



Hematologic Indices as Diagnostic Markers of Neonatal Sepsis: A Case-Control Study

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Abstract

Introduction: Neonatal sepsis is a significant cause of infant mortality, particularly in developing countries. Utilizing hematologic indices as easily accessible diagnostic markers for neonatal sepsis is considered a potential supplement to blood culture. This study investigated the association between hematologic indices and neonatal sepsis.

Methods: This case-control study was conducted on a sample of infants admitted to the neonatal intensive care unit of a tertiary hospital in southeastern Iran. Hematologic indices, including the total leukocyte count, platelet count, lymphocyte count, absolute neutrophil count, neutrophil-to-lymphocyte ratio, mean platelet volume (MPV), platelet-to-lymphocyte ratio, red blood cell distribution width (RDW), and RDW to platelet ratio, were compared between infants with positive blood culture and the control group.

Results: Sixty-eight infants (34 diagnosed with neonatal sepsis and 34 controls) with a mean age of 5.51 ± 4.76 days were enrolled. Of the total positive blood culture infants, 58.8% had late-onset, and 41.2% had early-onset sepsis. The most common microorganism was *Staphylococcus epidermidis* (50%). Among hematologic indices, MPV was significantly higher in the sepsis group, and a cut-off point of 9.65 fL resulted in a sensitivity of 73.5% and a specificity of 58.8% in predicting neonatal sepsis.

Conclusion: Overall, the present study demonstrated that among the hematologic indices, MPV can be considered a supplementary diagnostic marker of neonatal sepsis.

Keywords: Neonatal sepsis, Hematology, Hematologic Tests, Biomarkers, Mean platelet volume

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Introduction

Neonatal sepsis is described as a clinical syndrome with a systemic inflammatory response against pathogenic microorganisms that may be accompanied by hemodynamic disturbances and high morbidity and mortality (1). Neonatal sepsis is a major contributing factor to 30-50% of annual infant deaths in developing countries (2).

Based on the timing of clinical manifestations, neonatal sepsis is classified into early-onset sepsis (EOS) and late-onset sepsis (LOS), the former occurring within 72 hours of life and the latter beginning afterward. EOS can occur due to the infection transmitted from the mother, particularly with Group B *Streptococcus* (GBS), *Listeria monocytogenes*, and *E. coli* (3, 4). Factors including low birth weight, prematurity, asphyxia, prolonged rupture of membranes (PROM), and chorioamnionitis are among the most significant risk factors for EOS (2, 5).

Conversely, LOS mainly develops as a result of infection with microorganisms, including coagulase-negative staphylococci, enterobacteria, *E. coli*, *Pseudomonas*, *Klebsiella*, *Staphylococcus aureus*, and *Candida albicans*, while factors, including low birth weight, preterm birth, and in-hospital interventions, including central venous catheterization or long-term mechanical ventilation, are established risk factors of LOS (6, 7).

Neonatal sepsis is typically diagnosed with a laboratory culture of microorganisms from body fluids, such as blood, urine, pleural, cerebrospinal, or peritoneal fluid. Today, culture-independent methods are also used in the evaluation of sepsis. These measures include molecular assays, such as polymerase chain reaction (PCR), or evaluation of inflammatory markers, including C-reactive protein (CRP), procalcitonin, haptoglobin, and inflammatory cytokines (8, 9).

While culture-independent measures are not readily



available in most medical centers in developing countries, blood culture is routinely used as the gold standard for diagnosing neonatal sepsis. However, due to the fact that cultures are often time-consuming and expensive, while prior antibiotic therapy might also interfere with their results (10, 11), utilizing other laboratory assessments as supplementary diagnostic markers of sepsis has come to attention.

Among the laboratory-based approaches used in diagnosing neonatal sepsis, hematologic indices are derived from the patient's complete blood count (CBC) as easily accessible, quick, and inexpensive indicators. In general, hematologic reactions occur in response to several infectious and non-infectious agents. These reactions can result in alterations of different subsets of leukocytes along with indicators related to red blood cells in the patient's peripheral blood (12, 13). Therefore, several indices pertaining to these cellular changes have been suggested as useful diagnostic and prognostic markers for various diseases. In terms of neonatal sepsis, several recent studies have suggested that indices related to platelets, such as mean platelet volume (MPV) and platelet count, could be used to discriminate between septic and healthy infants (14). Other studies have used the proportion of various leukocyte subtypes, such as total leukocyte count (TLC), absolute lymphocyte count (ANC), absolute lymphocyte count (ALC), and neutrophil to lymphocyte ratio (NLR), as diagnostic markers for neonatal sepsis (15-17). Moreover, red blood cell distribution width (RDW) has been considered the most well-known index associated with red blood cells' size variability, which has been used as a marker of inflammation and cellular turnover (18). Additionally, several hematologic indices comprised of a compound subset of blood cells have also been proposed, which include platelet to lymphocyte ratio (PLR) or RDW to platelet ratio (RPR) (19, 20).

In this study, we explored the association of several hematologic indices with neonatal sepsis, as well as different types of microorganisms in a tertiary hospital in

the southeast of Iran.

Methods

Study population and design

This study utilized a case-control design and took place in the neonatal intensive care unit (NICU) of Afzalipour Hospital, a tertiary referral center in the southeast of Iran, during 2020-2022. The inclusion criteria for the case group were the presence of a positive blood culture on admission or during hospitalization in the neonatal period. The control group was recruited from age-, gender-, and gestational age-matched neonates with no suspected clinical signs of sepsis who had been admitted to the NICU for phototherapy. Patients were excluded if they were diagnosed with hypoxic-ischemic encephalopathy, severe congenital anomalies, congenital heart disease, or if they were born before 34th weeks of gestation. Of the total 590 infants who were suspicious for neonatal sepsis in the study period, a total of 137 had a positive culture, and 34 met the criteria of the study. Accordingly, 34 infants were enrolled as the control group (Figure 1).

Measures and procedures

To measure the hematologic indices, the CBC on the day the blood culture was obtained in the case group, as well as the CBC on the admission day of the control group, were analyzed. All CBC analyses were performed using the Kx21n Sysmex Hematology Analyzer at the laboratory of Afzalipour Hospital. Laboratory technicians were blinded to the patients' clinical diagnosis. Nine hematologic indices, including TLC (total leukocytes per mL blood sample), ANC (the absolute count of neutrophils per mL blood sample), ALC (the absolute count of lymphocytes per mL blood sample), NLR (neutrophil to lymphocyte ratio), PLR (platelet to lymphocyte ratio), platelet count, MPV, RDW, and RPR (RDW to platelet ratio) were derived from the laboratory results. Additionally, the type of microorganism according to the blood culture was recorded. EOS was considered a positive blood culture

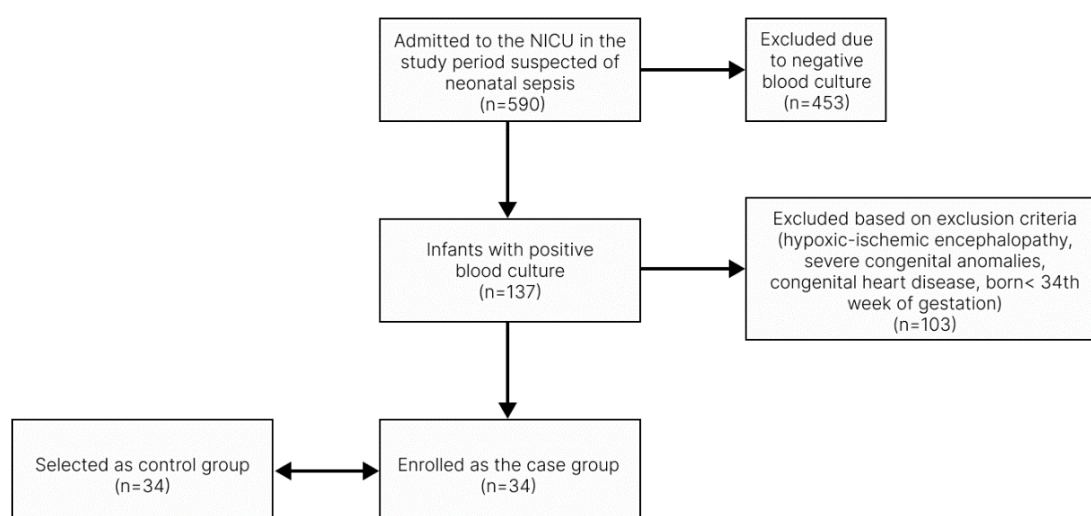


Figure 1. Flow chart of the study

within the first 72 hours of life, and LOS was defined as a positive blood culture obtained after 72 hours of the neonatal period.

Ethical considerations

Ethical approval for all study procedures was obtained from the Ethics Committee of Kerman University of Medical Sciences before initiation of the research (Ethical code: IR.KMU.AH.REC.1401.084).

Statistical analysis

The data were analyzed using the statistical package for the social sciences (SPSS) software (version 22.0. SPSS, Inc., Chicago, IL, USA). Descriptive data were presented as frequency, percentage, mean, and standard deviation (SD). Pairwise comparison of groups was made according to the Chi-square test, t-test (and Mann-Whitney if normality was not maintained). In case of multiple comparisons, ANOVA (alternatively, Kruskal-Wallis if normal distribution was not determined) was performed, followed by a Tukey's post-hoc test. As a measure of sensitivity analysis, the receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was measured. The Youden's index was utilized to determine the optimal cut-off point according to the ROC curve.

Results

A total of 68 infants (34 diagnosed with neonatal sepsis and 34 as controls) were enrolled in the study. The average age of participants was 5.51 ± 4.76 days, and 60.3% were female. Table 1 presents the demographic details of all enrolled participants (Table 1).

No significant difference was observed in gestational age ($P=0.901$), infant's age ($P=0.701$), mother's age ($P=0.717$), gender ($P=0.457$), and route of delivery ($P=0.209$) between the case and control groups. However, infants suffering from neonatal sepsis had a significantly lower birth weight ($P=0.002$) as well as lower weight at

Table 1. Demographic and anthropometric characteristics of participants

| Qualitative characteristics | N (%) |
|----------------------------------|---------------------|
| Gender | |
| Male | 27 (39.70) |
| Female | 41 (60.30) |
| Route of delivery | |
| NVD | 25 (36.77) |
| C/S | 43 (63.23) |
| Quantitative characteristics | Mean (\pm SD) |
| Gestational age at birth (weeks) | 36.83 (\pm 1.80) |
| Infant's age (days) | 5.51 (\pm 4.76) |
| Mother's age (years) | 29.38 (\pm 6.61) |
| Birth weight (g) | 2848 (\pm 579) |
| Current weight (g) | 2766 (\pm 592) |

Qualitative variables are presented as number (%) and quantitative variables are presented as mean (\pm standard deviation); NVD: Natural vaginal delivery; C/S: Cesarean section.

the time of study enrollment ($P=0.002$) (Table 2).

Among the case group, 41.2% were diagnosed with EOS and 58.8% with LOS. The most common microorganisms were *Staphylococcus epidermidis* (50.0%), *Pseudomonas* (11.8%), and *Citrobacter* (11.8%) (Table 3).

Comparison of the hematologic indices among case and control groups demonstrated that MPV was significantly higher in the sepsis group ($P=0.021$), while no significant difference in other indices was observed. Considering the onset of sepsis, none of the hematologic indices showed a statistically significant difference among the EOS, LOS, and control groups (Table 4).

According to the type of microorganisms, infants with neonatal sepsis were divided into those positive for *Staphylococcus epidermidis* (*S. epidermidis*) and those positive for other microorganisms (non-*S. epidermidis*). The results indicated that MPV significantly differed between the *S. epidermidis*, non-*S. epidermidis*, and control groups ($P=0.047$) (Table 5), where a post-hoc analysis showed that MPV was significantly higher in non-*S. epidermidis* group in comparison with the control group ($P=0.044$), while no significant difference was observed between *S. epidermidis*

Table 2. Comparison of demographic and anthropometric characteristics between septic and control infants

| Quantitative characteristics | Neonatal Sepsis Mean (\pm SD) | Control Mean (\pm SD) | P value | |
|----------------------------------|----------------------------------|--------------------------|-----------|-------|
| Gestational age at birth (weeks) | 36.76 (\pm 2.12) | 36.91 (\pm 1.44) | 0.901 | |
| Infant age (days) | 5.59 (\pm 5.21) | 5.44 (\pm 4.34) | 0.701 | |
| Maternal age (years) | 29.09 (\pm 6.44) | 29.68 (\pm 6.87) | 0.717 | |
| Birth weight (g) | 2636 (\pm 593) | 3059 (\pm 486) | 0.002* | |
| Current weight (g) | 2545 (\pm 598) | 2987 (\pm 503) | 0.002* | |
| Qualitative characteristics | Neonatal Sepsis N (%) | Control N (%) | P value | |
| Gender | Male | 12 (35.3) | 15 (44.1) | 0.457 |
| | Female | 22 (64.7) | 19 (55.9) | |
| Route of delivery | NVD | 15 (44.1) | 10 (29.4) | 0.209 |
| | C/S | 19 (55.9) | 24 (70.6) | |

Qualitative variables are presented as number (%) and quantitative variables are presented as mean (\pm standard deviation); NVD: Natural vaginal delivery; C/S: Cesarean section; * statistically significant ($P<0.05$).

Table 3. Distribution of sepsis onset and microbial culture in infants diagnosed with neonatal sepsis

| Variables | N (%) | |
|-----------------|-----------------------------------|-----------|
| Onset of sepsis | EOS | 14 (41.2) |
| | LOS | 20 (58.8) |
| Microorganism | <i>Citrobacter</i> | 4 (11.8) |
| | <i>E. coli</i> | 3 (8.8) |
| | <i>Klebsiella</i> spp. | 3 (8.8) |
| | <i>Pseudomonas</i> spp. | 4 (11.8) |
| | <i>Staphylococcus aureus</i> | 2 (5.9) |
| | <i>Staphylococcus epidermidis</i> | 17 (50.0) |
| | <i>Streptococcus pneumoniae</i> | 1 (2.9) |

Data are presented as number (%); EOS: Early-onset sepsis; LOS: Late-onset sepsis.

Table 4. Comparison of hematological indices between neonatal sepsis and control groups and according to the onset of sepsis

| Hematological index | Neonatal Sepsis Mean (\pm SD) | Control Mean (\pm SD) | P-value | EOS Mean (\pm SD) | LOS Mean (\pm SD) | P value |
|---------------------|----------------------------------|--------------------------|---------|------------------------|------------------------|---------|
| TLC | 11259 (\pm 5011) | 9141 (\pm 2174) | 0.073 | 12414 (\pm 6512) | 10450 (\pm 3594) | 0.110 |
| ANC | 5370 (\pm 4006) | 5246 (\pm 9961) | 0.121 | 5852 (\pm 5488) | 5032 (\pm 2640) | 0.237 |
| Plt | 301324 (\pm 157402) | 287000 (\pm 108926) | 0.664 | 261929 (\pm 109767) | 328900 (\pm 181226) | 0.332 |
| ALC | 4670 (\pm 2284) | 4089 (\pm 1612) | 0.508 | 5650 (\pm 2956) | 3984 (\pm 1741) | 0.182 |
| RDW | 18.3 (\pm 2.6) | 17.4 (\pm 1.4) | 0.357 | 18.4 (\pm 2.8) | 18.2 (\pm 2.6) | 0.611 |
| MPV | 10.1 (\pm 0.7) | 9.6 (\pm 0.9) | 0.021* | 10.0 (\pm 0.8) | 10.2 (\pm 0.6) | 0.061 |
| RPR | 15.7 (\pm 30.9) | 6.8 (\pm 2.4) | 0.677 | 18.2 (\pm 37.6) | 14.05 (\pm 26.1) | 0.812 |
| PLR | 82.6 (\pm 78.8) | 84.0 (\pm 49.4) | 0.556 | 60.6 (\pm 48.2) | 98.04 (\pm 92.7) | 0.182 |
| NLR | 1.4 (\pm 0.9) | 1.5 (\pm 2.2) | 0.418 | 1.2 (\pm 1.1) | 1.50 (\pm 0.8) | 0.266 |

Data are presented as mean (\pm SD); EOS: Early-onset sepsis; LOS: Late-onset sepsis; TLC: Total leukocyte count; ANC: Absolute neutrophil count; Plt: Platelet count; ALC: Absolute lymphocyte count; RDW: Red blood cell distribution weight; MPV: Mean platelet volume; RPR: RDW to platelet ratio; PLR: Platelet to lymphocyte ratio; NLR: Neutrophil to lymphocyte ratio; *Statistically significant ($P < 0.05$).

Table 5. Comparison of hematological indices according to the microbial culture

| Hematological index | Staphylococcus epidermis Mean (\pm SD) | Other microorganisms Mean (\pm SD) | Control Mean (\pm SD) | P value |
|---------------------|---|---------------------------------------|--------------------------|---------|
| TLC | 11718 (\pm 5320) | 10800 (\pm 4800) | 9141 (\pm 2174) | 0.143 |
| ANC | 5488 (\pm 3737) | 5252 (\pm 4370) | 5246 (\pm 9961) | 0.146 |
| Plt | 302706 (\pm 194858) | 299941 (\pm 114567) | 287000 (\pm 108926) | 0.909 |
| ALC | 4697 (\pm 2047) | 4642 (\pm 2563) | 4089 (\pm 1612) | 0.692 |
| RDW | 18.4 (\pm 2.9) | 18.1 (\pm 2.4) | 17.4 (\pm 1.4) | 0.648 |
| MPV | 10.0 (\pm 0.8) | 10.2 (\pm 0.7) | 9.6 (\pm 0.9) | 0.047* |
| RPR | 23.7 (\pm 42.2) | 7.8 (\pm 7.4) | 6.8 (\pm 2.4) | 0.914 |
| PLR | 67.2 (\pm 45.9) | 98.1 (\pm 101.0) | 84.0 (\pm 49.4) | 0.582 |
| NLR | 1.38 (\pm 0.8) | 1.4 (\pm 1.0) | 1.5 (\pm 2.2) | 0.663 |

Data are presented as mean (\pm SD); TLC: Total leukocyte count; ANC: Absolute neutrophil count; Plt: Platelet count; ALC: Absolute lymphocyte count; RDW: Red blood cell distribution weight; MPV: Mean platelet volume; RPR: RDW to platelet ratio; PLR: Platelet to lymphocyte ratio; NLR: Neutrophil to lymphocyte ratio; *Statistically significant ($P < 0.05$).

and non-S. epidermidis ($P = 0.645$).

To assess the diagnostic value of MPV in neonatal sepsis, the ROC curve yielded an AUC of 0.678 (95% C.I. 0.549-0.808). Considering an optimum cut-off point of 9.65 fL, MPV had a sensitivity of 73.5% and specificity of 58.8% in diagnosing neonatal sepsis (Figure 2).

Discussion

The neonatal period is often considered a critical period of life, where disturbances of physiological function can result in major adverse developmental outcomes. Accordingly, neonatal sepsis is a significant threat to the development of the newborn and is linked to high morbidity and mortality worldwide. Considering the high burden of neonatal sepsis, measuring the diagnostic utility of paraclinical indicators could equip the clinician with further insight for the timely diagnosis and treatment of sepsis. In this study, we evaluated the association of several hematologic indices with neonatal sepsis.

Our study demonstrated that among the studied hematologic indices, the MPV value was significantly higher in the neonatal sepsis group when compared to the control infants. This increase was more pronounced in non-Staphylococcus epidermis sepsis. Furthermore, an optimal cut-off point of 9.65 fL yielded a sensitivity

of 73.5% and a specificity of 58.8% for the diagnosis of neonatal sepsis. In line with our findings, several previous studies have suggested that MPV might provide acceptable diagnostic utility in neonatal sepsis. Similar to our observations, Wang et al. demonstrated that MPV was significantly higher in infants with neonatal sepsis compared to the healthy controls (21). In another recent study, Sagheb et al. have shown that MPV resulted in an AUC of 0.687 in neonatal sepsis (22), which is comparable to the findings of our study. Accordingly, using a diagnostic cut-off level of 9.2 fL, these authors indicated that MPV has a sensitivity of 80.5% and a specificity of 52% in diagnosing neonatal sepsis (22). Furthermore, another study has reported that MPV was significantly higher in preterm infants diagnosed with LOS, where an MPV cut-off of 9.2 fL was associated with a sensitivity of 63% and specificity of 73% in LOS prediction (23); however, the assessment of EOS was not performed in that study. On the other hand, another study on neonates with EOS has revealed that MPV had an AUC of 0.666 in the prediction of EOS, and using an optimal cut-off value of 9.3 fL, MPV had a sensitivity of 84% and a specificity of 32% in diagnosing EOS (24).

Similar to what is observed in neonatal sepsis, in settings other than neonatal sepsis, MPV has been

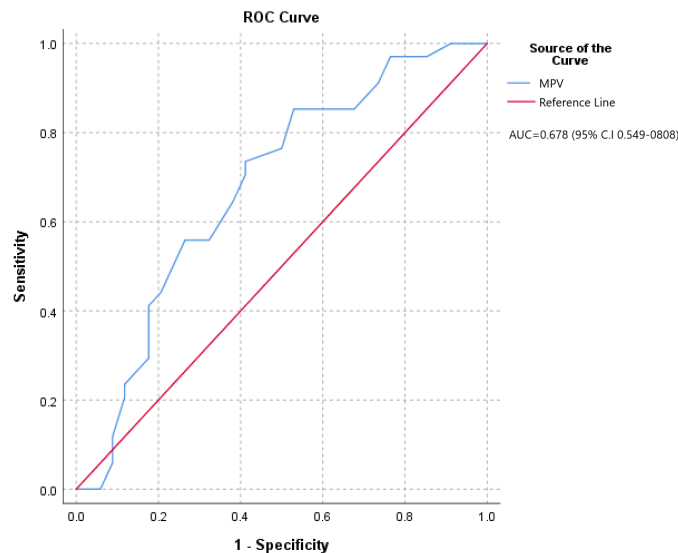


Figure 2. Receiver operating characteristic (ROC) curve representing area under curve (AUC) for mean platelet volume (MPV) in neonatal sepsis diagnosis.

proven to be a predictor of mortality among critically ill patients as well (25-27). Platelets generally play a pivotal role in inflammatory responses. It is hypothesized that following a serious systemic response, the release of proinflammatory cytokines, particularly IL-6, results in platelet activation and the massive production of young platelets in circulatory blood (27). MPV is an indicator of average platelet size and the maturity of circulating platelets. An increase in MPV reflects an increment in the number of young circulating platelets, which are in average, larger in size, and might be due to increased consumption of platelets that is followed by the overproduction of younger platelets (23, 28).

Although our study also showed that the mean level of a series of hematologic indices, including TLC, ANC, ALC, RPR, and RDW, were relatively higher in infants with neonatal sepsis, we observed no significant difference between the groups in this regard. Moreover, other leukocyte-related hematologic indices, such as the NLR and PLR, had no significant difference between the sepsis and control groups as well. These indicators also did not show any significant difference according to the onset of sepsis. Other studies have brought conflicting results regarding the association of indicators related to leukocyte and neonatal sepsis. For instance, in line with the findings of our study, Sumitro et al. did not observe a significant difference in TLC and ANC between the positive and negative blood culture groups; however, in contrast to the results obtained in the present study, these authors found that NLR was significantly higher in the group with positive blood culture (29). Nevertheless, these authors did not include a healthy control group in their study. Furthermore, Can et al. also reported that NLR and PLR showed a positive correlation with EOS among full-term neonates (16). These conflicting reports might be attributed to different characteristics of the studied sample (e.g., the maturity of infants, the onset of sepsis, and sample size), the gold standard used for definite sepsis diagnosis, or the analyzer device that has been used.

Therefore, further studies are needed to provide a more precise diagnostic utility of these hematologic markers in neonatal sepsis.

Conclusion

Overall, the findings of the present study demonstrated that among various hematologic indices, MPV was significantly higher in infants diagnosed with neonatal sepsis. These findings indicate that MPV might provide some utility in facilitating the prompt detection and clinical management of neonatal sepsis.

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None.

Authors' Contribution

Conceptualization: Fatemeh Sabzevari, Farzaneh Yazdi, Sarehsadat Ebrahimi, and Mozhdeh Poursalehi.

Data curation: Mozhdeh Poursalehi.

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Methodology: Fatemeh Sabzevari, Farzaneh Yazdi, and Sarehsadat Ebrahimi.

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Supervision: Fatemeh Sabzevari, Farzaneh Yazdi, and Sarehsadat Ebrahimi.

Validation: Fatemeh Sabzevari.

Visualization: Mehran Ilaghi.

Writing—original draft: Mehran Ilaghi.

Writing—review & editing: Fatemeh Sabzevari, Farzaneh Yazdi, and Sarehsadat Ebrahimi.

Competing Interests

The authors declare that there is no competing and financial interests.

Ethical Approval

All study protocols have been conducted under approval of the Ethics Committee of Kerman University of Medical Sciences (Ethical code: IR.KMU.AH.REC.1401.084). Informed consent was obtained from the legal guardians of subjects upon enrollment.

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