Assessment of vena cava inferior diameter with ultrasonography in patients undergoing spinal anesthesia and evaluation of postoperative hypotension

Ahmet Ozdemir¹, Sinan Yilmaz¹*, Mustafa Ogurlu¹

Abstract

Objective: Hypotension frequently occurs during spinal anesthesia and has serious complication that requires early and quick treatment. The goal of this study was to predict the state of fluids in the patient’s body through measurement via ultrasonography of the inferior vena cava diameter (IVCd) prior to administering the spinal anesthesia. This study also intended to investigate the presence or not of a relationship between the ultrasonography IVCd measurements before spinal anesthesia and hypotension.

Material and Methods: A prospective study was performed in which the patients (n:115) were grouped based on their ultrasonography IVC transverse diameter measurements: those with <1.5 cm diameters were categorized as Group 1 (n:22), those having 1.5-2 cm diameter in Group 2 (n:67) and those possessing >2 cm diameter in Group 3 (n:26). The hemodynamic parameters of all the patients were measured.

Results: Hypotension was observed in 23 (20%) patients, particularly in the 5th minute post administration of spinal anesthesia. Hypotension was especially high in Group 1 (52%) compared to Group 2 (34.8%) and Group 3 (13%) (p<0.001).

Conclusion: We concluded that IVCd measurement with ultrasonography is a significant indicator which helps to determine the volumetric status of the patient. However, considering the development of the complication of hypotension post SA, it is felt that the IVC diameter measurement by USG might not always be adequate by itself, and needs to be assessed together with the clinical parameters of old age and presence of chronic diseases.

Key words: hypotension, ultrasonography, spinal, anesthesia, vena cava inferior diameter

Introduction

Spinal anesthesia (SA) is a commonly implemented anesthetic procedure, as it is fast-acting and easy to perform. However, hypotension, a complication which frequently occurs during SA, necessitates early and fast treatment. Hypotension is the term applied when the systolic arterial pressure drops below 90 mmHg or decreases by 20-30% from the initially recorded value (1). The hypotension observed in patients under spinal anesthesia is usually a result of the vasodilatation in the arteries connected to the preganglionic sympathetic nerve block (2). Besides, hypotension is observed more frequently in patients with dehydration and hypovolemia because of the maximal dilatation in the veins with a sympathetic block, peripheral pooling of blood and decreased cardiac output due to the reduced venous circulation (3).

Although various methods (fluid infusion, vasopressor drugs and prophylaxis) are administered to circumvent this complication, no ideal treatment has yet been identified.

The inferior vena cava (IVC) is a vein whose elasticity enables it to vary in diameter according to respiration and total body fluids (4). The IVC diameter measured by ultrasonography (USG) was shown to have a correlation with the volume of circulating blood (4,5). Besides, it was also shown that the IVC diameter measured by USG in hypovolemic shock gave better results on the fluid situation within the body compared with the blood pressure and heart rate (4). Therefore, it is deduced that the IVC diameter measured via USG may be utilized to determine the volumetric composition of the body (4).
In this study, the main aim was to estimate the status of the body fluid through IVC measurement via USG prior to administering spinal anesthesia. The goal was also to investigate the presence of a relationship between the vena cava inferior diameter and hypotension which measurement of IVC diameter completed using ultrasound, prior to administering the spinal anesthesia and after the spinal anesthesia.

Material and methods

The prospective study was performed from 1st June, 2016 to 1st December, 2016. When the power analysis was done for the exploratory IVC diameter according to the study of Celebi Yamanoğlu, the effect size was 0.397, alpha 0.05, statistical power 90%, and at least 69 patients should have taken (6). A total of 115 patients (all volunteers) between 18 and 75 years of age who were applied SA prior to elective orthopedic surgery were included in the study. They were categorized according to the physical status classifications of I-II, based on the standards set by the American Society of Anesthesiology (ASA). Approval for the study was obtained from the local ethics board (Ethical code: 2016/898). Besides, all the patients gave signed consent.

Emergency surgical operations, cases of pregnancy, patients contra indicated for spinal anesthesia (brain tumor, hypovolemic shock, severe anemia, peripheral vascular disorders, autonomic disorders, heart failure, mental disorder, bleeding diathesis, anticoagulant treatment) and unacceptable cases for spinal anesthesia were excluded.

The demographic data of all the patients were recorded. Blood pressure measurements were taken using the oscillometric method. Preoperative systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), heart rate (HR) and oxygen saturation (OS) values were noted for all the patients. The IVC-USG measurement was performed by the same anesthesiologist, preferably possessing at least 4 years of ultrasonography experience. The inferior vena cava diameter was measured only once. However, with the patients kept supine the IVC diameter measurements were taken employing ultrasonography (Esaote MyLab Five) in B-mod, using a 1-8 MHz convex probe transducer only on the longitudinal plane, at the end of expiration. After the images were fixed, the IVC transverse diameter measurements were taken at 2 cm distal to the point where the hepatic veins joined with the IVC. All the patients showed USG diameter measurements to be median 1.77 (25-75 percentile 1.57-1.97). The patients were then grouped based on their USG IVC transverse diameter measurements. Group 1 included those below the 25th percentile (<1.5 cm), while Group 3 included those above the 75th percentile (>2 cm), and Group 2 were those with a diameter of 1.5-2 cm. A 20% or more decrease in the basal blood pressure measurement was accepted as hypotension (7). Bradycardia was defined as heart rate below 50 per minute (7). For all the patients the spinal anesthesia was administered using the 25-gauge Quincke needles and the same dose (12.5 mg 0.5%) of hyperbaric Bupivacaine (8) was given to all of them. Spinal anesthesia was administered with the patient in the sitting position. The patient was transferred in the supine position post the spinal anesthesia. The SBP, DBP, MAP, HR and OS values for all the patients were measured at the 0, 5, 10, 15, 20, 25 and 30th minute after the spinal anesthesia was given, and data were recorded. In fact, 5 minutes after the spinal operation, the degree of block was confirmed using the pinprick test. Besides, any complications that developed within 30 minutes were also noted. All the patients were given 3l min-1 of O2 through the nasal cannula during the operation. No pre-hydration was performed for any of the patients. During the operation, the patient’s preoperative time of fasting, maintenance of fluid and state of bleeding were calculated, based on which the patient was given a crystalloid fluid. The estimated maintenance fluid was calculated according to rule 4/2/1. At the first hour, half of the total maintenance fluid was applied, the remaining 1/4 fluid was applied at the second hour, and the last 1/4 fluid was applied at the third hour. (9,10). No record was taken because of the absence of significant blood loss during the first half-hour of the operation.

Statistical Analysis

The data were then analyzed using the SPSS statistics software. Percentages, means and standard deviations were used for the descriptive statistics. The normal distribution exhibit data was tested using the One-sample Kolmogorov Smirnov test. The normal distributed data were compared with the One Way ANOVA and Repeated Measures ANOVA. The non-normal distributed data were compared using the Kruskal-Wallis and Friedman variance analyses. The Pearson correlation analysis was used to determine the correlation between the numeric variables that indicated normal distribution. The Spearman rho correlation analysis was used to determine the correlation between the numeric variables showing non-normal distribution. Type 1 error level was accepted as 0.05. The Bonferroni correction was applied in the post-hoc analysis.

Results

The demographic and preoperative data of the 115 patients are shown in Table 1. Group 1 showed mean age significantly higher than that of the other two groups, and the mean body weight, height and heart rate of the male patients in Group 1 were significantly lower. No significant difference was evident among the groups in terms of the types of orthopedic surgery (p: 0.251). With respect to additional diseases, again Group 1 revealed a higher frequency of HT-DM and other diseases (Table 1). Besides, the preoperative SBP and MAP values were significantly higher in Group 1 compared to the other groups. It was evident that blood pressure increased with age. The amount of intraoperative fluid was higher in Group 1, and this difference was statistically significant (p: 0.013). Considering the block levels checked in the 3rd minute post spinal anesthesia, while the maximum of T6 block was observed in only one patient in Groups 1 and 2, blocks of T10 or lower were revealed in 40.9% in Group 1, in 49.3% in Group 2 and in 11.5% in Group 3 (p: 0.436).

Hypotension was observed in 23 (20%) patients, particularly in the 5th minute post administration of spinal anesthesia. Hypotension was especially high in Group 1.
(52%, n: 12) compared to Group 2 (34.8%, n: 8) and Group 3 (13%, n: 3) (p<0.001). The use of vasopressors in Group 1 (n: 5/22) was significantly higher than in the other two groups (respectively, n: 3/67, n : 1/26) (p<0.015).

On comparing the intergroup SBP values of the participants, the preoperative and 0th min mean SBP values of Group 1 were definitely higher than those of the other groups; however, the mean SBP values of Group 1 at the 10, 15, 20, 25 and 30th min were significantly lower (p<0.05) (Table 2) (Figure 1). From the intragroup repeated measures, it is clear that significant decreases in the SBP values were evident between the 0th and 5th min in Groups 1, 2 and 3 and between the 5th and 10th min in Groups 1 and 2 (p <0.001) (Figure 1). While the SBP values of Group 1 declined by more than 20% between the 0th and 5th min, the reduction in the other two groups was below 20% (Figure 1).

On comparison of the intergroup DBP values of the participants, the preoperative, 0th min and 5th min mean values were shown no significant difference (p>0.05). However, in the assessments done at the 10, 15, 20, 25 and 30th min, the DBP values of the Group 1 patients were significantly lower (p<0.05) (Table 2) (Figure 2). Besides, the DBP values showed a clear drop between the 0th and 5th min in Groups 1, 2 and 3 and between the 5th and 10th min in Groups 1 and 2 (p <0.001) (Figure 1). While Group 1 revealed a more than 20% drop in the DBP values between the 0th and 5th min, the decrease in the other two groups was below 20% (Figure 2).

When the intergroup MAP values of the participants were compared, the preoperative and 0th min mean values of Group 1 were observed to be much higher compared to the other groups; however, the mean MAP values of Group 1 at the 10, 15, 20, 25 and 30th min were significantly lower (p<0.05) (Figure 3). A significant decline in the MAP values were noted between the 0th and 5th min in Groups 1, 2 and 3 and between the 5th and 10th min in Groups 1 and 2 (p<0.001). While the Group 1 MAP values reduced by more than 20% between the 0th and 5th min, the reduction in the other two groups was below 20% (Figure 3). Positive correlation was detected between the IVC diameter and hemodynamic data [10th-15th-20th-25th minute of SBP (respectively, p: 0.002, r = 0.290, p: 0.004, r = 0.267, p: 0.026, r = 0.207, p: 0.029, r = 0.203), 10th-15th-20th-25th minute of DBP (respectively, p: 0.004, r = 0.269, p: 0.001, r = 0.312, p: 0.002, r = 0.286, p: 0.018, r = 0.220), 10th,15th, 20th minute of MAP (respectively, p: 0.001, r = 0.299, p: 0.002, r = 0.291, p: 0.021, r = 0.214)], age and hemodynamic data (0th minute of SBP-DBP-MAP) (respectively p: 0.001, r = 0.555, p: 0.001, r = 0.334, p: 0.001, r = 0.563).

No significant difference was observed among the groups in terms of heart rate and oxygen saturation values (p>0.05). Besides hypotension, other significant complications were evident after spinal anesthesia, more frequently in Group 1 (22.7%, n= 5/22) compared with the other groups (6%, 3.8%) (p: 0.033). Group 1 revealed complications like bradycardia in 13.6% (n= 3), nausea-vomiting in 4.5% (n= 1) and restlessness in 4.5% (n= 1).

Table 1: Comparison of patients’ demographic and preoperative data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (&lt;1.5 cm) (n:22)</th>
<th>Group 2 (1.5 - 2 cm) (n:67)</th>
<th>Group 3 (&gt;2 cm) (n:26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.7 ± 10.5</td>
<td>44.8 ± 15.7</td>
<td>47.1±14.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.8 ± 7.5</td>
<td>168.1±10.2</td>
<td>168.6±11.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72.1 ± 13.1</td>
<td>77.7 ± 13.6</td>
<td>86.3±17.0</td>
<td>0.003**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 ± 4.8</td>
<td>27.6 ± 5.6</td>
<td>30.6 ± 7.4</td>
<td>0.137</td>
</tr>
<tr>
<td>Sex (M/F) (n)</td>
<td>5/17</td>
<td>41/26</td>
<td>18/8</td>
<td>0.002*</td>
</tr>
<tr>
<td>ASA (I/II) (n)</td>
<td>4/18</td>
<td>46/21</td>
<td>18/8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Preoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>152.4±23.9</td>
<td>135.9±19.8</td>
<td>140.7±17</td>
<td>0.005*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.9±13.3</td>
<td>77±12.1</td>
<td>80.3±11.3</td>
<td>0.307</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>105.1±16.3</td>
<td>96.2±13.1</td>
<td>99.6±11.6</td>
<td>0.028*</td>
</tr>
<tr>
<td>Heart rate (min)</td>
<td>83.6±14.3</td>
<td>79.3±12.4</td>
<td>79.1±12.8</td>
<td>0.359</td>
</tr>
<tr>
<td>O2 saturation</td>
<td>95.8±1.5</td>
<td>96.7±1.7</td>
<td>96.5±1.8</td>
<td>0.120</td>
</tr>
<tr>
<td>Additional Diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT (%)</td>
<td>37</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>DM (%)</td>
<td>18</td>
<td>7</td>
<td>5</td>
<td>0.010</td>
</tr>
<tr>
<td>Other diseases (%)</td>
<td>27</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>None (%)</td>
<td>18</td>
<td>66</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Intraoperatively provided fluid</td>
<td>13.6</td>
<td>41.8</td>
<td>30.8</td>
<td></td>
</tr>
<tr>
<td>500-999 ml (%)</td>
<td>1000-1499 ml (%)</td>
<td>49.3</td>
<td>57.7</td>
<td>0.013*</td>
</tr>
<tr>
<td>&gt;1500 ml (%)</td>
<td>18.2</td>
<td>9</td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference caused by the group with IVC diameter of <1.5 cm.
** Significant difference caused by the group with IVC diameter of ≥2 cm.
Table 2: Blood pressure measurements of the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>p²</th>
<th>p &amp;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop</td>
<td>152±23.9</td>
<td>80.9±13.3</td>
<td>135.9±19.8</td>
<td>77±12.1</td>
<td>140.7±17.0</td>
<td>79.6±11.3</td>
<td>0.005*</td>
<td>0.307</td>
</tr>
<tr>
<td>0th min</td>
<td>163 (139.5-178.2)</td>
<td>79.4±11.5</td>
<td>136 (124-155.0)</td>
<td>77.7±11</td>
<td>143 (136.5-157)</td>
<td>79.6±10.2</td>
<td>0.003*</td>
<td>0.690</td>
</tr>
<tr>
<td>5th min</td>
<td>122 (104.7-147.2)</td>
<td>64.6±11.5</td>
<td>122 (115-141)</td>
<td>69.7±13.7</td>
<td>129 (118.2-137.2)</td>
<td>69.9±10.9</td>
<td>0.657</td>
<td>0.238</td>
</tr>
<tr>
<td>10th min</td>
<td>107 (96.0-124.2)</td>
<td>57.5±10.1</td>
<td>117 (109-130)</td>
<td>67.4±11.6</td>
<td>128 (112.7-134)</td>
<td>68.2±10.7</td>
<td>0.009*</td>
<td>0.001*</td>
</tr>
<tr>
<td>15th min</td>
<td>106 (95.7-116.2)</td>
<td>56.5±8.2</td>
<td>117 (106-131)</td>
<td>65.2±11.8</td>
<td>124 (108.7-131.2)</td>
<td>69.5±15.6</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
<tr>
<td>20th min</td>
<td>107.9±19.7</td>
<td>56.0±9.3</td>
<td>120.2±17.7</td>
<td>66.4±11.6</td>
<td>122.1±18.3</td>
<td>68.2±12.8</td>
<td>0.013*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>25th min</td>
<td>107.4±19.2</td>
<td>55.6±9.3</td>
<td>121.7±19.1</td>
<td>66.1±11.1</td>
<td>122.0±20.3</td>
<td>66.0±12.8</td>
<td>0.011*</td>
<td>0.001*</td>
</tr>
<tr>
<td>30th min</td>
<td>110±18.7</td>
<td>57.8±10.0</td>
<td>121.7±19.8</td>
<td>67.0±11.9</td>
<td>121.2±16.4</td>
<td>66.2±12.0</td>
<td>0.041*</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

Data mean ± SD / Median (25-75 P)
* Significant difference caused by the group with IVC diameter of <1.5 cm.
** Significant difference caused by the differences between the 0th min and 5th min, and between the 5th min and 10th min.
*** Significant difference caused by the difference between the 0th min and 5th min.

p² Intergroup SBP comparison, p& Intergroup DBP comparison, p Intergroup repeated measures

Figure 1: Comparison of mean SBP values of the groups

Figure 2: Comparison of mean DBP values of the groups
Discussion

This study investigated the relationship between the USG IVC diameter measurement in orthopedic patients and hypotension post SA. Very limited studies are available in the literature on the USG measurements of the IVC diameter in patients requiring SA (11). In the present study, the rate of hypotension post the administration of SA to patients having IVC diameters of <1.5 cm (52%) was higher than those in the other groups. Hence, it is concluded that the IVC diameter measurement may be a useful indicator in predicting the potential hypotension that may occur after spinal anesthesia.

Although spinal anesthesia is a widespread and safely used technique to administer regional anesthesia, it may cause significant complications, particularly hypotension (12,13). However, an objective assessment of the state of intravascular volume in patients needing spinal anesthesia may not be easy (9). Knowledge of the patient's fasting time or normal weight might not always produce accurate results. In our study, we observed that the Group 1 patients with IVC diameters of <1.5 cm had a higher rate of hypotension (52%) after SA application compared to both the literature (30-40%) (8) and the other two groups. Besides, the Group 1 patients more frequently experienced other complications.

In studies conducted on healthy volunteers, the IVC measurements made at the 2 cm distal of the point where the hepatic veins reach the IVC and on the level of the left renal vein were observed to be only slightly affected by the respiratory changes (13). Therefore, the measurements in this study were taken from the subxiphoid window, by directing a convex probe towards the right anterior-medial axillary line, and at 2 cm distal of the site where the hepatic veins reach the IVC on a longitudinal plane and at the end of expiration.

The IVC diameter measurements were noted to change between inspiration and expiration (4).

The negative pressure created by inspiration causes the IVC to collapse by increasing venous return to the heart, while expiration induces the IVC to return to its normal diameter by reducing the venous return to the heart. In the present study, an attempt was made to measure the normal diameter by taking the IVC diameter value at expiration. Additionally, while the veins show greater collapse at lower intravascular volumes (15), it was our assumption that the narrow diameter, in Group 1 in particular, which included older patients, may have a bearing on this issue.

Total body fluids decrease corresponding to increased age. Besides, it is known that during fluid distribution at older ages, the intracellular fluid increases and extracellular (intravascular and interstitial) fluid decreases (16). In the present study, Group 1 included the older patients with lower body weights, and fewer male patients. Therefore, it was felt that the Group 1 patients had less total body fluid due to their older age and low body weight. It was also understood that this group had less intravascular fluid compared to the other two groups.

Group 1 had significantly higher numbers of patients with HT and DM. Therefore, this study deduced that baseline blood pressure measurements of Group 1 patients are higher than that of the other groups. Post-SA hypotension risk has been noted to be higher in the older patients and those with chronic disease and a history of antihypertensive drug usage. It was reported that, with equal doses of spinal anesthesia, the drop in blood pressure and peripheral vascular resistance was higher in patients experiencing chronic hypertension compared to the normotensive ones (17). Also, in diabetic patients, noticeable hypotension may be observed post spinal anesthesia due to some autonomous nervous system dysfunction (18). In our study, more than 50% of the patients with hypotension post SA were receiving antihypertensive treatment, and 50% of them had diabetes mellitus.
Group 1 patients most frequently experienced hypotension after spinal anesthesia, and the reasons listed above (body fluid distribution, age, and chronic diseases) were deduced to have caused this hypotension. However, hypotension was observed post spinal anesthesia in 2 patients with chronic hypertension in Group 3, which included those with the widest IVC diameters. Thus, this study enabled the conclusion that the IVC diameter and presence of additional diseases may both exert some influence.

Several studies reported that IVC diameter measurement may prove to be a useful and non-invasive method for the diagnosis and monitoring of hypovolemic patients (13, 19-21). Besides, a correspondence was reported between the right atrium pressure measured by invasive venous catheterization and IVC diameter, and the IVC diameter is a good indicator of the volumetric status of the patient (5-21). Although hemodialysis patients revealed a weak relationship between the amount of fluid removed during ultrafiltration and post-dialysis blood pressure, it was clear that there was a strong relationship with a reduction in the IVC diameter (13, 20). The IVC diameter measurement is observed in the flow charts of the manuals of the departments like the intensive care and emergency services where critical patients are monitored, for the assessment and monitoring of these patient’s fluid requirements (22,23). Again, in the obstetrics patients experiencing rapid intravascular volume changes, the IVC diameter measurement may be a significant method to help to direct the treatment (10).

To prevent hypotension or minimize its prevalence, the most frequently used methods include intravenous fluid application, physical methods which boost the venous return, and the use of vasopressor related agents (24,25). Although these methods are frequently in use today, the debate continues on which fluid will be applied and the quantity, and the choice of vasopressor to be used. This study revealed that the intraoperatively provided fluid was in greater quantity in Group 1, where hypotension was observed more frequently, compared to the other groups.

The incidence of bradycardia post spinal anesthesia was reported at different rates in several studies (e.g. 20% and 33%) (26,27). The occurrence of bradycardia in the present study, in ASA I-II patients was 5.2%, a lower value than those reported in the literature. However, Group 1 showed the highest rate of bradycardia, and it must be noted that the sample in this study was smaller than the ones reported in the literature.

The present study included some limitations, as the patients were grouped based on their USG IVC diameter measurements. This is one reason, we feel, that the unequal numbers of patients in the groups might have affected the findings. Besides, the total number of patients in this study was low. Thirdly, while the caval index calculated by the expiration and inspiration measurements on the IVC diameter is a reliable assessment tool, the measurements in this study were taken only at expiration.

Conclusion
We concluded that IVC diameter measurement with ultrasonography, a non-invasive method that can easily be performed on patients, is a significant indicator which helps to determine the volumetric status of the patient. However, considering the development of the complication of hypotension post SA, it is felt that the IVC diameter measurement by USG might not always be adequate by itself, and needs to be assessed together with the clinical parameters of old age and presence of chronic diseases. Further studies in the future, involving a greater number of cases will bring more clarity to this issue.

Acknowledgement: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author’s Contributions: AO, SY, MO: Research concept and design, data collecting, analysis and interpretation of data. SY: Preparation of article and revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

References


