IV calcium infusion's role in ovarian hyperstimulation syndrome prevention

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ABSTRACT

Aim: to determine the prevalence of OHSS and clinical outcomes in high-risk women undergoing ART following calcium IV infusion.

Methods: Double-blinded randomized controlled trial included two hundred women at risk to develop OHSS undergoing IVF/ICSI treatment cycle subdivided randomly into two equal groups; group I (intervention group (study/calcium infusion group, n=100) received intravenous infusion of 10 mL 10% calcium gluconate in 100 mL 0.9% saline solution on the day of ovum pick-up (OPU) and days 1, 2, and 3 after, and group II (control/placebo group, n=100) received 100 mL 0.9% saline solution on the day of OPU and days 1, 2, and 3 after.

Main Outcome Measure: Incidence of OHSS.

Results: OHSS incidence was significantly lower in the calcium infusion group (group I) than in the placebo group (group II): 8 (8%) vs. 26 (26%); moderate OHSS was significantly lower in group I than in group II: 3 (3%) vs. 10 (10%); and severe OHSS was significantly lower in group I than in group II: 0 vs. 4 (4%).

Conclusion: IV calcium infusion has a significant preventive role against OHSS without adverse effect on pregnancy outcomes.

Key Words: OHSS, calcium infusion, IVF, ICSI

BACKGROUND

Ovarian hyperstimulation syndrome (OHSS), a major iatrogenic consequence of assisted reproductive technologies (ART), affects up to 30% of women receiving ART and has considerable morbidity and mortality risks. Women under the age of 35, those with a history of polycystic ovarian syndrome (PCOS), and those who have had prior OHSS are more likely to develop OHSS [1].

Increasing vascular permeability which results in extravascular space fluid collection, plays the main role in subsequent clinical presentations such as abdominal discomfort, abdominal distention, ovarian enlargements, and ascites [2-3].

According to the period of time after hCG administration, OHSS may present as one of two forms; the early-onset or the late-onset. After hCG injection, the early-onset form happens within 9 days, while the late-onset form occurs after 10 days. The late-onset form of OHSS may be due to hCG released by the trophoblastic tissues [4].

Although the pathophysiology of OHSS has not been completely understood, studies reported some vasoactive substances implicated in the increased vascular permeability and the subsequent pathologies in this condition [2]. Vascular endothelial growth factor (VEGF), which originally known as vascular permeability factor, seems to be an important factor causes fluid extravasation in OHSS [5]. In addition, renin levels in plasma and renin activity were found to be increased in OHSS, pointing out a role of renin-angiotensin system (RAS) [6]. Thus, higher levels of Angiotensin II in ascites fluid were reported in cases with OHSS than in other non-OHSS ascites[7]. Indeed, many of the angiogenic factors associating pathophysiology of OHSS may act through VEGF either directly or indirectly. Locally, angiotensin II stimulates secretion of VEGF, which in-turn complicates the condition leading to a viscous circle of cascade [8].

As calcium infusion was found to have an inhibitory effect on RAS, some studies suggested it can decrease levels of VEGF, and therefore it may reduce the incidence of OHSS. Gurgan el al. reported significant reduction in the rates of OHSS with

calcium infusion. They documented its successful impact in prevention of severe OHSS with no major side effects [9-10].

Our aim in this study was to assess the incidence of OHSS and clinical outcomes after calcium IV infusion in high-risk women undergoing ART.

MATERIALS AND METHODS

This was a double-blind randomized prospective controlled study included 200 women undergoing intra-cytoplasmic sperm injection (ICSI) cycles at Jeddah Fertility Center, Dr Erfan and Bagedo General Hospital. Institutional Review Board approval was obtained for the study.

We investigated for women who had ICSI between March 2018 and December 2020 and were at high risk of having OHSS. The following is how the risk of having OHSS was determined: Based on previous clinical observation, E2 level on day of hCG administration >2.500 pg/mL with at least 20 follicles >10 mm on day of hCG administration. The Research Ethical Committee decided to exclude women with E2 >6.000 pg/mL from this trial since it is unethical to administer just placebo to women with E2 levels >6,000 pg/mL in the control/placebo group with a high risk of severe OHSS.

Two hundred women at risk for OHSS were randomly assigned to either the study/calcium-infusion group (group I) or the control/placebo group (group II) using computer-generated random numbers (group II). To prevent prejudice, the ladies and the employees that followed up on them were blinded to the allocation.

Before participating in this trial, high-risk women for developing OHSS were given thorough written and spoken information about it. Those who accepted completed a formal permission form after being told.

Because calcium infusion might induce cardiac toxicity, all women in this trial were carefully evaluated by a cardiologist to rule out those having a history of digitalis usage, arrhythmias, or other heart problems. They had a medical history taken, a physical examination, an ECG, and an echocardiography testing.

On the day of ovum pickup and days 1, 2, and 3 following ovum pickup, 10 mL IV 10% calcium gluconate in 200 mL physiologic saline solution was delivered within 30 minutes in group I (calcium-infusion group), as previously reported by Gurgan et al., Yakovenko et al., and EL-Khyat et al., [9-12]. On the day of ovum pick-up and days 1, 2, and 3 following ovum pick-up, group II (control/placebo group) received IV 200 mL physiologic saline solution within 30 minutes.

All of the ladies in this research were undergoing ICSI cycles (antagonist protocol). Ovarian stimulation began at second or third day of woman's menstrual cycle by recombinant FSH (Gonal F, Merck) with a dose depending on the woman's age, antral follicle count, baseline FSH level, and past ovarian response . The first control (ultrasound and serum E2) was conducted regularly after 5 days of stimulation. Serial ultrasonography and serum E2 assays were used to track follicular development. The dosage of gonadotropin was adjusted as needed based on the follicular response. After follicle maturity was confirmed by ultrasound and at least three follicles attained the size of >18 mm, 5,000 IU hCG (Choriomon; IBSA) was used to trigger the pregnancy.

When at least three follicles with a mean diameter of >18 mm were found by transvaginal ultrasonography 36 hours after hCG injection, the oocytes were extracted. A commercially available sequential culture medium system was used to cultivate the embryos. Fertility testing was performed 16–18 hours following injection. The morphologic criteria were used to evaluate and select cleavage-stage embryos.

Under ultrasound supervision, embryo transfer was performed at the cleavage stage using the Labotect Embryo Catheter (Labor-Technik) or the Wallace Embryo Replacement Catheter (Irvine Scientific). Vaginal progestin suppositories were used to provide luteal support (400 mg Cyclogest; Actavis).

The OHSS rate was the major end measure, with secondary outcomes including OHSS (mild, moderate, or severe), fertilisation rate, implantation rate, chemical pregnancy, clinical pregnancy, continuing pregnancy, early miscarriage, and live birth rate as secondary outcomes. The existence of a gestational sac with foetal

heart activity was used to identify clinical pregnancy. The number of gestational sacs divided by the number of embryos transplanted yielded the implantation rate.

In this research, the diagnosis and categorization of OHSS were reported using Humaidan et al. criteria [13]. Pelvic pain, abdominal distention, ultrasound evidence of ascites in the Douglas pouch, and enlarged ovaries were all used to diagnosis mild OHSS. Pelvic pain, abdominal distention, ultrasound evidence of ascites in the pouch of Douglas and pelvis, enlarged ovaries, and aberrant hematologic profiles (hematocrit >45 percent) were all used to identify moderate OHSS.

The following objective (fluid in Douglas pouch, fluid around uterus (pelvis), fluid around intestinal loops, hematocrit >45 percent, leukocytic count >15,000/mL, and low urine output (600 mL/24 h) and subjective (pelvic discomfort, abdominal distention, breathing difficulty and ovarian enlargement) criteria should be present in the presence of severe OHSS.

RESULTS

Data were collected, revised, coded and entered to the statistical package for social science (SPSS) (Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.), quantitative data were presented as mean and standard deviation and compared using independent t-test while qualitative data were presented as numbers and percentages and compared using numbers and percentages. The confidence interval was set to 95% and the margin of error accepted was set to 5%, so, the p-value was considered significant at the level of <0.05.

This study included 200 women. They were divided randomly into two groups equal groups, each group included 100 women; group I (study group / calcium group) and group II (control / placebo group).

As shown in (Table-1), the demographic characteristics were of statistically insignificant differences. The range of age (years) of women in this study was (28.56+/- 5.74) and (28.12+/-4.96) in calcium group and placebo group respectively (t=0.580, p=0.563). The body mass index (kg/m2) in calcium group was (26.32+/-2.78) and in placebo group was (26.47+/-2.65) (t=0.391, p=0.697). The duration of infertility (years) in were (4.66+/-3.02) in calcium group and (4.82+/-3.64) in placebo group (t=0.338, 0.736).

Table (1): Demographic and laboratory characteristics in both groups.

	Ca group	Placebo group	T Test	P Value
	n=100	n=100		
Age (year)	28.56 ± 5.74	28.12 ± 4.96	-0.580•	0.563
Body mass index (kg/m2)	26.32 ± 2.78	26.47 ± 2.65	0.391•	0.697
Duration of infertility (year)	4.66 ± 3.02	4.82 ± 3.64	0.338•	0.736
Type of ifertility			0.080*	0.777
Primary infertility	54 (54)	52 (52)		
Secondary	46 (46)	48 (48)		
Cause of infertility (%)				
Male factor	11 (11)	13 (13)	0.189*	0.663
Anovulation	25 (25)	21 (21)	0.452*	0.501
Tubal factor	27 (27)	23 (23)	0.427*	0.513
Endometriosis	8 (8)	9 (9)	0.064*	0.800
Unexplained	29 (29)	34 (34)	0.091*	0.762
Basal FSH (mIU/mL)	6.68 ± 1.92	6.73 ± 1.69	0.195•	0.845
Basal LH (mIU/mL)	4.47± 1.59	4.49 ± 1.67	0.087•	0.931
Basal E2 (pg/mL)	36.02±12.66	37.87 ± 11.98	1.061•	0.289
Basal antral follicle count (n)	17.23± 1.83	16.69± 2.26	1.857•	0.065

•: Independent t-test; *: Chi-square test

Table-2 shows statistical insignificant differences between both two groups concerning type of infertility and causes of infertility. Regarding type of infertility, 54 women (54%) were presented by primary infertility and 46 women (46%) were presented by secondary infertility in calcium group, while 52 women (52%) were presented by primary infertility and 48 women (48%) were presented by secondary infertility in placebo group (t=0.080, p=0.777). Male factor of infertility was reported in 11 women (11%) in calcium group and in 13 women (13%) in placebo group (t=0.198, p=0.663). Anovulation was diagnosed as a the cause of infertility in 25 women (25%) in calcium group and in 21 women (21%) in placebo group (t=0.452, p=0.501). Tubal factor was detected in 27 women (27%) in calcium group

and 23 women (23%) in placebo group (t=0.427, p=0.513). Endometriosis was found in 8 women (8%) in calcium group and in 9 women (9%) in placebo group (t=0.064, p=0.800). Unexplained infertility was reported in 29 women (29%) in calcium group and in 34 women (34%) in placebo group (t=0.091, p=0.762).

Table-2: Type and Cause of infertility

	Ca group n=100	Placebo group n=100	T Test	P Value
Type of ifertility			0.080*	0.777
Primary infertility	54 (54)	52 (52)		
Secondary	46 (46)	48 (48)		
Cause of infertility (%)				
Male factor	11 (11)	13 (13)	0.189*	0.663
Anovulation	25 (25)	21 (21)	0.452*	0.501
Tubal factor	27 (27)	23 (23)	0.427*	0.513
Endometriosis	8 (8)	9 (9)	0.064*	0.800
Unexplained	29 (29)	34 (34)	0.091*	0.762

•: Independent t-test; *: Chi-square test

As shown in (Table-3), the basal serum hormonal levels (FSH, LH, E2) in both groups were of statistically insignificant differences. Basal serum FSH levels (mIU/mL) were (6.68+/-1.92) and (6.73+/-1.69) in calcium group and placebo group respectively (t=0.195, p=0.845). Basal LH (mIU/mL) were (4.47+/-1.59) and (4.49+/-1.67) in calcium group and placebo group respectively (t=0.087, p=0.931). Basal E_2 levels (pg/mL) were (36.02+/-12.66) in calcium group and (37.87+/-11.98) in placebo group (t=1.061, p=0.289).

In addition, basal antral follicle count in both groups had statistically insignificant differences (Table-3). Women in calcium group had basal antral follicle count (follicles) of (17.23+/-1.83) while in placebo group had (16.69+/-2.26) (t=1.857, p=0.065).

Table-3: Basal serum hormonal levels and basal antral follicle count in both groups

	Ca group n=100	Placebo group n=100	T Test	P Value
Basal FSH (mIU/mL)	6.68 ± 1.92	6.73 ± 1.69	0.195•	0.845

Basal LH (mIU/mL)	4.47± 1.59	4.49 ± 1.67	0.087•	0.931
Basal E2 (pg/mL)	36.02±12.66	37.87 ± 11.98	1.061•	0.289
Basal antral follicle count (n)	17.23± 1.83	16.69± 2.26	1.857•	0.065

•: Independent t-test; *: Chi-square test

Table-4 shows statistically insignificant differences between the two groups in amount of Gonadotropin (Gn) used for ovarian stimulation and in length of ovarian stimulation. The amount of total hMG (IU/L) used was (2,712.42+/-37734) and (2,685.23+/-329.45) in calcium group and placebo group respectively (t=0.543, p=0.588). The length of ovarian cycle stimulations (days) was (11.68+/-0.973) in calcium group and (11.84+/-0.942) in placebo group (t=1.181, p=0.239).

Furthermore, there were statistically insignificant differences between both groups regarding peak E_2 on hCG day, Endometrial thickness on hCG day, number of oocytes retrieved, and number of metaphase II oocytes retrieved (Table-4). Peak E2 on hCG day (pg/mL) was (3787.86 ± 730.04) and (3925.67 ± 729.13) in calcium group and placebo group respectively (t=1.336, p=0.183). Endometrial thickness (mm) in calcium group and in placebo group was (10.34+/-1.45) and (10.28+/-1.29) respectively (t=0.309, p=0.758). Number of oocytes retrieved was (22.59+/-5.12) and (22.41+/-5.01) in calcium group and in placebo group respectively (t=0.251, p=0.802). Number of MII oocytes retrieved in calcium group was (17.56+/-4.24) and in placebo group was (17.26+/-4.15), (t=0.506, p=0.614).

Also, fertilization rate, implantation rate, chemical pregnancy, clinical pregnancy, early miscarriage rate, ongoing pregnancy rate, and live birth rate were of statistically insignificant differences (Table-4). Fertilization rate was (77.63+/-7.74)% and (76.79+/-7.41)% in calcium group and placebo group respectively (t=0.784, p=0.434). Implantation rate was 28% in calcium group, and 30% in placebo group (t=0.097, p=0.755). Chemical pregnancy was reported in 60 women (60%) in calcium group, and in 65 women (65%) in placebo group (t=0.960, p=0.327). However, clinical pregnancy were diagnosed in 55 women (55%) and 60 women (60%) in calcium group and placebo group respectively (t=1.681, p=0.194). Early miscarriage happened in 8 women (8%) in calcium group and 11 women (11%) in placebo group, (t=0.491, p=0.483). Ongoing pregnancy and live birth were reported in 47 women (47%) in calcium group and in 49 women (49%) in placebo group (t=0.988, p=0,320).

Table-4: Ovarian stimulation and pregnancy outcomes in both groups

	Calcium group	Placebo group	T test	P Value
	n=100	n=100		
Amount of total hMG used (IU/L)	2,712.42 ± 377.34	2,685.23 ± 329.45	0.543•	0.588
Length of ovarian stimulation (days)	11.68 ± 0.973	11.84 ± 0.942	1.181•	0.239
Peak E2 on hCG day (pg/mL)	3787.86 ± 730.04	3925.67 ± 729.13	1.336•	0.183
Endometrial thickness on hCG day (mm)	10.34 ± 1.45	10.28 ± 1.29	0.309•	0.758
No. of oocytes retrieved (n)	22.59 ± 5.12	22.41 ± 5.01	0.251•	0.802
No. of MII oocytes retrieved (n)	17.56 ± 4.24	17.26 ± 4.15	0.506•	0.614
Fertilization rate (%)	77.63 ± 7.74	76.79 ± 7.41	0.784•	0.434
Implantation rate (%)	28 (28)	30 (30)	0.097*	0.755
Chemical pregnancy (%)	60 (60)	65 (65)	0.960*	0.327
Clinical pregnancy (%)	55 (55)	60 (60)	1.681*	0.194
Early miscarriage rate (%)	8 (8)	11 (11)	0.491*	0.483
Ongoing pregnancy rate (%)	47 (47)	49 (49)	0.988*	0.320
Live birth rate (%)	47 (47)	49 (49)	0.988*	0.320

•: Independent t-test; *: Chi-square test

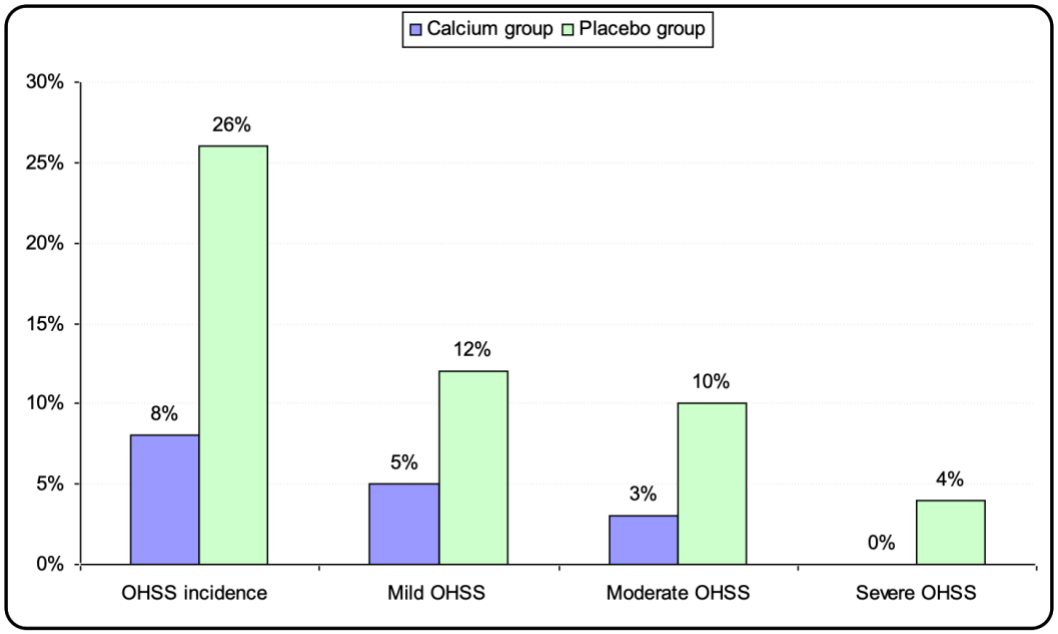
However, there was a statically significant difference between both groups incidence of OHSS as shown in (Table-5) and (Fig-1). The incidence of OHSS in calcium group was (8%) while it was (26%) in placebo group, making a statistical significant difference (t=11.481, p=0.001). Although there was an insignificant differences between both groups in incidence of mild OHSS, there were statistical significant differences in incidence of moderate and severe OHSS. Mild OHSS presented with incidence of (5%) in calcium group and (12%) in placebo group, with no statistical significant difference (t=3.150, p=0.075). There was a statical significant difference regarding incidence of moderate OHSS which was lower in calcium group (3%) than in placebo group (10%), (t=4.916, p=0.026). In addition, the incidence of severe OHSS recorded another statistical significant difference as it was nil in calcium group, while it was (4%) in placebo group (t=4.082, p=0.043).

Concerning side effects of calcium administration, there were no reported significant side effects; there were no flushing, headache, chalky taste or hypotension recorded in both groups.

Table-5: Incidence of Ovarian Hyperstimulation Syndrome in both groups

	Ca group n=100	Placebo group n=100	T Test	P Value
OHSS incidence (%)	8 (8)	26 (26)	11.481*	0.001
Mild OHSS (%)	5 (5)	12 (12)	3.150*	0.075
Moderate OHSS (%)	3 (3)	10 (10)	4.916*	0.026
Severe OHSS (%)	0 (0)	4 (4)	4.082*	0.043

•: Independent t-test; *: Chi-square test



DISCUSSION

The present study was a double-blind randomized control trial. We reported a reduction of the risk of OHSS development with using intravenous (IV) calcium

infusion in high-risk women. The incidence of OHSS was found to be significantly lower with IV calcium infusion (8%) when compared with placebo group (26%). In placebo group 26 women developed OHSS making a high incidence (26%), as 12 women(12%) developed mild OHSS, 10 women (10%) developed moderate OHSS, and 4 women (4%) developed severe OHSS [14-16].

IV calcium infusion demonstrated a significantly lower incidence of mild (5%) and moderate (3%) OHSS. In addition, calcium infusion did not show any negative impact on implantation rate and also had no effect on pregnancy rate.

Our study is in agree with a similar double-blind randomized controlled trial by El-Khayat et al. over 200 women to evaluate the preventive role of calcium infusion against OHSS in high risk women. El-Khyat et al. concluded significantly reduction of OHSS with calcium infusion. They reported that OHSS incidence was significantly higher in the placebo group 23% than in calcium group 7%; moderate OHSS was significantly higher in placebo group (8%) than in calcium group (1%); and severe OHSS was significantly higher in placebo group (4%) than in calcium group (0%). In addition, they stated no reduction in the pregnancy rate [12].

A study by Pundit et al. reported less incidence of OHSS with using antagonist protocol [17]. Triggering by GnRH agonist showed lower rates of ongoing pregnancy and live birth than triggering by hCG [18]. Tobler et al. studied 359 centers around 71 countries and concluded that hCG with antagonist protocol was the commonest drug used in triggering [19].

Many experimental studies assessed the preventive role of calcium infusion against OHSS. These studies concluded that increased calcium level surpasses cyclic adenosine monophosphate stimulated renin secretion. Stimulated renin decreases angiotensin II with depression in VEGF mRNA and protein expression in human granulosa lutein cells [21-23].

Although both angiotensin converting enzyme and angiotensin receptor antagonist can control renin-angiotensin system, they are teratogenic and not safe with pregnancy. Therefore, calcium infusion seems to be a safer choice [24].

Gurgan et al. studied the effect of calcium infusion in prevention of OHSS and stated its protective effect by suppressing renin secretion from granulose cells, which in turn suppress both angiotensin II synthesis and VEGF. Their retrospective study over 84 women received IV calcium infusion and 371 women as control reported a lower incidence of OHSS with calcium infusion (3.6%) than in control group (16%) [9].

Another randomized study on rat models by Kistou et al. suggested the efficacy of calcium in controlling vascular permeability [26].

Another randomized study by Naredi and Karunakaran over 202 women to assess the role cabergoline versus calcium infusion in preventing OHSS concluded similar protective effect for both of them 10.

The present study was a randomized controlled trial powered and having a double-blind design to minimize the influence of bias and to strengthen the study. However, the absence of measurement of serum levels of calcium, renin, and VEGF was a limitation of our study.

CONCLUSION

The present trial stated that IV calcium infusion has a significant preventive role against OHSS in high-risk women without adverse effect on pregnancy outcomes.

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