Research Article

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Changes in oxidative stress markers in pediatric burn injury over a 1-week period

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Abstract: The importance of oxidative stress in the pathogenesis of burn injuries has been shown in various studies. Glutathione (GSH) and thiols have important roles in antioxidant protection and detoxification. The study aimed to investigate the relationship between pediatric burn trauma and GSH and thiol homeostasis. Twenty-nine children with thermal-burn injuries and 29 healthy peers are included in this prospective randomized study. Children with burn wounds of 15–25% of total body surface area (TBSA) were included in the patient group. The control group was created from healthy peers of both sexes. All children were 1–10 years of age. Serum GSH, oxidized glutathione (GSSG), redox ratio (GSH/GSSG), and thiol–disulfide (SS) tests were conducted in both groups, and the changes between admission and day 7 were analyzed in patients with burn injuries. The mean age was 4.09 ± 2.54 years for the patient group and 4.28 ± 2.55 years for the controls (p > 0.05). Total thiol (TT), native thiol (SH), and SS levels were significantly lower in the patient group than in the controls (TT = 291.69 ± 7.93 vs 346.79 ± 18.89 μmol/L, SH = 259.39 ± 7.90 vs 297.64 ± 12.81 μmol/L, SS = 16.15 ± 4.68 vs 24.58 ± 5.76 μmol/L; p < 0.001). SH/TT ratio was higher in the patient group (89.05 ± 3.00 vs 85.93 ± 3.01 μmol/L; p < 0.001). The SS/SH and SS/TT ratios were significantly lower in the patient group, while the SH/TT ratio was significantly higher (p < 0.001). The patients had significantly decreased GSH levels (26.12 ± 2.42 vs 34.80 ± 2.26 μmol/L) and GSH/GSSG ratios (1.69 ± 0.12 vs 3.05 ± 0.29) and increased GSSG levels (16.09 ± 0.34 vs 11.48 ± 1.17, p < 0.001 for all). The GSSG level and GSSG/SH and GSSG/TT ratios were higher in the patient group than in the controls while the SH, TT, and SS levels, and SS/SH and SS/TT ratios were lower in the patient group. Analysis of serum GSSG levels, and ratios with SH and TT homeostasis, might be useful in order to determine burn damage in children.

Keywords: oxidative stress, glutathione, thiol, burn, children

1 Introduction

A burn is an inflammatory condition that can affect more than just the skin. The widespread inflammatory response it triggers can result in mortality and morbidity [1,2]. Early mechanisms due to burns include a decrease in cardiac output. It can cause metabolic acidosis, hyperventilation, and respiratory alkalosis. Skin and capillary endothelial cells can be further damaged due to the activation of toxic inflammatory mediators such as prostaglandin, proteases, and oxidants, leading to ischemic tissue damage [3]. The activation of complement and stimulation of neutrophils induce cytoxic reactive oxygen species (ROS). Elevated histamine level stimulates the catalytic activity of xanthine oxidase, resulting in enhanced vascular permeability. Skin structures may be directly damaged by xanthine oxidase toxic byproducts such as hydrogen peroxide (H₂O₂) and hydroxyl radical (OH) [4,5]. The cause of this damage is the oxidation of membrane molecules such as proteins, lipids, and nucleic acids by ROS and nitrogen species [5,6].

The glutathione reductase/glutathione peroxidase (GR/GPx) system comprising catalase and glutathione are enzymatic defenses against ROS. Tissues are protected from the effects of free radicals and lipid peroxides by superoxide dismutase (SOD) and GPx. Activities of SOD and GPx increase after free radical-mediated damage and lipid peroxidation [7]. One of the primary components of cellular defense systems against oxidative stress is glutathione (GSH). In the elimination of ROS, GSH plays a vital role in acting directly or interacting with GPx [8]. Usually, GR keeps the intracellular GSH/GSSG (oxidized GSH) ratio high, allowing GSH to function as an antioxidant [9,10]. Because of the excessive GSH usage during oxidative stress, intracellular GSH levels are reduced by tissue ischemia owing to burn injury [11,12].

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Thiols are an essential component of the body’s antioxidants. Cell function and structure are altered if proteins lose their thiol groups. In this context, many cell alterations occur. Erel and Neselioglu [13] tested serum native thiol (SH) and total thiol (TT) levels with their developed test kit. Measuring SH/TT ratios and parameters enables the evaluation of oxidative stress equilibrium. While SH and TT levels are expected to decrease under oxidative stress, SH/TT indices are predicted to rise [13,14]. Protein activity is dependent on disulfides (SSs), a reversible byproduct of thiol oxidation. Production and decomposition of SSs are in equilibrium [14]. Several metabolic processes (apoptosis, enzyme activity, immunological response, etc.) are regulated by a thiol–SS pair through the cell’s redox state [13]. Because of the antioxidant features of the thiols, the exchange of thiol–SS expresses oxidant/antioxidant levels. While GSH represents the most prominent thiol, named redox pair, the most important thiol–SS redox buffer is reduced GSH/GSSG [14]. GSH peroxidases, GSH S-transferases, and glutaredoxins act together with GSH to detoxify ROS and electrophiles and recycle the oxidized protein thiols [15].

This research aims to determine whether oxidative stress occurs following burn injury in children and changes over the first week. To the best of our knowledge, the values of GSH and thiol levels, glutathione ratio (GSH/GSSG), and thiol ratio (-SS/TT, -SS/-SH, and -SH/TT) values in pediatric burns will be discussed for the first time. It is aimed to show how the oxidant–antioxidant balances change in burn patients in the first week after trauma and to present a diagnostic parameter in pediatric burns.

2 Materials and methods

2.1 Study design and setting

This prospective study was conducted on burn patients who were hospitalized in MTU Training and Research Hospital, Turkey, for 3 months, from November 2022 to January 2023. The cardinal aim of this study was to determine the GSH and thiol–SS profiles and the associated ratios of reduced to oxidized children’s burn trauma. Additionally, our secondary aim was to evaluate the modification of the thiol–SS equilibrium following burn trauma. Using the data obtained during the first week, changes in GSH, GSSG, GSH/GSSG ratio, and SS/SH, SS/TT, and SH/TT ratios were measured. The Ethics Committee of the Human Clinical Researches of Malatya Turgut Ozal University (MTU) Medical Faculty (NO:2022/51) approved this study. This prospective randomized study was carried out as a single-center study in the Pediatric Burn Unit at Malatya Turgut Ozal University Training and Research Hospital.

2.2 Demographics and data collection

Fifty-eight children under 10 years of age who were admitted to MTU Training and Research Hospital Pediatric Surgery Clinic between November 2022 and January 2023 were included. There were 29 children with second-degree acute burns who were affected by 15–25% of total body surface area (TBSA) in the patient group. All cases were recorded with their age, gender, and % TBSA. Clinical wound assessments were performed by a single independent observer with more than 10 years of clinical expertise. A professional nurse collected all data. All patients were dressed with silver sulfadiazine-impregnated gauze. According to the standard protocol for treating burn patients, fluid resuscitation and analgesics (paracetamol) were administered. No antibiotic prophylaxis was given. A control group was formed with another 29 children of similar sex and age among the children who had applied for routine examination without any systemic disease. Patients who were hospitalized for more than 24 h after burn injury had a history or current hemato logical, metabolic, or autoimmune diseases, or burns with inhalation injuries, electrical burns, or chemical burns were not included in the study. History of any regular drug usage or any antioxidant drugs like multivitamins or vitamin C that might potentially impact the redox state was questioned and excluded.

2.3 Methods

After a detailed anamnesis and physical examination, parents were informed about the research content and signed informed consent documents were taken. Blood was drawn from burn patients within the first 6 h of injury and from their healthy peers in the control group during the participation in the study. Blood was drawn for the second time from both patient groups at the end of a week after the injury or participation date.

2.3.1 Blood samples

Blood samples were taken into a gel separator (serum) tube. Serum tubes after collection were incubated at
room temperature for 30 min, allowed to coagulate, and then centrifuged at 1,200g for 10 min. After centrifugation, serum samples were divided from the tubes and micro volumes were transferred to Eppendorf tubes. After biochemical analysis, serum samples were preserved at –80°C.

2.3.2 GSH and thiol levels

On the day of analysis, the TT (RelAssay, Cat. No. RL0192, Gaziantep, Turkey), SH (RelAssay, Cat. No. RL0185, Gaziantep, Turkey), total GSH, and GSSG (Elabscience, Cat. No. E-BC-K097, Wuhan, China) levels from serum samples stored at –80°C were studied using kits based on colorimetric analysis (Cloud-CloneCorp, Cat. No. SED076Hu, China) in accordance with the manufacturer’s recommendations. All samples were thawed at the same time after collection. Blood thiol–SS characteristics were analyzed using the technique published by Erel and Neselioğlu [13].

2.4 Statistical analysis

The normality of data was evaluated by the Shapiro–Wilk test, which was applied to evaluate the distribution of each data. While evaluating the study data, descriptive statistics (mean ± standard deviation) were used for numerical variable. In order to compare the gender and age groupings for the patient and control groups, cross tables and test statistics of number (n), percentage (%), and chi-square ($\chi^2$) were performed. The differences between the two independent groups were analyzed using the independent samples t-test or Mann–Whitney U test. For analyzing the differences between the two dependent groups, dependent sample t-tests were used for normally distributed parameters, and the Wilcoxon Signed Rank test was used for non-normally distributed parameters. Cut-off points for quantitative variables were determined by ROC (receiver operation characteristics) analysis.

3 Findings

3.1 Demographic characteristics of patients with burn injuries and the control group

In this study, there were 25 (43.1%) boys and 33 (56.9%) girls in the patient group. There was no statistically significant difference between the patient and control groups according to gender ($p > 0.05$, Table 1). While the mean patient age was 4.09 ± 2.54, it was 4.28 ± 2.55 in the control group, and no statistically significant age-related difference was observed between the groups ($p > 0.05$). While the burn rate was below 20% in 79.3%, it was between 20 and 25% in 20.7% of the patient group.

3.2 Laboratory markers of burn patients and control group

In Tables 2 and 3, all individuals’ thiol–SS and GSH parameters are given. When thiol–SS profile tests of burn and control groups on day 1 blood samples were compared,

| Table 1: Comparison of the groups by gender, age, and TBSA distribution |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Variable                 | Patient (n = 29)          | Control (n = 29)          | Total (n = 58)            | Test                     |
|                         | n (%)                    | n (%)                    | n (%)                    | $\chi^2$; z; p            |
| Gender                  | Girl                     | 16 (55.2)                | 17 (58.6)                | 33 (56.9)                |
|                         | Boy                      | 13 (44.8)                | 12 (41.4)                | 25 (43.1)                |
| Age (y)                 | 1–2                      | 12 (41.4)                | 11 (37.9)                | 23 (39.6)                |
|                         | 3–4                      | 5 (17.2)                 | 6 (20.7)                 | 11 (19.0)                |
|                         | 5–6                      | 7 (24.2)                 | 5 (17.3)                 | 12 (20.7)                |
|                         | 7/older                  | 5 (17.2)                 | 7 (24.1)                 | 12 (20.7)                |
| TBSA (%)                | 15–20                    | 23 (79.3)                | —                       | —                       |
|                         | 20–25                    | 6 (20.7)                 | —                       | —                       |
| Age Mean ± SD           | 4.09 ± 2.54              | 4.28 ± 2.55              | 4.18 ± 2.52              | z = 0.340; p = 0.734      |

n: frequency; %: percent; SD: standard deviation; $\chi^2$: Chi-square test value; z: Mann–Whitney test value; p: p value < 0.05 considered significant; TBSA (%): total body surface area percent; y: years.
Dependent sample t signif. p total GSH/GSSG ratio were found to be signifi-
ficantly higher than the control group. In addition, the SS/SH and SS/TT ratios of the burn group were found to be significantly lower than the control group (p < 0.001). In the burn patient serum, total GSH level and total GSH/GSSG ratio were found to be significantly lower, while GSSG levels were higher; a statistically significant difference was found between all values of the burn and control groups based on the day 1 sample (p < 0.001). When compared to the control group, an increase was observed in the GSSG/TT and GSSG/SH ratios on the day 1 sample (p < 0.001).

Although SH, TT, GSH, and SS levels and SS/SH, SS/TT, and total GSH/GSSG ratios were found in increasing trends, these were found to be lower than the control groups (p < 0.001) on day 7. GSSG levels and SH/TT, GSSG/TT, and GSSG/SH ratios were found in significantly decreasing trends (p < 0.001) over the week. The values of those variables were found to be higher still on day 7 than the control group.

### Table 3: Comparison of the mean measurement values on day 1 and day 7 of the patient group and the day 1 measurement values of the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patient mean ± SD</th>
<th>Control mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (µmol/L)</td>
<td>291.69 ± 7.93</td>
<td>346.79 ± 18.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SH (µmol/L)</td>
<td>259.39 ± 7.90</td>
<td>297.64 ± 12.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SH/TT (%)</td>
<td>89.05 ± 3.00</td>
<td>85.93 ± 3.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>26.12 ± 2.42</td>
<td>34.80 ± 2.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSSG (µmol/L)</td>
<td>16.09 ± 0.34</td>
<td>11.48 ± 1.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total GSH/SH</td>
<td>1.69 ± 0.12</td>
<td>3.05 ± 0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSSG/TT (%)</td>
<td>5.58 ± 0.19</td>
<td>3.32 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSSG/SH (%)</td>
<td>6.26 ± 0.25</td>
<td>3.86 ± 0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dynamic SD (µmol/L)</td>
<td>16.15 ± 4.68</td>
<td>24.58 ± 5.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dynamic SD/TT (%)</td>
<td>5.47 ± 1.50</td>
<td>7.03 ± 1.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dynamic SD/SH (%)</td>
<td>6.25 ± 1.95</td>
<td>8.25 ± 1.97</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, p value <0.05 considered significant.

### 3.3 ROC analysis

ROC analysis results are given in Table 4, and ROC curves for all analyzed parameters are given in Figures 1 and 2. According to ROC analysis results in the groups, the TT and SH level evaluation result of the area under the curve (AUC = 0.966; AUC = 0.969) was found to be statistically significant (p < 0.001). The cut-off point of the TT variable was determined as 319.48. For TT and SH, sensitivity was 100.00% and selectivity was 96.60%. According to ROC analysis, total GSH value (AUC = 0.987) with the most valuable prediction in pediatric burns sensitivity scale was 93.10 and specificity was 100. GSSG evaluation result
and AUC (0.966) were statistically significant ($p < 0.001$). The cut-off point of the GSSG variable was determined to be 13.70, for which sensitivity was 100.00% and selectivity was 96.60% (Table 4, Figures 1 and 2).

Determination of the cut-off point according to ROC analysis is shown in Figures 1 and 2.

### 4 Discussion

A burn injury is a significant cause of local and systemic inflammation in the body [16]. Thermal shock and oxidative stress can contribute to tissue damage following burns [17]. Oxidative stress is connected with disturbances of homeostatic balancing, and the release of free radicals causes cell and tissue damage [18]. Antioxidant and defense mechanisms resist oxidative stress while inflammation improves [19]. Oxidative stress is the process that leads to high oxidant production in pathophysiological conditions [19]. If not appropriately treated, systemic total oxidant levels are greatly elevated in burn patients, whereas systemic total antioxidant capacity levels are significantly reduced [20].

GSH is a tripeptide, which is composed of three amino acids: lysine, l-cysteine (Cys), and glycine, and

Table 4: ROC analysis of variables in patient and control groups

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>AUC</th>
<th>Asymptotic 95% confidence interval</th>
<th>Cut off</th>
<th>$p$</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (S)</td>
<td>0.966 ± 0.034</td>
<td>0.899–1.000</td>
<td>319.48</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>96.60</td>
</tr>
<tr>
<td>SH (S)</td>
<td>0.969 ± 0.031</td>
<td>0.909–1.000</td>
<td>276.30</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>96.60</td>
</tr>
<tr>
<td>SH/TT (%) (L)</td>
<td>0.803 ± 0.061</td>
<td>0.683–0.922</td>
<td>87.18</td>
<td>&lt;0.001*</td>
<td>82.80</td>
<td>72.40</td>
</tr>
<tr>
<td>Total GSH (S)</td>
<td>0.987 ± 0.012</td>
<td>0.964–1.000</td>
<td>31.32</td>
<td>&lt;0.001*</td>
<td>93.10</td>
<td>100.00</td>
</tr>
<tr>
<td>GSSG (L)</td>
<td>0.966 ± 0.034</td>
<td>0.899–1.000</td>
<td>13.70</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>96.60</td>
</tr>
<tr>
<td>Total GSH/GSSG/(S)</td>
<td>0.998 ± 0.003</td>
<td>0.991–1.000</td>
<td>2.34</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>96.60</td>
</tr>
<tr>
<td>GSSG/TT (%) (L)</td>
<td>0.999 ± 0.001</td>
<td>0.999–1.000</td>
<td>5.04</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>GSSG/SH (%) (L)</td>
<td>0.999 ± 0.002</td>
<td>0.995–1.000</td>
<td>5.03</td>
<td>&lt;0.001*</td>
<td>100.00</td>
<td>96.60</td>
</tr>
<tr>
<td>Dynamic SS (S)</td>
<td>0.885 ± 0.050</td>
<td>0.787–0.982</td>
<td>21.17</td>
<td>&lt;0.001*</td>
<td>93.10</td>
<td>79.30</td>
</tr>
<tr>
<td>Dynamic SS/TT (%) (S)</td>
<td>0.803 ± 0.061</td>
<td>0.683–0.923</td>
<td>6.41</td>
<td>&lt;0.001*</td>
<td>82.80</td>
<td>72.40</td>
</tr>
<tr>
<td>Dynamic SS/SH (%) (S)</td>
<td>0.800 ± 0.061</td>
<td>0.680–0.920</td>
<td>7.35</td>
<td>&lt;0.001*</td>
<td>82.80</td>
<td>72.40</td>
</tr>
</tbody>
</table>

L: Larger test result indicates more positive test, S: Smaller test result indicates more positive test; * statistically significant difference ($p < 0.05$); AUC: area under the curve; 95% CI, confidence interval.

Figure 1: ROC curve of TT, SH, total GSH, total GSH/GSSG, dynamic SS, dynamic SS/TT (%), and dynamic SS/SH (%) variables.
works as a major cellular antioxidant [21]. An elevation in serum GSH levels signifies a rise in the cellular oxidative stress response [21]. Beiraghi-Toosi et al. examined GSH levels on day 1, day 2, and day 7 after a burn injury in adults and found that the levels decreased during the time [22]. However, the GSH/GSSG ratio increased during the 1-week follow-up, which was statistically significant, according to these results [23]. Based on our study, in the first week after the burn injury, total GSH levels increased over time compared to day 1 and day 7, while GSSG levels decreased. It was observed that the ratio of GSH/GSSG increased in the first week of the burn. Although GSH/GSSG showed an upward trend in the 1-week follow-up, it was lower than the control group.

Due to their -SH group, thiols are intracellular and extracellular non-enzymatic free-radical scavengers sensitive to oxidation. The metabolic response to oxidative stress usually changes intracellular GSH content, which is depleted by cell-protecting reactions. When the oxidant-antioxidant balance is disturbed, the reduced GSH is oxidized to the GSSG form. Reconversion to the GSH form occurs by enzymatic reduction of disulfide (GSSG) or by de novo synthesis [23,24]. GSSG levels were found to be higher than in control groups, and GSH decreased in this research. During oxidative stress, a thiol is oxidized to SS; in this process, thiol decreases oxidant levels, thus preventing oxidative stress-induced tissue damage [14,25]. In burn patients, the initial “hypodynamic” phase is mostly pro-inflammatory and characterized by increased reactive species production [26]. The second phase mostly includes an anti-inflammatory process known as the “hyperdynamic” phase [26]. In our study, GSH, TT, and SH levels and ratios of S's to thiols decreased over time compared with control groups; these values also increased on day 7 in the patient group (Tables 2 and 3). These results reflected the acute metabolic response in pediatric burn trauma.

It is known that oxidative stress increases from physical injuries. Although many studies have shown that trauma causes an increase in ROS and a decrease in antioxidant levels [27–29], a statistical difference in TT, SH, SS, and SS/TT rate on day 2 and day 5 of children with trauma was not found [30]. The SS/SH and SS/TT ratios were substantially lower in the patient group, but the SH/TT ratio was significantly greater in children with head trauma [31]. Research by Erel and Neselioglu [13] hypothesized that the SH/SS ratio did not change in the SS direction because the trauma did not exceed the oxidative stress threshold.

According to the ROC results obtained in our study, GSSG/TT and GSSG/SH capacities (AUC = 0.999) were found to be statistically significant as the most valuable predictors in pediatric burns ($p < 0.001$). In our study, the sensitivity of GSSG/TT and GSSG/SH values was calculated as 100 and specificity as 100 and 96.60, respectively (Table 4, Figure 2). Increased GSSG and decreased SH levels in pediatric burn patients compared to the control group indicate intracellular oxidative stress. According to

![Figure 2: ROC curve of SH/TT (%), GSSG, GSSG/TT (%), and GSSG/SH (%)](image-url)
the results of this study, the intracellular GSSG/TT ratio could be a good marker in terms of sensitivity and specificity in the follow-up period of pediatric burn patients.

In this research, the increased oxidation parameters of GSH and thiols in burn patients indicated that antioxidants may be necessary to reduce oxidative stress in pediatric burn patients. Studies on oxidative stress in pediatric burn injuries are limited, and this research may contribute new data to the literature on this subject.

5 Conclusion

In this study, we researched the changes in the oxidative stress process in middle-grade burns in a week period. The GSSG level and GSSG/SH and GSSG/TT ratio were higher in the patient group than in the controls while the SH, TT, and SS levels, and SS/SH and SS/TT ratios were lower in the patient group. Analysis of serum GSSG levels, and ratios with SH and TT homeostasis, might be useful in order to determine the burn damage in children. Additionally, antioxidants can be studied topically or as a dressing for burn treatment in children. Since the compensatory mechanism and regeneration rate are higher in children, the need for antioxidants while treating pediatric burns might be discussed again and investigated with more comprehensive studies.

6 Limitations

Because the oxidative stress after burn injury begins immediately, the first samples were taken as soon as possible. Patients admitted to the hospital within 6 h of burn trauma were included in the study. Because wide exclusion criteria were established to make certain of the normal health status of the subjects enrolled in this study, the number of total cases remained limited. Despite a wide spectrum of exclusion criteria any undetected factor that might affect the results is still not to be ruled out.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki and the study protocol was approved by the ethical committee of Malatya Turgut Ozal University Faculty of Medicine Ethics Committee (no: 2022/51).

Informed consent: Informed consent was obtained from the parents of all individual participants included in the study.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References