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The Effect of Magnesium Sulfate Administration at Day of Embryo Transfer on Improving Subendometrial Blood Flow, Endometrial Receptivity, Implantation Rate and Clinical Pregnancy Rate.

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ABSTRACT

For successful implantation, a high-quality embryo, receptive endometrium, and tight communication between the two are required. The lack of uterine contractions during embryo transfer is one of the characteristics that greatly affects endometrial receptivity. This is because it has been observed that ET may cause uterine contractions, which may be harmful to embryonic apposition and endometrial blood flow .The aim of this study was to assess how magnesium sulfate administration on the day of embryo transfer improve sub endometrial blood flow, endometrial receptivity, and increase implantation rate and clinical pregnancy rate . Eighty infertile women undergoing ART cycles participated in this prospective. Recombinant FSH (Gonal-F) was used in a controlled ovarian hyperstimulation technique. The first group A (n=40) got a 4gm infusion of magnesium sulfate, while the second placebo control group B (n=40) received 200ml of 5% glucose water as a placebo, respectively. While there were no significant changes in body mass index (BMI), endometrial thickness, number of transferred embryos, or embryo transfer day, significant variations were seen in patient mean age ($p<0.001$) and mean number of grades I embryo ($P=0.003$). It was significantly different after magnesium sulfate administration with the lowest value in magnesium sulfate group ($P=0.024$; $P <0.001$ and $P <0.001$ respectively). Implantation rate and clinical pregnancy rate both increased after drugs administration although it was non- significant ($P= 0.106$ and $P= 0.348$ respectively). It appears that magnesium sulfate injection before to embryo transfer can significantly increase sub endometrial vascularity and endometrial receptivity and improving implantation rate and clinical pregnancy rate non significantly in ICSI cycles by lowering uterine muscular

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KEYWORD

Endometrial Receptivity, Sub endometrial blood flow, Magnesium Sulfate, Pregnancy ,2D Power Doppler

1. *Introduction*

Successful implantation requires a high-quality embryo, a receptive endometrium, and close communication between the two. Despite the fact that high-quality embryos were transferred (as specified by conventional embryological procedures), the majority of embryos do not implant, making the assessment of endometrial receptivity crucial **(Sardana D et al., 2014 [1])**. Embryo quality, endometrial receptivity, and using proper embryo transfer procedures are the main factors that impact conception. An important step in assisted reproduction is endometrial evaluation. Numerous studies have been conducted in this area to increase endometrial receptivity **(Eftekhar M et al., 2020 [2])** .

To increasing endometrial receptivity, four policies were recommended; it was recommended to avoid the endometrium during stimulated cycles, to promote uterine vascularization, to treat the pathology, and to establish ovarian stimulation protocols that produce a minimum drop in endometrial receptivity or may even increase it **(Elnashar and Aboul-Enein, 2004 [3]; Jain S et al., 2018 [4])**. The lack of uterine contractions during embryo transfer is one of the elements that greatly affect endometrial receptivity since ET may cause uterine contractions, which might be harmful to embryonic apposition and endometrial blood flow **(Saravelos and Chiu Li., 2019 [5])**. Endometrial disruption from uterine hyperperistalsis can result in decreased blood flow, which can cause tissue hypoxia and severely impact endometrial receptivity and embryo implantation **(Munro MG., 2019 [6])**.

The endometrium's good blood supply is recognized as a need for implantation **(Prasad S et al., 2017 [7])**. Studying endometrial blood flow has become a key method of determining endometrial receptivity using 2D or 3D power Doppler ultrasonography because it more accurately represents uterine receptivity than the uterine artery **(Altriagey et al., 2019 [8]; Lv H et al., 2020 [9]).** Regardless of the endometrium's morphological appearance, endometrial vascularity offers a useful prognostic value for the implantation rate in IVF cycles **(Choudhary M et al. 2017 [10])**. Different methods were used in order to improve endometrial receptivity and improve ICSI outcomes. One of them was the use of a tocolytic drug, namely atosiban by **(Yuan C et al., 2019 [11])**; our novel research try to study the effect of magnesium sulfate in improving sub-endometrial blood flow, endometrial receptivity, and ICSI outcomes. In obstetrics, magnesium sulfate prevents myometrial contractions in preterm labor. It is believed to directly affect the smooth muscle of the uterus by opposing calcium both intracellularly and extracellularly **(Di Renzo GC et al., 2017 [12])**. Magnesium may also influence calcium channels by having a conflicting impact. Together, these actions lower intracellular calcium levels, preventing the actin and myosin complexes from activating **(Kuijsters N et al., 2017 [13])**.

2. Patients and Methods

A prospective randomized clinical trial of 80 infertile women 20 to 39 years old who having undergone their first IVF-ET with a GnRH antagonist protocol and stimulation by recombinant follicle-stimulating hormone (FSH) (rFSH) was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University; all cases were under the age of 40, had normal uterine cavities on hysterosalpingiography, saline infusion sonography, and ultrasound scanning, had body mass indices of 18 to 30 kg/m2, and had ART recommended by an infertility specialist because of ovulation disorders, tubal factors, unexplained infertility, mild cases of endometriosis, male factor, or a combination of male and female factors with the transfer of the good quality embryo. While uterine abnormalities instances were disregarded. To prevent selection bias, all patients were enrolled for just one cycle .

The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/AlNahrain University's Ethical Committee approved the trial's ethical procedures and required that all patients provide written informed permission before taking part.

1- Controlled ovarian stimulation and IVF-ET procedures

For regulated ovarian stimulation, all patients received a GnRH agonist regimen. All patients were enrolled in the flexible antagonist regimen, which began on day 2 of the menstrual cycle. An ultrasound examination was performed to rule out any patients who had ovarian cysts and to measure the endometrial thickness. It entailed starting the menstrual cycle with ovarian stimulation with gonadotropin (rFSH) (Gonal-F; Merk - Serono, Switzerland), followed by an injection of a GnRH antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland). The adaptable approach was injecting 0.25 mg of Cetrorelix acetate into the biggest follicles when they reached 13-14 mm in size. Transvaginal ultrasound was done on the 5th day of stimulation, and subsequent scan was done every 2-3 days as necessary. Transvaginal ultrasonography and the serum E2 level were used to monitor follicle development up to the day that hCG was administered. Subcutaneous injection of (Ovitrelle) 250 microgram/0.5 ml s.c. (Ovitrelle®; Merck Serono, Switzerland) was used to initiate ovulation. Oocytes were retrieved approximately 34–36 hours after Ovitrelle administration by aseptic oocyte aspiration transvaginal ultrasound-guided, under general anesthesia. Preparation of semen was done by the swim-up method. All patients had intracytoplasmic sperm injection (ICSI) within 4-6 hours of oocyte retrieval. After ICSI for 12 to 17 hours, the presence of two pronuclei with two fragmented or distinct polar bodies indicated normal fertilization. The development of the embryo, including the number of cells and level of fragmentation, was evaluated 48- or 72 hours following insemination or ICSI (day 2 or 3). On the day of ET, therapeutic treatments were carried out following microinjection and embryo development, and the study group (N=80) was randomly split into two groups. Half an hour before embryo transfer, Group A(n=40) got a 4-gm infusion of magnesium sulfate over 20 minutes (Magnesium sulfate ampoule 50% 2.5 gm in 5 ml, Pioneer, Sulaymaniyah, Iraq), whereas placebo control Group B(n=40) received glucose water 5% 200 ml (Glucose 5%(W/V) 500 ml, B. Braun, Germany). Transvaginal color and power Doppler ultrasonography was used to measure blood flow indices shortly before (ET), before and after receiving medication. Two or three high-quality embryos were transplanted after the cultivated embryos' quality was evaluated. The luteal phase has been supported with vaginal progesterone (400 mg twice daily). A serum ß-HCG test was carried out 14 days after the embryo transplantation. An ultrasound was performed on the lady, who subsequently got a positive result to confirm the presence of one or two gestational sacs, indicating clinical pregnancy **(Pandey S et al., 2012 [14])**.

2- Two-Dimensional Power Doppler Ultrasound of Endometrial and Sub endometrial Zones

Transvaginal ultrasound scanning was performed to assess the endometrial thickness, regularity, and echogenicity, as well as color Doppler indicators of sub-endometrial blood flow (S/D ratio, PI, and RI). The zones of vascular penetration had previously been identified as follows:

Zone 1: The zone of myometrium adjacent to the subendometrium

Zone 2: The outer hyperechogenic sub-endometrial zone

Zone 3: The inner hypoechogenic zone

Zone 4: The endometrium

The SonoAce-X6 Ultrasound Set's 6 MHz vaginal probe was used to do the transvaginal scan (Medison, Seoul, South Korea). While the patient was in the dorsal lithotomy posture, ultrasound scans were completed. The endometrial thickness was determined by measuring the maximum thickness between the strongly reflective endometrial-myometrial junction contacts with the uterus in the sagittal plane. Three measurements were done, and the average value was recorded. The measurement encompassed both endometrial layers but excluded the nearby low-amplitude echo layer. Following the B-mode test, blood flow analysis was performed using pulsed Doppler equipment. At the endometrial periphery, sub-endometrial arteries were visible; on rare occasions, they even broke through the hyperechogenic endometrial edge and entered the endometrial cavity **(Elnaggar A et al., 2017 [15]; Jasim et al., 2021 [16])**. The pulsed Doppler function was turned on when the Doppler gate was over the colored region at Zone 2 to get the blood flow velocity waveforms from the sub-endometrial arteries. A recording was regarded as satisfactory when at least three consecutive uniform waveforms were made, each of which displayed the maximum Doppler shift. Each parameter was subjected to three measurements, with the average value being recorded. Using the following formula, the ultrasonography automatically calculated the Resistance Index (RI) and Pulsatility Index (PI) for both sub-endometrial vasculatures:

RI=**PSV**−**EDV**/PSV

PI=**PSV**−**EDV**/**mv**

mv=**PSV**+**EDV**/2

(Perfusion Index) =S/D ratio

PSV: Peak Systolic Velocity, EDV: End-Diastolic Velocity, mv: mean velocity .

3- **Statistical Analysis**:

The data was analyzed by the Statistical Package for Social Sciences (SPSS) version 22.0. ANOVA, independent sample t-tests, chi-square. The findings were considered statistically significant whenever the p-value was equal to or less than 0.05. The study's primary objective was to assess the effect of magnesium sulfate administration on blood flow indices and compare the two groups' blood flow indices and ICSI results (implantation rate and clinical pregnancy rate).

3. Results

The study's eligibility requirements were satisfied by a total of 80 cycles, which were classified at random, split into two groups (N=40), and made accessible for analysis. These cycles employed a brand-new GnRH antagonist technique. Magnesium sulfate infusion was given for group A, and 200ml glucose water 5% was given for group B (placebo-control group). Characteristics of both groups at the day of ET were illustrated in Table (1). There was a significant difference in the mean ages between the two groups; group A was older than group B (31.75±4.01 and 27.70±4.35 years) respectively and ($p<0.001$). While there was no significant difference in mean BMI between the two groups ($p=0.571$), the values were for A (24.42±3.06) and B (24.77±2.49 kg/m2). There was also a significant difference in the mean number of grade I embryos (P=0.003) but no significant difference in the mean number of grade II embryos (P=0.275), number of transplanted embryos (P=0.723), and endometrial thickness between the research groups (P=0.857), hormonal level at day of ET regarding E2(P= 0.094) and P4 level (p= 0.180) and day of embryo transfer whether transferring of embryos done at day 2 or 3 (p=0.217).

When we compare the two groups according to the blood flow indices at the day of ET before drug administration and categorize them regarding to the S/D ratio, RI, and PI, as shown in (table 2), There were no significant variations in S/D ratio between research groups before receiving medication (P=0.902). Also, there were no significant variations in RI (P=0.544) or PI (P=0.786) before receiving medication.

When we compare the two groups according to the blood flow indices at the day of ET after drug administration and categorize them regarding to S/D ratio, RI, and PI, as shown in (table 3), there were significant variations in S/D ratio across research groups following medication delivery (P=0.024). Also, there were significant variations in RI (P <0.001) and PI (P <0.001) following receiving medication, as shown in Figure (1).

When we compare the two groups based on ICSI-ET results in terms of implantation rate and clinical pregnancy rate, as shown in Figure (2), there were increasing in IR (20 % versus 11% with P= 0.106) and CPR (40 % versus 30 % with P= 0.348) in magnesium sulfate group more than control group respectively although it was non-significant.

Table (1): Characteristics of the study groups at the day of embryo transfer

Variables		Magnesium Sulfate group (A)	Control group (B)	*P*-value
Age (years) (Mean ±SD)		31.75±4.01	27.70±4.35	<0.001* t-test
BMI (kg/m^2) (Mean ±SD)		24.42±3.06	24.77±2.49	0.571 t-test
EMT (mm) at the day of ET (Mean ±SD)		8.71 ± 0.92	8.68 ± 0.93	0.857 t-test
Grade I embryo (Mean ±SD)		2.48± 1.33	1.70 ± 0.85	0.003*
Grade II embryo (Mean ±SD)		1.85 ± 1.05	2.10 ± 0.98	0.275
Number of transferred embryos (Mean ±SD)		3.33 ± 0.57	3.28 ± 0.67	0.723 t-test
Hormonal level at the day of ET (Mean±SD)	Estradiol (pg/ml)	754.73±415.25	962.82±652.63	0.094 t-test
	Progesterone (ng/ml)	13.18±14.51	17.72±15.52	0.180 t-test
ET Day Number (%)	Day 2	14 (60.9%)	9 (39.1%)	0.217 Chi-square test
	Day 3	26 (45.6%)	31 (54.4%)	

SD: Standard deviation; BMI: Body mass index; EMT: Endometrial thickness ;ET: Embryo transfere *: p value ≤ 0.05 (significant)

Table (2): Comparison of the blood flow indices at the day of ET before drug administration between study groups

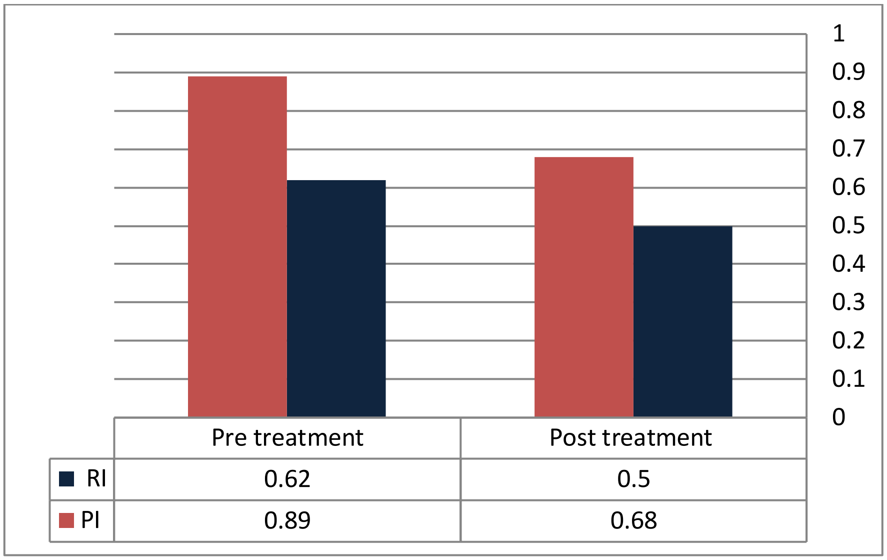
Variables	**Magnesium Sulfate group (A)**	**Control (B)**	***P*-value (Independent sample t-test)**
	Mean± Standard deviation		
S/D ratio	3.56± 3.98	3.67 ± 3.87	0.902
RI	0.62 ± 0.11	0.60 ± 0.13	0.544
PI	0.89 ± 0.23	0.91 ± 0.32	0.786

*: p value ≤ 0.05 (significant)

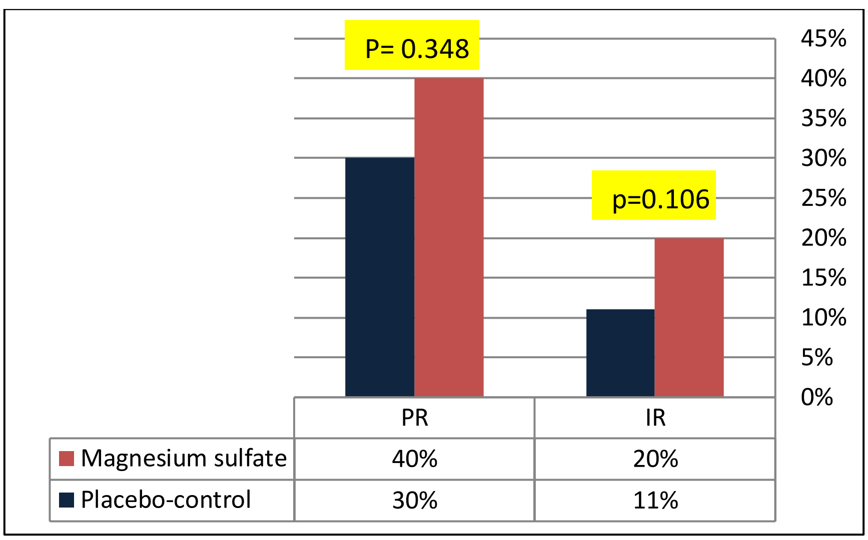
Table (3): Comparison of the blood flow indices at the day of ET after drug administration between study groups

Variables	**Magnesium Sulfate group (A)**	**Control (B)**	***P*-value (Independent sample t-test)**
	Mean± Standard deviation		
S/D ratio	2.11± 0.47	3.32 ± 3.22	0.024 *
RI	0.50 ± 0.09	0.59 ± 0.12	<0.001*
PI	0.68 ± 0.17	0.89 ± 0.30	<0.001*

*: p value ≤ 0.05 (significant)



(Figure 1): Pre and Post-treatment effects with magnesium sulfate on RI and PI



(Figure 2): ICSI outcomes (implantation rate and clinical pregnancy rate) among study groups

4. Discussion

The study's eligibility requirements were satisfied by a total of 80 cycles, which were classified at random, split into two groups (N=40), and made accessible for analysis. These cycles employed a brand-new GnRH antagonist technique **(Sharma N et al., 2019 [16]; Robert CA et al., 2019 [17])**. Blood flow of the endometrium can be studied by 2D color Doppler ultrasound. Proper vascular development at the time of embryo implantation is vital for a successful pregnancy. Abnormal angiogenesis leads to reproductive failure **(Chen et al., 2017 [18]).** Many researchers try to improve endometrial receptivity by different measures, one of them by the use of tocolytic drugs in order to decrease uterine contraction and improve sub-endometrial vascularity. The current novel study uses magnesium sulfate and try to investigate its effect on improving endometrial receptivity. Because women were assigned to the research groups at random and there was no bias in the selection of study participants by ultrasound, our study found no changes in S/D ratio, RI, or PI between the study groups prior to medication administration. Sub-endometrial Doppler blood flow indices (S/D ratio, RI, and PI) after drug administration, however, demonstrate significant differences between study groups, with magnesium sulfate having the lowest value. This indicates that magnesium sulfate's tocolytic effect on uterine contractility was significantly effective in lowering the S/D ratio as well as systolic blood pressure, RI, and PI. This drug's lowering action improves sub-endometrial blood flow, enhanced endometrial perfusion, and increases endometrial receptivity, implantation rate, and clinical pregnancy rate, all of which have an impact on the success of ICSI. $MgSO_4$ has a tocolytic effect by acting as a competitor for calcium in the sarcoplasmic reticulum, decreasing the interaction of calcium with the actin-myosin complex and interfering with myometrial repolarization. It is a direct effect on the smooth muscle of the uterus by opposing calcium both intracellularly and extracellularly **(Di Renzo GC et al., 2017 [12]; Osaghae BE et al., 2020 [19])**. MgSO4 reduces striated muscle contractions and blocks peripheral neuromuscular transmission by reducing acetylcholine release at the myoneural junction, slowing contractions. However, MgSO4 inhibits calcium-dependent myosin light-chain kinase phosphorylation by competitively blocking the calcium influx through the voltage-dependent calcium channels **(Catterall WA., 2011 [20])**. Since this study is the first to suggest the potential advantages of magnesium sulfate in enhancing endometrial receptivity and ICSI-ET outcomes in the ART cycle, to the best of our knowledge, no prior comparative information was available with the current literature concerning the impact of magnesium sulfate on sub endometrial vascularity in ICSI-ET cycle.

The small sample size in this study is another drawback. According to the findings of this investigation, a trial that is considerably bigger would be necessary to show that the suggested therapy has a therapeutic advantage, given that magnesium sulfate has not yet been tested in ART in any other published research. Numerous research has looked at different additional pharmacological substances in an effort to enhance endometrial receptivity and so improve ET outcomes. By reducing intrauterine prostaglandin synthesis and enhancing uterine blood flow, atosiban lowers UC. Both elements might be advantageous for embryo implantation **(Pierzynski P., 2011 [21]; Lan VT et al., 2012 [22]; Schwarze JE et al., 2020 [23])**. Additionally, Moraloglu et al. shows that using tocolytics prior to ET alters the endometrial environment by reducing uterine contractile activity and increasing endometrial perfusion and receptivity, which enhances IVF-ET pregnancy outcomes **(Moraloglu O et al., 2010 [24])**. In recent IVF cycles, significant endometrial blood flow on the day of embryo transfer has been associated with improved endometrial receptivity; it is possible that this is correlated with high pregnancy success **(Kim A. et al., 2014 [25]) .**

In the current study, the characteristics of ET day were evaluated, such as age, BMI, EMT, and embryo transferred numbers and day of embryo transfer. There was a significant difference in mean age with the older women in the magnesium sulfate group. Although they are older but, the effect of magnesium sulfate counteract this differences and improves ICSI outcome, although the effect of age in some studies suggested that poor pregnancy outcome and decrease fertilization rate may be associated with increase maternal age **(Pierce & Mocanu, 2018 [26])**. Additionally, there were changes in the mean number of grades I embryos that were higher in the magnesium sulfate group than the control group that were statistically significant, and this put our study in agreement with Sivrikoz et al. conclusion's that High-quality embryos have a beneficial effect on pregnancy rates and ICSI results **(Sivrikoz et al., 2021 [27])**. The current study demonstrated no statistically significant difference in E2 and P4 levels between the two groups on the day of ET. On the day of ET, no correlation between serum E2 levels and the results of ICSI were found to be significant. According to Mackens S. et al., there was no discernible correlation between the blood E2 levels on the ET day and the success rates of ICSI. They contend that this may be explained by the fact that at the time of ET, women had supra-physiological E2 concentrations due to COH. As a result, during the follicular phase of COH, the maximum effects of E2 had already been attained, and during the early luteal phase, the uterus had stopped responding to changes in E2 level

(Fanchin et al., 1998 [28]; Mackens S et al., 2020 [29]) which was similar to current study while Li Q et al., suggests that elevated E2 levels may negatively affect pregnancy outcomes during cleavage-stage embryo transfers **(Li Q et al., 2022 [30]).**

There was no discernible difference in progesterone levels between the magnesium sulfate group, which had the lowest amount, and the other groups. When evaluated on the transfer day, progesterone levels were shown to have no significant effects on pregnancy and delivery rates in related research by Metello et al.; high P4 levels have also been linked to poorer outcomes, but less consistently. On the other hand, progesterone was discovered to be crucial for the endometrial support, implantation of an embryo, and pregnancy maintenance in natural cycles, fresh IVF cycles, and frozen embryo transfer (FET) cycles. As a result, Fanchin et al. and Labarta E et al. discovered that a P4 value below 9.2 ng/ml on the day of ET was associated with a decreased rate of ongoing pregnancy **(Fanchin et al., 1998 [28]; Labarta E et al., 2021 [31])**.

6- Conclusion

It appears that magnesium sulfate injection before to embryo transfer can significantly increase sub-endometrial vascularity and endometrial receptivity and improving implantation rate and clinical pregnancy rate non significantly in ICSI cycles by lowering uterine muscular spasm.

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Author Contribution

Iqbal A. Fahad performed the study, and Lubna A. Al-Anbari Muayad S. Abood supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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