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Original Research Article

Myriad of hematological manifestations in COVID-19 infected paediatric age group: our experience

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ABSTRACT

Background: The incidence of COVID-19 is remarkably less in pediatric population compared to the adult population. Little information on the association of various hematological abnormalities with disease severity, clinical course and age exists in children. There is also paucity of data regarding peripheral smear findings in children with SARS-CoV-2 infection.
Methods: A retrospective-cum-prospective review of the hematological parameters and peripheral smear findings of children diagnosed with COVID-19 infection admitted in tertiary care hospital over a period of two months was conducted. The study was conducted after obtaining approval from the Institute's Ethics committee. Data was recorded in a pre-designed form and analyzed anonymously.
Results: A total of 72 SARS-CoV-2 positive cases were included. Age of patients ranged from 2 days-18 years with male to female ratio of 1.6:1. Patients were grouped, based on age, into <1 year (21%), 1-5 years (15%) and 5-18 years (64%). Mild disease was noted in 82.1% cases. Anemia was the commonest hematological abnormality noted in 62.5% cases followed by leucopenia in 21% cases. Neutrophil to lymphocyte ratio (NLR) ranged from 0.1-11 and was raised in 40% cases of <1 year of age, 27% of 1-5 years and 28% of 5-18 years age group. Abnormalities in RBC and WBC morphology on peripheral smear were noted in 45.8% and 41.6% each. Peculiar finding was the presence of covicytes on peripheral smear showing Sandglass pattern in WBC scattergram.
Conclusions: The present study showed that the majority of children with COVID-19 had a mild disease with absence of significant lymphopenia.

Keywords: COVID-19, Pediatric, Hematological abnormalities

INTRODUCTION

Coronaviruses are a diverse group of viruses which tend to cause mild to severe respiratory infections in humans. Severe acute respiratory syndrome coronavirus, a novel coronavirus (SARS-CoV-2) emerged in the city of Wuhan, China, in 2019 and caused a pandemic outbreak of unusual viral pneumonia posing extraordinary threat to global healthcare. While the virus causes varying severity of respiratory infection and systemic inflammatory response in adult population even leading to significant morbidity and mortality, pediatric population has largely been protected or mildly affected with better prognosis as compared to the adults. The incidence ranges between 1-5% among the pediatric age groups.[1,2] Multitude of studies have been conducted worldwide assessing the hematological manifestations of COVID-19 in adults along with their prognostic significance but there is paucity of data regarding hematological abnormalities and their association with disease severity, clinical course, age group in case of pediatric population. The present study was thus undertaken to describe the hematological manifestations and peripheral smear findings of SAR-CoV-2 infection in COVID-19 positive

symptomatic children and their association with patient age, disease severity and outcomes.

METHODS

A retrospective cum prospective review of the hematological parameters and peripheral smear findings of the children diagnosed with COVID-19 positive status admitted in the emergency room, wards or intensive care unit of the department of pediatrics at Maulana Azad Medical College, New Delhi from September 2020 to December 2020 (over two months) was conducted. The study was conducted after obtaining approval from the Institute's Ethics committee. All pediatric patients less than 18 years of age, with laboratory confirmed SARS-CoV-2 infection and symptoms of COVID-19 disease whose hemogram and peripheral smear were done as per the departmental management policy were included. Cases with negative SARS-CoV-2 status as confirmed by reverse transcription polymerase chain reaction (RT-PCR) were excluded. Also, SARS-CoV-2 infected cases which did not have hemogram and peripheral smear findings were excluded. EDTA blood samples were analysed using Sysmex XN 1000 analyser (Sysmex Corporation, Japan). Peripheral smears were prepared and stained by May Grunwald Giemsa stain. Following data was collected: age, severity of COVID-19 disease at admission, outcome (discharge/death), hemoglobin levels, total leucocyte count, differential leucocyte count, platelet count, peripheral smear findings, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin content. The data was entered in MS excel and stored in password protected computers, shared only between researchers. Strict patient confidentiality was maintained.

Sample size calculation

In a study of 486 hospitalized children, the most common hematological abnormalities detected in pediatric inpatients with COVID-19 were lymphocytosis (22%) and leukopenia (21%).[3] Expecting the proportion of symptomatic children with hematological abnormalities to be around 20% with precision of 5% and confidence of 95%, sample size calculated was 246. However, a sample size of convenience of 72 patients was taken.

Study definitions

Confirmed COVID-19 case: A patient with laboratory confirmed SARS-CoV-2 infection, detected in nasopharygeal or oral swab by RT-PCR (reverse transcriptase polymerase chain reaction) or rapid antigen test, irrespective of clinical signs and symptoms.[4]

Disease severity classification; mild: uncomplicated upper respiratory tract infection (fever, cough, sore throat, nasal congestion, malaise, headache) without any evidence of respiratory distress or hypoxia (normal saturation of ≥ 95% on room air). Moderate*:* pneumonia with features of tachypnea, respiratory distress and/ or hypoxia of 90-94% on room air but no signs of severe disease. Severe: severe pneumonia with features of tachypnea, severe respiratory distress or SpO2 <90% on room air. In a child with severe pneumonia, the following if present were taken as manifestations of severe respiratory distress: central cyanosis, refusal to feed or drink, lethargy, convulsions. Presentation as acute respiratory distress syndrome or sepsis or septic shock were included in the severe disease category.[5] Hematological parameters: Age appropriate reference ranges were considered.[6]

Outcome variables

Primary outcome variable: proportion of children with any hematological abnormality. Secondary outcome variables: Comparison of proportion of children with abnormal hematological parameters across various age groups (<1 year vs. 1-5 years vs. 5-18 years). Comparison of proportion of children with abnormal hematological parameters in different disease severity classification (mild vs. moderate vs. severe).

Statistical analysis

The data obtained was analysed using MS Excel and Statistical Package for the Social Sciences (SPSS) software, version 26. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 72 SARS-CoV-2 infection positive cases were included. Age of patients ranged from 2 days-18 years. Mean age was 8years. Majority of the patients were male with male to female ratio of 1.6:1. At admission, mean hemoglobin and WBC counts were 10.3 and 9,265/mm^3 respectively. Mean RBC count was 4million/mm^3, mean platelet count was 2.5 l/mm^3, mean absolute neutrophil count (ANC) was 4,723/mm^3, mean absolute lymphocyte count (ALC) was 9,448/mm^3 and mean absolute monocyte count (AMC) was 723/mm^3. Patients were grouped, based on age, into <1 year (15 cases), 1-5 years (11 cases) and 5-18 years (46 cases). According to disease severity at admission in <1 year age group, mild disease was present in 12 cases and moderate disease in 03 cases. No case with severe disease was present in this group. In 1-5 years of age, mild disease was present in 09 cases and moderate in 02 cases. Similarly, in 5-18 years of age, mild disease was present in 41 cases, moderate in 04 cases and severe in 01 case. Anemia was the most common hematological abnormality, noted in 45 cases (62.5%). It was seen in 80% cases belonging to <1 year of age group, 55% in 1-5 years and 59% in 5-18 years. The predominant anemia across all age groups was microcytic hypochromic. It was seen in 67% cases in <1 year age and 1-5 years age groups, and 41% cases in 5-18 years age group. Hemolytic anemia was also noted among the children in addition to other nutritional

deficiency anemias. Mild disease was present in 75% anemic cases in <1 year age group, 67% cases in 1-5 years of age and 81% cases of 5-18 years age, while moderate disease was present in 25% cases in <1 year of age, 34% cases of 1-5 years of age and 15% cases of 5-18 years of age. None of the patients had severe manifestations of SARS-CoV-2 infection in <1 year and 1-5 years of age and was only seen in 4% cases belonging to 5-18 years of age.

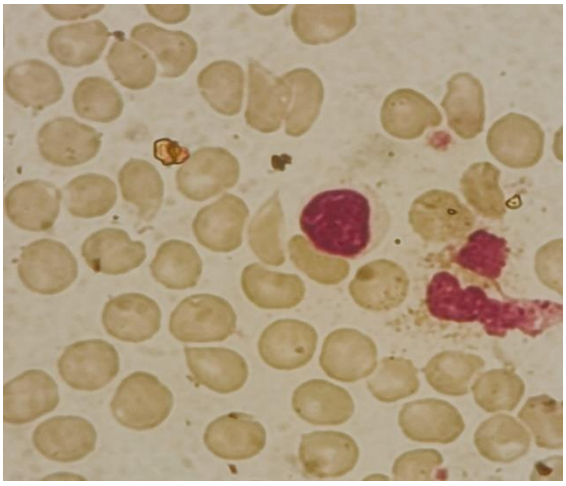

Figure 1: (P/S:Giemsa) monocytoid transformed lymphocyte.

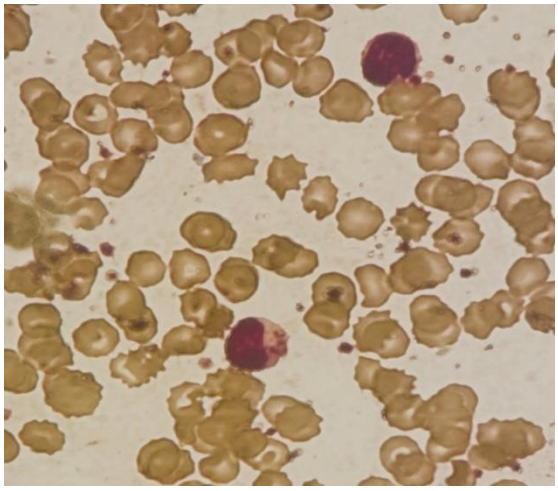

Figure 2: (P/S:Giemsa) large granular lymphocyte.

The next common hematological abnormality seen was leucopenia. It was noted in 15 cases (21%). Leucopenia was seen in 7% cases belonging to <1 year of age, 18% cases of 1-5 years of age and 26% cases of 5-18 years of age. All cases with leucopenia across all age groups had only mild infection. Lymphopenia was noted in 13 cases (18%) of which 20% cases belonged to <1 year of age, 55% cases to 1-5 years age and 9% cases to 5-18 years of age. Mild disease was present in 67% lymphopenic patients of <1 year of age and 1-5 years of age while it was seen in 75% patients of 5-18 years of age. Moderate disease was seen in 33% patients of <1 year of age and 1-5 years of age and 25% patients of 5-18 years of age. None of the lymphopenic patients had severe disease. Neutropenia was also seen in 13 cases (18%) out of which 20% were in <1 year age group, 18% were in 1–5-years age group and17% in 5-18 year age group. Mild disease was seen in 92% neutropenic patients, rest had moderate disease. Lymphocytosis was seen in only 5% cases, belonging to 5-18yrs of age, and majority had mild disease.

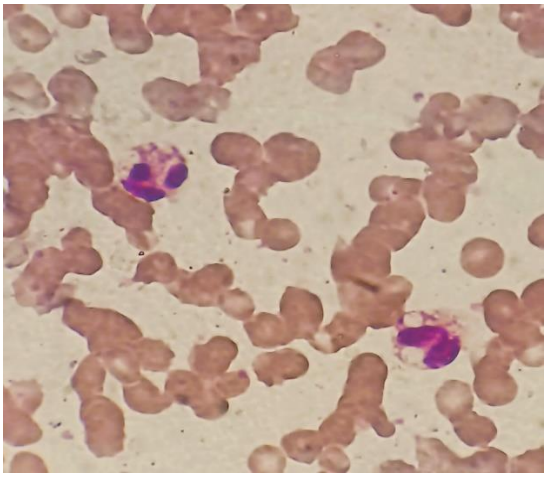

Figure 3: (P/S:Giemsa) dyspoietic, hypolobated polymorph with cytoplasmic vacuoles and coarse sparse granules.

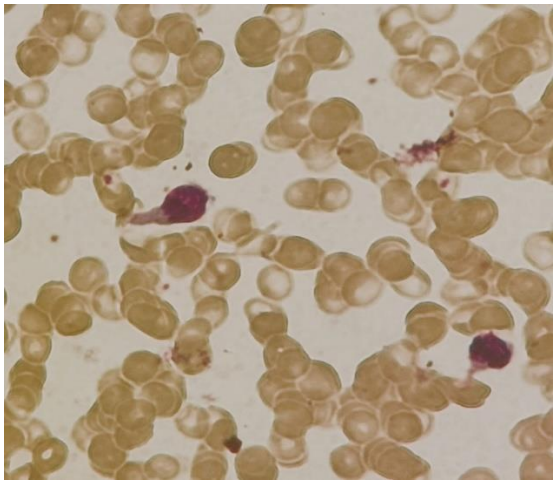

Figure 4: (P/S:Giemsa) large atypical, bizarre looking mononuclear cells having irregular nuclear membrane, dense chromatin, scant to moderate cytoplasm.

In the present study neutrophil to lymphocyte ratio (NLR) ranged from 0.1-11 and was raised in 40% cases of <1 year of age with majority being mild (67%) followed by moderate (34%) disease. Among 1-5 years age group, NLR ranged from 0.1-8.8 and was raised in 27% cases with all having mild disease. In 5-18 year age group, NLR ranged from 0.6-10.8 and was raised in 28% cases, out of which mild disease was seen in 77% cases,

moderate in 15% and severe in 8%. Of the 72 cases included, abnormalities in RBC morphology on peripheral smear were noted in 33/72 cases (45.8%), with microcytic hypochromic RBCs (MCHC) being the commonest, seen in 23/72 cases (32%) followed by macrocytes. Schistocytes as part of hemolytic blood picture were noted in 05/72 cases (7%).

Table 1: Clinico-hematological profile of patients.

Characteristics		Age group (years)			P value
		<1 (n=15)	1-5 (n=11)	5-18 (n=46)	-
Male:female		2:1	1.8:1	1.4:1	
Hb (g/dl)	Range	4.4-14.9	8.5-12.1	5.7-17.7	0.87
	Mean	9.8	10.5	10.7	
	Median	10.7	10.7	11.1	
	SD	3.4	1.06	2.5	
TLC (/mm$^{3)}$	Range	4,780-62,120	2,590-18,020	2,190-18,050	0.002
	Mean	13,626	11,009	6,841	
	Median	8,740	13,070	5,985	
	SD	14325	5,014	3,168	
ANC	Range	411-54,666	405-9,551	307-9,328	0.93
	Mean	7,567	3,298	3,304	
	Median	3,212	1,568	2,613	
	SD	13720	3,381	2,203	
ALC	Range	1,276-7,929	1,166-7,929	608-8,336	0.00003
	Mean	4,581	4,102	2,296	
	Median	5,293	4,382	2,009	
	SD	1869	2,209	1,890	
NLR	Range	0.1-11	0.1-1.9	0.2-10.8	0.048
	Mean	2.17	0.81	2.12	
	Median	0.65	0.6	1.3	
	SD	3.5	0.6	2.2	
MCV	Range	57.5-102.5	63.2-94.6	66.5-109.9	0.053
	Mean	78.53	79.46	86.18	
	Median	75.25	79.5	86.7	
	SD	14.67	10.1	9.4	
MCH	Range	14.7-31.9	16.4-30.3	20.9-34.2	0.038
	Mean	24.3	25.38	28.25	
	Median	23.7	25.6	28.4	
	SD	5.6	4.1	5.1	
MCHC	Range	25.6-35.2	26-33.4	27.4-36.4	0.047
	Mean	30.62	31.83	32.38	
	Median	30.85	32.2	32.35	
	SD	2.5	15.4	1.9	
Platelet (l/mm$^{3)}$	Range	0.14-5.14	0.75-4.7	0.33-4.73	0.14
	Mean	2.6	2.8	2.1	
	Median	2.56	2.95	1.9	
	SD	1.44	1.27	1.05	
Transformed lymphocytes n/N (%)		5/15 (33.3)	2/11 (18.2)	23/46 (50.0)	0.12

Abnormalities in WBC morphology on peripheral smear were noted in 30 cases (41.6%). The most common abnormality noted was presence of transformed lymphocytes (TL) (Figure1) seen in 30/72 cases (41.6%), followed by eosinophilia seen in 07/72 cases (9.7%), activated monocytes in 06/72 cases (8.3%) and large granular lymphocytes (Figure 2) seen in 01/72 case (1.4%). Other abnormalities included polymorphs having dyspoietic features such as hypolobated forms, ring shaped nucleus, cytoplasmic vacuolations, coarse granules mimicking toxic granules as well as hypogranular polymorphs (Figure 3). Peculiar finding

was the presence of covicytes which are bizarre looking mononuclear cells having a cell size of 2-3 times that of an RBC, high N:C ratio, irregular nuclear membrane and basophilic cytoplasm (Figure 4). Atypical aspects in WBC scattergram were noted in patients which had covicytes on peripheral smear. These included >4 dots in upper graduation of WBC scattergram representing discontinuous cluster of lymphocytes aka "sandglass" pattern and have not been described widely in the available literature (Figure 5).

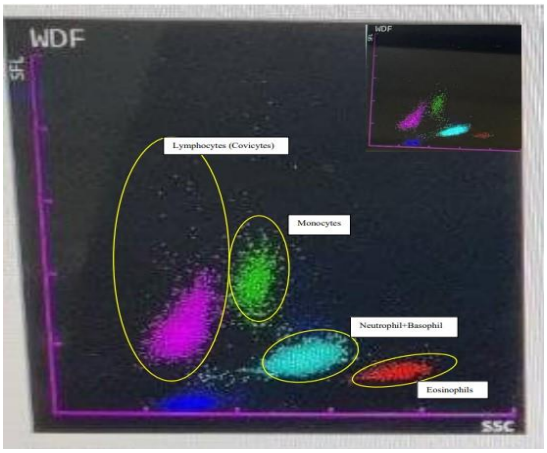

Figure 5: Atypical WBC scattergram (sandglass pattern), inset: Normal WBC scattergram.

DISCUSSION

Since the start of SARS-CoV-2 pandemic in December 2019, multiple studies on the clinical characteristics of the affected patients have focused mainly on adult patients. Only limited data is available analyzing effects of SARS-CoV-2 infection in pediatric age group patients. The present study has evaluated the haematogical abnormalities associated with COVID-19 disease in pediatric and adolescent age group, and the association of these abnormalities with disease severity. In the present study, anemia was the most common hematological abnormality, noted in 62.5% cases with majority (80%) belonging to <1 year of age group. This is in contrast to study by Kosmeri et al in which no abnormalities in red blood cell (RBC) count or level of hemoglobin (Hb) were noted.[7] This could be already existing anaemia in this population as this hospital caters to population of lower to lower middle class economic strata,this not directly attributed to disease in question.

The next common hematological abnormality seen was leucopenia noted in 21% cases. Majority of the leucopenic patients (26%) were of 5-18 years of age. All cases with leucopenia across all age groups had only mild infection. Studies by Patel et al and Meena et al confirmed that majority of children with COVID-19 had a normal TLC, and that the most common abnormality was leukopenia.[8,9]

Table 2: Association of hematological parameters with clinical status of the patients.

Parameter (n/N)	Mild COVID-19 (n=62/72)	Moderate/severe COVID-19 (n=10/72)	One way ANOVA	
			F	P
Leucopenia (15/72; 20.8%)	15	0	2.836	0.097
Neutropenia (13/72; 18.0%)	12	1	0.649	0.423
Lymphopenia (13/72; 18%)	9	4	2.071	0.155
Abnormal NLR (22/72; 31%)	17	5	0.339	0.562
Transformed lymphocytes (30/72; 41.6%)	27	3	0.638	0.427

They however did not provide any information on the association of the various WBC abnormalities (leukopenia, lymphopenia) with the disease severity or clinical course. In a systematic review by Ma et al of 486 hospitalized children, lymphocytosis (22%) and leukopenia (21%) were found to be the commonest abnormalities and most had mild clinical manifestations with milder disease course than adults. In the present study, however lymphocytosis was found in only 5% cases, belonging to 5-18 years of age, and majority had mild disease.

Multiple studies in adult patients have shown lymphopenia to be the commonest hematological abnormality of the infection and was found to be associated with severe disease and poorer outcome.[10,11] In studies by Yang et al and Chen et al lymphopenia was detected in 80% of critically ill adults and only 25% of adults with mild disease.[12,13] It has been postulated that direct attachment of SARS-CoV-2 to lymphocyte surface receptor, viz, angiotensin converting enzyme 2 (ACE 2) receptor is responsible for lymphopenia in these patients.[7] Another theory suggests role of apotosis mediated lymphocyte decrease in response to marked increase in cytokines and inflammatory mediators in critically ill adults.[7,14] Pediatric age group seems to be protected against this viral infection and severe disease due to less developed ACE 2 receptor along with a less mature immune system.[7] In a systematic review by Henry et al including 66 children with COVID-19 from 12 studies, a normal leukocyte count was seen in majority of children while lymphopenia was reported in only two infants

(3%), neither of which had severe disease.[15] In the present study, lymphopenia was noted in 18% cases, with majority (55%) cases belonging to 1-5 years age group. Mild disease was present in 69% lymphopenic patients. None of the lymphopenic patients had severe disease. This is similar to the findings of a meta-analysis including 160 pediatric patients by Raba et al wherein they detected lymphopenia in 16% of the infants and neonates.[16]

Neutrophil to lymphocyte ratio (NLR) has been shown to be an independent prognostic biomarker associated with virus triggered inflammation and severe pneumonia in adult population infected with COVID-19. An optimal threshold of ≥3.3 was found to be of superior prognostic value in predicting disease progression from mild to severe disease.[17] However, given the physiological variability with age in various hematological parameters among pediatric patients no definite cut-off has been assigned to NLR in pediatric age group. In the present study NLR was raised in 31% cases, out of which 77% had a mild disease and only 8% had severe disease course. While assessing the peripheral smears, many morphological changes were observed especially in the WBC series. The most peculiar finding was of large bizarre atypical looking cells with basophilic cytoplasm, *aka* a "covicyte". Polymorphs with hypogranular cytoplasm as well as showing coarse granules resembling toxic granules were seen in a few cases. Polymorphs also showed abnormalities of nuclear shape, cytoplasmic vacuolations and dyspoeitic features such as hypolobated forms.[18] Also noted were large granular lymphocytes, reactive monocytoid and plasmacytoid forms and blastoid forms, activated monocytes with large bizarre forms, vacuoles and granules.[18,19] In a study by Zhang et al by analysing monocyte subset it was shown that the peripheral smear in COVID-19 is different from any other viral illness.[20] More recent updates suggest that cytokine storm and hyperinflammation which is seen in COVID-19 infection can lead to such quantitative and qualitative abnormalities in hematological parameters, possibly in the form of secondary hemophagocytic lymphohistiocytosis, leading to possibility of relapse or fatal multi-organ failure.

Limitations

Smaller sample size and heterogenous data were the limitations of the study.

CONCLUSION

The present study showed that the majority of children with COVID-19 had a normal WBC count. The absence of significant lymphopenia in children may be explained by the milder disease in this population. The most common WBC abnormality in children with COVID-19 was leucopenia. Little information on the association of the various hematological abnormalities (leukopenia, lymphopenia, NLR) with the disease severity, clinical course and age exists in children. Likewise, there is paucity of data regarding the peripheral smear findings in children with SARS-CoV-2 infection. Thus, this study aimed to describe the hematological manifestations and peripheral smear findings of SARS-CoV-2 in symptomatic children with COVID-19 so as to understand the disease better and be better prepared to manage patients from different age strata.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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