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Can propofol procedural sedation implementation increase the acceptance of spinal anesthesia during cesarean section?



Alaa Mazy^{1*}, Nadia Madkour³ and Hesham Shaalan²

Abstract

Background: Parturients are highly anxious preoperatively. The worries of spinal anesthesia may preclude its acceptance despite being recommended. Procedural sedation is not a routine during regional blocks, but it is sensible that anesthesiologists should provide their blocks comfortably. The proposal is that implementing the propofol procedural sedation (PPS) may increase the acceptance rate of spinal anesthesia for cesarean section.

Methods: In this prospective observational study, the patients who refused spinal anesthesia primarily were interrogated to implement PPS for painless comfortable spinal anesthesia. Their acceptance rate was the primary outcome. In the sitting position, propofol 0.7 mg/kg and 20 mg increments were used as required. Patients were well supported and monitored. Data were compared by Mann-Whitney, chi-square, Fisher's exact, and Friedman's ANOVA tests as appropriate.

Results: The acceptance rate of spinal anesthesia increased from 17 to 93%. During PPS, the mean values of minimal mean blood pressure were not significantly decreased, while the mean values of the heart rate slightly increased. The minimal values of oxygen saturation showed no significant reduction compared to the basal values. Patients expressed a marked relief of anxiety and high satisfaction.

Conclusion: The use of propofol procedural sedation was effective in increasing the acceptance rate of spinal anesthesia during CS with safety and high patient's satisfaction.

Keywords: Spinal anesthesia, Cesarean section, Anxiety, Propofol, Conscious sedation, Sitting position, Maternal satisfaction

Introduction

Patient's safety and satisfaction are major concerns of anesthesiologists and obstetricians. According to evidence, guidelines, and quality markers, regional anesthesia is preferred over general in obstetric surgery (Apfelbaum et al. 2016). Already a high level of anxiety is present in obstetric patients preoperatively (Akildiz et al. 2017). The anxious patients tend to prefer general anesthesia (GA) for cesarean section (CS) (Maheshwari and Ismail 2015). The patient refusal is the main contraindication for applying spinal anesthesia (SA) during CS (Gülhaş et al. 2012). There is an evolving trend towards the judicious

¹Department of Anesthesia, Surgical Intensive Care and Pain Management, Faculty of Medicine, Mansoura University, Mansoura, Egypt



The anxiety can be reduced variably by nonpharmacologic and pharmacologic methods including preoperative visit (Akildiz et al. 2017), information (Tulgar et al. 2017), music (Lee et al. 2017), listening to Holy Quran recitation (Ghiasi and Keramat 2018), hypnosis (Romain et al. 2017), nitrous oxide (Gerhardt et al. 2001), and benzodiazepines (Danielak-Nowak et al. 2016). Intravenous sedation can control the anxiety in 90% of patients subjected to spinal procedures (Kim et al. 2007). While the evidence supports the benefits of procedural sedation for distressed, anxious pregnant women (Neuman and Koren 2013), our hypothesis is that PPS may increase the acceptance rate of SA during CS.



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^{*} Correspondence: alaa_mazy@yahoo.com

Full list of author information is available at the end of the article

Methods

This prospective observational study included 216 patients scheduled for elective CS at Delta Hospital, Mansoura. The patients were included consecutively from February to July 2018 after approval by the institutional review board (ID: R.18.02.23) and clinical trial registration (ID: NCT03437980).

A priori G power analysis, version 3.01 (Franz Faul, Christian-Albrechts-Universität Kiel, Germany) was used to determine the difference between two dependent means of the rate of acceptance as the primary outcome. Considering an effect size 0.3, α error 0.05, with a power 95%, a sample size of at least 147 patients was required.

The inclusion criteria were age 18-40 years and American Society of Anesthesiologists (ASA) I-II, of any parity. The exclusion criteria were patients with absolute contraindication to spinal anesthesia, a known psychiatric disease, sleep apnea syndrome, severe systemic disease, emergency CS, and high-risk pregnancy, such as placenta previa, uterine rupture, umbilical cord prolapse, eclampsia, fetal distress, intrauterine fetal death, severe anemia, and antepartum hemorrhage.

Before shifting the patient to the theater, the surgeon and the anesthetist discussed the exclusion criteria, and then discussed the information about spinal and general anesthesia with the illegible patients. The first decision for anesthesia, either spinal or general, was recorded. Then, patients who refused SA and preferred general anesthesia (GA) were consulted again as regards SA under propofol sedation for painless and comfortable spinal procedure. Their last choice is the second decision that determines the final type of anesthesia to be implemented. The acceptance rate of SA under PPS was the primary outcome. A written consent was taken.

The number of crying patients and anxiety causes were documented just before SA. The anxiety score was assessed by the visual analog score (VAS) from 0-10, where 10 is the maximum anxiety level. The anxiety scores were recorded, preoperatively, 10 min after resuming supine position following SA procedure, and before shifting from the recovery room. All patients received 10 ml/kg Ringer's solution preoperatively.

Procedural sedation

For spinal injection, patients were in the sitting position in the middle of the operating table facing towards the table's foot (Fig. 1). The table's foot was dropped 45° for a comfortable chair position. The back of patients faced towards the head of the table for an easy lay down after injection.

The applied monitors were a pulse oximeter for oxygen saturation (SpO₂) and heart rate (HR), non-invasive blood pressure automatic measuring every 1 min, and capnography tube in contact with the nostrils (Datex-Ohmeda, Aspire View, USA). The basal data were recorded. The anesthesia machine was shifted to the right side of the table for easy monitoring during injection.

One theater staff supports the patient on the right side, and an anesthesia resident or nurse injects propofol and supports the patient on the other side. Propofol 0.7 mg/kg was given initially over 30 s. This dose is based on a study provided sedation titrated to a level of 65-85 using bispectral analysis (Verma et al. 2013). Additional increments of propofol 20 mg were given with any of the following conditions: the patient extended the back, moved the arms towards the back, and expressed pain sounds rather than that of the skin puncture. Under sterile condition, a 25-G Quincke spinal needle was used at the paramedian plane without local infiltration. After injection, patients were laid down on bellows under the head. The procedural time and total dose of propofol were recorded. Neck extension and airway support were planned if oxygen saturation dropped less than 90% or the apnea alarm was initiated.

The premature procedure termination is assigned if the total dose of propofol exceeded 150 mg; failure of intrathecal accesses after five trials or exceeding 5 min; excessive movement or excitability not controlled by four consecutive doses of 20 mg propofol; desaturation (SpO2 \leq 90%) not alleviated by airway support and oxygen face mask; hypotension defined as mean arterial blood pressure (MAP) less than 60 mmHg or systolic blood pressure less than 90 mmHg; bradycardia defined as HR less than 60 b/min; and vasovagal attacks as manifested by sudden bradycardia, loss of muscular tone, and sweating, where atropine 0.01 mg/kg is scheduled. General anesthesia was considered in case of procedural termination ensuring a sleeping dose of propofol and succinylcholine 1 mg/kg, followed by an oral endotracheal intubation.

Sedation score was assessed by the Ramsay scale (Ramsay et al. 1974). It was determined 1 and 10 min after resuming supine position. The minimal MAP, HR,

upporting nurs pulse oximeter Leas down



and SpO₂ during PPS were recorded. In addition, SpO₂ values were measured 5, 10, and 15 min after resuming the supine position. After delivery, patients were asked about spinal injection pain as present or not. The Apgar score was noted at 1 and 5 min, twins were excluded (17 patients). The patient satisfaction as regards their choice of SA was assessed at recovery room by (0–10) visual analog score (VAS), where 10 is the maximum satisfaction. All patients were asked about their choice of anesthesia the next time doing CS.

All data were analyzed using Statistical Packages for Social Science version 17 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test assigned the data distribution. The numerical data were compared by Wilcoxon signed rank test for paired data and Mann-Whitney test for unpaired data, while Friedman's ANOVA test was used for multiple paired comparisons. Chi-square and Fisher's exact tests were used for unpaired qualitative data and McNemar's chi-square test for paired data. Data were displayed in median and range or frequency and percentage. The $P \leq 0.05$ value is the significance level.

Results

This study population showed a strong positive impact of PPS upon the acceptance rate of SA (Fig. 2). The rate changed from 17% without sedation to 93% with PPS. The demographic, obstetric data and preoperative anxiety scores for the patients who preferred SA or GA were compared (Tables 1, 2, and 3). The first decision regards the type of anesthesia without sedation correlated positively with the younger age (P = 0.001 by Spearman correlation) and gravidity (P = 0.048). The second decision regards the type of anesthesia after PPS significantly correlated with the ASA status (P = 0.045) and body mass index (P = 0.007). The women who refused spinal anesthesia under PPS (7%) elucidated their satisfaction with a previous GA or they absolutely refused being awake during CS.

The PPS was successful in all trials. It was easy to perform spinal anesthesia in absence of pain. The median dose of propofol was 70 mg. The mean body weight in SA patients was 72 ± 14 kg. Patients were sometimes talking, showing movements or expressed pain sounds during injection (Table 4). However, nearly all patients



ltems	Spinal group, n = 166	General group, n = 13	Р	
Age (years)	29.8 ± 5.4	27.9 ± 3.9	0.252	
BMI (kg/m²)	26 (22–54)*	24 (24–26)	0.008	
Diseases				
Hypertension	20 (12%)	0	0.192	
Bronchial asthma	18 (11%)	0	0.226	
Diabetes mellitus	8 (5%)	0	0.546	
Hypothyroidism	5 (3%)	0	0.687	
Hepatic disease	3 (2%)	0	0.742	
ASA				
I	126 (76%)*	13 (100%)	0.035	
II	40 (24%)*	0		
Education				
Low	10 (6%)	0	0.65	
Medium	27 (16%)	2 (15%)		
High	129 (78%)	11 (85%)		
Operative duration (min)	55 (30–110)	45 (43–60)	0.088	

Table 1 The demographic data according to the primary decision (spinal or general anesthesia)

Data are in mean \pm SD, median (range), and number (percent) BMI body mass index

*Significant difference ≤ 0.05

Table 2 The obstetric data according to t	the primary decision
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ltems	Spinal group, n = 166	General group, n = 13	Р	
Gravidity				
1	66 (40%)	3 (25%)	0.797	
2	42 (25%)	5 (38%)		
3	30 (18%)	5 (38%)		
> 3	28 (17%)	0		
Parity				
0	75 (45%)	3 (25%)	0.409	
1	41 (25%)	5 (38%)		
2	41 (25%)	5 (38%)		
> 2	10 (6%)	0		
Previous CS				
No	75 (45%)	3 (25%)	0.16	
GA once	38 (23%)	2 (13%)		
GA twice	30 (18%)	5 (37%)		
Spinal once	5 (3%)	3 (25%)		
Spinal twice	7 (4%)	0		
Vaginal delivery	8 (5%)	0		
One GA, one spinal	3 (2%)	0		
ICSI	33 (20%)	3 (23%)	0.801	
Twin pregnancy	17 (10%)	0	0.221	

Data are in number (percent)

ICSI intra cytoplasmic sperm injection

Items	Spinal group, n = 166	General group, n = 13	Ρ
Anxiety score			
Preoperative	10 (5-10)*	10 (8–10)	0.613
10 min after SA	0 (0–6)	-	-
At recovery room	0 (0-1)	-	-
Number (%) of patients with maximum anxiety score (= 10)	123 (74%)	11 (83%)	0.384
Number of patients with preoperative crying	22 (13%)	3 (25%)	0.278
Anxiety causes			0.370
About herself	133 (80%)	13 (100%)	
About her baby	18 (11%)	0	
Both fears	12 (7%)	0	
Web site worries	3 (2%)	0	

Data are in median (range), or number (percent)

*Significant (P = 0.001) difference between anxiety scores preoperative and 10 min after SA or at recovery by Friedman's ANOVA test

denied pain during spinal injection. They mostly forgot their talks and sometimes forgot for a while the situation of surgery. Four patients started loud euphoric laughing after PPS. There was no vomiting or any vasovagal attack.

The mean oxygen saturation maintained above 97% during PPS, but decreased significantly in comparison to basal values 5 and 10 min after the patients resumed the supine position post spinal injection (Fig. 3). Head support was required in five patients when saturation dropped to \leq 90%. The minimal MAP during PPS was not different from the basal value, while the minimal HR slightly increased (Fig. 4).

The anxiety score was near maximum (= 10) for all patients preoperatively. The anxiety score significantly alleviated 10 min after SA, and many patients asked for photos with their babies on delivery. Also, anxiety score was minimal at recovery (Table 3, Fig. 5). There was no statistical difference in the number of crying patients for SA and GA groups (Table 3). There was no correlation

Table	4 Data	of	propofol	procedural	sedation	(PPS)	in	the
spinal	group							

Items	Description	Value
Total propofol doses during PPS (mg)	Median (range)	70 (50–150)
Pain sounds during PPS, n (%)	Yes	33 (20%)
Movement during PPS, n (%)	Yes	46 (28%)
Spinal injection time (min)	Median (range)	1 (1–5)
End tidal CO_2 during PPS (cmH ₂ O)	Median (range)	22 (17–32)
Satisfaction score (0–10) at recovery	Score = 10, <i>n</i> (%)	163 (98%)
Next CS anesthesia choice, n (%)	PPS with spinal	163 (98%)

Data are expressed in median (range) or the number of patients (percent); n = 166 PPS propofol procedural sedation



between the first decision of anesthesia and preoperative anxiety (P = 0.615, Spearman correlation test) or crying (P = 0.280). The sedation score significantly decreased 10 min after resuming supine position following spinal injection (Fig. 6), where most of the patients were communicating. The median values of sedation scores were 3 (1–4) and 1 (0–3) at 1 and 10 min consecutively (P = 0.001). The Apgar score significantly increased at 5 min (Table 5).

Discussion

In this study; the use of PPS increased the acceptance rate of SA from 17 to 93% during CS. In our community, the first decision as regards the choice of anesthesia revealed that 83% of parturients preferred GA. The rate of SA acceptance differs between countries and may be linked to social cultures (Altiparmak and Koseoglu 2017). A Nigerian study found an equally preferred SA

as GA (51.6% vs. 48.4%) that was explained by searching of safety (44.3%), fear of death (41.2%), and the desire for being awake during the procedure (14.5%) (Rabiu et al. 2019). Another study in Nigeria revealed that most respondents preferred GA due to fear from the conduct of anesthesia (Bukar et al. 2010). In Turkey, a study showed a higher rate of selecting GA (64.2% vs. 35.8% for SA); the increased choice of SA correlated with the level of education and increased income (Arslan et al. 2019). In the UK, the rate of regional anesthesia for elective CS rose from about 70 to 95% within 10 years (1992-2002); SA was used in 86.6% of cases (Jenkins and Khan 2003). In the USA, the frequency of GA dropped from 35 to 12% from 1981 to 1992 (Hawkins et al. 1998) to 7.3% recently (Cobb et al. 2019). The rate also showed differences in private, university, and state hospitals (Töre et al. 2009).





Most of the included parturients in this study showed a high degree of preoperative anxiety. That was confirmed by many studies (Maheshwari and Ismail 2015, Altiparmak and Koseoglu 2017). In this study, there was no correlation between the first choice of anesthesia and anxiety level or the number of crying patients. Anxiety was present in approximately all patients, so the statistical difference may not be apparent between the patients who preferred SA or GA. Altiparmak and Koseoglu also found no influence of anxiety on patient's choice of anesthesia (Altiparmak and Koseoglu 2017). Many factors may contribute to the anxiety and refusal of SA such as needle phobia, the fear that anesthesia will not work, and being awake during surgery. In association between the first choice of anesthesia and the demographic and obstetric characteristic, there was a positive correlation with the younger age and lower gravidity. Most of the populations in this study (about 80%) were highly educated; however, the fears and cultural effects may explain the preference of GA. In a previous study, the anxiety correlated with the younger age, higher education, low parity, and non-anesthetist information source (Maheshwari and Ismail 2015). In contrast, Mitchell et al reported that the rate of SA selection increases as the level of education and income increases (Sosis et al. 1995). Nonetheless, the second choice of anesthesia (under PPS) correlated significantly with the higher level of ASA and body mass index (BMI).



Table 5 The Apgar score for the spinal group babies,

 excluding twins

	Score value	1 min after delivery	5 min after delivery	Ρ
Apgar score	7	2 (1%)	0	
	8	5 (3%)	2 (1%)	
	9	60 (36%)	3 (2%)	
	10	99 (60%)	161 (97%)	
Total Apgar score	Median (range)	10 (7–10)	10 (8–10)*	0.00

Data are presented in number (percent); n = 149

*Significant difference, $P \le 0.05$

In this study, the implication of PPS improved the acceptance rate of SA from 17 to 93% after insuring painless comfortable spinal injection. This rate is comparable to that in the developed countries (Cobb et al. 2019). The PPS markedly alleviated the anxiety and provided a high satisfaction that nearly all patients selected SA for the next possible CS.

Most anesthesiologists may omit the procedural sedation to avoid drug side effects. We may resort to the preoperative visit to decrease the anxiety. Controversially, that may not be effective (Panjabi et al. 2017). Lidocaine infiltration is used, but it is already painful and may increase anxiety (Bakshi et al. 2015). Most of the fears from drug side effects were from hypoxemia and hypotension.

Procedural sedation is a hospital-wide protocol. It is sensible that anesthesiologists should implement their procedures comfortably. Traditionally, using sedation is unusual during regional anesthesia. In a meta-analysis, the neuraxial blocks were performed in 83.4% awake, 15.2% sedated, and 1.4% anesthetized patients (Kubulus et al. 2016). Surprisingly, the items of fear from sedation were comparable in sedated and awake patients that may include the rate of premature termination and the incidence of postoperative paresthesia. In agreement with this study, patient's satisfaction was higher with sedation; therefore, procedural sedation is highly recommended (Kubulus et al. 2016).

The initial dose of propofol was 0.7 mg/kg, and the mean body weight in SA patients was $72 \pm 14 \text{ kg}$. So, the mean initial dose was 50 mg. The median total dose was 70 mg that most of patients required one incremental dose (20 mg). The incremental doses may be more suitable than the infusion due to the short time of the SA procedure (1–5 min). Clinically, some patients were talking, moving (28%), and expressing pain sounds (20%), and no one showed flaccidity. All trials were successful.

In this study, the PPS was not a restraint. However, propofol provided painless spinal injection. In addition, the unpleasant emotions of anxiety altered into calm sedation, euphoria, logorrhea (talkativeness), and grave emotions of happiness on seeing their babies. Propofol displayed also many adventitious properties including short onset, amnesia that patients denied feeling pain during SA. Furthermore, there were no vomiting or vasovagal reactions in any patient, a rapid predictable recovery with mild sedation for 10 min after resuming supine position. In pregnant women, the total body clearance of propofol is more rapid that may be explained by blood loss and fetal and placental delivery (Gin et al. 1990). Many studies confirmed the pharmacological and beneficial effects of propofol (Patki and Shelgaonkar 2011, Kennedy et al. 2015, Danielak-Nowak et al. 2016). Furthermore, the sub-hypnotic doses of propofol have an antiemetic, antipruritic, analgesic, and antihyperalgesic properties (Bandschapp et al. 2010). Generally, brief, low-dose PPS can be delivered safely in pregnancy (Neuman and Koren 2013).

Comparatively, pre- or post-spinal propofol trials during CS are few. Cheng et al. used pre-spinal propofol 0.3 mg/kg bolus, then 3 mg/kg/h infusion. It was safe for mothers and babies. There was no hypoxemia or hypotension compared to non-sedation patients (Cheng et al. 1997). The use of propofol after umbilical cord clamping in doses 0.3, 0.4, and 0.5 mg/kg followed by infusion of 3, 4, or 5 mg/kg/h showed a post-delivery hemodynamic and respiratory stability, low nausea, and high satisfaction (Wang et al. 1996). Also, propofol after baby extraction in a dose 0.5 mg/kg then infusion of 6–8 mg/kg/h increased the acceptance of regional anesthesia (Danielak-Nowak et al. 2016).

The newly applied PPS in the sitting position in this study was safe for mothers and babies. Additionally, the SA technique is easier in sitting than lateral position, with no difference in hemodynamics, final distribution, patient comfort, and muscle relaxation (Chevuri et al. 2015).

The sedation in the sitting position is used in many procedures. During shoulder surgery under interscaline block, Soeding et al. showed a stability and maintenance of the MAP, HR, and cerebral blood flow. Their sedation was through fentanyl 80 ± 20 mcg, midazolam 4 ± 1 mg and propofol infusion at 50 to 200 mg/h in a mean dose of 39 ± 21 mg, and bispectral index of 81 ± 9 . The level of sedation aimed to maintain the response to verbal communication (Soeding et al. 2011).

During PPS, oxygen saturation did not decrease, except in five patients where larger doses of propofol were given (range 50–150 mg), and head support was sufficient to improve oxygenation. After resuming supine position, oxygen saturation significantly decreased for 10 min. However, the SpO₂ level (97%) was far from hypoxia. The maintained saturation in spite of the effects of propofol may reflect the better respiratory dynamics during sitting position. In the third trimester, oxygen saturation is significantly higher compared to non-pregnant in sitting position (Revathi and Neelambikai 2018). Also, the mean arterial oxygen tension in the supine position is around 90.5 mmHg, while it is about 97.5 mmHg in the sitting position (Spiropoulos et al. 2004).

During PPS, the MAP showed non-significant decrease, while the HR mildly increased. It may be suspected that the preload will decrease during sitting position. Paradoxically, the preload, stroke volume, and blood pressure increased, while systemic vascular resistance reactively decreased late in pregnancy using invasive or noninvasive cardiac output monitor (Clark et al. 1991; Guy et al. 2018). The slow injection of propofol is associated with lower hemodynamic deterioration (Rather et al. 2018).

The patient satisfaction and preference of SA in the next CS were high with PPS in this study (98% each). The convenience may change the attitude towards SA in our locality. Generally, SA is satisfactory for almost all patients (Rabiu et al. 2019). However, this satisfaction is a collaboration between inclusion of the patient in anesthesia choice, good conduct of SA procedure, and proper management of side effects, in addition to the baby welfare. Therefore, with the respiratory and hemodynamic stability, we can expect that PPS in the sitting position may become a routine during neuraxial blocks.

During preoperative visit counseling, the authors found that patients easily accept SA if associated with PPS. Already counseling may impact the choice of anesthesia. Both the anesthetist and obstetrician have favorable contributions to the selection of SA (Arslan et al. 2019). In a study concluded 250 women, the rate of SA acceptance had changed from 37.6% to 68% after counseling (Imtiaz et al. 2018). It seems that PPS is more motivating for SA.

The median value of Apgar score was 10 at 1 min, with more improvement at 5 min. Propofol sedation was safe for babies. There was no difference in umbilical blood gas analyses between the propofol sedated (0.3 mg/kg bolus followed by 3 mg/kg/h) and the nonsedated groups and no adverse effects on the neurological and adaptive fetal outcomes (Cheng et al. 1997). Although propofol can cross the placenta (Sanchez-Alcaraz et al. 1998) and may cause hypotension but without effects on the fetus, it provides fetoplacental vasodilation that maintains placental blood flow (de Moura et al. 2010).

In preferential comparison with propofol (a bolus of 0.5 mg/kg, followed by 5–8 mg/kg/h), midazolam has a slower onset, difficult control of sedation level, less control of nausea and vomiting, less hypotension and recall, less euphoria and logorrhea, and less patients' satisfaction (Danielak-Nowak et al. 2016). Midazolam/fentanyl also has lower sedation and patient satisfaction effects than propofol (Malekmakan et al. 2018). Ketamine triggered more agitation and longer recovery, so it may not be preferred (Neuman and Koren 2013). Ketofol (ketamine + propofol 0.25 mg/kg each) provide more stability

and fewer side effects than propofol but longer recovery (Baykal Tutal et al. 2016). Dexmedetomidine has a comparable sedation to propofol with systolic hypotension using a loading dose of $1 \mu g/kg$ (Karanth et al. 2018).

The limitations of this study may comprise the lake of bispectral monitoring or plasma level. Extra staffs are needed to support the sedated patients during spinal injection. The SA in this study was executed by senior staff, so the juniors' performance needs to be evaluated. The unusual PPS in the sitting position during CS may encounter the resistance of the traditional practice, but it deserves further evaluation in different communities.

Conclusion

The use of propofol procedural sedation increased the acceptance rate of spinal anesthesia during CS in our community.

Abbreviations

ASA: American Society of Anesthesiologists; BMI: Body mass index; CS: Cesarean section; GA: General anesthesia; HR: Heart rate; MAP: Mean arterial blood pressure; PPS: Propofol procedural sedation; SA: Spinal anesthesia; SpO₂: Oxygen saturation; VAS: Visual analog score

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Authors' contributions

The idea, design, and data analysis were prepared by Dr. AM. Literature search and manuscript editing were done by Dr. AM and NM. All authors shared the clinical data acquisition and the final manuscript review and approval.

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Availability of data and materials

The analyzed data are included in the tables. The details are available from the corresponding author upon a reasonable request.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board with the reference number (R/18.02/23). A written consent was signed by all participants. The clinical trial registration number is NCT03437980 at ClinicalTrials.gov.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Anesthesia, Surgical Intensive Care and Pain Management, Faculty of Medicine, Mansoura University, Mansoura, Egypt. ²Department of Obstetric and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt. ³Department of Obstetric and Gynecology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

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