Calcium gluconate infusion is not as effective as dopamine agonists in preventing ovarian hyperstimulation syndrome

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Abstract. – OBJECTIVE: The aim of the study was to compare the effectiveness of calcium gluconate and cabergoline therapy in the prevention of ovarian hyperstimulation syndrome (OHSS).

PATIENTS AND METHODS: Eight hundred and forty-five women who underwent GnRH antagonist protocol and at high risk for developing OHSS were divided into two groups, those given cabergoline (n=435) or calcium gluconate (n=410). In cabergoline group, 0.5 mg of cabergoline was administered once daily p.o. starting on the day of ovulation trigger and continued until the following 8 days. In calcium gluconate group, intravenous calcium gluconate was administered daily for four days starting on the day of oocyte pickup (OPU). 10 ml of 10% calcium gluconate solution was dissolved in 200 ml of physiological saline and administered by intravenous route within 40 minutes. Infusion was started within the first 30 minutes following the OPU and continued on the 1st, 2nd and 3rd days after OPU.

RESULTS: Mild OHSS was developed in 367 (89%) patients receiving calcium gluconate infusion, while 251 patients (57%) in the cabergoline group developed mild OHSS. The frequency of mild OHSS in the calcium group was significantly higher than the cabergoline group (p<.001). Moderate OHSS was observed in 32 people (7.8%) in the calcium gluconate group, while it was observed in 184 people in the cabergoline group (42.3%). Calcium gluconate infusion significantly reduced the development of moderate OHSS compared to cabergoline therapy (p<.001). Severe OHSS developed in 11 patients (2.7%) in the calcium gluconate group, while severe OHSS did not develop in those given cabergoline (0%, p<.001). Clinical pregnancy, live birth and abortion rates were similar in the two groups. When logistic regression analysis was performed, a significant correlation was found between age, BMI, AMH, the number of antral follicle count, OHSS history, paracentesis, progesterone on the day of hCG, 2 PN zygotes, and HbA1c levels and the development of OHSS. No correlation was found between the use of metformin or cetrotide and the development of OHSS.

CONCLUSIONS: Calcium gluconate treatment is not as effective as dopamine agonists in preventing ovarian hyperstimulation syndrome.

Key Words: OHSS, Calcium gluconate, Cabergoline, PCO.

Introduction

Although assisted reproductive technologies (ART) have helped many infertile couples get pregnant it paved the way for ovarian hyperstimulation syndrome (OHSS) which is iatrogenically created by the clinician and puts maternal life at risk. OHSS is a systemic disease characterized by abdominal swelling and tenderness secondary to a sudden increase in vascular permeability due to volume overgrowth of the ovaries that occur during controlled ovarian stimulation cycles. The main causes OHSS are unknown. It is characterized by an increase in the synthesis of vascular endothelial growth factor (VEGF). Additionally, due to multi-follicular, intravascular fluid in the ovarian vessels is directed to the third space and forms the typical findings of the OHSS1-3. The increase in the amount of fluid shifting to the third spaces also determines the outcome of the OHSS. The follow-up and treatment of the syndrome becomes more difficult as a result of pre-renal azotemia and deterioration in hematological parameters due to the accumulation of fluid in the third spaces. Although spontaneous development of OHSS depending on FSH receptor mutation has been reported, it is believed that the main mech-
Calcium gluconate and OHSS prevention

The mechanism underlying OHSS is the use of high-dose gonadotropins for controlled ovarian stimulation (COS). The use of human chorionic gonadotropin (hCG) also increases the risk of OHSS. Supraphysiological levels are another risk factor for OHSS.

Patients in the risk group for OHSS should be well managed. Otherwise, thromboembolic events, respiratory distress, liver or renal failure may occur. Women with polycystic ovary syndrome (PCOS) or having many follicles during COS are in the high risk for OHSS. The development of OHSS can be prevented by using the following methods in patients in the risk group for OHSS: (i) individualization of COS protocols and individual adjustment of gonadotropin dose, (ii) ovulation trigger with GnRH agonists, (iii) coasting by reducing or cutting the dose of gonadotropins, (iv) cycle cancellation or freezing embryos, (v) use of plasma expanders.

A recent comprehensive review has shown that dopamine agonists are effective in reducing the occurrence of OHSS. In full compliance with this, cabergoline, a dopamine agonist, is highly effective in the treatment of OHSS. By inhibiting the phosphorylation of VEGF receptor, cabergoline decreases vascular permeability and prevents new vessel formation. However, since cabergoline acts only through VEGFR, VEGF secretion continues and a complete recovery in the course of OHSS is not possible. The increase in VEGF synthesis due to the activation of the renin-angiotensin-aldosterone system contributes to the formation of OHSS by increasing the permeability of the ovarian vessels. Abnormality in the fluid-electrolyte balance leads to a decrease in intracellular calcium, leading to the continuation of VEGF production by increasing adenylyl cyclase-mediated cyclic adenosine monophosphate (cAMP)-renin synthesis. Due to all these mechanisms, both VEGF synthesis and VEGFR-2 activation must be blocked in order to prevent the development of OHSS.

Increasing intracellular calcium blocking cAMP-mediated VEGF secretion is a new method to prevent OHSS. Hence, there are several studies investigating the effect of calcium gluconate therapy in patients with OHSS. The most important limitation of previous studies is the small number of participants. Therefore, this study was planned to determine the incidence of early onset OHSS development in 845 high-risk patients for OHSS who received calcium gluconate infusion or cabergoline. Clinical pregnancy, miscarriage, and live birth rates were recorded.

Patients and Methods

This study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all participants at the time of enrollment. The sample size of the study was calculated with the Mann-Whitney test with the effect size of 0.5%, power of 80% and maximum acceptable type I error of 5%. A total of 845 patients at high risk for developing OHSS were included in the study. Participants were divided into two groups as those who received calcium gluconate (n=410) or those who received cabergoline treatment (n=435). Women with PCOS had a high antral follicle (>24) or AMH (> 3.4 ng/ml). Development of 18 or more follicles larger than 12 mm in diameter was set as the criterion for inclusion in the high-risk group for OHSS. Those with a history of OHSS in their previous trials were also considered in the risk group for OHSS and included in the study. Patients with overt endocrinopathy of the thyroid and adrenal glands, especially diabetes mellitus, were excluded. Likewise, patients with a history of acute or chronic renal or liver disease, chronic obstructive pulmonary disease, hypertension, bleeding diathesis, and patients with a systemic disease were excluded.

In cabergoline group, 0.5 mg of cabergoline was administered once daily p.o. starting on the day of ovulation trigger and continued until the following 8 days. Calcium gluconate infusion was started within the first 30 minutes following the oocyte pick-up in the other 410 patients in the high-risk group for OHSS. Then, infusion was continued on the 1st, 2nd and 3rd days after OPU. 10 ml of 10% calcium gluconate solution was dissolved in 200 ml of physiological saline and administered by intravenous route within 40 minutes. A detailed protocol on dissolution and administration of calcium gluconate can be found elsewhere.

The diagnosis and classification of OHSS was made as described by Golan et al. The presence of enlarged ovaries accompanied by nausea, vomiting, abdominal distension and pelvic pain was defined as mild OHSS. Ovarian size varying between 5 and 12 cm in sonographic measurement was accepted as enlargement of the ovaries. In...
addition to mild OHSS findings, the presence of ascite in the pelvis and abdomen in ultrasonography was considered as moderate OHSS. Severe OHSS was diagnosed in patients with clinically evident ascitic fluid in pleural and pericardial spaces and respiratory distress in addition to moderate OHSS findings. Patients with increased hematocrit levels (>45%), decreased renal perfusion and urine output (<600 mL/24 h), and increased liver enzymes were also considered to have severe OHSS and were hospitalized. Participants in both groups managed on an out-patient basis with daily ultrasonographic and clinical examination to evaluate the development of OHSS after OPU.

Biochemical and hematological parameters were followed closely. Patients hospitalized for severe OHSS were observed in terms of fluid, electrolyte balance, and their urine output was recorded. The amount of ascitic fluid evacuated in cases who underwent paracentesis was recorded.

Women with mild or moderate OHSS were hospitalized if clinical and laboratory findings worsened. Patients were kept under follow-up until complete improvement in OHSS symptoms was achieved. In order to prevent development of OHSS, no embryo transfer was performed to the patients in both groups. Embryos of the participants were frozen and stored on the 3rd or 5th day. Frozen embryo transfer was performed in patients recovering from OHSS.

Primary outcome of our study was to determine the incidence and type of OHSS development in the treatment groups. Secondary outcome was to compare clinical pregnancy, miscarriage, and live birth rates between the groups. Clinical pregnancy was defined as evidence of a gestational sac, confirmed by ultrasound examination. The loss of fetus before 20 weeks of gestation was defined as miscarriage. After recording the previous OHSS history of the women in both groups, whether they used metformin or antagonists, whether abdominal paracentesis was performed, and the number of collected oocytes were also recorded. Following the recording of the demographic data, the laboratory and embryological data, especially the hematocrit and AMH values, were also recorded. The contributions of demographic, laboratory, clinical and embryological findings to the development of OHSS were analyzed one by one using multiple logistic regression analysis (OR, 95% - CI).

**Statistical Analysis**

SPSS 23.0 (IBM Corp., Armonk, NY, USA) package program was used for statistical analysis of the data. The normal distribution properties of the variables were examined using the Shapiro-Wilk test. Chi-square and Fischer’s exact test were used to compare categorical variables. The Mann-Whitney-U test was used in groups that did not fit the normal distribution. Multiple logistic regression analysis was used to determine the factors affecting the development of OHSS. Categorical measurements were given as numbers and percentages, while continuous measurements were given as median and minimum-maximum. 

*p <0.05* was considered statistically significant in all tests.

**Results**

Median age (*p* <0.001), platelet counts (*p* <0.001), and HTC values (*p* <0.001) of cabergoline group were significantly lower than those treated with calcium gluconate. However, in addition to the number of follicle (*p* <0.001) and oocyte (*p* <0.001), the number of IVF attempt (*p* <0.001), BMI (*p* <0.001), AMH (*p* <0.01), HbA1C values (*p* <0.001), gonadotropin usage time (*p* <0.001) and dose (*p* <0.001), E2 and progesterone values on the day of hCG (*p* <0.001, *p* <0.001) MI (*p* <0.001) and MII oocyte (*p* <0.001), 2 PN zygote (*p* <0.001), cleavage stage (*p* <0.001) and blastocyst stage embryo (*p* <0.001) were significantly higher in those treated with cabergoline than those treated with calcium treatment (Supplementary Tables I and II).

The history of OHSS was significantly higher in the cabergoline group than in the calcium group (*p* <0.001). The need for paracentesis was higher in those given cabergoline than those given calcium (*p* <0.001). The number of patients using metformin due to PCOS was significantly higher in the cabergoline group than in the calcium group (*p* <0.001). Similarly, GnRH antagonist use after OPU was significantly higher in those taking cabergoline than those taking calcium (*p* <0.004). During OPU, the number of eggs collected below 20 and between 20-40 was higher in the calcium group, while the number of eggs between 40-60 and above 60 was significantly higher in the cabergoline group (*p* <0.001) (Supplementary Table II).

When the groups were compared in terms of the incidence of OHSS (Supplementary Table II), the frequency of mild OHSS was significantly higher in the calcium group than in the cabergoline group (89.5% vs. 57.7%, *p* <0.001). Moderate OHSS was observed in 184 people in the cabergoline group (42.3%), while it was observed in
32 women (7.8%) in the calcium group, and the difference between the two groups was statistically significant ($p<.001$). Severe OHSS was detected in 11 individuals in the calcium group (2.7%), while it was not detected in anyone in the cabergoline group (0%). The difference between the two groups in terms of severe OHSS was statistically significant ($p<.001$). There was no significant difference between the two groups in terms of clinical pregnancy (52.4% vs. 55.2%, $p=.44$), frequency of miscarriages (2% vs. 3.4%, $p<.208$) and live birth rates (47.8% vs. 50.8%, $p<.409$).

Two different methods were used to examine the factors affecting OHSS (Supplementary Tables III and IV). In the first method, multiple logistic regression analysis was performed by including all parameters, including the OHSS history, into the model. In the second method, other parameters were included without adding the OHSS story to the model. In the regression analysis by adding the history of OHSS (Supplementary Table III), age 0.8 fold (OR=0.851), number of follicle 1.0 fold (OR=1.077), BMI 1.0-fold (OR=1.076), AMH 0.8-fold (OR=0.869), the number of MI oocyte 1.3 fold (OR=1.342), the number of MII oocyte 1.2-fold (OR=1.221), OHSS history 177-fold (OR=177.304), paracentesis 152-fold (OR=152.050), number of oocyte (<20) 18-fold, number of oocyte (20-40) 72-fold, and severity of OHSS 5.3 fold contributed to the development of OHSS. In addition to use of metformin or cetrotide after OPU, clinical pregnancy, live birth and miscarriage rates did not affect the development of OHSS.

In the regression analysis without adding OHSS history (Supplementary Table IV), age 0.8-fold (OR=0.830), number of follicles 1.0-fold (OR=1.086), number of trials 1.4-fold (OR=1.475), BMI 1.1-fold (OR=1.125), AMH 0.8-fold (OR=0.872), HbA1C 1.3-fold (OR=1.328), progesterone on the day of hCG 1.1-fold (OR=1.140), MI oocyte 1.3-fold (OR=1.310), MII oocyte 1.2-fold (OR=1.256), 2 PN zygotes 0.8-fold (OR=0.855), paracentesis 138-fold (OR=138.057), the number of oocytes (<20) 19-fold, the number of oocyte (20-40) 83-fold, and OHSS severity 4.9-fold (OR=4.927) were contributing to the development of OHSS. Clinical pregnancy and live birth rates did not contribute to the development of OHSS. In the regression analysis without OHSS, the number of IVF attempt, HbA1c, hCG day P4 value, and the number of 2 PN zygotes also contributed to the development of OHSS.

**Discussion**

OHSS is an iatrogenic complication of assisted reproductive techniques, characterized by increased arteriolar vasodilation and impaired permeability, resulting in a shift of intravascular fluid to the extravascular space. Since mild form OHSS is seen in approximately one third of IVF cycles, preventive measures are directed towards preventing the development of moderate and severe OHSS. Severe OHSS develops about 2-3% of cycles. A recent Cochrane review has listed the following seven methods for preventing the development of OHSS; Use of metformin in women with PCOS, use of GnRH antagonist protocol or clomiphene citrate for COS, use of dopamine agonist for VEGFR-2 blockade, use of volume expanders for hypovolemia, coasting, and use of progesterone for luteal phase support. However, neither method alone or in combination has been able to provide complete prevention. For this reason, research continues to develop new methods and medications to prevent OHSS.

By making hypovolaemic hyponatraemia and haemoconcentration OHSS reduces intracellular calcium and increases Ca^2+/-mediated-renin-angiotensin II-VEGF release. The continuation of VEGF synthesis due to hypocalcemia is also an obstacle to fully recover from OHSS. Calcium gluconate infusion has been introduced to prevent VEGF synthesis and development of OHSS by blocking the renin-angiotensin II-VEGF pathway. When the literature was reviewed, five studies aiming to prevent the development of OHSS were found by infusing calcium gluconate in patients at high risk for OHSS (Supplementary Table V). While calcium infusion was used in all of these five studies, either cabergoline or placebo was used in the patients in the control group. In only one study, calcium infusion was used in combination with other preventive methods. As a common result of all studies calcium gluconate infusion prevented the moderate and severe OHSS, equivalent to cabergoline. When compared with placebo, calcium gluconate infusion significantly reduced the progression of both moderate and severe OHSS.

Our results, on the other hand, were similar to the literature data at some points and completely different at other points. Mild OHSS was developed in 367 patients receiving Calcium gluconate infusions, while 251 patients in the cabergoline group developed mild OHSS. Mild OHSS frequency detected in the calcium group was sig-
nificantly higher than the cabergoline group. In previous studies\(^{20-22}\), moderate and severe OHSS incidences were compared and mild OHSS was not taken into account because it occurs in approximately 30% of normal cycles. In our study, mild OHSS was detected in 89% of the cases in the calcium group and in 73% of the cases in the cabergoline group, and these data are significantly higher than the literature data (30%). The high incidence of mild OHSS, despite calcium and cabergoline treatment, can be interpreted as both drugs reduce the transition from mild form OHSS to moderate or severe OHSS.

Moderate OHSS was detected in 32 women (7.8%) in the calcium gluconate group, while moderate OHSS was observed in 184 people (42.3%) in the cabergoline group. Calcium infusion significantly reduced the development of moderate OHSS compared to cabergoline therapy \((p < .001)\). The reason why cabergoline cannot sufficiently reduce moderate OHSS can be attributed to its partial inhibition of VEGFR2. Cabergoline inhibits VEGFR2 phosphorylation in the ovaries, thus preventing VEGF from binding to this receptor to some extent. However, since this is not a complete receptor blockade, luteal angiogenesis continues despite decreased permeability, which prevents a complete recovery in OHSS\(^{23}\). In the group given calcium gluconate, increased calcium levels may have reduced the development of moderate OHSS by preventing the release of cAMP-mediated-renin-angiotensin II-VEGF\(^{10}\).

While severe OHSS developed in 11 patients (2.7%) in the calcium gluconate group, severe form of OHSS did not develop in the cabergoline group (0%, \(p < .001)\). Since the frequency of moderate OHSS is low in those taking calcium gluconate, some of the mild forms may have turned into severe OHSS. Despite Calcium treatment, we can explain the development of severe OHSS in 11 patients with juxtaglomerular cells (JG) dysfunction. Calcium and cAMP regulate the release of renin in the JG apparatus. Increase in cAMP and decrease in calcium in JG cells increase the release of renin. Calcium reduction activates calcium-inhibitable isoforms of adenylyl cyclase (types V and/or VI) in JG cells. Type V or VI adenylyl cyclase firstly increases cAMP level and subsequently both renin and VEGF secretion\(^{10}\). Calcium gluconate, which we use in the prevention of OHSS, inhibits calcium-inhibitable isoforms of adenylyl cyclase by increasing JG cell calcium and prevents the release of cAMP-mediated-renin-angiotensin II-VEGF\(^{10}\). We do not know whether calcium levels in the circu-

Conclusions

Our results were quite different when compared with the other five studies using calcium gluconate for the prevention of OHSS in the literature. Although the results of patients in the cabergoline group were consistent with the literature we showed
that calcium gluconate treatment did not prevent the development of severe OHSS. Considering the high number of participants, we can say that calcium gluconate infusion is not very effective in the prevention of OHHS. However, our results need to be confirmed with extensive studies.

**Ethics Approval and Consent to Participate**

The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and ethical approval was received. Informed consent was obtained from all participants at the time of enrollment.

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**Authors’ Contributions**

N.T., C.K. and R.O. conceived, designed and performed the study procedures; C.K., N.T. and R.O analyzed the data and contributed reagents and materials; N.T. wrote the paper.

**Conflicts of interest**

The authors declare no conflicts of interest.

**References**


17) Golan A, Ronel R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome:


