Original Research Article

Stress response and safety of lidocaine combined with dexmedetomidine in patients undergoing laparoscopic hysterectomy

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Abstract

Purpose: To investigate the anesthetic effect and safety issues when lidocaine is combined with dexmedetomidine in laparoscopic hysterectomy.

Methods: The data for 100 patients from the Anesthesiology Department of Changzhou Second People's Hospital Affiliated to Nanjing Medical University who underwent laparoscopic total hysterectomy were randomly but equally divided into a control group and a combination group. Lidocaine hydrochloride was administered by infusion in the control group, while dexmedetomidine and lidocaine were administered together in the combination group. Inflammatory factors and stress hormone levels before and after the operation, as well as the incidence of adverse reactions following the operation, hemodynamic index, visual pain simulation (VAS) score, and sedation score at various times after the operation were evaluated in the two groups. Furthermore, the incidence of postoperative adverse reactions were also recorded and compared between the two groups.

Results: The combination group's recovery time was significantly longer than that of the control group (p < 0.05). At the point of extubation and 10 min after, mean arterial pressure (MAP) and heart rate (HR) in the combination group were significantly lower than those of the control group, respectively (p < 0.05), while the combination group's VAS and Ramsay scores were statistically lower than those of the control group at 30 min, 24 h, and 48 h following surgery, respectively (p < 0.05). The incidence of nausea and vomiting in the combination group was significantly lower than that in the control group (28.0% vs 58.0%, p < 0.05).

Conclusion: Lidocaine and dexmedetomidine, when combined, enhance hemodynamics, postoperative analgesia, and sedation in laparoscopic hysterectomy patients. The combination also lowers inflammatory stress and stress hormone levels, as well as the risk of nausea and vomiting, leading to better safety in the patients. However, multicenter trials are recommended to validate these findings prior to its use clinical practice.

Keywords: Lidocaine, Dexmedetomidine, Anesthetic, Body stress, Safety, Inflammatory factors

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INTRODUCTION

Laparoscopic total hysterectomy is highly favored by women who wish to undergo total hysterectomy because of its minimal impact on the patient's ovarian function, minimal invasiveness, and rapid postoperative recovery [1]. Previous clinical studies have found that the choice of perioperative anesthesia protocol not only affects the anesthetic effect but also effectively reduces the occurrence of postoperative cognitive dysfunction, and improves the overall quality of life of patients [2]. As a result, improving the anesthetic regimen in laparoscopic complete hysterectomy has become a focus of clinical studies in recent years.

Postoperative pain and adverse reactions are important indicators for assessing postoperative recovery. Poor pain control not only causes some serious complications but also nausea and vomiting, and other manifestations of delayed recovery of gastrointestinal function, which seriously impacts postoperative recovery [3]. Lidocaine is a more commonly used amide local anesthetic in clinical practice, and intravenous infusion of this agent not only has an analgesic effect, but also reduces the need for postoperative anesthetics in patients receiving lidocaine compared with those receiving other anesthetic agents. This shortens the duration of postoperative intestinal obstruction, contributing to the recovery of postoperative bowel function [4].

Some studies have confirmed that dexmedetomidine is an anesthetic drug with multiple effects such as sedation, analgesia, and anti-inflammation, especially in suppressing inflammatory responses and improving postoperative cognitive function [5]. Several studies at worldwide have reported the anesthetic effect of lidocaine when combined with dexmedetomidine [6]. However, few studies have been reported fever as a consequence of total hysterectomy. For this reason, this investigation aims to assess the effect of the combined regimen on perioperative stress response of patients.

METHODS

General patient data

Patients who underwent laparoscopic total hysterectomy under general anesthesia for uterine fibroids or adenomyosis, and admitted to the Department of Obstetrics and Gynecology of Changzhou Second People's Hospital Affiliated to Nanjing Medical University from January 2021 to January 2023 were selected as study subjects.

Inclusion criteria

(1) Preoperative diagnosis of uterine fibroids or adenomyosis, and the surgery was performed by the same treating surgeon; (2) American Association of Anesthesiologists (ASA) classification I-II; (3) laparoscopic total hysterectomy confirmed by examination indications; (4) normal spirit, clear consciousness, and a high degree of cooperation; (5) completed clinical information and voluntarily signed the informed consent form.

Exclusion criteria

(1) allergy to anesthetic drugs and drug components involved in the study; (2) acute cardiogenic ischemic syndrome, stress syndrome, severe bradycardia or hypotension; (3) severe respiratory dysfunction or other severe organ function impairment; (4) severe mental illness, unable to communicate normally, thus affecting the study data collection; (5) preoperative presence or preoperative history of opioid use; (6) unwillingness to join the clinical study or to sign the informed consent form.

A total of 100 patients from the Anesthesioloogy Department of Changzhou Second People's Hospital Affiliated to Nanjing Medical University who underwent laparoscopic total hysterectomy were selected as the study sample according to the study criteria. Age range: 27 ~ 61 years old. The patients were divided into control group and combination group, with 50 patients in each group. General information on the subjects is shown in Table 1. The study was approved by the Ethics Committee of Changzhou Second People's Hospital Affiliated to Nanjing Medical University, Changzhou, China (approval no. 20210212), and carried out in accordance with the guidelines of 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects [7].

Treatments

The subjects all fasted for 6 to 8 h and went without drink for 4 h. One hour before the procedure, 0.1 g of midazolam injection (Jiangsu Enhua Pharmaceutical Co. Ltd, National Drug Quantifier H19990027, 5 mg/ml) was given intramuscularly.
Table 1: Patient profile (mean ± SD, n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (years)</th>
<th>Body mass index (kg/m²)</th>
<th>ASA(Ⅰ /Ⅱ)</th>
<th>Uterine fibroids/uterine adenomyosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.68 ± 6.20</td>
<td>23.31 ± 2.87</td>
<td>31/19</td>
<td>36/14</td>
</tr>
<tr>
<td>Combination</td>
<td>47.90 ± 7.02</td>
<td>23.84 ± 2.47</td>
<td>35/15</td>
<td>38/12</td>
</tr>
</tbody>
</table>

Patients in the control group were given a 2% lidocaine hydrochloride injection (Hubei Tianshing Pharmaceutical Co. Ltd, State Drug Administration permit no. H20201839, specification, 0.5 g/5 ml) diluted with saline in a 20-ml syringe and 20 ml saline pumped simultaneously for 10 min before induction of anesthesia at a dose of 1.5 mg/kg. This was followed by continuous infusion of lidocaine 1.5 mg/kg/h and saline 20 ml/h at a constant rate until the end of the abdomen.

Patients in the combination group were treated with a combination of dexmedetomidine (Nanjing Zhengda Tianqing Pharmaceutical Co. Ltd, China, National Drug Administration permit no. H20213542, specification, 0.2 mg/2ml) and lidocaine. The lidocaine loading dose was the same as the control group, with a dexmedetomidine loading dose of 0.5 μg/kg, also pump for 10 minutes. Subsequent continuous infusion of lidocaine 1.5mg/Kg/h and dexmedetomidine 0.4 μg/kg/h. Pump at a rate of 20 ml/h at a rate of g/Kg/h until the surgical closure is complete.

**Administration of anesthesia**

All patients breathed in pure oxygen through a mask before being put under anesthesia. Remifentanil and propofol injections (Yichang Renfu Pharmaceutical Co. H20054171, specification, 50 μg/ml; Sichuan Guorui Pharmaceutical Co. Ltd, State H20030114, specification, 0 mg/ml) were used to put the patients to sleep. Target-controlled infusion approach, in which the initial plasma target concentration of propofol was set at 3 μg/ml, and the patient received benzene sulfonyl 3 min after receiving remifentanil at an initial concentration of 5 ng/ml, was adopted. The patient was given an injection of 0.15 mg/kg benzene sulfonyl aztreonam (Jiangsu Hengrui Pharmaceutical Co. Ltd, State Pharmacopoeia H20183042, specification, 2 mg/ml) after going unconscious. The anesthesia machine was connected for mechanical ventilation after successful endotracheal intubation, and the target-controlled concentration of propofol (2 - 3 μg/ml) was changed intraoperatively to maintain the EEG dual-frequency index between 45 and 60. Remifentanil (3 - 5 ng/ml) was adjusted to keep the heart rate and mean arterial pressure within 20 % of the basal value; ephedrine was administred when mean arterial pressure (MAP) fell below 60 mmHg, while atropine was administered when the heart rate (HR) fell below 50 bpm. The tracheal tube was removed after the patient's spontaneous breathing returned, and the infusion of propofol and remifentanil was terminated after suturing. Each patient was then led to the anesthesia recovery room.

**Evaluation of parameters/indices**

**Surgical status**

Surgical time, anesthesia time, and awakening time of the patients in the two groups were counted.

**Hemodynamic indices**

The MAP and HR of patients were determined at three-time points before induction, at the time of extubation, and 10 min after extubation, respectively.

**Pain and sedation scores**

Resting visual pain simulation score (VAS) and sedation score (Ramsay sedation scores) were postoperatively determined in the patients at 30 min, 24 h, and 48 h respectively. Ramsay sedation score is usually based on the patient's clinical status and is graded on 6-point scale, viz: 1: anxious, agitated, or restless; 2: calm and able to communicate and follow instructions normally; 3: sleepy and indifferent during the visit, non-smoking and able to follow instructions; 4: continuously asleep during the visit, unable to follow general instructions and only reacts when the patient's name was called out, or the patient responded when touched by force; 5: the patient's response to strong stimuli was only based on 4 levels of performance; and 6: the patient is in a sleepy state without any response to strong external stimuli. On this 6-point scale, 1 is an evidential deficiency, 3 to 4
are appropriate sedation, and 5 or 6 indicates that the patient is over-sedated.

**Inflammatory factors**

Peripheral venous blood was collected from patients before and 24 h after surgery, and the levels of tumor necrosis factor-α (tumor necrosis factor-α, TNF-α) and interleukin-6 (IL-6) in the patients were determined by chemiluminescence immunoassay.

**Stress index levels**

Early morning fasting peripheral venous blood was collected before and 24 h after surgery, and the epinephrine (E), cortisol (Cor), and norepinephrine (NE) levels were measured by enzyme-linked immunosorbent assay after centrifugation. The incidence of nausea, vomiting, bradycardia, hypotension, drowsiness, and pruritus in the patients were assessed.

**Statistical analysis**

The data obtained were processed by SPSS 23.0 software, and count data expressed as percentage following statistical analysis using chi-square test. Measurement data were tested for normal distribution and analyzed by independent sample t-test. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Surgical conditions of the patients**

The surgical conditions of the patients in the two groups are shown in Table 2. As can be seen from the table, the surgical time and anesthesia time in the two groups were similar, and any differences were not statistically significant \((p > 0.05)\). Compared with the awakening time of the control group, the awakening time of the combination group was significantly longer \((p < 0.05)\).

**Hemodynamic indices**

The levels of MAP and HP before induction were similar between the two groups, and the difference was not statistically significant. As shown in Table 3, the hemodynamic indices (MAP and HR) of the two groups before induction were similar, and the difference was not statistically significant \((p > 0.05)\). However, at 10 min after extubation, the combination group's HR levels were significantly lower than those of the control group \((p > 0.05)\).

**Inflammatory factors**

The results of inflammatory factor levels before and after surgery in the two groups of subjects are shown in Table 5. The levels of TNF-α and IL-6 of the combination group were significantly lower than those of the control group at 24 h after surgery \((p < 0.05)\).

**Stress hormone levels**

The results of E, Cor and NE levels in the two groups of subjects are shown in Table 6. The levels of E, Cor and NE of the combination group were significantly lower than those of the control group at 24 h after surgery \((p < 0.05)\).

**Adverse reactions**

The occurrence of perioperative adverse reactions in the subjects of both groups is shown in Table 7. Compared with the control group, the incidence of nausea and vomiting was significantly lower in the combination group \((28.58\% , p < 0.05)\). The incidence of bradycardia, hypotension, and skin pruritus was relatively similar in both groups,

<table>
<thead>
<tr>
<th>Table 2: Comparison of surgical parameters (mean ± SD, n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Combination</td>
</tr>
<tr>
<td>( t )-value</td>
</tr>
<tr>
<td>( P )-value</td>
</tr>
</tbody>
</table>

Trop J Pharm Res, January 2024; 23(1): 170
Table 3: Comparison of hemodynamic indices between the two groups at different points (mean ± SD, n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mmHg) Before induction</th>
<th>HR (min⁻¹) Before induction</th>
<th>10min after extubation</th>
<th>HR (min⁻¹) 10min after extubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.82 ± 6.40</td>
<td>75.88 ± 5.02</td>
<td>83.95 ± 6.15</td>
<td>81.27 ± 8.33</td>
</tr>
<tr>
<td>Combination</td>
<td>86.83 ± 6.14</td>
<td>77.19 ± 5.90</td>
<td>81.87 ± 6.72</td>
<td>75.72 ± 7.75</td>
</tr>
<tr>
<td><em>t</em>-value</td>
<td>1.586</td>
<td>2.01 ± 0.29</td>
<td>1.92 ± 0.39</td>
<td>1.46 ± 0.15</td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>0.116</td>
<td>0.000</td>
<td>0.028</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Table 4: Comparison of VAS scores and Ramsay scores at different postoperative times (mean ± SD, n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>VAS score Before surgery</th>
<th>24 h</th>
<th>48 h</th>
<th>Ramsay score Before surgery</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.46 ± 0.58</td>
<td>1.97 ± 0.43</td>
<td>2.67 ± 0.46</td>
<td>2.01 ± 0.29</td>
<td>1.63 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>1.86 ± 0.62</td>
<td>1.77 ± 0.47</td>
<td>2.41 ± 0.33</td>
<td>1.92 ± 0.39</td>
<td>1.46 ± 0.15</td>
<td></td>
</tr>
<tr>
<td><em>t</em>-value</td>
<td>4.927</td>
<td>2.225</td>
<td>3.184</td>
<td>1.174</td>
<td>6.856</td>
<td></td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
<td>0.243</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Comparison of inflammatory factor levels between the two groups (ng/L, mean ± SD, n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α Before surgery</th>
<th>24 h after surgery</th>
<th>IL-6 Before surgery</th>
<th>24 h After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.45 ± 0.51</td>
<td>17.37 ± 1.73</td>
<td>217.52 ± 32.86</td>
<td>285.52 ± 28.83</td>
</tr>
<tr>
<td>Combination</td>
<td>4.54 ± 0.36</td>
<td>13.60 ± 1.25</td>
<td>210.44 ± 34.97</td>
<td>234.40 ± 25.78</td>
</tr>
<tr>
<td><em>t</em>-value</td>
<td>1.022</td>
<td>12.448</td>
<td>1.043</td>
<td>9.381</td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>0.310</td>
<td>0.000</td>
<td>0.299</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 6: Comparison of stress hormone levels between the two groups (mean ± SD, n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>E (ng/ml) Before surgery</th>
<th>24 h after surgery</th>
<th>Cor (ng/ml) Before surgery</th>
<th>24 h after surgery</th>
<th>NE (pg/ml) Before surgery</th>
<th>24 h after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46.59 ± 5.47</td>
<td>185.16 ± 263.90</td>
<td>225.20 ± 30.10</td>
<td>305.81 ± 23.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>45.49 ± 6.69</td>
<td>188.95 ± 221.98</td>
<td>231.59 ± 215.90</td>
<td>276.47 ± 20.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>t</em>-value</td>
<td>0.898</td>
<td>0.397</td>
<td>0.151</td>
<td>6.688</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>0.372</td>
<td>0.000</td>
<td>0.134</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Incidence of adverse reactions (n = 50)

<table>
<thead>
<tr>
<th>Group</th>
<th>Nausea &amp; vomiting</th>
<th>Bradycardia</th>
<th>Hypotension</th>
<th>Skin pruritus</th>
<th>Drowsiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29 (58.00)</td>
<td>4 (8.00)</td>
<td>0</td>
<td>2 (4.00)</td>
<td>10 (20.00)</td>
</tr>
<tr>
<td>Combination</td>
<td>14 (28.00)</td>
<td>6 (12.00)</td>
<td>1 (2.00)</td>
<td>1 (2.00)</td>
<td>18 (36.00)</td>
</tr>
<tr>
<td><em>χ²</em>-value</td>
<td>9.18/0</td>
<td>0.444</td>
<td>1.010</td>
<td>0.344</td>
<td>3.175</td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>0.002</td>
<td>0.505</td>
<td>0.315</td>
<td>0.558</td>
<td>0.075</td>
</tr>
</tbody>
</table>

but the number of patients with drowsiness was higher in the combination group than in the control group (36 vs. 20 %), but the differences between the groups were not statistically significant (p < 0.05).

**DISCUSSION**

Both uterine fibroids and adenomyosis are common gynecological conditions, but uterine fibroids are benign tumors of the female reproductive organs, while adenomyosis is a difficult condition that is best treated surgically. For patients who do not require fertility treatment, a total hysterectomy is an option for removing the lesion completely and prevent a recurrence from any leftover lesions [8]. With the development and clinical application of minimally invasive techniques, physicians and patients have widely accepted laparoscopic total hysterectomy. Although surgery can solve a variety of gynecological diseases, treating the disease as an invasive treatment, even if minimally invasive, may cause some damage to the patient and result in surgery-related complications, seriously affecting treatment outcome and prognosis [9].
In addition, studies have found that intraoperative anesthesia and pneumoperitoneum induces immune suppression in the body, and that patients undergoing total hysterectomy are prone to agitation during the awakening period, the occurrence of which may be related to numerous factors such as the stimulation of the trachea and catheter by anesthetic agents or intraoperative pain caused by surgery [10]. Patients are subjected to high levels of oxidative stress during surgical operations as well as anesthesia due to several reasons, including surgical trauma, bleeding, development of a pneumoperitoneum, and hypoxia, which result in oxidative stress injury [11]. Although laparoscopic total hysterectomy is less traumatic for patients, studies have found that different anesthetic protocols have different degrees of impact on the stress state of the organism [12].

In addition to the oxidative stress indicators levels, serum inflammatory factors and stress hormone levels are also important indicators for assessing the stress state of the body. Surgical trauma triggers abnormally high levels of inflammatory factors and lead to a high state of stress in the body [13]. An increased release of inflammatory factors, typically TNF- and IL-6, two essential cytokines in the acute inflammatory response, is brought about by surgical trauma [14]. A low-dose intravenous infusion of lidocaine also functions as an antinociceptive allergy and anti-inflammatory drug, as was discovered in previously published studies. Lidocaine was found to be beneficial in preventing the release of oxidative free radicals from patients' neutrophils during clinical anesthesia [15]. Pharmacological studies have found that lidocaine impairs the initiation of neutrophils and reduces the activation of certain inflammatory factors [16].

Dexmedetomidine is a highly selective, specific, and effective α2 adrenergic receptor agonist, and this agent is not only effective in analgesia and inhibition of sympathetic activity but also does not cause respiratory depression [17]. The anti-inflammatory effect of dexmedetomidine is mainly based on some indirect evidence [18].

The present study was intended to investigate the anesthetic effect of dexmedetomidine combined with a lidocaine regimen by looking at various aspects of patients’ surgical parameters, hemodynamics (MAP and HR), postoperative VAS and Ramsay sedation scores, and organismal serum inflammatory factors.

The results of this study showed that the surgery duration and anesthesia time were similar in both groups, but the awakening time was significantly longer in the combination group compared with the control group. However, MAP and HR levels at different time-points were significantly lower in the combination group at the time of extubation and post-extubation. Furthermore, HR level 10 min after extubation was also significantly lower than that of the control group. The combination of dexmedetomidine and lidocaine may cause delayed postoperative awakening in patients, but does not trigger significant respiratory depression. Postoperative VAS and Ramsay scores in both groups revealed that lidocaine effectively reduced postoperative VAS scores in both groups, and also reduced capsaicin-induced secondary nociceptive hypersensitivity through its central action [19]. In contrast, the analgesic effect of both agents was better after combining with dexmedetomidine.

Inflammatory factors and stress hormone levels play an important role in the summary of emergency processes such as trauma to the organism. Comparing the results of the two groups, it can be seen that TNFα, IL-6, E, Cor, and NE levels in the subjects of both groups increased to varying degrees compared with presurgery values, suggesting that the patients were in a significant state of stress after surgery. However, comparing the levels of the parameters for the two groups 24 h after surgery, it was found that the increase in the levels in the combined group was significantly smaller than in the control group. In previous studies it was confirmed that lidocaine inhibits the activation of neutrophils and reduces the release of inflammatory factors, indicating its significant anti-inflammatory effect [20]. In the present study, it was found that combining dexmedetomidine with lidocaine further reduced the inflammatory factor levels, and relieved the stress state of the body, suggesting that dexmedetomidine also plays a certain role in anti-inflammation. This may be related to the inhibition of inflammatory factor release and relief of elevated stress hormone levels. The specific mechanism still needs to be further investigated.

The incidence of postoperative adverse reactions has always been a key clinical concern, as they seriously affect the recovery status and prognosis of patients [21]. The results of the present study show that the incidence of nausea and vomiting was significantly lower in the combination group. The incidence of drowsiness in this group significantly increased, but the incidence of bradycardia, hypotension, and skin pruritus were relatively similar, indicating that combining dexmedetomidine with lignocaine did not increase the incidence of serious adverse reactions, but actually reduced the incidence of nausea and vomiting. This may be related to the
The anesthetic regimen of lidocaine combined with dexmedetomidine is effective in laparoscopic total hysterectomy, and it enhances the hemodynamic status of patients in the perioperative period, relieves postoperative pain, and improves sedation. It also reduces the level of inflammatory factors and stress hormones in patients, thus relieving the stress state of the body, and accelerating their postoperative recovery.

CONCLUSION

The anesthetic regimen of lidocaine combined with dexmedetomidine is effective in laparoscopic total hysterectomy, and it enhances the hemodynamic status of patients in the perioperative period, relieves postoperative pain, and improves sedation. It also reduces the level of inflammatory factors and stress hormones in patients, thus relieving the stress state of the body, and accelerating their postoperative recovery.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Jiao Zhang and Jun Chen designed the study and carried them out, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. Both authors read and approved the manuscript for publication.

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