Novel Insights into the Hematological Parameter Abnormalities in Pediatric COVID-19 Cases: Observation from A Preliminary Study of 11 Pediatric COVID-19 Cases in a Tertiary Care Center of North India

Dr. Neema Tiwari¹, Dr. Devajit Nath¹, Dr. Jyotsna Madan¹, Prashant Bajpai², Dr. Ujjwal Madan³, Dr. Savitri Singh¹

¹Dept. of Pathology, Superspeciality Pediatric Hospital & Postgraduate Teaching Institute, Sector 30, Noida-201301, India
²Dept. of Biostatistics, University of Lucknow, Lucknow-226025, India
³University College of Medical Sciences, New-Delhi-110095, India

Abstract

Introduction: The Corona Virus Disease 2019, caused by novel Coronavirus SARS-CoV-2 is an acute respiratory and systemic illness, and is declared as a global pandemic by WHO on the 11th of March 2020. Only 2.2% to 1.7% of pediatric COVID-19 cases have been reported from USA and China respectively. The hematological parameter alteration in the pediatric COVID-19 cases needs to be addressed in the Indian population. Aim: To assess the alteration of the baseline hematological parameters in the paediatric COVID-19 cases and to compare the values with the adult COVID-19 cases for any statistically significant difference. Methods: A single Centre retrospective study of 11 COVID-19 asymptomatic pediatric patients and asymptomatic gender-matched adult cases admitted to SSPH&PGTI NOIDA from March to April, were enrolled for the study. The demographic date, baseline hematological findings of both groups were recorded and analyzed statistically. Results: Demographic characterization shows a mean age of 8.7 years in the pediatric population. Baseline CBC findings of all the pediatric cases show mild anemia, normal leucocyte, normal absolute lymphocyte count, and neutrophilia. Majority of the pediatric cases have NLR value below the cut-off point of 3.13. PLR was slightly higher in pediatric cases. Comparison between two groups shows statistically significant differences for the hemoglobin and platelet value only. Conclusion: The comparative analysis of baseline hematologic parameters did not show any significant derangement of the CBC parameters in pediatric cases. Significance in the difference of Hemoglobin level and platelet count is observed in comparison to the adult cases.

Key words: Corona virus, COVID-19, Pediatric, CBC, NLR, PLR.

INTRODUCTION

The Corona Virus disease (COVID-19) is an acute respiratory and systemic illness, caused by novel Coronavirus SARS-CoV-2 and is declared as a global pandemic by WHO on 11th March 2020. Humans of all ages are susceptible to the virus. However, as compared to adults, the number of reported Covid-19 cases in the pediatric population is low. Globally the Chinese literature has reported a percentage of 2.2% of Pediatric COVID-19 out of 44,672 confirmed cases as on 11th February 2020[1]. The United States has reported a percentage of 1.7% of Pediatric COVID-19 out of 149082 confirmed cases between 12th February and 02nd April 2020[2]. Italy and Spain reported a 1.2% [3] and 0.8%[4] of confirmed pediatric COVID-19 Cases, respectively[5].

As the pandemic is progressing over time, India has reported over 56000 cases so far with as many as 1886 death as of 8th May 2020 as per Ministry of Health and Family Welfare Government of India (MOHFWGOI) website data, however, there is a paucity of data with limited information on the incidence of pediatric COVID-19 in the Indian population.

Various hematological parameters alteration has been documented in the current literature review in SARS-Cov-2 infection primarily from the adult cases in the Chinese and the Western population, which signifies the severity and the prognosis of the patient outcome. As per the Diagnosis and Treatment Guidelines (trial version 7) [6] review, in the early stage of the disease onset, total leukocyte count is normal, and the lymphocyte count is reduced. From a study...
conducted by Li et al. [7] the lymphocyte percentage (Lym%) was found to be inversely related to the severity and prognosis of patients, which in turn could be used to predict the severity and prognosis of patients with COVID-19. The hematomal alteration has the potentiality to serve as indicators to provide guidance for the diagnosis, treatment, and prognosis for COVID-19 patients. The documentation of the hematological parameter alteration is very limited in the pediatric COVID-19 cases worldwide and in India as a whole owing to the relatively lower incidence of the disease in the pediatric population.

The present study was undertaken with the aim to assess the characteristic changes in the value of hematologic routine parameters in the pediatric COVID-19 cases in the Indian scenario. In order to explore the value of hematologic routine parameters, we retrospectively analyzed the baseline hematologic test results of 11 asymptomatic pediatric COVID-19 cases. The secondary objective of the study was to compare the baseline hematologic parameters of the pediatric cases with the adult COVID-19 cases to look for any statistically significant difference between the two groups.

**METHOD**

**Patient selection**
A single-center retrospective study of 11 pediatric patients with laboratory-confirmed COVID-19 admitted to Super Speciality Pediatric Hospital & Post Graduate Teaching Institute (SSPH & PGTI), NOIDA from March to April, were enrolled for the study. The 11 pediatric cases were asymptomatic on the day of admission. For comparative analysis, gender-matched 11 asymptomatic laboratories confirmed adult cases were also enrolled for the study. The diagnosis of COVID-19 was according to the Ministry of Health and Family Welfare (MOHFW) Government of India (GOI) guidelines and confirmed by RTPCR performed on nasopharyngeal and throat swab samples of the patient. Patients with preexisting hematological or underlying disorders were excluded in both the pediatric and the adult group.

**Baseline data Collection**
The demographic data and hematological findings of the pediatric and the adult group were recorded. The samples for complete blood count (CBC) for both the group were collected on the day of admission. All patients didn’t receive any treatment before blood sampling. The samples were tested for complete blood count on 5 part hematology analyzer Abbott Cell Dyn Ruby, and values were noted. The hematology test results were divided into two groups based on the sources of the samples as pediatric and adult on which retrospective and comparative analysis was performed.

**STATISTICAL ANALYSIS**
Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS version 15.0, SPSS Inc., Chicago, IL, USA) software. Normally distributed continuous variables were presented as mean, median, Standard deviation (SD), Minimum, maximum, and 95% C.I (Confidence Interval). The respective hematology parameters of the two groups were compared using two-sample t-Test at a 5% level of significance.

**RESULTS**

**Demographic characteristics**
The mean age of presentation in adult cases is 37.3 years, while in pediatric cases, the mean age is 8.7 years. The youngest case we had was of 4 months of age. Each cohort had 4 (36.3%) females and 7 males (63.6%) out of 11 cases (Fig-1).

**Hematology findings**
Baseline hematologic finding in the pediatric cases and the comparative analysis with the adult cases are presented in Table 1 and Table 2. The boxplots for these parameters are presented in Figure 2-11 (Legend: Composite analysis of variables under study for baseline values between the pediatric and the adult group (box and whisker plots) show range of variation in different parameters. The lines show variation beyond the upper and lower ranges with each plot with random outliers. Each plot has a central line showing the range of dispersion of values and in the composite plots there is variation in the value of Hemoglobin and the platelet count.)

Anemia was found in 54.5% (6/11) of children while in 45.5% (5/11) cases have a hemoglobin level less than 10mg/dl. 18.2% (2/11) of adult cases have anemia, while only 1 case (9.1%) has a hemoglobin level of less than 10mg/dl. (Figure 2). Hemoglobin level is higher in Adult patients as compared to pediatric patients, and there is a statistically significant difference (p<0.05) in the Hemoglobin between Pediatric & Adult patients (Table 2). RBC count is higher in Adult patients as compared to pediatric patients, but there is no statistically significant difference in the RBC count between the Pediatric & the Adult patients. (Figure 3, Table 2).

WBC count is higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the WBC count between Pediatric & Adult patients (Figure 4, Table 3). Neutrophilia is observed in 36.3% (4/11) cases and children younger than 10 years, showing more predominant neutrophilia as compared to children above 10 years. In adult cases, 27.3% (3/11) showed neutrophilia. The neutrophil count is higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the Neutrophil count between the Pediatric & the Adult patients.
Of the pediatric cases, 27.3% (3/11) showed lymphopenia, and 27.3% (3/11) cases showed lymphocytosis. Mild monocytosis is observed in only 1 (9.1%) adult case. Monocyte count is slightly lower in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the Monocyte count between Pediatric & Adult patients (Figure 6, Table 2).

Monocyte count was reduced to the lower normal limit in 72.7% (8/11) of the pediatric cases, with 18.2% (2/11) cases showed monocytosis. Mild monocytosis is observed in only 1 (9.1%) adult case. Monocyte count is slightly lower in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the Monocyte count between Pediatric & Adult patients (Figure 7, Table 2).

Eosinophil count is slightly lower in Pediatric patients, while in adults, 27.3% (3/11) cases showed Eosinophilia with remaining values were within the normal range, but there is no statistically significant difference in the Eosinophil count between Pediatric & Adult patients (Figure 8, Table 2).

Platelet count is higher in Pediatric patients as compared to adult patients, and there is a statistically significant difference in the Platelet count between Pediatric & Adult patients (Figure 9, Table 2).

Table 1: Basic statistical values for different variables in the Pediatric and the adult population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>4.4</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Hb</td>
<td>10.6</td>
<td>10.9</td>
<td>1.8</td>
</tr>
<tr>
<td>WBC</td>
<td>11.5</td>
<td>11.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>7.2</td>
<td>6.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>3.0</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Monocyte</td>
<td>.42</td>
<td>.39</td>
<td>.34</td>
</tr>
<tr>
<td>eosinophil</td>
<td>.013</td>
<td>.06</td>
<td>0.15</td>
</tr>
<tr>
<td>platelet</td>
<td>230.0</td>
<td>245.0</td>
<td>102.6</td>
</tr>
<tr>
<td>NLR</td>
<td>4.1</td>
<td>1.8</td>
<td>5.25</td>
</tr>
<tr>
<td>PLR</td>
<td>109.3</td>
<td>68.3</td>
<td>49.7</td>
</tr>
</tbody>
</table>

Table 2: T-test for comparison of onetime baseline haematological parameters of 11 paediatric and the adult cases on the day of admission

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>-0.218</td>
<td>0.36744</td>
<td>-0.79, 0.75</td>
</tr>
<tr>
<td>HB</td>
<td>-2.13545</td>
<td>0.94015</td>
<td>-4.09, -0.17</td>
</tr>
<tr>
<td>WBC</td>
<td>3.08273</td>
<td>2.24188</td>
<td>-1.59, 7.76</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>2.33182</td>
<td>2.22832</td>
<td>-2.32, 6.98</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>.991545</td>
<td>.732422</td>
<td>-0.56, 2.52</td>
</tr>
<tr>
<td>Monocyte</td>
<td>-0.032636</td>
<td>.165785</td>
<td>-0.38, 0.32</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>-1.78364</td>
<td>.118714</td>
<td>-0.43, 0.07</td>
</tr>
<tr>
<td>Platelet</td>
<td>101.07091</td>
<td>42.60493</td>
<td>12.19, 189.94</td>
</tr>
<tr>
<td>NLR</td>
<td>1.36455</td>
<td>1.77981</td>
<td>-2.35, 5.08</td>
</tr>
<tr>
<td>PLR</td>
<td>37.78909</td>
<td>30.35321</td>
<td>-25.53, 101.11</td>
</tr>
</tbody>
</table>

Table 2: T-test for comparison of onetime baseline haematological parameters of 11 paediatric and the adult cases on the day of admission

The Neutrophil-Lymphocyte Ratio (NLR) is taken as a cut-off of 3.13 as a low-risk group stratification for age less than 50 years. The neutrophil-lymphocyte ratio (NLR) in the pediatric cases showed that 27.3% (3/11) cases had raised NLR above the cut off value of 3.13. NLR is slightly higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the NLR between Pediatric & Adult patients (Figure 10, Table 2). PLR is slightly higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the PLR between Pediatric & Adult patients (Figure 11, Table 2).
Fig-1: Hemoglobin

Fig-2: RBC Count

Fig-3: WBC count

Fig-4: Absolute Neutrophil Count

Fig-5: Absolute Lymphocyte Count
Fig.1-11(Legend: Composite analysis of variables under study for baseline values between the pediatric and the adult group (box and whisker plots) show range of variation in different parameters. The lines show variation beyond the upper and lower ranges with each plot with random outliers. Each plot has a central line showing the range of dispersion of values and in the composite plots there is variation in the value of Hemoglobin and the platelet count.)

DISCUSSION

Coronavirus disease caused by coronavirus SARS-CoV-2 belongs to the β-coronavirus cluster and is the third known cause of zoonotic coronavirus disease after severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS)\(^8\) Early reports from Wuhan, China showed that all of the COVID-19 patients were middle-aged and elderly (http://2019ncov.chinacdc.cn as accessed on 10\(^{th}\) of May 2020)[8]. As the disease enters into the epidemic stage, pediatric patients (even newborns) have been reported increasingly [9, 10]. According to the latest report from the Chinese Center for Disease Control and Prevention (CCDC), 965 confirmed patients below 19 years (2.16%, 965/44,672) were reported nationwide[1].
The relatively lower percentage of cases in the pediatric population as compared to adults follows a similar epidemiological pattern that was observed in the SARS and MERS outbreak [11]. Chinese literature suggest that median age of pediatric cases is 6.7 years (Range 1 day-15 years) with clinically pediatric COVID-19 cases might be less severe than cases in adults and the children might experience different symptoms than do adults[12,13].

Demographic data from other studies document that most of the patient’s age ranged from 2 weeks to 17 years, and females accounted for 54.5% of cases[11].

In our study, the patients are asymptomatic, and the age ranged from 4 months to 19 years (median age 9.0 years), and most were males (64%). Males seemed to be more susceptible to SARS-CoV-2 infection, which was similar to previously reported study[14-17].

Of the hematological parameters, lymphopenia had been the most common documented findings on complete blood count panel for adult patients with COVID-19 infection[14,18], whereas this finding has not been seen consistently in the pediatric population. Majority of the pediatric study showed normal white blood cell counts with lymphopenia ranging from 35% patients in a case series of 20 patients [19] to 3.5% in a study describing 171 children[19].

Wang et al. [19], in their study, documented that 3.0% of infants presented with lymphopenia, which included only patients with mild, moderate, or asymptomatic disease. The lack of significant lymphopenia may, in part, explain the limited number of severe COVID-19 in children. Yang et al. [25] reported that 80% of critically ill adult COVID-19 patients had lymphopenia, whereas Chen et al. [20] reported that only 25% of patients with mild COVID-19 had lymphopenia, suggesting that lymphopenia may correlate with the severity of the infection. In both SARS and MERS, lymphopenia was a predominant feature, due to a combination of viral particle-induced cytoplasmic damage and apoptosis [21-23]. In very young children; however, lymphopenia may not occur due to the relative immaturity of their immune system and differences in their immune response compared to adults [24]. Reports from some previous studies indicated a decreased lymphocytes count in severe cases of infection [14, 20]. As a result of viral infection, white blood cell and lymphocyte counts can be reduced by consumption.

Results from our study showed that 27.3% (3/11) of the pediatric cases had lymphopenia, 27.3% (3/11) cases had lymphocytosis, and 45.5% (5/11) cases had normal absolute lymphocyte count while only 9.1% (1/11) of the adult cases showed lymphopenia. Lymphocyte count was slightly higher in Pediatric patients as compared to Adult patients, but there was no statistically significant difference in the Lymphocyte count between the Pediatric & the Adult patients. The finding of lymphopenia in our study is correlating with various previous studies conducted elsewhere; however, the finding of lymphocytosis in the pediatric cases needs further research to document the pathogenesis.

The total leucocyte count did not show any consistent derangements in the various studies conducted earlier in adult cases. A normal leucocyte count was found in 69.6%, with leucocytosis in 15.2% and leucopenia in another 15.2% relative to the locally defined reference limits [13]. In our study, a normal total leucocyte count was found in 36.3%(4/11), leucopenia in 9.1%(1/11), and leucocytosis in another 54.5%(6/11) of cases. WBC count is higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the WBC count between Pediatric & Adult patients.

The neutrophil count from the earlier pediatric studies showed that 89.4% cases had normal neutrophil count, 4.6% of cases had neutrophilia, and 6.0% cases had neutropenia. These findings were in contrast to adult study whereby increased leucocyte and neutrophil counts were common in patients with unfavorable COVID-19 progression[25].

In another study by Cai et al. [26] in 10 symptomatic children of COVID 19 infection with radiological features of pneumonia, only one of the three cases had alterations in leucocyte and neutrophil counts. In our study, Neutrophilia is observed in 36.3% cases and children younger than 10 years showing more predominant neutrophilia as compared to children above 10 years. In adult cases, 27.3% showed neutrophilia. The neutrophil count is higher in Pediatric patients as compared to Adult patients, but there is no statistically significant difference in the Neutrophil count between the Pediatric & the Adult patients.

A study conducted on adult cases from China highlights eosinopenia as a significant prognosticating factor [27]. The study proposes Eosinopenia as a potentially more reliable laboratory predictor of SARS-CoV-2 infection than recommended “leukocyte counts” and “lymphopenia[27]”.

In our study, Eosinophil count is slightly lesser in Pediatric patients, while 27.3% of the adult cases showed Eosinophilia. Eosinopenia was seen in 36.3% (4/11) of the pediatric cases and 27.3% (3/11) of the adult cases, but there was no statistically significant difference in the Eosinophils count between Pediatric & Adult patients. No consistent eosinophil count abnormality is observed in our study for pediatric cases, unlike the adults where eosinopenia has been described in the studies.
In the present study, monocyte count was normal in 81.8% of the pediatric cases, while only 18.2% cases showed mild monocytosis, whereas mild monocytosis is noted in 9.1% of the adult cases. The finding of monocytosis in the minority of the pediatric cases has not been documented in other studies.

The platelet count from the pediatric cases is within the normal limit in 81.8% of cases, while 18.2% of cases showed some degree of thrombocytopenia, while 36.3% of adult cases had thrombocytopenia. Platelet count is higher in Pediatric patients (mean=2.3L) as compared to Adult patients (mean=1.29L), and there is a statistically significant difference in the Platelet count between Pediatric & Adult patients.

Findings from a study of 30 adult COVID-19 cases conducted in Huizhou, China showed that the patients with markedly elevated platelets and longer average hospitalization days might be related to the cytokine storm.

The Platelet Lymphocyte Ratio (PLR) is a parameter for the degree of cytokine storm, which might provide a new indicator in the monitoring of patients with COVID-19 [27,28].

The PLR in our study was slightly higher in the Pediatric patients (mean=109.31) as compared to Adult patients (mean=71.52), but there is no statistically significant difference in the PLR between Pediatric & Adult patients.

The Neutrophil-to-Lymphocyte ratio (NLR) is a biomarker for the assessment of the severity of bacterial infections and the prognosis of patients with pneumonia and tumor. NLR may serve as a surrogate marker of early risk identification in COVID 19 infection. In a study by Forget et al. [29] identified that normal NLR values in an adult, non-geriatric population in good health are between 0.78 and 3.53. Whereas, in another study by Jingyuan Li [30], it was concluded that Patients with age ≥50 having NLR ≥3.13 are at risk of severe illness, and they should get rapid access to the intensive care unit, if necessary.

The neutrophil-lymphocyte ratio(NLR) in the pediatric cases showed that 27.3% of cases had raised NLR above the cut off value of 3.13. The mean NLR of the adult cases was 2.7 below the cut-off value of 3.13. NLR is slightly higher in Pediatric patients(Mean=4.1) as compared to Adult patients(Mean=2.7), but there is no statistically significant difference in the NLR value between Pediatric & Adult patients.

In a recently discussed paper, it is suggested that in addition to various mechanisms, SARS-CoV-2 viral protein infects hemoglobin by the immune hemolysis of red blood cells[31].

In our study, 54.5% of children have anemia, with 45.5% cases have hemoglobin level less than 10mg/dl, while only 18.2% of adult cases have anemia with only 1 case (9.1%) has hemoglobin level less than 10mg/dl. Hemoglobin level is higher in adult patients as compared to pediatric patients, and there is a statistically significant difference (p≤0.05) in the Hemoglobin between Pediatric & Adult patients. RBC count is higher in Adult patients as compared to pediatric patients, but there is no statistically significant difference in the RBC count between the Pediatric & the Adult patients.

CONCLUSION

The study of the 11 pediatric asymptomatic COVID-19 cases of the Indian population shows an age range of 4 months to 19 years with a male predominance.

- Baseline CBC findings of all the pediatric cases show mild anemia, normal leucocyte, and absolute lymphocyte count in the majority of the cases and neutrophilia in a minority of the cases. The normal count of Eosinophil, monocyte, and platelet, were noted.
- Majority of the pediatric cases had a NLR below the cut-off point of 3.13.
- PLR was slightly higher in pediatric cases.
- The comparative analysis of the baseline CBC parameters between the pediatric and the adult cases show statistically significant differences for the hemoglobin and for the platelet value while all other parameters did not show any statistical difference between the two groups.

SUMMARY

Results from the baseline hematologic parameters of pediatric COVID-19 cases and its comparative analysis with the adult cases did not show any significant derangement of the CBC parameters in the pediatric COVID-19 cases in the Indian scenario. Significance in the difference of Hemoglobin level and platelet count is observed in comparison to the adult cases. In summary, unlike in adults, a consistent pattern of hematology parameter alteration has yet to be observed in children with confirmed COVID-19. The laboratory alternations reported in children with SARS-CoV-2 infections worldwide are not consistent with the early observations in cases of COVID-19 infection in India.

LIMITATION OF THE STUDY

There are some limitations to our findings. Only 11 asymptomatic pediatric cases were included. Moreover, laboratory methods and reference ranges may have differed between centers and were not always
clearly defined. Importantly, a lack of linearity of data prevents comparison of laboratory values between symptomatic and asymptomatic children or analysis based on the severity of illness. The cut-off value of NLR for risk stratification with age (≥50,<50) is set at 3.13; however, a standard cut-off value for a pediatric age group may have to be defined with a large cohort study. A lack of follow up CBC Parameters in the study group is also a limitation for statistical inference.

**RESEARCH IN CONTEXT**

Special considerations for pediatrics

Importantly, unlike adults, the laboratory profile in asymptomatic to severe COVID-19 pediatric patients is not clear and does not appear to be consistent with earlier infection like in SARS. Recent guidelines recommend clinicians to monitor lymphocyte and neutrophil count as a prognostic indicator to assess the severity of the infection. A preliminary description of pediatric U.S. COVID-19 cases was published by the CDC on April 10th, 2020. In line with data from China, the CDC reports that pediatric COVID-19 cases might be less severe when compared to adults and that children may experience different symptoms. Additionally, although most cases reported among children to date have not been severe, the CDC recommends clinicians maintain a high index of suspicion for COVID-19 infection in children and monitor for progression of illness, particularly among infants and children with underlying conditions. Importantly, interpretation of pediatric laboratory tests based on known adult trends may not be recommended due to lack of pediatric information and characteristics and need more research in the area.

**Known facts about the topic**

Sparse literature is available on the hematology laboratory parameter alteration among the pediatric covid-19 cases in the Indian population, where the pandemic is spreading with gradual and greater involvement of the pediatric population.

**Added information from the study**

The study obtained data from 11 pediatric COVID-19 cases in NOIDA, India, to explore the epidemiology and the baseline hematological parameters of the patients. The pilot study provides further information on the comparative hematological parameters of pediatric patients with adult cases, thereby adding substantial inputs to the knowledge database.

**Conflict of Interest**

All authors declare no competing interests.

**Author contribution**

The authors of the manuscript declare an equal contribution to the study.

**Ethics committee approval**

No Patient identifiers were collected, and approval of exemption for informed consent was applied.

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