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Original Article

Evaluating the Effect of Oxytocin and Zinc Sulphate on Reproductive Indices in Opiate-Addicted Men Receiving Methadone Maintenance Therapy: A Randomized, Double-Masked Clinical Trial

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Abstract

Background: Despite considerable advances in comprehending the neurobiology of drug addiction, available treatment options remain limited in terms of effectiveness and are often accompanied by adverse side effects that contribute to relapse. Notably, oxytocin administration has demonstrated potential in influencing neurobehavioral changes related to drug abuse. Furthermore, the effect of zinc on male fertility has been established. This study aimed to assess the impact of combining oxytocin with zinc sulfate on reproductive parameters in men addicted to opioids who are undergoing methadone maintenance therapy.

Methods: This research followed a double-masked randomized controlled clinical trial design and involved 40 men with opioid dependence receiving methadone treatment. Patients were randomly assigned to one of two groups: a control group receiving a saline nasal spray and a zinc sulfate placebo, and an intervention group receiving 40 international units of oxytocin nasal spray in combination with 220 mg zinc sulfate capsules, daily. Baseline evaluations of testosterone, oxytocin, sperm characteristics, and zinc levels were conducted three days after a 5–10 mg reduction in methadone dosage. Subsequently, a two-week treatment regimen was administered, followed by post-intervention assessments.

Findings: The age of participants was comparable in the control and intervention groups. Zinc levels in the control and treatment groups showed no significant difference before the intervention, while a significant increase was seen after the intervention in the treatment group ($P=0.023$). A significant increase was reported in testosterone levels ($P=0.002$) and the active motility of sperm ($P=0.015$) in the treatment group after the intervention. The intervention led to a significant reduction in the total abnormality of sperm ($P<0.001$). Prescription of nasal oxytocin spray with zinc supplementation can be an effective remedy in improving reproductive indices in opiate-addicted men and could be considered in addiction management guidelines. The present study is one of the first studies evaluating the effects of combined oxytocin and zinc supplementation on improving the reproductive indices in opiate-addicted men.

Conclusion: According to the results, the combination of oxytocin and zinc supplementation had a positive and significant effect on sperm parameters in opioid-dependent men undergoing methadone treatment.

Keywords: Opiate addiction, Oxytocin, Zinc, Testosterone, Sperm indices

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Introduction

Drug addiction poses a substantial challenge to male reproductive health, impacting critical sperm parameters. Chronic drug use, particularly opioid use, can disturb the intricate balance of hormonal regulation and neurobiological systems, consequently affecting sperm quality and function. These disruptions often lead to decreased sperm count, motility, and morphology, vital determinants of male fertility.[1,2] Moreover, the commonly employed methadone maintenance therapy, while indispensable for managing addiction, may contribute to sexual dysfunction.[3,4] The complex interplay between drug addiction and sperm parameters is well-documented in studies.[1,2,5,6] Paradoxically, the use of substances such as cannabis, opioids, and heroin has been associated with decreased sperm motility, and the movement of sperm toward the egg is impaired. Moreover, it reduces sperm concentration and increases the number of morphologically abnormal spermatozoa; in particular, heroin increases the probability of protamine-deficient spermatozoa, which is necessary for chromatin condensation. They also indicate opioids' ability to decrease semen quantity and quality by restricting the generation of seminal fluid. Moreover, substances like crack and cocaine affect sperm DNA fragmentation, thus making it easy for men to become impotent or have genetic alterations in their children.[6-9]

Oxytocin, a neuropeptide renowned for its multifaceted physiological and behavioral effects, has emerged as a promising intervention in substance abuse. It not only mitigates the reward-seeking factor in drug addiction but also ameliorates withdrawal-related stress and social consequences, ultimately preventing relapse.[10] Clinical studies have demonstrated oxytocin's potential in reducing the abuse of substances like heroin, cocaine, alcohol, and hashish.[11-13] Notably, it decreases drug-seeking behaviors, possibly by modulating the reward circuitry, involving glutamate and dopamine, thereby diminishing the desire for and motivation toward reward.[10] Accepting oxytocin as an important regulator in male reproductive function relies on identifying oxytocin receptors in male reproductive tissues. *In vitro* evidence supports oxytocin's role in spermatogenesis and enhancing seminal fluid output and sperm count. It facilitates the transport of sperm through the vas deferens and ejaculation. Additionally, oxytocin contributes to male sexual arousal and penile erection.[14]

The homeostasis of trace elements in the human body is pivotal for overall health. These elements encompass compounds essential for physiological functions and natural development in living organisms. Elements like copper, zinc, and iron mainly act as catalysts for vital enzymatic processes. Zinc, the second most abundant trace element in the human body after iron, plays structural, catalytic, and regulatory roles. In the central nervous system, it influences synaptic plasticity, hormone secretion, and neuronal activity at the postsynaptic level. Studies suggest that zinc may alleviate specific opioid withdrawal symptoms in animals.[15-17]

Sufficient zinc content in semen is essential for normal sperm function and fertility; however, excessive zinc levels can harm sperm quality.[18] While zinc deficiency in semen may not directly cause infertility, studies have established a relationship between zinc levels in semen and both normal and pathological aspects of sperm function and male fertility.[18] Adequate zinc levels can counteract the adverse effects of smoking on sperm quality, particularly in smokers and individuals facing increased oxidative stress due to increased levels of reactive oxygen species (ROS).[18]

Although studies have indicated that zinc can alleviate opioid withdrawal symptoms in mice,[14] the generalization of such findings to humans remains uncharted territory. Moreover, the combined effect of oxytocin and zinc in opioid-dependent individuals under methadone maintenance has not been thoroughly investigated. Notably, drug addiction and methadone maintenance treatment may result in sexual dysfunction. The efficacy of oxytocin in improving sexual dysfunction, combined with the positive effect of zinc on male fertility, provided a foundation for the present study, which explored the impact of oxytocin and zinc sulfate on fertility indicators in drug-dependent men. Hence, this research aimed to investigate the impact of oxytocin in combination with zinc sulfate on individuals undergoing methadone maintenance treatment.

Methods

Study design

This research was structured as a double-masked, randomized controlled clinical trial. The present study was conducted on individuals who had an opioid addiction and were undergoing methadone treatment at the Educational & Treatment Center of Shahid Beheshti Hospital in Zanjan, Iran. The patients were randomly allocated to either the intervention or control group. This study acquired ethical approval from the Iranian National Committee for Ethics in Biomedical Research (ethical code: IR.ZUMS.REC.1401.096) and has been registered in the Iranian Registry of Clinical Trials (20221022056266N1) (https://irct.behdasht.gov.ir/trial/66785).

Study population and sample size

The study's population comprised men with opioid addiction undergoing methadone maintenance treatment in Zanjan city. Considering that it was a single-center study with a limited number of patients and the potential cultural considerations, we encountered some limitations in recruiting and following up the participants. In this study, the participants were selected using a convenience sampling method and underwent initial screening based on specific inclusion and exclusion criteria. Each group was initially intended to include 22 participants, but, ultimately, the study assessed 19 subjects in the control group and 21 in the treatment group.

Eligible patients met the following criteria: a DSM-5 diagnosis of opiate dependence, male sex, age between 20 and 50 years, current participation in methadone maintenance treatment (MMT), and a three-day interval following a recent methadone dose reduction of 5 to 10 mg before testing (in order to induce drug withdrawal symptoms in patients and test whether the intervention would reduce the withdrawal symptoms or not). Additionally, written consent was obtained from all participants. However, individuals who missed two sessions, used any narcotic or stimulant substances, consumed serotonergic drugs or other antidepressants, or used drugs known to affect the function of the hypothalamic-pituitary-adrenal (HPA) axis were excluded from the study.

Randomization

In this research, the patient allocation involved a random assignment to either the intervention or placebo group through a random number table. This random allocation method ensured that variables such as age and other potential confounding factors, both known and unknown, were evenly distributed among the groups at the study's outset. Consequently, we can assert that any observed associations are attributable to the intervention itself, rather than being influenced by these confounding variables.

Study interventions and outcomes

Following random allocation, the intervention group received a package containing oxytocin spray and zinc sulfate. Conversely, the control group received a comparable package based on the randomization code, which included a normal saline spray and placebo capsules.

The patients in the intervention group were administered 40 units of oxytocin spray, following the study by Moeini et al,[19] and 220 mg of zinc sulfate (comprising 50 mg of zinc, the maximum permissible dose without side effects) per session for two weeks. Conversely, the control group received a daily dose of normal saline spray and a placebo capsule.

The study duration for each patient spanned two weeks, and participants who missed two sessions, engaged in the use of any narcotic or stimulant substances, or exhibited non-cooperation were excluded. Blood and sperm samples were collected from each patient once three days after a 5–10 mg reduction in the methadone dosage and following urine tests for illicit substances and once after the intervention.

Before and after the intervention, assessments were made through the evaluation of blood serum zinc levels, blood testosterone levels, and spermogram tests.

Statistical analysis

The primary outcome of this study was the impact of the combined administration of oxytocin and zinc sulfate in the context of men's reproductive indices among opiate-dependent patients undergoing methadone maintenance therapy. This was declared in the study's objective and is quantified in terms of assessments of the semen characteristics, such as the semen volume, total sperm count, progressive motile sperm, and the percentage of abnormal spermatozoa. Secondary outcomes were biochemical changes, thus forming part of the study's secondary measures, and these are the blood concentrations of zinc and testosterone resulting from the treatment. These outcomes contribute to the main objective by offering an understanding of the bodily functions presumably affected by the treatment course. These biochemical parameters consist of zinc and testosterone levels that were tested and compared before and after the intervention, which consisted of oxytocin and zinc sulfate administration.

Data were collected using SPSS version 26 software. Descriptive statistics were generated to summarize the data. Frequency (number) and relative frequency (percentage) were reported for qualitative variables, while quantitative variables were summarized by reporting the mean and standard deviation.

The independent t test was employed to compare the control and treatment groups. Paired t test or Wilcoxon test was applied to analyze the data before and after intervention in each group (intergroup analysis) based on the normality of the data. Additionally, analysis of covariance (ANCOVA) was utilized to assess the impact of the prescribed treatment on the enhancement of sperm parameters during the intervention period (between-group). The results were reported as mean ± SD, and $P < 0.05$ was considered significant.

Results

The CONSORT diagram of the present study is shown in Figure 1.

Study population

The mean age of participants in the control and intervention groups was 34.32 ± 8.39 years and 33.81 ± 5.90 years, respectively, with no statistically significant difference between the two groups ($P = 0.825$). Regarding marital status, 47.4% of participants in the control group, compared to 52.6% of those in the treatment group, were single, and this difference was not statistically significant ($P = 0.554$). Furthermore, regarding educational attainment, 78.9% of the control group and 61.9% of the treatment group had high school diplomas, with no significant difference between the two groups ($P = 0.502$, Table 1).

Evaluation of zinc and testosterone levels

Table 2 illustrates the results related to zinc and testosterone levels. The initial zinc levels before the intervention exhibited a mean ± SD of 109.21 ± 31.35 in the control group and 99.45 ± 15.56 in the treatment group ($P = 0.222$, independent t-test).

Conversely, post-intervention zinc levels showed a significant difference, with a mean ± SD of 99.37 ± 25.01 in the control group and 119.33 ± 27.84 in the treatment group, indicating a notable increase in zinc levels in the treatment group ($P = 0.023$, independent t test).

The baseline testosterone levels before the intervention were 3.48 ± 1.98 in the control group and 2.53 ± 1.33 in the treatment group ($P = 0.080$, independent t test). After the intervention, testosterone levels were 2.74 ± 1.53 in the control group and 3.57 ± 1.35 in the treatment group; these values were not significantly different ($P = 0.076$, independent t test).

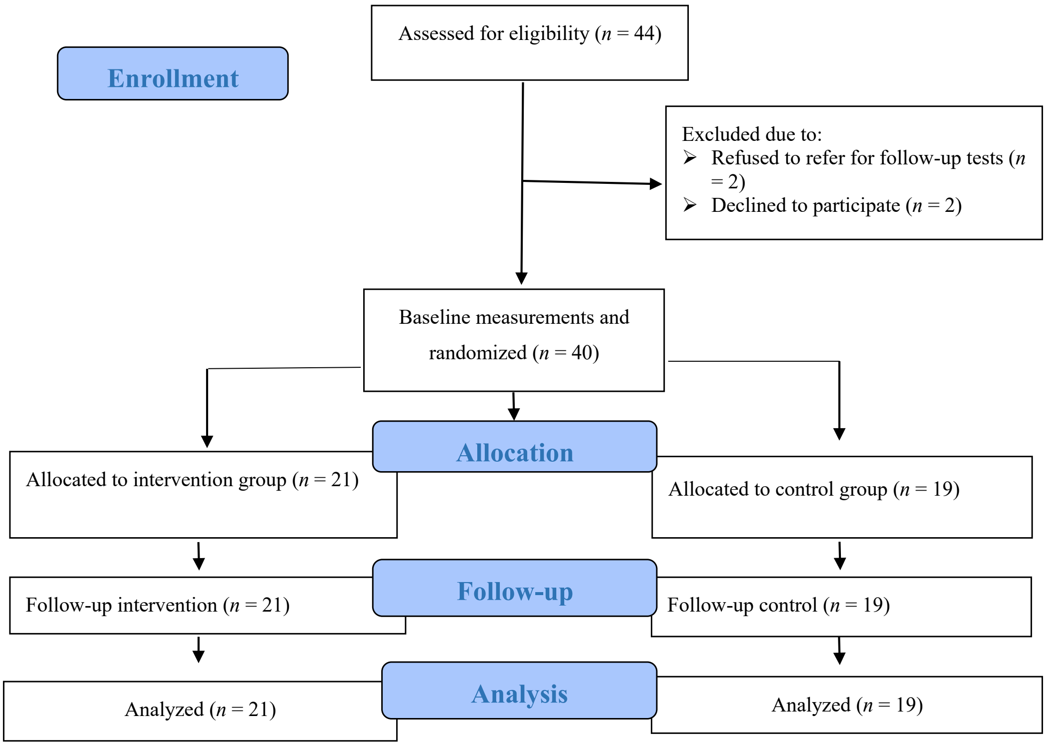

Figure 1. The study flowchart

Table 1. Demographic characteristics of evaluated patients

	Category	Control No. (%)	Treatment No. (%)	*P* value
Marital status	Single	9 (47.4)	8 (38.1)	0.554
	Married	10 (52.6)	13 (61.9)	
Education level	None, below high school diploma	15 (78.9)	13 (61.9)	0.502
	High school diploma	2 (10.5)	4 (19.0)	
	Higher education	2 (10.5)	4 (19.0)	

Furthermore, changes in zinc levels within the control group were not statistically significant compared to the baseline values (~10% decline, $P=0.293$, paired *t*-test). In contrast, the treatment group exhibited a substantial increase in zinc levels compared to the baseline state (~20% rise, $P<0.001$, paired *t* test). Similarly, the testosterone levels in the control group did not exhibit significant changes in comparison to baseline (20.57% decline, $P=0.115$, paired *t* test), while the treatment group displayed a significant increase in testosterone levels compared to baseline (29.1 % rise, $P<0.001$, paired *t* test).

The ANCOVA test results demonstrated that changes in zinc levels relative to the baseline were significant between the two groups ($P=0.001$). Additionally, the ANCOVA test indicated a significant post-intervention increase in testosterone levels in the treatment group compared to the control group ($P=0.002$).

Evaluation of sperm parameters

The results of sperm parameters are shown in Table 3. Semen volume in the control and treatment groups before the intervention was 2.92 ± 1.57 and 2.96 ± 1.08, respectively ($P=0.932$, independent *t*-test). Semen volume in the control and treatment groups after the intervention was 2.70 ± 1.42 and 3.77 ± 1.15, respectively, which was significantly different ($P=0.048$, independent *t*-test). The results showed that changes in semen volume in the control group compared to their baseline states were not significant; however, semen volume substantially increased in the treatment group ($P<0.001$; independent *t* test). Meanwhile, the results of the ANCOVA test showed that the changes in semen volume between the two groups compared to the baseline were significant ($P=0.007$). Moreover, total sperm count significantly increased in the treatment group after intervention ($P=0.001$, paired *t* test); however, no significant differences were observed between groups ($P=0.157$, ANCOVA).

The percentage of active motile sperm in the control and treatment groups before the intervention was 48.18 ± 25.23 and 47.50 ± 15.90, respectively ($P=0.935$, independent *t* test). The percentage of active motile sperm in the control and treatment groups after the intervention was 45.91 ± 20.71 and 58.57 ± 12.16, respectively, indicating no significant difference ($P=0.068$, independent *t* test). The results of the ANCOVA test showed that the intervention significantly improved the active motile condition after the intervention in the treatment group compared to the baseline and the control group ($P=0.015$).

The percentage of total abnormality in the control and treatment groups before the intervention was

Table 2. Zinc and testosterone levels in patients before and after treatment

	Control group			Treatment group			
	Before treatment	After treatment	*P* value[a] Intergroup	Before treatment	After treatment	*P* value[a] Intergroup	ANCOVA[b] Between groups
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		
Zinc	109.21 ± 31.35	99.37 ± 25.01	0.135	99.45 ± 15.56	119.33 ± 27.84	< 0.001	0.001
Testosterone	3.48 ± 1.98	2.74 ± 1.53	0.115	2.53 ± 1.33	3.57 ± 1.35	< 0.001	0.002

[a] Paired *t*-test; [b] ANCOVA.

Table 3. Evaluation of sperm parameters in patients before and after treatment

	Control group			Treatment Group			
	Before treatment	After treatment	*P* value Intergroup	Before treatment	After treatment	*P* value Intergroup	ANCOVA[c] Between groups
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		
Semen volume (mL)	2.92 ± 1.57	2.70 ± 1.42	0.552[a]	2.96 ± 1.08	3.77 ± 1.15	< 0.001[a]	0.007
Semen pH	7.84 ± 0.50	7.82 ± 0.06	0.341[a]	7.82 ± 0.10	7.80 ± 0.08	0.426[a]	0.637
Active motile (%)	48.18 ± 25.23	45.91 ± 20.71	0.730[a]	47.50 ± 15.90	58.57 ± 12.16	< 0.001[a]	0.015
Total sperm	44.48 ± 38.78	39.85 ± 30.40	0.449[b]	40.14 ± 43.06	51.07 ± 42.38	0.001[b]	0.157
Total abnormality %	20.18 ± 5.60	.55 ± 5.68	0.470[b]	28.21 ± 16.36	19.65 ± 11.34	0.001[b]	< 0.001
Grade III	23.91 ± 16.25	19.09 ± 11.36	0.258[b]	18.57 ± 11.17	23.64 ± 8.39	0.041[b]	0.097
Grade IV	9.55 ± 9.61	7.14 ± 9.75	0.726[b]	18.57 ± 11.17	1236 ± 6.02	0.014[b]	0.153

[a] Paired *t*-test; [b] Wilcoxon test; [c] ANCOVA.

20.18 ± 5.60 and 28.21 ± 16.36, respectively, which were not significantly different ($P = 0.134$, independent *t*-test); and the values in the control and treatment groups after the intervention was 19.55 ± 5.68 and 19.65 ± 11.34, respectively ($P = 0.980$, independent *t* test). The results of the ANCOVA test showed that the intervention significantly reduced the level of total abnormality after the intervention in the treatment group compared to the baseline and the control group ($P < 0.001$).

Discussion

In the present study, a comprehensive investigation was performed to evaluate the multifaceted effects of the intervention, combined administration of oxytocin and zinc sulfate, on sperm parameters in men undergoing methadone maintenance therapy for opioid addiction. The research findings yielded valuable insights into the manifold effects of this intervention.

The study examined the demographic characteristics of the participants. Notably, the age distribution within the control and intervention groups displayed no significant disparities, establishing a solid foundation for further analyses. Of particular significance, the assessment of zinc levels before and after the intervention revealed a significant increase in the intervention group, underscoring the intervention's effectiveness in altering the biochemical profiles of the participants. Equally noteworthy, the intervention was associated with a substantial elevation in testosterone levels, enhanced sperm activity, and correlation with a reduction in sperm abnormalities, suggesting improvements in reproductive parameters.

The notable increases in testosterone levels and enhanced sperm motility observed in the treatment group following the intervention align with the established roles of oxytocin and zinc in modulating reproductive functions. Testosterone, a pivotal hormone in male reproductive health, is crucial to spermatogenesis and sperm motility.[20] The effect of oxytocin on the hypothalamic-pituitary-gonadal axis, which regulates testosterone production, potentially accounts for this increase. It seems that the use of oxytocin enhances the production of testosterone, especially in young animals. On the other hand, extended exposure to oxytocin leads to a decrease in testosterone levels but an increase in dihydrotestosterone production. It is important to note that the increase in androgen production following oxytocin administration is primarily influenced by the effect of oxytocin on the pituitary gland.[21] Furthermore, a relationship between zinc and testosterone levels in men has been indicated.[22] In a systematic review by Te et al, the collected data demonstrated that zinc deficiency reduced testosterone levels, while zinc supplementation enhanced testosterone levels.[22]

The enhancement in sperm motility may be attributed to the synergistic effects of oxytocin and zinc. Zinc is recognized as a vital element for spermatogenesis, affecting both spermatozoa's structural and functional aspects.[23] Zinc supplementation has been associated with improved sperm quality, including motility.[24] The interaction between zinc and testosterone underscores their roles in supporting male reproductive function.[18] Omu et al[25] investigated the effect of zinc supplementation on sperm parameters in infertile men and demonstrated improvements in sperm motility and morphology. Akbari et al have also reported that zinc supplementation reduced the abnormalities in the neck, head, and tail of sperm in infertile diabetic men.

Additionally, zinc administration improved sperm motility, resulting in a reduction in abnormal sperm morphology.[26] Moreover, Chitme et al reported that infertile patients had notably lower sperm concentration, fewer sperm with normal morphology and motility, and more abnormally structured sperm compared to the fertile group of patients. They also demonstrated a significant correlation between serum oxytocin levels and semen volume and sperm concentration and motility.[27] These studies confirm the findings of the present study regarding the potential effect of the intervention on improving sperm parameters in addicted men.

While the exact mechanism remains to be fully elucidated, numerous studies underscore the critical role of zinc ions in male fertility. The involvement of zinc in sperm motility, capacitation, and acrosomal exocytosis, processes crucial to successful fertilization, has been reviewed extensively. These findings underscore the multifaceted impact of zinc on male fertility, especially its effects on sperm function and fertilization processes. As a hormone regulator, zinc contributes to testosterone steady state, prostate health, and sexual function while acting as an antibacterial agent in the male urinary system. Its role in maintaining epithelial integrity underscores its importance in preserving the lining of reproductive organs and its potential involvement in capacitation and acrosome reaction processes. Conversely, zinc deficiency impedes sperm production, leading to sperm abnormalities and detrimentally affecting serum testosterone levels.[18] Another study highlighted the role of testosterone in regulating spermatogenesis and sperm motility, reinforcing the observed positive effects in the treatment group.[28]

The reduction in the level of total sperm abnormality is consistent with the literature related to zinc and reproductive health. Zinc plays a protective role in safeguarding sperm DNA integrity and mitigating oxidative stress, contributing to a lower incidence of sperm abnormalities.[29] This finding is consistent with the results of the research by Wong et al[24] and Kothari et al,[30] who reported a reduction in sperm abnormalities following zinc supplementation.

Conclusion

The substantial improvements in testosterone levels and sperm motility and the reduction of sperm abnormalities observed in the treatment group can be attributed to the synergistic effects of oxytocin and zinc. The interplay between the regulatory impact of oxytocin on the hypothalamic-pituitary-gonadal axis and the role of zinc in spermatogenesis and sperm quality provides a scientifically sound basis for these results. These findings expand our understanding of the potential advantages of oxytocin and zinc interventions in enhancing male fertility indicators in individuals undergoing MMT for drug addiction.

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Authors' Contribution

Conceptualization: Ehsan Saboory, Nasser Mirzai, Alireza Faridi.
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Software: Ehsan Saboory, Nasser Mirzai.
Supervision: Ehsan Saboory.
Validation: Ehsan Saboory, Nasser Mirzai, Alireza Faridi.
Visualization: Ehsan Saboory, Nasser Mirzai.
Writing–original draft: Ehsan Saboory, Nasser Mirzai.
Writing–review & editing: Ehsan Saboory, Nasser Mirzai, Alireza Faridi.

Competing Interests

There are no possible conflicts of interest.

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