10 ml of 10% calcium gluconate solution in 200 ml physiologic saline within 30 min of ovum pick up and continued thereafter on day 1, day 2 and day 3. **RESULTS:** The occurrence of OHSS was seen in only nine patients (in the calcium infusion group, when compared with 16 patients (9.2% vs. 15.4%) who were administered Cb2, but it was not statistically significant. However, only one had severe OHSS in Group I, whereas two women were diagnosed as severe OHSS belonging to the Cb2 arm. **CONCLUSION:** Our results document that calcium infusion can effectively prevent severe OHSS and decreases OHSS occurrence rates when used for high-risk patients, but does not suggest its superiority over Cb2. With comparable success rates, either of them can be employed as a preventive strategy for OHSS.

KEY WORDS: Cabergoline, calcium gluconate, ovarian hyperstimulation syndrome

INTRODUCTION

The prevention and treatment of ovarian hyperstimulation syndrome (OHSS), an iatrogenic and potentially life-threatening disease process, which may occur in healthy young women undergoing controlled ovarian hyperstimulation (COH) for assisted reproduction has been the subject of maximum research and innovation since the inception of assisted reproductive technology (ART). As the exact etiopathogenesis of this syndrome is still elusive, the treatment is largely empirical and thus prevention forms the mainstay of management. The preventive strategies aim, to target women at high risk of developing OHSS and institution of various pharmacological and non-pharmacological interventions on them. The pharmacological tools being used are: low-dose follicle stimulating hormone (FSH) or gonadotropin-releasing hormone antagonist protocol during stimulation, albumin infusion at the time of oocyte recovery, dopamine agonist cabergoline (Cb2) started from the day of ovulation

trigger and institution of an insulin sensitizer like metformin, whereas the non-pharmacologic modalities incorporated are: Coasting, cycle cancellation, cryopreservation of all embryos for future transfer or use of *in vitro* maturation.[1,2] Although these preventive measures have not been able to completely eliminate this iatrogenic complication but have definitely brought about a reduction in the severity of OHSS as, it is the, severe OHSS, which is the cause of maximum concern.[3] Severe forms of OHSS may complicate 0.5-5% of *in vitro*-fertilization (IVF) cycles and can lead to severe morbidity and even mortality if not timely and appropriately intervened.[4]

As the search for an ideal preventive therapy continued, a new and innovative therapy in the form of calcium gluconate infusion was introduced in the armamentarium of reproductive specialists to prevent this potentially dreadful complication, however, data and literature on its effectiveness is presently limited.[5] In the present study, we aimed to evaluate the effectiveness of intravenous (IV) calcium infusion in comparison to the dopamine agonist Cb2 in preventing OHSS in high risk patients undergoing assisted reproductive technique cycles.

MATERIALS AND METHODS

Patients and study design

A total of 202 patients at risk for developing OHSS while undergoing IVF cycle at our center during the period of 01 January 2011-31 May 2012 were included in the study. It was a comparative study wherein the 202 high risk patients after meeting the strict inclusion and the exclusion criteria, were randomly divided into two groups with 98 subjects in Group I and 104 in Group II. The women with even registration numbers were in Group I and were administered IV calcium gluconate infusion while the remaining 104 who had odd registration numbers belonged to Group II and received the dopamine agonist Cb2. Evaluation of the subjects for the development of OHSS was the primary outcome measure and requirement of hospitalization and abdominal paracentesis or pleural tap in the event of significant fluid collection because of severe OHSS was the secondary outcome measure.

The inclusion criteria or patients who were at risk for OHSS were: Known case of polycystic ovarian disease as diagnosed by the Rotterdam criteria (2004), development of 18 or more follicles larger than 12 mm in diameter and history of OHSS in the previous IVF cycle if any.[6] The patients were excluded from the study if they had other endocrinopathies in the form of diabetes mellitus, hyperprolactinemia or congenital adrenal hyperplasia. Women with systemic diseases like bronchial asthma, hypertension or bleeding disorders were also excluded. If an antagonist cycle was instituted for COH the patients were not included in the study.

Stimulation protocol and oocyte retrieval

All 202 patients were administered oral contraceptive pills (tablet Loette; Pfizer) from the 5th day of their menstrual cycle prior to their down regulation by the luteal long protocol. Leuprolide acetate (LUPRIDE; Abbott Cedex) 1 mg subcutaneously was started from the 21st day of the cycle, i.e., from the 17th day of oral contraceptive pill treatment. This dose was reduced to 0.5 mg/day once down regulation was confirmed both sonologically and by serum estradiol levels (<50 pg/ml). After the documentation of ovarian quiescence, recombinant FSH 150 IU/d (Gonal F; Merck Serono) was administered for next 4 days and doses adjusted thereafter as per the ovarian response as evident on the trans vaginal sonography. Human chorionic gonadotropin (hCG) 10,000 IU IM (Pregnyl; Organon) was administered if greater than three follicles reached a mean diameter of 18 mm. Oocyte retrieval was carried out transvaginally under ultrasound guidance 36 h after hCG administration. Conventional IVF or Intracytoplasmic sperm injection was performed on the retrieved oocytes depending on the couple's history. The embryos were graded on day 3 according to a 1-4 scoring system with 1 being the best, which was based on fragmentation, cell symmetry and blastomere number.[7] Embryo transfer was not carried out in any of the high risk study group patients and all Grade 1 and Grade 2 embryos were cryopreserved for future transfer while Grade 3 and Grade 4 embryos were discarded after informing the patients. The patients were informed about the status of their embryos and fertilization failure if any. All the high risk subjects were managed on an out-patient basis with daily clinical and sonological monitoring and hospitalized only if they went into severe OHSS or their clinical condition mandated close supervision. A baseline hematological and biochemical profile on the day of pick up and daily thereafter was also carried out for the study group subjects as a part of monitoring for OHSS.

Cabergoline institution

The 104 patients belonging to Group II were started on tablet Cb2 (Tab Dostinex 0.5 mg; Pfizer) 0.5 mg/day from the day of ovulation trigger and continued until the next 8 days.

Calcium gluconate infusion

The 98 high risk patients from Group I were started on calcium gluconate injection after ovum pick up as per the protocol mentioned by Yakovenko *et al*.[5] It was prepared by dissolving 10 ml of 10% calcium gluconate solution in 200 ml of physiologic saline and instituted over a period of 40 min. This infusion was administered within 30 min of oocyte retrieval on the day of ovum pickup and on day 1, day 2 and day 3 thereafter.

Diagnosis of ovarian hyperstimulation syndrome

OHSS was diagnosed and classified as per Golan's classification.[8] Mild OHSS was described as the presence of

pelvic discomfort, abdominal distension, nausea vomiting and/or diarrhea and enlarged ovaries as seen on sonography (5-12 cm). Moderate OHSS was described as the presence of features of mild OHSS plus ultrasonic evidence of ascites in the pouch of Douglas and pelvis and, enlarged ovaries. In the presence of severe OHSS, a lady had features of moderate OHSS plus evidence of ascites and/or hydrothorax and breathing difficulties. In addition, there were changes in the blood volume, with increased blood viscosity due to hemoconcentration (hematocrit > 45%), coagulation abnormality and diminished renal perfusion and function (low urine output < 600 ml/24 h).

All the patients (from both the study groups) after the day of oocyte recovery were monitored daily by clinical examination and a transvaginally sonography. Calcium gluconate infusion in the first group was instituted thereafter until the next 3 days. Hematological and biochemical profile was also carried out. Any patient requiring any active intervention or who went into severe OHSS was hospitalized. The volume of ascitic fluid drained daily and the changes in hematological parameters in the hospitalized subjects were recorded. The patients even after completion of calcium gluconate infusion protocol or Cb2 protocol were monitored on an out-patient basis until complete resolution of signs and symptoms.

Statistical analysis

The primary aim of this study was to evaluate the occurrence of OHSS in both the treatment groups. Assuming the incidence of OHSS as 5% with 90% power along with 6% permissible error, the minimum required sample size was estimated as 191 to detect the true difference between the two groups. The χ^2 test and Student's *t*-test were used for statistical analysis with Statistical Package for the Social Sciences software, version 18.0 for Windows. The $P < 0.05$ was taken as statistically significant.

RESULTS

There were 202 women included in the study of which 98 were in Group I and 104 belonged to Group II. The mean female age was 28.1 ± 3.3 years in Group I whereas it was 28.2 ± 3.3 years, in Group II ($P > 0.05$). It was observed that there was no significant difference in terms of female age (years), body mass index (kg/m^2), duration of infertility (years), basal hormone levels (FSH [mIU/ml]), basal antral follicle count, length of ovarian stimulation (days), number of follicles on the day of hCG trigger and the number of oocytes retrieved in both the study groups [Table 1]. Fertilization rate, cleavage rate and implantation rate among the groups have not been evaluated and included as it was not part of the study as all the high risk patients underwent frozen embryo transfer.

Statistical analysis also revealed that the number of women undergoing the first or the second IVF cycle were also comparable so was the history of OHSS in the previous cycle in patients undergoing second cycle in both the study groups (24.5% vs. 26%) [Table 2].

The occurrence of OHSS was seen in only nine patients (9.2%) in the calcium infusion group, as compared to 16 patients (15.4%) who were administered Cb2. However, the rate of occurrence of OHSS in both the study group was not found to be statistically significant. Among the nine patients in the calcium infusion group who developed the complication only one had severe OHSS, whereas two women were diagnosed as severe OHSS belonging to the Cb2 arm [Table 3]. All the three women (from both the study groups) with severe OHSS required hospitalization. Two patients from the Cb2 group required ascitic tap due to massive ascites and breathing difficulties and one woman

Table 1: Comparison of clinical and laboratory characteristics in calcium gluconate-administered group (Group I) and cabergoline group (Group II)

Parameter	Group I (*n*=98)	Group II (*n*=104)	*P* value
Female age (years)	28.1±3.2	28.2±3.3	0.779
FSH (mIU/mL)	6.0±1.8	6.1±1.7	0.884
Duration of infertility (years)	5.7±2.1	5.9±2.1	0.831
AFC	14.7±2.0	14.7±2.0	0.965
BMI (kg/m^2)	23.4±2.2	23.3±2.2	0.689
Length of stimulation (days)	8.6±1.5	8.8±1.5	0.610
Retrieved oocytes	19.6±7.3	19.6±7.3	0.925

FSH=Follicle stimulating hormone; AFC=Antral follicle count; BMI=Body mass index

Table 2: Comparison of cycle characteristics in the two treatment group

Cycle characteristics	*n* (%)		*P* value
	Calcium *n*=98	Cabergoline *n*=104	
Cycle number			
First	55 (56.1)	59 (56.7)	0.931
Second	43 (43.9)	45 (43.3)	
History of OHSS in previous cycle			
Absent	74 (75.5)	77 (74.0)	0.809
Present	24 (24.5)	27 (26.0)	
Presence of OHSS in present cycle			
Absent	89 (90.8)	88 (84.6)	0.181
Present	9 (9.2)	16 (15.4)	

OHSS=Ovarian hyperstimulation syndrome

Table 3: Severity of OHSS in the two study group (*n*=number of patients with OHSS in two arms)

	Calcium (*n*=9)	Cabergoline (*n*=16)
Mild	6	10
Moderate	2	4
Severe	1	2

OHSS=Ovarian hyperstimulation syndrome

with severe OHSS from Group I required abdominal paracentesis. These patients' condition improved with supportive therapy and interventions such as abdominal paracentesis and were discharged once asymptomatic. The patients with mild and moderate OHSS from both the study groups were monitored on an out-patient basis until the resolution of signs and symptoms. None of the patients who were started on calcium gluconate injection developed any allergic reactions, anaphylaxis, symptoms or signs of hypercalcemia, or other side effects.

DISCUSSION

Of the various pathophysiological mechanisms implicated for the causation of OHSS, it is the angiogenic molecule, vascular endothelial growth factor (VEGF), which has been found to be the biggest mediator of this potentially dreadful complication.[9] It has been proven that VEGF stimulates new blood vessel development and vascular hyper permeability by interacting with its VEGF receptor 2 (VEGFR-2).[10] Thus various studies were carried out, which have proven that Dopamine agonists can inhibit phosphorylation of the receptor VEGFR-2 and can thus reduce the vascular permeability and various presentations of OHSS in the ART cycles.[11] In pursuance, Cb2 was therefore extensively studied and was found to bring about a decrease in the severity or incidence or both of OHSS.[12-16]

Apart from the increased capillary permeability brought about by VEGF which results in a fluid shift from the intravascular space to third space compartments[10] the other contributing factors elucidated in the pathophysiology of OHSS are: Increased secretion or exudation of protein-rich fluid from enlarged ovaries or peritoneal surfaces, increased follicular fluid levels of prorenin and renin and increased angiotensin-mediated changes in capillary permeability.[17,18] Thus the effectiveness and safety of dual renin-angiotensin system blockage for prevention of OHSS in over stimulated patients undergoing IVF was evaluated in a study. However, this new strategy for use in patients at high risk for OHSS did not completely eliminate the development of the syndrome.[19] In addition, there were concerns about the safety of the treatment, as angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists were associated with possible teratogenic effect in humans.[20] Nevertheless, this study did suggest the possible role of an altered renin angiotensin system in the development of OHSS. Another observation found in a separate study was the stimulatory role of low intracellular calcium on adenylyl cyclase, which resulted in cyclic adenosine monophosphate (cAMP) synthesis and thus, renin release.[21] It was also postulated by Beierwaltes that renin secretion is inversely related to the extracellular and intracellular calcium concentrations and therefore, calcium may modify the amplitude of cAMP-mediated renin-signaling pathways.[22] Thus it was inferred that although calcium does not directly control renin secretion, increased calcium inhibits and decreased calcium amplifies cAMP-stimulated renin secretion.[22] Gurgan *et al*. in their retrospective study also researched and found that calcium infusion successfully prevents the development of severe OHSS and significantly decreases OHSS occurrence rates without any major adverse effect when used for high-risk patients such as those with polycystic ovary syndrome (PCOS).[23] With the background of success, demonstrated by both Cb2 and calcium for the prevention of this iatrogenic complication, we compared their efficacy.

Identifying patients who are at-risk is the most critical step in the prevention of OHSS as it guides a clinician to make changes to the ovarian stimulation regimen and to add other preventative measures. Predictive factors for OHSS can be primary risk factors, which confer an increased risk of OHSS on patients and secondary risk factors, which become apparent during ovarian stimulation when patients with no known predisposing factors experience an excessive response to treatment.[24] In this study too, we incorporated these risk factors and targeted them to either of the preventive strategy.

It is noteworthy that in this study we compared the two drugs which ultimately targeted the same key molecule: VEGF. The pathway of reaching the target and the mode of administration of both the drugs might be different but the preventive mechanism was same, i.e., either antagonizing VEGF receptor as in Cb2 or decrease VEGF levels as with calcium gluconate infusion.

It has been hypothesized that calcium infusion for patients with high-risk factors for OHSS, initially prevent renin secretion. Reduced renin production results in decreased angiotensin II synthesis. As a consequence, the stimulatory effect of angiotensin II on VEGF production is ameliorated.[23] All these pathophysiologic mechanisms (decreased synthesis of renin, angiotensin II and VEGF) which occurs from calcium gluconate infusion, therefore prevents the development of OHSS in such high risk patients undergoing ART cycles. Our results also document that calcium infusion can effectively prevent the development of severe OHSS and decreases OHSS occurrence rates without any major adverse affect when used for high-risk patients such as those with PCOS. It also needs to be reiterated that very few studies have been carried out on the efficacy of this new strategy of calcium gluconate infusion and comparison between this novel protocol and the established role of dopamine agonist is the first of its kind.

Our observations in this present study was only limited to the occurrence of early onset of OHSS in contrast to the

findings of Carizza *et al*. as we carried out freezing of all the embryos so late onset of OHSS was not evaluated.[13]

CONCLUSION

We can say that even though both the drugs were found to be equally effective for the prevention of OHSS and most importantly in decreasing the severity of this potentially life-threatening complication, their effect on the implantation process and the comparison of the pregnancy rate, implantation rate and miscarriage rate was not computed which accounts for the limitation of this study. Larger well designed trials need to be carried out incorporating the aforementioned factors as well as measurements of VEGF levels in both the study groups. Nevertheless, as both the drugs are safe, cheap and have comparable success rates either of them can be employed as a treatment strategy for patients with high risk factors for OHSS undergoing ART cycles.

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