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# Hematological Profile of Covid-19 in Children

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***Abstract***
***Background****: Literature available on pediatric COVID-19 infection emphasizes on the clinical presentation and radiological changes. Studies on hematological profile of children in COVID- 19 infection are very few, especially in India.* ***Aim****: To study the hematologic profile of COVID-19 infection in children.* ***Methods****: This is a cross sectional observational study conducted over a period of 12 months. Children diagnosed to have COVID-19 infection by rapid antigen test or RT-PCR between 1 month and 12 years of age were included in the study. Laboratory data was collected from online portal of the institute, this was entered and analyzed in Microsoft Excel sheet and SPSS version 2.0. Bivariate logistic regression was done in R software version 4.0.5.* ***Results****: The mean age of presentation was 4.7 +/=3.7 years. Major presenting complaint was fever (n=44, 44%) followed by cough (n=27, 27%). On admission CBC showed anemia in 68% (n=67). Mean Hb was 10.7 +/-2.4 gm/dl. NLR was raised in 12% (n=12). D-dimer was high in 37% (n=37). High NLR had statistically significant association with positive CRP and leukocytosis (p value 0.07, 0.007 respectively. Leukocytosis and thrombocytopenia were found to have statistically significant association with death (p value 0.032, 0.006 respectively). Binary logistic regression study conducted with hospital stay for more than 10 days as the dependent variable showed high d-dimer as the only surviving variable.* ***Conclusion****: The study highlights the prevalence of anemia in COVID-19 infected children. None of the hematological parameters studied were associated with hospital stay of more than 10 days. However, thrombocytopenia and leukocytosis can be used to predict death in COVID -19 positive children.*

***Keywords:*** *COVID-19, children, hematology, pediatrics*

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## I. Introduction

Corona virus disease, the novel threat to the mankind and the health system had its outbreak from Wuhan city in China. There was a sudden spread of the disease across the globe resulting in rapid increase in the number of cases. On March 11, 2020, the disease was declared as a pandemic by the World Health Organization (1). The global prevalence of covid 19 was more than 4 million cases by March 2021 and India had more than eight lakh cases with more than 4500 deaths reported in the country (2).

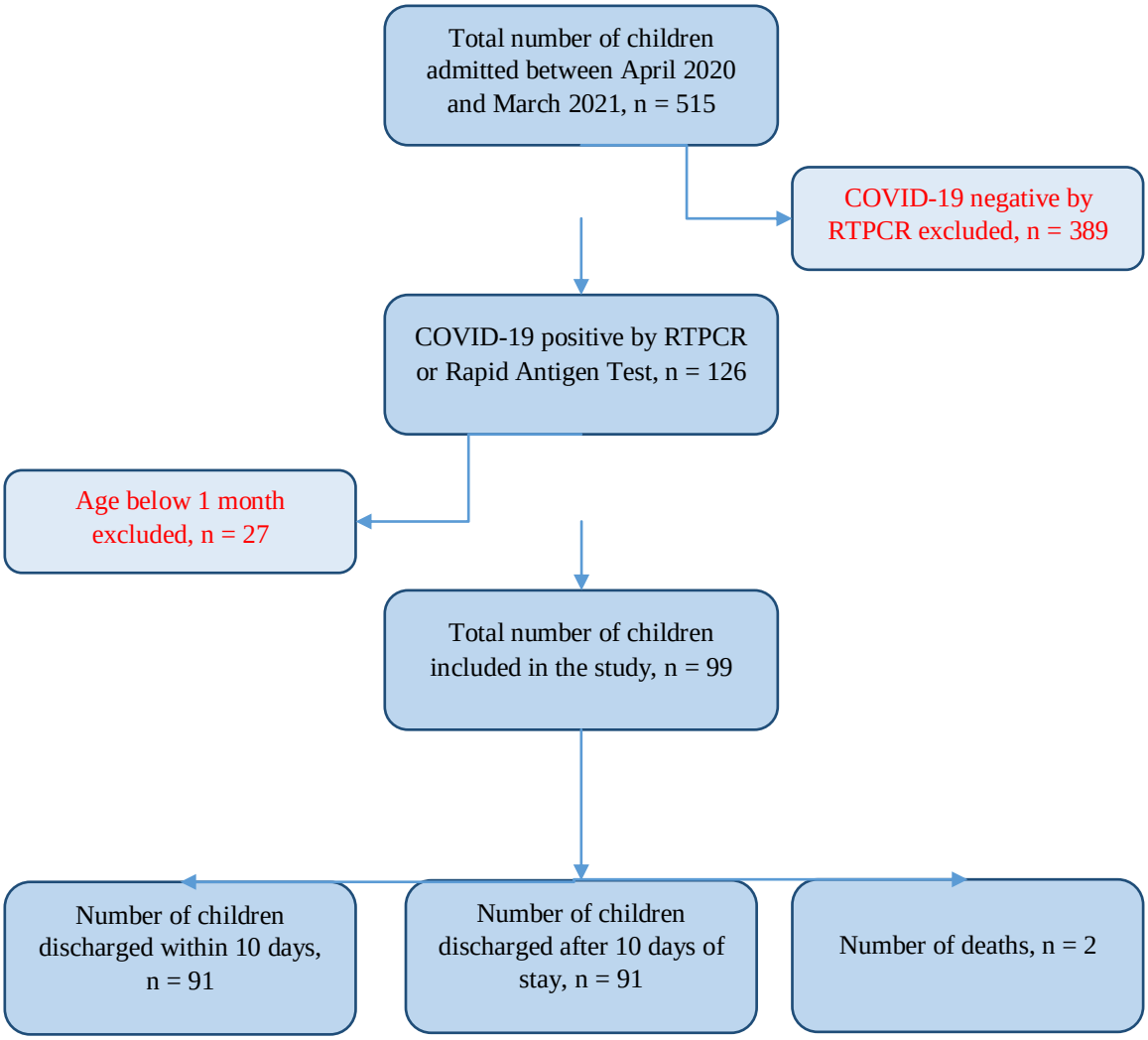
Mode of spread of the virus is via droplet infection. COVID-19 mainly affects the respiratory system and cause substantial pulmonary disease which includes pneumonia and acute respiratory distress syndrome (ARDS). But this virus also affects other systems resulting in a multisystem disease. Sepsis and septic shock has been reported in covid infected adult population. COVID 19 can also complicate underlying cardiovascular disease and cause myocardial injury, infarction, arrhythmias and heart failure. The virus can cause pancreatic beta cell dysfunction causing transient Type-2 Diabetes mellitus. Hyperinflammatory condition characterized by hypercytokinemia caused by the virus can cause pulmonary involvement in the form of ARDS, unremitting fever, cytopenias and increased ferritin levels. Lymphocytopenia, thrombocytopenia and leukopenia or normal total leucocyte count are seen in COVID-19 infection. Following coagulation activation from sepsis, cytokine storm and multiorgan failure, coagulopathy can be seen in severe disease (3).

Although studies have been conducted on the systemic manifestations of COVID -19 in children, studies on hematological manifestations are scarce. Considering majority of the families in the country do not have access to quality health care, we need to rely on basic blood cell indices to triage the infected children. Hence, we decided to study the spectrum of hematological manifestations in children affected with COVID-19.

## II. Methodology

This was a cross sectional observational study conducted in a tertiary care hospital in Pune city of Maharashtra. The hospital is considered as a COVID-19 treatment centre and has been admitting both adult and pediatric covid patients since the beginning of the epidemic. All the children who were brought to the emergency room with symptoms or signs suggestive of COVID-19 infection as well as children who were already admitted to the in-patient department who later developed respiratory complaints suggestive of COVID-19 infection were screened routinely using covid RT-PCR. Covid antigen tests were performed in those children who required urgent interventions to decide on further management. Covid RT-PCR tests were done in this group of children if the antigen test turns out negative. Among the admitted children, none had a positive covid RT-PCR following a negative covid antigen test. Children who turned out COVID-19 positive were isolated. Only those children between the age of 1 month to 12 years who were positive for COVID-19 infection by either antigen test or RT-PCR from April 2020 to March 2021 were included the study. Children less than 1 month and more than 12 years of age were excluded. Radiological tests like chest X-ray was done in all children. Blood investigations which included Complete blood count, peripheral blood smear, renal function tests, liver function tests, CRP, Blood culture, coagulation profile, d-dimer, IL- 6 , ferritin, Vitamin B12 levels were sent routinely for every COVID-19 positive child. Reports were analyzed. Abnormal reports were assessed based on the clinical condition of the patient. In this study only the initial hematological reports and CRP were taken for analysis. Children were followed up during their hospital stay for the treatment involved and the outcome. Data were entered in Microsoft Excel sheet. Continuous data was expressed as mean, median and standard deviation and categorical data was expressed as proportion. Bivariate logistic regression was done in R software version 4.0.5. The package 'Resource Selection' was used to do Hosmer Lemeshow goodness of fit test in R software(4) (5). Institutional Ethics Committee Clearance has been obtained.

**Figure 1:** Flowchart of patient selection in the study



## III. Results And Analysis

Ninety-nine COVID-19 positive children admitted in twelve months from April 2020 to March 2021 were included in the study. Patient characteristics and demographic data are summarized in Table 1.

**Table 1:** Demographic data and other patient characteristics

| Parameters | **Frequency (n)** | **Percent (%)** |
|---|---|---|
| Sex | | |
| Male | 55 | 55 |
| Female | 44 | 44 |
| Age 1 month to 1 year | 32 | 32 |
| >1 year to 5 years | 22 | 22 |
| >5 year to 10 years | 39 | 39 |
| >10 years to 12 years | 06 | 06 |
| Socio-economic status* | | |
| Upper | 03 | 03 |
| Upper middle | 21 | 21 |
| Lower middle | 27 | 27 |
| Upper lower | 48 | 48 |
| Lower | 00 | 00 |
| Symptoms | | |
| Fever | 44 | 44 |
| Cough | 27 | 27 |
| Sorethroat | 02 | 02 |
| Bodyache | 03 | 03 |
| Headache | 07 | 07 |
| Vomiting | 07 | 07 |
| Loose stools | 06 | 06 |
| Breathlessness | 11 | 11 |
| Asymptomatic | 37 | 37 |
| Congenital heart disease | 03 | 03 |
| Chronic kidney disease | 01 | 01 |
| Nephrotic syndrome | 01 | 01 |
| Bronchial asthma | 01 | 01 |
| Thalassemia | 00 | 00 |
| Immunocompromised | 00 | 00 |
| Nutritional status | | |
| Moderate acute malnutrition | 05 | 05 |
| Severe acute malnutrition | 04 | 04 |
| Normal | 90 | 90 |

*Modified Kuppuswamy classification

Male children outnumbered female children with a male: female ratio of 5:4. Mean age of presentation was 4.7± 3.7 years. Six among the 99 children had a pre-existing co-morbidity in the form of congenital heart disease (n=3), chronic kidney disease (n=1), Nephrotic syndrome (n=1) and bronchial asthma (n=1). Severe acute malnutrition was diagnosed in 4% (n=4). None of the studied children had any form of immunocompromised condition. Thirty seven percent (n=37) of the children were asymptomatic at admission and were diagnosed as COVID-19 positive as part of contact screening. Among the rest, fever was the most common symptom at presentation (n=44, 44%) followed by cough (n=27, 27%).

The hematological parameters are shown in Table 2.

**Table 2:** Laboratory results

| Parameter (unit) | Frequency | Percentage (%) | Mean | Median | SD |
|---|---|---|---|---|---|
| Hemoglobin (gm /dl) | | | | | |
| < 12 | 67 | 68 | 10.7 | 11.2 | 2.4 |
| >/- 12 | 32 | 32 | | | |
| MCV (fl) | | | | | |
| < 74 | 53 | 54 | 72.6 | 72.5 | 9.3 |
| >/= 74 | 37 | 37 | | | |
| Not available | 09 | 09 | | | |
| MCH (pg) | | | | | |
| < 25 | 53 | 54 | 23.9 | 23.65 | 3.85 |
| >/= 25 | 37 | 37 | | | |
| Not available | 09 | 09 | | | |
| MCHC (gm /dl) | | | | | |
| < 33 | 59 | 60 | 28.75 | 32.1 | 5.9 |

| | | | | | |
|---|---|---|---|---|---|
| >/= 33 | 31 | 31 | | | |
| Not available | 09 | 09 | | | |
| RBC (x 10 $^{12}$/L) | | | | | |
| < 4.5 | 37 | 37 | 4.42 | 4.6 | 0.9 |
| >/=4.5 | 53 | 54 | | | |
| Not available | 09 | 09 | | | |
| RDW (%) | | | | 13.9 | 5.5 |
| <18 | 77 | 78 | 15.7 | | |
| >/=18 | 13 | 13 | | | |
| Not available | 09 | 09 | | | |
| Total leucocyte count (x10$^9$ cells/L) | | | 9.3 | 7.7 | 4.8 |
| <4 | 03 | 03 | | | |
| 4 to 12 | 78 | 79 | | | |
| >12 | 18 | 18 | | | |
| Platelet count (lakhs/microL) | | | | | |
| <1.5 | | | 303.15 | 276 | 125.7 |
| 1.5 to 4.5 | 8 | 08 | | | |
| >4.5 | 77 | 78 | | | |
| | 14 | 14 | | | |
| D dimer (mg/L) | | 14 | | | |
| <0.5 | 32 | 32 | 3.08 | 0.59 | 5.09 |
| >0.5 | 37 | 37 | | | |
| Not available | 30 | 30 | | | |
| NLR | | | | | |
| <1 | 49 | 50 | 1.55 | 1.05 | 1.78 |
| 1-3 | 38 | 38 | | | |
| >3 | 12 | 12 | | | |
| CRP | | | | | |
| Positive | 04 | 04 | | | |
| Negative | 95 | 96 | | | |

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin concentration; RBC: Red Blood Cell count; RDW: Red cell Distribution Width; NLR: Neutrophil-lymphocyte Ratio; CRP: C- Reactive Protein

Anemia was the most common abnormality in the complete blood count reports (n=67, 68%). A relatable number of children had similar picture (lower than normal range) in their MCV and MCHC values (n=53/90, 53% and n=59/90, 59% respectively). A higher than normal RDW was seen only in 13 %. Total leucocyte count and platelet counts were normal in majority of the children (n=78, 79%, n=77, 78% respectively). D-dimer was raised above the normal level in 37% (n=37/69). Neutrophil-lymphocyte Ratio (NLR) was raised in 12% (n=12) of the children.

**Table 3:** Outcome of patients and the supportive care required

| Parameter | Frequency (n) | Percentage (%) |
|---|---|---|
| Outcome | | |
| Discharged | 97 | 98 |
| Death | 02 | 02 |
| Inotropes | 08 | 08 |
| Ventilator support | 03 | 03 |
| Other O2 support | 14 | 14 |

Among 99 children, 8% (n=8) required inotrope support and 3% (n=3) required intubation. Other methods of oxygen delivery devices including CPAP and nasal prongs were used for 14% (n=14) of the children. Out of 99 children, 2 children (2%) died. Rest of the children (n=97, 98%) were discharged.

**Table 4:** Association of positive CRP, NLR and prolonged inpatient stay or death with other parameters:

| PARAMETER | FREQUENCY (N=99) | P VALUE | ODDS RATIO(95% CI) |
|---|---|---|---|
| **ASSOCIATION WITH POSITIVE CRP** | | | |
| Anemia | 4 | 0.301 | 4.61(0.24-88.2) |
| Leukocytosis | 2 | 0.15 | 4.94(0.65-37.68) |
| Thrombocytopenia | 1 | 0.29 | 4.19(0.38-45.75) |
| High D- dimer | 3 | 0.18 | 4.32(0.43-43.34) |
| High NLR | ***2*** | ***0.07*** | ***8.5(1.08-67.13)*** |
| **ASSOCIATION WITH HIGH NLR >3** | | | |
| Anemia | 10 | 0.327 | 2.63(0.54-12.79) |
| Leukocytosis | ***6*** | ***0.007*** | ***9.29(1.73-22.6)*** |
| Thrombocytopenia | 2 | 0.250 | 1.36(0.48-15.32) |

| **ASSOCIATION WITH PROLONGED HOSPITAL STAY >10 DAYS** | | | |
|---|---|---|---|
| Anemia | 5 | 0.66 | 2.58(0.289-23.091) |
| Leukocytosis | 2 | 0.257 | 2.75(0.459-16.477) |
| Thrombocytopenia | 0 | 1.00 | 0.934(0.884-0.996) |
| High NLR | 2 | 0.137 | 4.556(0.730-28.444) |
| Positive CRP | 1 | 0.229 | 5.867(0.513-67.037) |
| High D-dimer | ***5*** | ***0.07*** | ***8.167(0.910-73.306)*** |
| **ASSOCIATION WITH DEATH** | | | |
| Anemia | 2 | 1.000 | 1.031(0.988-1.075) |
| Leukocytosis | ***2*** | ***0.032*** | ***1.125(0.955-1.325)*** |
| Thrombocytopenia | ***2*** | ***0.006*** | ***1.333(0.894-1.989)*** |
| High NLR | 1 | 0.229 | 7.818(0.456-134.078) |
| Positive CRP | 0 | 1.000 | 0.979(0.951-1.008) |
| High D-dimer | 2 | 0.178 | 1.057(0.979-1.142) |

Anemia: Haemoglobin <12gm/dl; Leukocytosis: Total leucocyte count >12x$10^9$ /L; Thrombocytopenia: platelet count >4.5 lakhs/microL; High D- dimer >0.5 mg/dl; High NLR >3; CRP: C reactive peptide

To study the significance of various hematological parameters in predicting the severity and prognosis of the infection, statistical analysis using Fisher exact test was carried out. Positive CRP was found to have statistically significant association with high NLR. Out of the 12 children with high NLR, 4 had positive CRP. Other indices like leukocytosis, thrombocytopenia which are considered as markers of infection did not show statistical association with positive CRP. High NLR also showed a positive association with leukocytosis. (Table 4)

Analysis was carried out to study the role of hematological parameters in predicting the outcome of COVID-19 infected children. Outcomes considered were in patient stay for more than 10 days and death of the patient. This is because the median duration of stay for COVID 19 infected children in the study population was 10 days. None of the variables showed significant association with prolongation of inpatient care for more than 10 days. However leukocytosis and thrombocytopenia were found to have statistically significant association with death ( p value 0.032, 0.006 respectively)

Binary logistic regression using backward elimination method was done to know the role of hematological variables in predicting poor outcome defined as hospital stay more than 10 days. A model was created with anemia, leukocytosis, thrombocytopenia, positive CRP, high NLR and high d-dimer as independent variables and hospital stay more than 10 days as dependent variable. High d-dimer was the only surviving variable in the final reduced model.

Table 5: Binary Logistic Regression for prolonged stay >10 days

| **VARIABLE** | **ADJUSTED ODDS RATIO** | **P value** |
|---|---|---|
| High d-dimer | 8.17 | 0.910 |
| High NLR | 5.636 | 0.756 |
| Thrombocytopenia | 0.000 | 0.999 |

**Hosmer and Lemeshow Test: Chi- square 1.795, p- value 0.180**

## IV. Discussion

In this single centre cross sectional observational study, total of 99 children of the age group 1 month to 12 years who were diagnosed to have COVID-19 infection were included. The mean age of presentation was 4.7+/-3.7 years. The mean age of presentation was 8 years in the study conducted in May 2020 by Tiwari et al(6) and 4 years in a study conducted in Paris, France. (7). Male children outnumbered female children in our study with a ratio of 5:4. This possibly reflects the preference of male children and their health by the families which is still prevalent in some parts of India due to educational backwardness of the society This is further substantiated by the fact that majority of the study population belonged to upper lower socioeconomic classification. This is in contrast to the study by Oualha et al in which female children outnumbered male children.(7) However in studies by Tiwari et al and Han et al male children were more compared to female children (6) (8). Only 10% (n=10) had underlying medical conditions, most common being malnutrition (n=4,

4%) followed by congenital heart disease (n=3, 3%) followed by equal cases of chronic kidney disease, Nephrotic syndrome and Bronchial asthma (n=1, 1% each). Oualha et al had neurological conditions as the most common co-morbidity (n=7) followed by respiratory (n=4) and sickle cell disease (n=4) (7). Most of the children belonged to the upper lower socioeconomic classification (n=48, 48 %) as per the modified Kuppuswamy classification. No children from the lower socioeconomic class was admitted to our hospital during the study period. This could be the due to ignorance of the lower class towards the disease or the apprehension regarding the disease, testing methods or financial issues on admission. This emphasizes the need for improved awareness among the general population about the COVID-19 disease. Also only three children who were admitted during the study period belonged to the upper class. This could be because of the preference of private hospitals over the government hospital for admission or because of the home isolation facilities available among this class.

Hematological reports showed anemia with Hb <12 gm/dl as the most common finding (n=67, 68%). Similar results were shown by Tiwari et al with 54.5% (n=6/11) among which 45.5 % (n= 5/11) had Hb <10 gm/dl. The mean WBC count was 11.5 in the study. Neutrophilia was seen in 36.3% (n=4/11) in study conducted in Noida(6). In a study from China, out of 244 confirmed cases of COVID-19, 193 symptomatic patients had anemia. (13). Mean Hb among the study population was 10.7+/-2.4 gm/dl. Since there was no statistical significance between anemia and either of in hospital stay more than 10 days or death, the anemia could be a result of poor nutrition. Most of the children had normal WBC count (n=78, 79%) and platelet count (n=77, 78%). Leukocytosis (n=18, 18%) was more common than leucopenia (n=3, 3%). Similarly thrombocytosis (n=14, 14%) was more common than thrombocytopenia (n=8, 8%). NLR was high in 11% (n=12) of the study population. Though NLR is considered as a prognostic marker for COVID-19, in our study 92% (n=11/12) of the children with high NLR were discharged whereas one child had expired. Qin et al, in a study in adult population from China showed higher NLR in those with severe infection than in those without. (11). Our study similarly showed statistically significant association between high NLR and leukocytosis (p value 0.007). High NLR however didn't show significant association with prolonged hospital stay or death. Tiwari et al shows 27.3% (n=3/11) cases with high NLR above the cut off values. However the association between high NLR and clinical outcome of the children were not taken into consideration in this study(6). A study from adult population conducted in China showed significantly elevated NLR among non-survivors compared to survivors of the disease (p<0.001) (9).

In a study conducted in adult population in Italy in 2020, it was found that CRP was raised in the initial phases of severe COVID infection. The same study states that CRP values were positively associated with the CT severity score. (10). In our study, CRP showed no significant association with hematological parameters as well as other parameters like high d-dimer and high NLR. Although both high NLR and CRP are considered markers of infection, this could be due to the lesser number of study population. A study conducted in COVID 19 infected children which was published in April 2021 shows an association between positive CRP and the need for critical care. However this study has considered a wider age group of population upto 21 years.(11) D-dimer was another parameter considered in our study. Being a predictor of thromboembolism, d-dimer was elevated in 37% (n=37/69). Mean d-dimer level was 3.08+/- 5.09 mg/dl. Even though statistically non-significant, high d-dimer showed closest association with prolonged hospital stay in binary logistic regression study with p value of 0.061. Administration of LMW heparin in those children with high d-dimer until it becomes normal could have resulted in this.

A model was created with anemia, leukocytosis, thrombocytopenia, high d-dimer, high NLR, positive CRP. Although none of the parameters were statistically significant, high d-dimer had the closest association among the parameters. Hosmer- Lemeshow test showed a p value of 0.180. Statistically significant association was found between thrombocytopenia and leukocytosis with death. A multicenter study conducted in Kuwait, Saudi Arabia and Egypt shows a significant association of thrombocytopenia (p 0.001) with death in children admitted to ICU. (12)

To the best of our knowledge, there are very few studies conducted in India about the hematological parameters in COVID-19 infected children as well as their role in predicting the final outcome. Studies conducted in pediatric population as well as in adult population with COVID-19 infection are mostly centered on the respiratory infection. However most of the COVID-19 infected children are asymptomatic and parental ignorance regarding the disease especially during the initial months of the epidemic has necessitated a study on the baseline, less expensive laboratory tests in predicting the prognosis of the disease. This study not only explored the profile of hematological parameters in children affected with COVID-19 infection but also tried to arrive at an association of these parameters with poor outcome defined as either prolonged in hospital stay for more than ten days or death. However the major limitation of the study lies with the less number of study population and hence the chances of erroneous analysis. Number of deaths in the study population were two and this has affected the logistic regression analysis results and hence these results on death cannot be extrapolated to a larger population.

## V. Conclusion

Our study showed that anemia is the most common hematological finding in COVID-19 infected children. Significant association was found between high NLR and positive CRP as well as high NLR and leukocytosis. None of the studied hematological parameters were associated with in hospital stay for more than ten days. However thrombocytopenia and leukocytosis could be used to predict death in COVID-19 infected children. Due to the small population in this study, larger studies are required to confirm our findings and to understand the profile of hematological parameters and their role in predicting the outcome in COVID-19 infected children.

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