Better Hemodynamic Profile of Laryngeal Mask Airway Insertion Compared to Laryngoscopy and Tracheal Intubation

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Background: Laryngoscopy and tracheal intubation can cause serious cardiovascular responses in patients such as hypertension, tachycardia, and arrhythmias. Alternative airway maintenance techniques may attenuate these hemodynamic stress responses.

Objectives: This study aimed to compare the immediate hemodynamic effects of the insertion of laryngeal mask airway supreme (LMA-S) and classic (LMA-C) with laryngoscopy and Endotracheal Intubation (ETT).

Patients and Methods: This study was a prospective, double-blind, and randomized clinical trial conducted on 150 patients aged 18 to 50 years with ASA I (American Society of Anesthesiologists), in the general operating room of Shahid Mohammadi hospital, Hormozgan university of medical sciences, Bandar Abbas, Iran. In the ETT group, endotracheal intubation was performed using the Macintosh laryngoscope; while for the LMA-C and LMA-S groups, LMA Classic and LMA Supreme were inserted, respectively. The induction and maintenance of anesthesia were similar in all patients. The hemodynamic parameters such as heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured before (baseline) and after induction of anesthesia at 4 different time points. The statistical analysis was done and P value less than 0.05 was considered significant.

Results: Participants in all groups were similar in terms of gender, age, weight, height, and Mallampati class. The mean ± SD of SBPs (105.62 ± 12.12, 112.90 ± 12.2, and 112.48 ± 15.14 mm Hg, respectively for ETT, LMA-C, and LMA-S) and DBPs (64.64 ± 10.23, 73.78 ± 9.70, and 71.20 ± 12.27 mm Hg, respectively for ETT, LMA-C, and LMA-S) were significantly lower in the ETT group compared to LMA groups 5 minutes after device insertion (P < 0.01 for SBPs and P < 0.001 for DBPs); however these values were lower than the baseline values in all groups. There were no differences in the mean SBP and DBP between the three groups at the other time points. The mean ± SD heart rates in the ETT group, compared to the LMA-C and LMA-S groups, were considerably higher in the first minute (100.06 ± 18.27, 82.50 ± 10.52, and 82.00 ± 13.60 bpm, respectively for ETT, LMA-C, and LMA-S) and fifth minute (85.82 ± 16.01, 75.78 ± 11.73, and 75.04 ± 13.90 bpm, respectively for ETT, LMA-C, and LMA-S) after intubation (P < 0.01 for SBPs and P < 0.001 for DBPs); however these values were lower than the baseline values in all groups. There were no significant differences between the LMA-C and LMA-S groups in terms of hemodynamic parameters.

Conclusions: Maintaining the airway using laryngeal mask airway is associated with less cardiovascular responses compared to direct laryngoscopy and tracheal intubation.

Keywords: Laryngeal Masks; Airway Management; Intubation-Intratracheal; Hemodynamics; Blood Pressure; Heart Rate

1. Background

The safest and most common method for maintaining the airway during general anesthesia is direct laryngoscopy and endotracheal intubation. Hemodynamic changes, such as tachycardia, hypertension, and arrhythmias can occur during intubation, which can cause myocardial ischemia. These serious complications can be a threat for the patients with underlying cardio-cerebrovascular diseases (1-4). In order to prevent adverse cardiovascular responses, laryngeal mask airway can be used instead of laryngoscopy and tracheal intubation during airway management (5). In a study conducted on controlled hypertensive patients, Bhattacharya et al. had reported an increase in hemodynamic responses following tracheal intubation compared to the insertion of laryngeal mask airway classic (LMA-C). They concluded that the LMA-C insertion can be an effective method for preventing high blood pressure responses in the airway management of patients with hypertension (6).

So far, many studies have been conducted on the use of laryngeal masks available in different forms: LMA-C, LMA for intubation (ILMA), LMA-Supreme (LMA-S), LMA-ProSeal and i-gel (7,8). LMA-S was developed in April 2007 by Intavent Orthofix Co. (Maidenhead, UK). It is a disposable airway instrument made of latex-free PVC, adapted for medical use, which has the specifications of LMA-ProSeal (enjoying the privilege of accessing the stomach...
through the esophageal port) as well as LMA Fastrach (enjoying the privilege of a curved shaft body that allows its easy insertion in the mouth and throat). The oval shape of its anatomic airway tube allows easy insertion without inserting fingers into the patients’ mouth (9, 10).

2. Objectives

In the review of medical literature, we could not find any other study on comparing hemodynamic responses between laryngoscopy for tracheal intubation (ETT), LMA-C, and LMA-S. The purpose of this study was to perform a clinical trial to compare the effects of 3 airway management instrumentations on blood pressure and heart rate.

3. Patients and Methods

3.1. Ethics

This study was approved by anesthesiology, intensive care and pain management center, and deputy for research and medical ethics committee of Hormozgan university of medical sciences (ethical committee code: HEC-92-11-2-2). Implementation of the research project and possible side effects were explained to the patients and they were included in the study after obtaining written informed consent from each patient in accordance with the Helsinki declaration.

3.2. Study Type

This clinical trial was performed as a single center, prospective, randomized, double blind study.

3.3. Sample Size Calculations

To calculate the required number of patients for 3 groups, the mean values for SBP, DBP and HR were considered based on a previously published research (7). One-way analysis of variance was used to calculate the sample size with the parameters of \( \mu_1 = 75.4, \mu_2 = 78.4, \) and \( \mu_3 = 81.9 \). In this regard, the following formulas were used:

\[
\Delta = \frac{1}{\kappa} \sum_{i=1}^{k} \left( \mu_i - \mu \right)^2
\]

\[
\Delta = \frac{1}{K} \sum_{j}^{k} \mu_j
\]

\[
\chi^2_{k-1} \left( \chi^2_{x,k-1} | \lambda \right) = \beta
\]

\[
n = \frac{\lambda}{\Delta}
\]

The sample size \( n \) was calculated from \( \lambda \) considering that \( a = 0.05, \beta = 0.1, \sigma = \sqrt{\text{MSE}} = 9.01, \) and an uneven distribution of \( k \), which was two times 12.66. By calculating \( \Delta \), the size of sample for each group was found as 50 (NCSS software). A number of 180 patients were assessed eligible and enrolled into the study, but 30 participants were excluded according to the flow chart (Figure 1).

Random allocation software version 1.0.0 was used for randomization. A random allocation sequence permutated into a single block was designed for three groups. In case of exclusion of any patient from the study, she or he was replaced by another patient with the same block code. This study was double blinded, as the other researchers who recorded the data were unaware of the type of applied airway instrument. The researcher who was assigned to collect the hemodynamic data was a medical student familiar with the airway devices and had no information about the type and groups of the study. Besides, this researcher was unable to see the scene of device insertion due to a curtain located between the head of patient and the monitor-observing site.

3.4. Inclusion and Exclusion Criteria

A number of 150 patients (aged 18 - 50 years) with ASA I and candidates for elective general surgery were included in the study. Patients with ASA > I, those with the probability of difficult intubation and Mallampati class > II, emergency surgery, full stomach, morbid obesity (BMI \( \geq \) 40 kg/m\(^2\)), cardiovascular diseases, and high intra-cranial pressure (ICP) were excluded.

3.5. Study Design

Patients were randomly assigned into one of three groups of 50 participants; the airway access and maintenance was provided with an endotracheal tube by direct laryngoscopy in the ETT group, by a classic laryngeal mask airway in the LMA-C group, and by a supreme laryngeal mask airway in the LMA-S group. After patients’ arriving at the operating room and placing on the operating table, standard monitoring devices, including capnograph, ECG, NIBP, and pulse oximeter (S/5 anesthesia monitor [Datex-Ohmeda, Finland]) were used for monitoring. The researcher who was in charge of the data collection recorded all the hemodynamic parameters of the study (baseline, pre-device insertion, and post-device insertion at first, third and fifth minute). The time points and duration of device insertion were recorded using a clock software version IOS 7.0.6 Apple Inc on an iPhone 4S device. Preinduction hydration was done with 5 ml/kg Ringer solution for all patients. Premedication was provided with intravenous midazolam (0.05 mg/kg) and fentanyl (2 pg/kg). All patients were preoxygenated with 100% oxygen through a face mask for 3 minutes before induction of general anesthesia.
Anesthesia was induced with propofol (2 mg/kg) and atracurium (0.5 mg/kg) in a similar way for all patients. In all patients, airway instrumentations were applied after a sufficient relaxation based on the neuromuscular response monitoring (TOF = 0). All the laryngoscopy and tracheal intubations were performed by one experienced anesthesiologist and all the laryngeal mask insertions were performed by another experienced anesthesiologist. After appropriate placement and adequate ventilation, airway devices were fixed in place.

In the ETT group, intubation of the trachea was attempted with a cuffed endotracheal tube (ID 7 mm for women and 8 mm for men) using direct laryngoscopy. In the LMA groups, the size of LMA was determined by the same attending anesthesiologist based on the patient’s body weight and according to the manufacturers’ instructions.
The surface of LMA-C and LMA-S were lubricated with a water base gel. The ETT and LMA cuffs were inflated to get an adequate seal according to the standards of the size used and manufactures’ guidelines (12).

If it was not possible to ventilate the lungs, the following airway maneuver would be performed: chin lift, jaw thrust, head extension, or flexion on the neck. The position was also allowed to be adjusted by gently pushing or pulling the device. After each maneuver, the adequacy of ventilation was re-assessed. Patients’ lungs were mechanically ventilated and minute ventilation was set to maintain end-tidal CO\textsubscript{2} at a range of 30 - 35 mm Hg. For LMA a maximum airway pressure of 20 cm H\textsubscript{2}O was applied. The duration of device insertion was recorded from the starting of patient’s head sniff positioning till the returning of head to neutral position after removal of the laryngoscope in ETT group, and till the return of head to neutral position after inflation of LMA cuff.

In addition to measuring the baseline values before anesthesia, hemodynamic parameters were measured at 4 subsequent stages; i.e. after the induction of anesthesia (before airway instrumentation) and at the first, third, and fifth minute after device insertion. Patients with prolonged tracheal intubation (> 30 seconds) or failure of LMA insertion at the first try were excluded from the study. In all patients, anesthesia was maintained with N\textsubscript{2}O/O\textsubscript{2} (60%/40%) and propofol infusion at 100 - 200 μg/kg/min. Fluid administration was standardized to 10 mL/kg/h of Ringer’s lactate solution.

3.6. Statistical Analysis

Demographic data, including age, sex, height, weight, and Mallampati class, along with above collected information were recorded using a checklist. Data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Variables were tested for normality using the Kolmogorov-Smirnov test (P > 0.05). All data were expressed as mean (SD) or number (%). Results of descriptive statistics were illustrated through frequency distribution tables and charts. The qualitative data were analyzed by the Chi-square test. Also we used the ANOVA test for statistically analyzing quantitative data. P value < 0.05 was considered statistically significant.

4. Results

4.1. Baseline Demographic and Clinical Characteristics

Patients in all groups were similar in terms of gender, age, weight, and height, but the duration of tracheal intubation in the ETT group was significantly higher than the LMA-C and LMA-S groups (Table 1).

The male distribution among the groups was as follows; ETT Group, 44 (88%); LMA-C Group, 46 (92%); and LMA-S Group, 46 (92%).

4.2. Hemodynamic Data

There were no significant differences between the groups in terms of Mallampati class. The percentage distribution of Mallampati class I/II were as follows: ETT Group, 80/20; LMA-C Group, 78/22; LMA-S Group, 86/14. The baseline hemodynamic parameters were comparable between the three groups. The mean SBP and DBPs values after anesthesia induction were lower than the baseline values in all groups. There was no difference in the mean SBP and DBPs among the study groups at subsequent time points, except in the fifth minute in the LMA groups (LMA-C and LMA-S) in which the mean SBP and DBPs were significantly higher than those of the ETT group. Comparing the LMA groups with the ETT group, the mean heart rate (HR) was significantly higher in the ETT group at the first, third, and fifth minutes after insertion (Table 2, Figures 2-4).

| The Group Indicator | Age, y          | Weight, kg | Height, cm | Duration of Insertion, s |
|---------------------|----------------|------------|------------|--------------------------|
| Group 1 (ETT)       | 27.60 ± 7.95   | 65.30 ± 12.49 | 168.04 ± 7.47 | 14.30 ± 6.87              |
| Group 2 (LMA-C)     | 26.76 ± 9.42   | 65.24 ± 12.07 | 170.32 ± 7.66 | 9.56 ± 4.05               |
| Group 3 (LMA-S)     | 27.36 ± 7.01   | 65.12 ± 10.27 | 171.12 ± 7.18 | 7.34 ± 3.33               |
| Average total       | 27.24 ± 8.14   | 65.22 ± 11.57 | 169.83 ± 7.51 | 9.73 ± 5.99               |
| P Value             | 0.87           | 0.99        | 0.10       | < 0.001 \textsuperscript{b} |

\textsuperscript{a} The values are presented as (Mean ± SD).
Table 2. Hemodynamic Changes Between the 3 Groups at Five Different Time Points

|                              | Group 1 (ETT)       | Group 2 (LMA-C)    | Group 3 (LMA-S)  | P Value |
|------------------------------|---------------------|--------------------|------------------|---------|
| **Systolic Blood Pressure, mmHg** |                     |                    |                  |         |
| **Baseline**                 | 119.90 ± 10.93      | 123.38 ± 8.91      | 122.66 ± 10.18   | 0.19    |
| **Before insertion**         | 102.88 ± 14.92      | 107.26 ± 11.12     | 108.00 ± 11.41   | 0.09    |
| **After insertion**          |                     |                    |                  |         |
| First minute                 | 113.26 ± 18.55      | 108.72 ± 13.94     | 110.22 ± 20.20   | 0.43    |
| Third minute                 | 110.86 ± 14.09      | 110.94 ± 12.94     | 111.54 ± 15.34   | 0.96    |
| Fifth minute                 | 105.62 ± 12.12      | 112.90 ± 12.29     | 112.48 ± 15.14   | < 0.01 b |
| **Diastolic Blood Pressure, mm Hg** |                     |                    |                  |         |
| **Baseline**                 | 76.86 ± 8.83        | 77.60 ± 4.38       | 76.82 ± 7.48     | 0.82    |
| **Before insertion**         | 60.28 ± 12.70       | 63.60 ± 7.90       | 64.28 ± 9.67     | 0.11    |
| **After insertion**          |                     |                    |                  |         |
| First minute                 | 70.96 ± 15.05       | 67.76 ± 11.98      | 66.96 ± 15.33    | 0.33    |
| Third minute                 | 67.78 ± 11.21       | 71.64 ± 11.23      | 69.38 ± 13.69    | 0.28    |
| Fifth minute                 | 64.64 ± 10.23       | 73.78 ± 9.70       | 71.20 ± 12.27    | < 0.001 b |
| **Heart Rate, bpm**          |                     |                    |                  |         |
| **Baseline**                 | 87.96 ± 61.41       | 83.46 ± 11.35      | 81.76 ± 13.64    | 0.07    |
| **Before insertion**         | 86.02 ± 16.44       | 82.90 ± 10.54      | 83.72 ± 13.50    | 0.50    |
| **After insertion**          |                     |                    |                  |         |
| First minute                 | 100.06 ± 18.27      | 82.50 ± 10.52      | 82.00 ± 13.60    | < 0.001 b |
| Third minute                 | 91.04 ± 17.12       | 78.84 ± 11.23      | 78.90 ± 13.41    | < 0.001 b |
| Fifth minute                 | 85.82 ± 16.01       | 75.78 ± 11.73      | 75.04 ± 13.90    | < 0.001 b |

a All values are expressed as mean ± SD.
b P < 0.05 is significant.

Figure 2. Mean Systolic Blood Pressure of Patients at Different Time Points

Baseline, before, first minute, third minute, fifth minute insertion.

Figure 3. Mean Diastolic Blood Pressure of Patients at Different Time Points

Baseline, before, first minute, third minute, fifth minute insertion.
5. Discussion

5.1. General Consideration

Direct laryngoscopy and tracheal intubation can cause exaggerated cardiovascular responses such as hypertension and tachycardia. Increase in blood pressure usually starts 5 seconds after laryngoscopy, and culminates in the next 1 to 2 minutes. It returns to the baseline level in the fifth minute. Most patients with normal heart function may tolerate such changes without serious complications while in patients with altered cardiac reserves, these hemodynamic turbulences may be hazardous.

Various methods and techniques such as increasing the depth of anesthesia, administration of intravenous opioids, local anesthetics, beta blockers, and tracheal intubation by flexible fiberoptic laryngoscopy have been used to minimize hemodynamic responses, but none of them are ideal (13).

5.2. Interpretation and Comparing the Findings for Blood Pressures

Several studies have been conducted on the use of laryngeal mask insertion. Siddiqui et al. compared hemodynamic responses after tracheal intubation by the 2 methods; laryngoscopy and intubating laryngeal mask airway (ILMA). They concluded that the use of laryngeal mask for intubation is associated with minimal hemodynamic changes; therefore, it can be used for patients in whom a marked pressor response would be deleterious (8).

From a different perspective, it seems that the comparison of laryngeal masks alone with laryngoscopy and tracheal intubation may not provide a completely similar condition for stimulation response estimation. Because according to the rank order of noxious stimuli, tracheal intubation is the most powerful affecter, which requires the deepest level of anesthesia (14). In a study by Kahl et al. on patients who had coronary artery bypass graft (CABG) surgery, ILMA was compared to conventional laryngoscopy for tracheal intubation. The heart rate, blood pressure, and noradrenaline levels in the ILMA group were statistically lower (15). This again indicates how and to what extent tracheal intubation by using supraglottic airway devices can be effective in reducing the cardiovascular responses.

In the study by Eghbal et al. laryngoscopy and endotracheal intubation was compared to LMA-C in the patients undergone dacryocystorhinostomy; their LMA-C group had less hemodynamic changes, coughing, straining, and a lower incidence of nausea and vomiting at the end of anesthesia (16). Bennett SR et al. compared the hemodynamic effects of LMA versus ILMA and laryngoscopy for tracheal intubation in patients with coronary disease and concluded that LMA allows airway management without hypertension and tachycardia (17). The results of the previous studies are consistent with the results of the current study. The only exception was SBP and DBP in the fifth minute that was significantly lower in the ETT group compared to the LMA groups (5, 6, 8, 15-17).

In the analysis of the sequence of changes in SBP and DBP compared to the baseline, no significant changes were found in any of the time points. This was also in contradiction with the findings of the previous study (7). In justifying the possible reasons for the blood pressure changes, it can be noted that in this study, propofol was used for both the induction and maintenance of anesthesia which provided somewhat greater hypotension compared to sodium thiopental used in most other studies (6, 18). Additionally this finding may be due to the loss of stimulatory effects of direct laryngoscopy and intubation, which is usually transient and returns to the control status within 5 minutes after the first insult (13). Another speculation for the higher blood pressure in the fifth minute in the LMA groups is attributed to the mechanical stimulus to the supralaryngeal area, which is rich in nociceptive receptors, and can cause strong hemodynamic responses (19). This is supported by the study by Sener EB et al. They concluded that the further increase in hemodynamic parameters with ILMA compared to laryngoscopy and tracheal intubation could be due to the production of excessive pressure on the oral laryngeal structures by ILMA, which could exceed the larynx capillary perfusion pressure and overstimulate the local structures of the larynx (20). Another possible explanation for the findings of Sener EB et al. could be due to the longer duration for insertion of ILMA compared to laryngoscopy and tracheal intubation (20).

But the results of the study conducted by Kihara et al. are also worth noting. They concluded that devices other than Macintosh laryngoscope such as the Trachlight™ lightwand (LW) and the intubating laryngeal mask airway Fastrach™ (ILM) cause less hemodynamic changes during intubation. This benefit was only seen in hypertensive patients. Surprisingly in the patients with normal blood pressure, the hemodynamic changes with these devices were similar to the ETT group (21). However, the
effect of blood pressure (normal or high) in determining the hemodynamic responses to the method used in the management of the airway is still uncertain and there are many different and conflicting results (2, 21-24)

5.3. Interpretation and Comparing the Findings for Heart Rate

Heart rate is the main determinant of myocardial oxygen consumption, and there is so much evidence that suggests patients with coronary artery disease have low tolerance to tachycardia. When the heart rate goes higher than 110 bpm, the incidence of myocardial ischemia during surgery increases (25).

The results of examining the mean HRs of the patients in 3 groups at 5 different time points indicate that there is no significant relationship between these 3 groups at baseline and before intubation. However, in the first, third, and fifth minute after intubation, the mean HRs in the ETT group showed a significant increase compared to the LMA groups. This finding was consistent with the results of previous studies (5, 6, 15-17, 26). Moreover, these findings were in contrast with the results of Siddiqui et al. (8) and Sener EB et al. (20).

5.4. Interpretation and Comparing the Findings for Duration of Insertion

Duration of laryngoscopy can also have significant impact on the hemodynamic responses. As Bucx et al. showed laryngoscopy alone, and only for 3 seconds, has less hemodynamic effect but significant changes compared to laryngoscopy with endotracheal intubation (27). In contrast, Shirbman et al. showed that an increased duration of laryngoscopy causes longer sympathetic stimulation, and as a result, they recorded equal hemodynamic responses to laryngoscopy (alone) performed only within 10 seconds, compared to laryngoscopy with endotracheal intubation (ETT) (4). In the current study, the duration of insertion for ETT, LMA-C, and LMA-S groups were 14.30, 9.56, and 7.34 seconds, respectively. As more time was needed for ETT insertion, hemodynamic changes got more prominent compared to the LMA groups. Our device insertion time for LMA-S was shorter (7.34 vs. 13.5 seconds) compared to the time reported by Belena et al. (28). Besides, they did not explain in detail how they measured the duration of LMA-S insertion.

In the study conducted by Chloros et al. on manikins of cardiopulmonary resuscitation (CPR), insertion of LMA-C was compared to LMA-S by healthcare professionals who did not have previous experience in this procedure. The time required for successful insertion in non-CPR scenario for LMA-S compared to LMA-C was 10.4 ± 2.7 seconds vs. 13.4 ± 3.2 seconds, respectively, and the difference was significant (29). This timing was longer than the timing of our study, which could be due to the insertion of airway devices by an experienced anesthesiologist in contrast to the healthcare personnel of Chloros et al. study (29).

Because it has been proven that the hemodynamic changes are associated with the duration of tracheal intubation, i.e. manipulation of the upper airway (8), in our study, the duration of LMA insertions was much less than laryngoscopy with tracheal intubation. Especially the LMA-S insertion time was almost half of that for laryngoscopy with tracheal intubation (7.34 vs. 14.30 seconds) so an easier and faster insertion of LMA compared to laryngoscopy with tracheal intubation has milder possible hemodynamic effects.

5.5. Conclusions

We did not find any other related study in the literature comparing endotracheal intubation by laryngoscopy with LMA-S and their hemodynamic effects. In our study, changes in SBP, DBP, and HR in the first minute after intubation were equal to 10.08%, 17.71%, and 16.32%, respectively in the ETT group; 13.6%, 6.54%, and -0.68%, respectively in the LMA-C group and 2.05%, 4.17%, and -2.05%, respectively in the LMA-S group. The amount of increase in SBP and DBP in the first minute was comparable for the LMA groups, but unexpectedly the HR decreased in the LMA groups. If a range of 15% change rate in hemodynamic indexes is considered acceptable (30), no unstable hemodynamic parameters are observed in the LMA groups of our study, where the least changes occurred in the LMA-S group.

Therefore, considering the more favorable hemodynamic changes following insertion of LMA compared to intubation with ETT in this study, especially with regard to the changes in the heart rate and lesser duration of insertion, we recommend using LMA, particularly LMA-S as a useful airway instrumentation in patients undergoing surgery, especially those who suffer from ischemic heart disease.

One of the limitations of the present study is the exclusion of patients with airway problems. In these patients, airway problems require a longer intubation time, which can result in different outcomes. On the other hand, the adverse effects of using LMA and tracheal intubation were not examined in the present study. This issue could be addressed in the future studies. Also, this study was conducted on patients with ASA I. Making such a comparison on patients with underlying cardiac diseases may bring out different results. Further studies should be conducted in this regard and on hemodynamic effects of different types of LMA in future.

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**Authors’ Contributions**

Hashem Jarineshin: Designing the study, supervising and coordinating all stages of the study. Saeed Kashani: Performing the laryngoscopy and endotracheal intubation procedures. Majid Vatankhah: Performing the classic laryngeal mask airway procedures and the laryngeal mask airway supreme procedures. Alireza Abdulhazade Baghaee: Supervising the procedures and statistical analysis. Sahar Sattari: Collecting and analyzing the data and computer workups. Fereydoon Fekrat: As the corresponding author, writing the manuscript, editing the final statistical analysis and checking data. All authors read and approved the manuscript.

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