A dilemma for women: having many children risks deterioration of diastolic functions

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Background. Echocardiography is the most widely used diagnostic tool for detecting changes in cardiac function. Pregnancy is a dynamic process that affects the cardiovascular system and recent studies have shown that increased parity may cause irreversible changes in the cardiovascular system. In this study, we aimed to evaluate echocardiographic changes in women, especially grand multiparous (6 to 9 parities) and great grand multiparous (more than 9 parities) women, after all their pregnancies had finished. Methods. This was a cross-sectional study and contained 195 female patients. Women with one delivery were defined as primiparous (PP), 2 to 5 deliveries were defined as multiparous (MP), 6 to 9 deliveries were defined as grand multiparous (GMP) and more than 9 deliveries were defined as great grand multiparous (GGMP). Results: The mean age at cardiac evaluation was 59.6 ± 16.3 and mean parity was 6.5 ± 4.2. Diastolic dysfunction was grouped as grade 1–3 and this was determined according to the E/e’ ratio. Spearman correlation analysis showed that diastolic dysfunction had positive correlations with parity, age, hypertension, and diabetes mellitus. Receiver-operating curve (ROC) analysis showed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity. Discussion: In the present study, we showed that diastolic dysfunction significantly increased as the number of pregnancies increased. Additionally, the cut-off value of parity for diastolic dysfunction was 6.5 which is higher than other studies.

Keywords
Pregnancy, Echocardiography, Left ventricular dysfunction

1. Introduction

Echocardiography is the most widely used diagnostic tool for detecting changes in cardiac function [1] and innovations in assessment of cardiac ventricular functions are ongoing [2]. The systolic and diastolic functions of the heart can be affected by many variables [3, 4] and systemic chronic diseases (diabetes mellitus, hypertension, hyperlipidemia) in particular negatively affect these functions. However, in healthy populations, some conditions such as pregnancy may also change cardiovascular mechanisms.

Pregnancy is a dynamic process that affects the cardiovascular system. During pregnancy maternal cardiac output, preload and maternal blood volume increase and systemic vascular resistance decreases [5]. These changes are necessary for the continuation of pregnancy and the health of the fetus. Most of the changes that occur during pregnancy return to normal after pregnancy [6].

Recent studies have shown that increased parity may cause irreversible changes in the cardiovascular system [7, 8]. Left ventricular diastolic functions deteriorate during pregnancy and this is associated with increased cardiovascular mortality [9–11]. Diastolic function and other cardiovascular changes tend to return to normal postpartum; however as parity increases, diastolic parameters are affected and these reversible changes may become permanent [6, 12].

In this study, we aimed to evaluate echocardiographic changes in women, especially grand multiparous (6 to 9 parities) and great grand multiparous (more than 9 parities) women, after all their pregnancies had finished.

2. Patients and methods

This was a cross-sectional study and contained 195 female patients. Exclusion criteria were patients under 18 years-of-age, a history of coronary artery disease, heart failure, structural heart diseases, rhythm disorders, renal or hepatic disorders and women who were currently pregnant. Inclusion criteria were patients with a history of one or more deliveries and completion of their pregnancy with a living birth. Also, patients with a history of hypertension and diabetes mellitus that may affect left ventricular diastolic functions were included in the study. For all patients, the time since the last birth was at least one year. A written consent form was signed by all the participants. The study was designed in accordance with the Helsinki Declaration’s ethical standards.

Women with a history of one delivery were defined as primiparous (PP), 2 to 5 deliveries were defined as multiparous (MP), 6 to 9 deliveries were defined as grand multiparous (GMP) and more than 9 deliveries were defined as great grand multiparous (GGMP).
Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Primiparous 1 delivery n = 16</th>
<th>Multiparous 2 to 5 deliveries n = 73</th>
<th>Grand multiparous 6 to 9 deliveries n = 46</th>
<th>Great grand multiparous &gt;9 deliveries n = 60</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (± SD)</td>
<td>30.8 ± 9.0</td>
<td>39.4 ± 8.8</td>
<td>52.8 ± 11.9</td>
<td>67.7 ± 9.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parity number, n</td>
<td>1.9 ± 0.0</td>
<td>2.9 ± 1.0</td>
<td>7.5 ± 1.2</td>
<td>11.7 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>2.12%</td>
<td>3.14%</td>
<td>8.17%</td>
<td>19.31%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>4.25%</td>
<td>4.29%</td>
<td>32.69%</td>
<td>55.91%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>105.6 ± 15.9</td>
<td>115.6 ± 19.4</td>
<td>132.4 ± 22.0</td>
<td>137.5 ± 23.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>67.1 ± 9.6</td>
<td>75.5 ± 12.0</td>
<td>83.0 ± 11.3</td>
<td>83.2 ± 12.9</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abb. BP, blood pressure; SD, standard deviation.

Table 2. Echocardiographic parameters of the study groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PP</th>
<th>MP</th>
<th>GMP</th>
<th>GGMP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E, cm/s</td>
<td>87.7 ± 15.4</td>
<td>89.1 ± 26.2</td>
<td>102.4 ± 19.8</td>
<td>90.7 ± 25.3</td>
<td>0.017</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>66.6 ± 11.8</td>
<td>78.2 ± 20.4</td>
<td>101.6 ± 21.4</td>
<td>101.6 ± 20.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lateral e’, cm/s</td>
<td>14.0 ± 3.3</td>
<td>12.7 ± 3.4</td>
<td>10.6 ± 3.0</td>
<td>8.6 ± 2.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lateral s’, cm/s</td>
<td>10.1 ± 0.9</td>
<td>10.3 ± 2.0</td>
<td>9.7 ± 2.1</td>
<td>9.3 ± 2.4</td>
<td>0.027</td>
</tr>
<tr>
<td>Septal e’, cm/s</td>
<td>10.5 ± 2.3</td>
<td>9.5 ± 2.4</td>
<td>7.4 ± 2.1</td>
<td>6.2 ± 1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Septal s’, cm/s</td>
<td>8.1 ± 1.1</td>
<td>8.2 ± 1.5</td>
<td>8.1 ± 2.0</td>
<td>7.0 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>44.7 ± 2.4</td>
<td>44.4 ± 3.5</td>
<td>44.8 ± 4.4</td>
<td>44.5 ± 6.0</td>
<td>0.964</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>28.7 ± 2.3</td>
<td>27.4 ± 3.8</td>
<td>27.1 ± 4.9</td>
<td>27.2 ± 6.5</td>
<td>0.733</td>
</tr>
<tr>
<td>EF, %</td>
<td>62.3 ± 3.1</td>
<td>62.4 ± 3.0</td>
<td>58.9 ± 5.4</td>
<td>56.2 ± 6.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The bold P values are statistically significant.

Echocardiographic (Vivid 7 system with 3S echocardiography probe, GE Vingmed Ultrasound AS, Horten, Norway) evaluation was done by trained cardiology specialists for patients that referred to cardiology clinic with cardiac complaints. The evaluated parameters were peak early filling velocity before atrial systole (E), peak filling velocity during atrial systole (A), left ventricular ejection fraction (LVEF), left ventricular end-systolic diameter, left ventricular end-diastolic diameter, lateral e’ velocity, lateral s’ velocity, septal e’ velocity, septal s’ velocity, tricuspid S velocity. E/A was calculated as the ratio of E to A. E/e’ ratio was calculated as the ratio of E velocity to mean e’ (as average of lateral e’ wave and septal e’ wave).

Septal e’ ≥ 8 cm/sec, lateral e’ ≥ 10 cm/sec were designated as normal diastolic function. Diastolic dysfunction was determined as septal e’ < 8 cm/sec, lateral e’ < 10 cm/sec. Stage 1 diastolic dysfunction was defined as the mitral E and A wave velocity ratio (E/A) < 0.8 and the ratio of E to the mean early diastolic mitral annular velocity (E/e’) ≤ 8. Stage 2 diastolic dysfunction was defined as the E/A between 0.8–1.5 and the E/e’ ratio between 9 and 12. Stage 3 diastolic dysfunction was defined as the E/A ratio being ≥ 2 and the E/mean e’ ratio ≥ 13. All these parameters were obtained from the American Society of Echocardiography and European Association of Cardiovascular Imaging (ASE/EACVI) guidelines recommendations [13].

Hypertension was defined as systolic pressure greater than 140 mm Hg or diastolic pressure greater than 90 mm Hg or a history of hypertension with the use of antihypertensive medication [14]. Diabetes mellitus was defined as a fasting blood glucose level of 126 mg/dL, a random glucose measurement of 200 mg/dL, hemoglobin A1c > 6.5%, or a previous diagnosis with any use of anti-diabetic medication [15].

Data were presented as mean ± standard deviation (SD) for continuous variables and as numbers and proportions for categorical variables. Distribution of the data for normality was tested by the Shapiro-Wilk test and homogeneity of group variances were tested by the Levene test. The t-test or Chi-square test was used for comparisons of continuous and categorical variables, respectively. For the parameters which are not normally distributed, the Mann Whitney U test was used. More than two independent groups with normal distribution were compared with the ANOVA test. Binary logistic regression analysis was used to identify the associations of diastolic dysfunction presence to other variables. Multinomial regression analysis was used to evaluate the associations of diastolic dysfunction grades to other variables. The data analyses were performed with SPSS 23.0 (IBM SPSS Ver. 23.0, IBM Corp, Armonk, NY, USA). A P-value of <0.05 was considered significant.

3. Results

The study population consisted of 195 women with a history of at least one delivery. PM women constituted 8.2% (n = 16), MP women constituted 37.4% (n = 73), GMP women constituted 23.6% (n = 46) and GGMP women constituted 30.8% (n = 60) of the study population. The mean age at cardiac evaluation was 50.6 ± 16.3 and mean parity was 6.5 ± 4.2. Mean height and weight were 159 ± 5.3 centimeter and 64 ± 7.1 kilogram, respectively. Body mass index (BMI) was...
Table 3. Binary comparison of parity groups according to parameters.

<table>
<thead>
<tr>
<th></th>
<th>PP vs MP</th>
<th>PP vs GMP</th>
<th>PP vs GGMP</th>
<th>MP vs GMP</th>
<th>MP vs GGMP</th>
<th>GMP vs GGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.844</td>
<td>0.009</td>
<td>0.779</td>
<td>0.004</td>
<td>0.714</td>
<td>0.011</td>
</tr>
<tr>
<td>A</td>
<td>0.025</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.987</td>
</tr>
<tr>
<td>Lateral e'</td>
<td>0.147</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lateral s'</td>
<td>0.623</td>
<td>0.442</td>
<td>0.026</td>
<td>0.084</td>
<td>0.005</td>
<td>0.350</td>
</tr>
<tr>
<td>Septal e'</td>
<td>0.138</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>Septal s'</td>
<td>0.830</td>
<td>0.907</td>
<td>0.018</td>
<td>0.645</td>
<td>&lt; 0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>EF</td>
<td>0.892</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.027</td>
</tr>
</tbody>
</table>

The bold P values are statistically significant.

Table 4. Diastolic function classification among the study population.

<table>
<thead>
<tr>
<th></th>
<th>PP (n=16)</th>
<th>MP (n=73)</th>
<th>GMP (n=46)</th>
<th>GGMP (n=60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diastolic function</td>
<td>14 (87.5%)</td>
<td>52 (71.2%)</td>
<td>26 (56.5%)</td>
<td>17 (28.3%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DD Grade 1</td>
<td>1 (6.25%)</td>
<td>9 (12.3%)</td>
<td>5 (10.9%)</td>
<td>20 (33.3%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DD Grade 2</td>
<td>1 (6.25%)</td>
<td>12 (16.4%)</td>
<td>15 (32.6%)</td>
<td>23 (38.3%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 5. Spearman correlation analysis between the presence of diastolic dysfunction and parity number, age, hypertension and diabetes mellitus.

<table>
<thead>
<tr>
<th>Parity number</th>
<th>Age</th>
<th>Hypertension</th>
<th>Diabetes mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.404</td>
<td>0.614</td>
<td>0.448</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The bold P values are statistically significant.

Table 6. Binary logistic regression analysis for the presence of diastolic dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity number</td>
<td>0.805</td>
<td>0.692–0.938</td>
<td>0.005</td>
</tr>
<tr>
<td>Age</td>
<td>1.166</td>
<td>1.103–1.234</td>
<td>0.000</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.968</td>
<td>0.359–2.611</td>
<td>0.949</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.520</td>
<td>0.176–1.536</td>
<td>0.237</td>
</tr>
</tbody>
</table>

The bold P values are statistically significant.

Table 5. Spearman correlation analysis between the presence of diastolic dysfunction and parity number, age, hypertension and diabetes mellitus.

Parity number | Age | Hypertension | Diabetes mellitus |
-------------|-----|--------------|-------------------|
| r           | 0.404 | 0.614 | 0.448 | 0.331 |
| P           | 0.000 | 0.000 | 0.000 | 0.000 |

The bold P values are statistically significant.

Table 6. Binary logistic regression analysis for the presence of diastolic dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
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<th>P</th>
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</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.520</td>
<td>0.176–1.536</td>
<td>0.237</td>
</tr>
</tbody>
</table>

The bold P values are statistically significant.

25.3 ± 2.5. BMI, height and weight parameters were not statistically significant different among groups. The characteristics of the study population were given in Table 1.

The E velocity (P = 0.017), A velocity (P < 0.0001), lateral e’ velocity (P < 0.0001), lateral s’ (P = 0.027), septal e’ (P < 0.0001), septal s’ (P < 0.0001), and EF (P < 0.0001), values were significantly different among all parity groups. The results were shown in Table 2. Binary comparison of the study groups evaluating the echocardiographic parameters can be seen in Table 3.

Diastolic dysfunction classification was done according to the echocardiographic parameters. For the PM group, 87.5% (n = 14) had normal diastolic function, 6.25% (n = 1) had grade 1 diastolic dysfunction and 6.25% (n = 1) had grade 2 diastolic dysfunction. For the MP women, 71.2% (n = 52) had normal diastolic function, 12.4% (n = 9) had grade 1 diastolic dysfunction and 16.4% (n = 12) had grade 2 diastolic dysfunction. For the GMP women, 56.5% (n = 26) had normal diastolic function, 10.9% (n = 5) had grade 1 diastolic dysfunction and 32.6% (n = 15) had grade 2 diastolic dysfunction. For the GGMP women, 28.6% (n = 17) had normal diastolic function, 33.2% (n = 20) had grade 1 diastolic dysfunction and 38.2% (n = 23) had grade 2 diastolic dysfunction (Table 4). There were no women with grade 3 diastolic dysfunction among the study population.

Spearman correlation analysis showed that diastolic dysfunction has significant positive correlations with parity, age, hypertension, and diabetes mellitus (Table 5).

Table 6 and Table 7 report the findings of the binary and multinomial logistic regressions. Explanatory variables in both models were age, parity number, hypertension and diabetes mellitus. The differences among the models stems from how the dependent variable is handled. In the binary logistic regression, dependent variables are grouped into two categories: the existence of diastolic dysfunction or normal diastolic function. On the other hand, multinomial logistic regression in this study separates the patients into three groups: patients without diastolic dysfunction, patients with grade 1 and with grade 2 diastolic dysfunction. Both models show that only parity number and age are statistically significant.

ROC analysis showed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity (Fig. 1).

4. Discussion

In the present study, we have shown that diastolic dysfunction significantly increased as the number of pregnancies increased. Additionally, the cut-off value of parity for diastolic dysfunction was 6.5, which is higher than other studies [6, 12].

Previous studies have shown that cardiovascular mortality increases along with increased parity [7]. Changes in the renin-angiotensin-aldosterone (RAAS) system explain this mechanism [16]. Estrogen secreted by the placenta increases the release of angiotensinogen. Angiotensinogen produces...
Table 7. Multinomial logistic regression analysis for diastolic dysfunction grade.

<table>
<thead>
<tr>
<th>Diastolic dysfunction grade</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity number</td>
<td>0.760</td>
<td>0.625–0.925</td>
<td>0.006</td>
</tr>
<tr>
<td>Age</td>
<td>1.198</td>
<td>1.118–1.283</td>
<td>0.000</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.885</td>
<td>0.217–3.614</td>
<td>0.865</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.525</td>
<td>0.152–1.815</td>
<td>0.309</td>
</tr>
<tr>
<td>Grade 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity number</td>
<td>0.829</td>
<td>0.705–0.976</td>
<td>0.024</td>
</tr>
<tr>
<td>Age</td>
<td>1.150</td>
<td>1.084–1.220</td>
<td>0.000</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.006</td>
<td>0.334–3.032</td>
<td>0.991</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.516</td>
<td>0.164–1.621</td>
<td>0.257</td>
</tr>
</tbody>
</table>

Fig. 1. Receiver-operating curve (ROC) analysis. ROC analysis revealed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity (Area Under the Curve: 0.734; 95% CI 0.663 to 0.805; P < 0.000).

Angiotensin-2 that activates the RAAS system. The RAAS system induces sodium and water retention. As a result, increased afterload is observed during pregnancy. Also, decreased relaxin levels affect cardiovascular mortality during pregnancy [17]. However, these changes continue only during pregnancy and their effects after pregnancy are still not clear. In our study, we hypothesized that repeated pregnancy exposed the cardiovascular system to the above-mentioned mechanisms for longer periods of time. Therefore, even if hormonal levels return to normal after pregnancy, changes in the cardiovascular system can become permanent.

We also found that diastolic function deteriorated as parity increased. Aggarwal et al., performed the first published study on this issue and they found the same results [18]. Other studies similarly showed that diastolic dysfunction increases with parity [6, 12]. However, these studies examined up to 7 pregnancies (grand multiparity). In our study, women that have a history of 9 and more pregnancies (great grand multiparity) were also included. The present study has the highest range of parity numbers in the literature.

There is a lack of evidence about the relationship between parity and the severity of diastolic dysfunction. Kim et al. found that a parity number of 2.5 and above significantly increased diastolic dysfunction [6]. A study performed by Keskin et al. showed that a parity number of 4 and above significantly increased diastolic dysfunction [12]. In our ROC curve analysis, the cut-off value for diastolic dysfunction severity was 6.5 pregnancies, higher than in previous studies.

Aortic stiffness is a prognostic risk factor for cardiovascular mortality. In the present study, binary logistic regression analysis showed that the presence of hypertension and diabetes mellitus did not make a significant difference in terms of diastolic dysfunction; however, the number of pregnancies and age did make a significant difference. This can be attributed to increasing aortic stiffness. In our study, we showed the same results as previous studies in the literature [19–22].

In the present study, a significant decrease in ejection fraction was observed as the number of pregnancies increased. However, this decrease did not reach the systolic dysfunction range (less than 50%). Although Kim et al. found the same results as ours, other studies have not shown this correlation [6, 12, 18]. Our findings on diastolic dysfunction could be attributed to more participants in our study population having longer exposure due to higher pregnancy numbers.

In conclusion, we showed that parity number is significantly correlated with diastolic dysfunction. Therefore, we recommend that physicians discuss with their patients about multiparity’s negative effect on the cardiovascular system. In addition, we recommend that patients with a parity of six or greater receive more cardiology screening.

5. Limitations
This study has limitations that should be considered. First, our study population had a limited number of patients. Second, our findings do not represent the healthy population, because our study only studied patients that were referred to the cardiology clinic with cardiac complaints. Third, we performed this study in a lower socio-economic area that could affect cardiovascular status independently. Fourth, we...
showed by multinominal logistic regression that parity num-
ber and age were significant risk factors for diastolic dys-
function. In future studies, patient’s age should be selected to be
homogeneous among groups. Finally, due to lack of facil-
ities, we did not perform cardiac magnetic resonance mea-
surements or measure brain natriuretic peptides that could
give more detailed information about diastolic dysfunction.

Author contributions
Concept and design: MO; Data analysis/interpretation:
MO, OTY, MSK; Drafting article: MO, OTY, MSK; Critical
revision of article: MO, MAA; Statistics: MO, MAA; Data
collection: MO. All authors contributed to editorial changes
in the manuscript. All authors read and approved the final
manuscript.

Ethics approval and consent to participate
All subjects gave their informed consent for inclusion be-
fore they participated in the study. The study was conducted
in accordance with the Declaration of Helsinki, and the pro-
tocol was approved by the Ethics Committee of Osmangazi
University (approval number: 2020-398).

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all the peer reviewers for their opinions and suggestions.

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Conflict of interest
The authors declare no competing interests.

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