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# Haematological Parameters in Covid-19 Patients-An East Nimar Hospital Based Study

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## ABSTRACT

*Background: The Coronavirus disease 2019 (Covid-19) is caused by the virus SARS COV-2 and is declared as a global pandemic by WHO. It is known that in SARS COV-2 virus infection causes haematological changes and often present the potential to optimize the monitoring of infectious process or to indicate the suspicion of their severity. The present study was conducted to study the routine haematological parameters including NLR (Neutrophil lymphocyte Ratio) in Covid-19 patients and to assess their utility in identifying severity of the disease.*

*Methods: This retrospective study was conducted on 257 Covid-19 RT PCR positive cases. The cases were divided into mild, moderate, and severe category as per MoHFW. Haematological parameters were measured by Fully Automated Hematology cell counter using blood sample collected in EDTA vacutainers. MS Excel was used for data analysis and ANOVA tests were applied to test statistical significance. P <0.05 was considered statistically significant.*

*Results: Degree of severity of Covid-19 cases could be correlated with older age group. Most important haematological parameters noted in adults are Eosinopenia in 84%, Monocytopenia in 64%, NLR >3 in 18.32 %, and Leucopoenia (17.5%). Severe category showed higher proportion of NLR >3 in 40.6%, Neutrophilia in 31.2%, Leucocytosis in 28.2% and lymphocytopenia in 12.5%.*

*Conclusions: Haematological parameters in Covid-19 positive cases could help to predict a patient risk and outcome in the Indian scenario that will provide guidance to subsequent clinical practice.*

**Key words:** Category, Neutrophil lymphocyte ratio (NLR), SARS COV-2

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## INTRODUCTION

The Coronavirus disease 2019 (COVID-19) is caused by the virus SARS COV-2 and is declared as a global pandemic by WHO. It is known that in SARS COV-2 virus infection causes hematological changes and often present the potential to optimize the monitoring of infectious process or to indicate the suspicion of their severity [1]. Laboratory abnormalities particularly hematological changes allow checking the status of SARS COV-2 infection, since the hematopoietic system and haemostasis suffer significant impacts during the evolution of Covid-19 [2].

Patients suffering from Covid-19 that is caused by the SARS CoV-2 can present with severe lung damage, ARDS and have a significant mortality risk [3]. Coronavirus infection primarily manifested as a respiratory tract infection and data emerging from studies indicate that it should be regarded as a multisystem including cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic and immune system. Some patients only show a mild course of a disease with good prognosis and other suffers greatly with difficult treatment and high mortality. The cytokines storm is characterized by markedly increased levels of Interleukins (Mostly IL-6, 2, 7), G-CSF, Interferon-γ inducible protein-10, MCP-1, MIP-1 and TNF-α which may promote lymphocytes apoptosis [4-6]. Hence, classification of disease severity is very important to guide the right treatment and care.

Elevated WBC Count, Absolute Neutrophil Count (ANC) and depleted Absolute Lymphocyte Count (ALC) have been repeatedly observed in Covid-19 patients with severe outcome and Neutrophil-Lymphocyte Ratio (NLR) have been proposed as a prognostic biomarker [7-9].

Thus, identification of routine haematological parameters that can guide classification between mild, moderate and

severe Covid-19 cases could help to predict a patient risk and outcome in the Indian scenario that will provide guidance to subsequent clinical practice. Therefore, present study was conducted to study the routine haematological parameters including NLR in Covid-19 patients and to assess their utility in identifying severity of the disease.

## MATERIALS AND METHODS

This Retrospective study was conducted on patients who were admitted to Covid Care Hospital associated with Government Medical College, Khandwa (East Nimar), Madhya Pradesh, India. The duration of study was from July 2020 to August 2020.

The study included 257 cases of Covid-19 positive cases admitted in CCC, DCHC, DCH/ICU ward of Covid Care Hospital. Demographic details of the patients noted from pathology records. Pediatric (n=6) and adult (n= 251) cases of both genders were included in the study. The cases were divided into three groups-mild, moderate, and severe cases based on the MoHFW, Government of India, guidelines on clinical management of Covid-19.

The laboratory diagnosis of Covid-19 was according to the guidelines by MoHFW and confirmed by RT-PCR performed on respiratory samples of the patients such as Nasopharyngeal and Oropharyngeal swab. RT-PCR is gold standard for SARS-COV-2 detection, and it is the laboratory test of choice for the diagnosis of symptomatic patients in acute phase (9).

In our study haematological analysis was performed on Fully Automated Hematology cell counter using blood sample collected in EDTA vacutainers.

All the data were entered on excel sheet. Qualitative data was summarized as number and percentage and Quantitative data was summarized in the form of mean± SD. To check the statistical significance of the data, statistical test like chi square, ANOVA test were used, P-value <5% was considered as statistically significant and p-value >5% considered as statistically insignificant. ANOVA test was applied to test the significance of difference in mean value of various haematological parameters in Covid-19 positive cases.

The reference range for the Hemoglobin concentration in Adults and Pediatric age group taken from Shirish et al. Essential of Clinical Pathology 2nd Edition [10] [Hb in Adult males: 13-17 gm/dl, in adult females: 12-15 gm/dl, at birth: 13.6-19.6 gm/dl, at 2 to 6 months: 9.5-14.0 gm/dl, at 6 months to 6 years: 11.0- 14.0 gm/dl, at 6 to 12 years: 11.5-15.5 gm/dl]. Following values were taken as normal for haematological parameters (Table 1):

**Table 1: Haematological parameters.**

| Haematological parameters* | 1-2 Years | 2-6 Years | 6-12 Years | Adults |
|---|---|---|---|---|
| Total Leukocyte Count (TLC) / cumm | 6000-16000 | 5000-15000 | 5000-13000 | 4000-10000 |
| Absolute Neutrophil Count (ANC) /cumm | 1000-7000 | 1500-8000 | 2000-8000 | 2000-7000 |
| Absolute Lymphocyte Count (ALC) /cumm | 3500-11000 | 6000-9000 | 1000-5000 | 1000-3000 |
| Absolute Eosinophil Count (AEC) /cumm | 100-1000 | 100-1000 | 100-1000 | 20-500 |
| Absolute Monocyte Count (AMC) /cumm | 200-1000 | 200-1000 | 200-1000 | 200-1000 |
| Platelet count lakh/cumm | 2.0 - 5.5 | 2.0 - 4.9 | 1.7 - 4.5 | 1.5 – 4 |

*Dacie and Lewis Practical Haematology 12thEdition [11]

Low count=below lower limit of normal range, High count=above higher limit of normal range.

Normal NLR in adult, non-geriatric population in good health ranges between 0.78 and 3.53 (12). Patient with age >50 years having NLR >3.13 are at risk of severe illness [5].

All the procedures adhered to the ethical guidelines given by the Declaration of Helsinki (2013 revision).

## RESULTS

Total Adult patients showing anemia were 61 out of 251 (24.3%), in which adult males were 31 out of 166 (18.66%), and adult females were 30 out of 85 (35.2%).

Among all three groups we found eosinopenia in 216 out of 251 cases (84%), Monocytopenia seen in 64.5%, Leukopenia in 17.52%, Lymphocytosis in 12.7%, Lymphopenia in 7.5%, Leucocytosis in 6.37%, Neutropenia in 8.7 %, Neutrophilia in 7.1 %, Thrombocytopenia in 13.54 %, Thrombocytosis in 6.37% cases.

Among severe category showed maximum proportion of lymphocytosis cases, i.e. 6/32 cases (18.7%), Leucocytosis 28.2%, Neutrophilia in 13.2% and Thrombocytosis in 15.6%.

In moderate category maximum proportion of monocytopenia 25/30 (83.3%), thrombocytopenia 7/30 (23.3%).

In adults 46 out of 251 cases (18.32%) had NLR>3, in children age group between 0-12 years none of the cases had NLR ratio >3. Among all three categories NLR ratio >

3 maximum Proportion of cases seen in severe category 13 out of 32 cases (40.6%). Maximum number cases with NLR ratio >3 was seen in the age group of 51-60 years that is 22.7%.

2 out of 6 cases (33.3%) showed monocytopenia, 1 out of 6 cases (16.6%) showed lymphocytopenia and 2 out of 6 cases (33.3%) showed eosinopenia. None of case had NLR >3. 3 out of 6 cases (50%) showed anemia of mild degree (Tables 2-7).

**Table 2: Comparison of baseline demographic characteristics of covid-19 patients among three study groups.**

| Total no. of Covid -19 cases= 257 | | Mild category n=195 (75.8%) | | Moderate category n=30 (11.6%) | | Severe category n=32 (12.4%) | |
|---|---|---|---|---|---|---|---|
| Adult=251 | Paediatric= 6 | Adult =189 | Paediatric=6 | Adult=30 | Paediatric=0 | Adult=32 | Paediatric=0 |
| Mean Age (52.09 Years) | | 33.86 Years | | 56.83 Years | | 65.6 Years | |
| Male (n=168) to Female (n=89) ratio-1.88:1) | | Male (n=128) to Female (n=67) ratio-1.91:1 | | Male (n=16) to Female (n=14) ratio-1.14:1 | | Male (n=24) to Female (n=8) ratio-3:1 | |

**Table 3: Comparison of grading of Anemia in adult patients among three groups.**

| Grading of anemia | Mild Category n=40 | | Moderate Category n=15 | | Severe Category n=6 | | Total cases N=61/251 (24.03%) |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | |
| Severe (Hb<7gm%) | 0 | 1 | 0 | 1 | 1 | 0 | 3 |
| Moderate (Hb 7-10 gm%) | 3 | 10 | 0 | 2 | 0 | 0 | 15 |
| Mild -Hb 10-12.9 gm% in males, 10-11.9 gm% in females | 15 | 11 | 8 | 4 | 4 | 1 | 43 |

**Table 4: Comparison of haematological parameters in adult Covid-19 patients among three groups (Showing number of cases in each group).**

| | Mild category | | Moderate category | | Severe category | | Total patients | |
|---|---|---|---|---|---|---|---|---|
| No. of cases | n=189 | | n=30 | | n=32 | | n=251 | |
| Hematological parameters* | Low count | High count | Low count | High count | Low count | High count | Low count | High count |
| TLC | 29 | 5 | 8 | 2 | 7 | 9 | 44 (17.52%) | 16 (6.37%) |
| ANC | 18 | 7 | 1 | 1 | 3 | 10 | 22 (8.76%) | 18 (7.17%) |
| ALC | 9 | 22 | 6 | 4 | 4 | 6 | 19 (7.56%) | 32 (12.74%) |
| AMC | 123 | 0 | 25 | | 14 | 0 | 162 (64.54%) | 0 |
| AEC | 164 | | 29 | | 23 | 0 | 216 (84%) | 0 |
| Platelet Count | 23 | 9 | 7 | 2 | 4 | 5 | 34 (13.54%) | 16 (6.37%) |

*Dacie and Lewis Practical Haematology 12th Edition (11)

**Table 5: NLR comparison according to age group among three groups of Covid-19 cases.**

| Age Range | Mild Category (n=195) | | Moderate Category (n=30) | | Severe Category (n=32) | |
|---|---|---|---|---|---|---|
| | NLR <3 | NLR >3 | NLR <3 | NLR >3 | NLR <3 | NLR >3 |
| 0-12 years | 6 | 0 | 0 | 0 | 0 | 0 |
| 12-20 years | 23 | 2 | 0 | 0 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21-30 years | 62 | 6 | 0 | 1 | 0 | 1 |
| 31-40 years | 38 | 2 | 2 | 0 | 1 | 0 |
| 41-50 years | 24 | 4 | 6 | 2 | 0 | 1 |
| 51-60 years | 22 | 5 | 7 | 1 | 5 | 4 |
| 61-70 years | 0 | 0 | 7 | 1 | 5 | 3 |
| 71-80 years | 0 | 0 | 1 | 1 | 7 | 3 |
| 81-90 years | 0 | 0 | 1 | 0 | 0 | 1 |
| 91-100 years | 0 | 0 | 0 | 0 | 1 | 0 |
| Total | 166 (85.2%) | 29 (14.8%) | 24 (80%) | 6 (20%) | 19 (59.1%) | 13 (40.6%) |

**Table 6: Age wise haematological parameter in Pediatric age group Covid-19 positive cases (only in mild category).**

| **Haematological parameter** | **Anemia*** | | | **TLC** | | **ANC** | | **AMC** | | **ALC** | | **AEC** | | **NLR ratio** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age group | Mild Degree | Moderate Degree | Severe Degree | Low | High | Low | High | Low | High | Low | High | Low | High | <3 | >3 |
| <1 year | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-2 year | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 2-6 year | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 6-12 Year | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 3 | 0 |

*Shirish M Kawthalkar Essential of Clinical Pathology 2nd Edition (10)

**Table 7: Comparison of Haematological parameters of Covid-19 positive patients among three groups.**

| **Haematological Parameters** | **Total Covid -19 positive cases n= 257** | | | | | | **P-value (ANOVA)** |
|---|---|---|---|---|---|---|---|
| | **Mild (n=195)** | | **Moderate (n=30)** | | **Severe (n=32)** | | |
| | **Mean** | **± SD** | **mean** | **± SD** | **mean** | **± SD** | |
| Hb gm% | 13.2 | 2 | 12.3 | 2.36 | 13.1 | 2.1 | 0.085 |
| TLC/cumm | 6016 | 2114 | 5796 | 2308 | 8868 | 5275 | 0 |
| ANC/cumm | 3649 | 1620 | 3725 | 1686 | 6332 | 4332 | 0 |
| ALC/cumm | 2055 | 934.5 | 1802 | 876 | 2139 | 1321 | 0.34 |
| AEC/cumm | 130 | 69.1 | 111.1 | 49.4 | 160 | 93.67 | 0.022 |
| AMC/cumm | 190 | 100 | 156 | 64.8 | 242 | 158 | 0.005 |
| NLR | 2.03 | 1.15 | 2.27 | 0.75 | 3.33 | 2.14 | 0 |
| Total Platelet count/cumm | 2.36 | 1.59 | 2.57 | 0.64 | 2.64 | 1.19 | 0.507 |

## DISCUSSION

In our study total 257 cases studied, admitted in Covid care Hospital –mildly symptomatic in CCC ward, moderate cases in DCHC ward and severe cases in DCH/ICU ward. Both males and females' patients were included in the study.

We studied haematological parameters separately in adults (n=251) & pediatric patients (n=6). Demographic data from our study shows that most of the patients are mildly symptomatic (n=195 i.e., 75.8%), this correlates with the Indian study and other studies in the China and the western population [12,13].

In our study Pediatric patients presented only in mild category. Margekar et al [14] also found same in an Indian study.

In our study higher mean age correlated with increasing severity of cases. Patients in the moderate groups were older than the cases in the mild category noted by Surveillances et al. [15]. No significant difference in mean age and sex between the three groups noted by Pomilla Singh et al [16].

In our study males are affected more than females, ratio of male: female is 1.88:1. This is in accordance to other studies conducted by Guan et al [17] and Zhang et al [18]. Male to female ratio of 3:1 according to Pomilla Singh et al [16]. The reduced susceptibility of females to viral infections could be attributed to the protection from X chromosome, which play important role in innate and adaptive immunity [19].

In our study mean age of moderate cases was 56.83 Years and Mean age of severe cases was 65.6 Years. Our results were correlated with study by Pomilla Singh et al [16].

In our study most important haematological parameters observed in Covid-19 adult cases are Eosinopenia (84%), Monocytopenia(64%).Other haematological changes are NLR >3 (17.89%), Leucopenia (17.5%) with Lymphocytosis (12.7%) followed by Lymphopenia (7.5%) and Neutrophilia (7%) and Thrombocytopenia(13.2%) noted. Most important haematological parameter abnormalities observed in Covid-19 patients which may predict the progression toward severe or critical forms of Covid-19 were Leukocytosis, Neutrophilia and Lymphopenia according to Neema Tiwari et al [13].

It was identified that normal NLR value in an Adult, Non-geriatric population in good health are between 0.78 and 3.53 according to Forget et al [12]. Patient with age >50 years having NLR >3.13 are at risk of severe illness noted by Liu et al [8]. In our study in adults 46 out of 251 cases (18.32%) had NLR >3.Maximum number cases with NLR ratio>3 was seen in the age group of 51-60 years that is 22.7%. We found maximum number of cases with NLR ratio >3 was seen in severe category that is 13 out of 32 cases (40.6%). Liv et al [8] found patients with age >50yrs having NLR >3.13 are at risk of severe illness. High NLR may be indicative of the patient's response to inflammatory insult, with neutrophils rising in response to stress which when overwhelming induces lymphocytes apoptosis [20-23]. NLR was found to have the most efficient screening parameter by Pomilla Singh et al [16].

In our study maximum proportion of cases showing leucocytosis were seen in severe category i.e., 9 out of 32 cases (28.2%). Increased WBCs count observed in the severe Covid-19 patients when compared to healthy control or mild Covid-19 patients [7,24].

According to Surveillance et al [15] 12.9% cases showed Neutrophilia. In our study 7% adult cases, out of 251 showed Neutrophilia and maximum proportion 10 out of 32 cases (31.2%) were seen in severely ill patients.

According to Surveillance et al [15] showed Lymphopenia in 9.6% cases. In our study 7.5% of cases in adults showing Lymphopenia. The cytokine storm is characterized by markedly increased levels of interleukins which may promote lymphocyte apoptosis [4,6].

In our study 4 out of 32 cases (12.5%) of lymphopenia seen in severe category. Disparity in the percentage of cases showing lymphopenia may impart be reflective of the epidemiological variation of the Indian population [13].

In our study we found Monocytopenia in 162 cases (64.5%). Maximum percentage of cases showing monocytopenia were seen in moderate category (83.3%) followed by mild category (65.1%) and then severe category (43.7%). Bloome et al noted Monocyte counts lower in the deceased group compared to the recovered group but remained between normal reference ranges (2–10%) making this finding not usable in the assessment of Covid-19 patients at risk. [25].

In our study 84% Covid 19 cases showed Eosinopenia. No association of eosinopenia could be noted with the severity of disease. Bloome et al also noted Eosinophil counts significantly lower in deceased patients compared to recovered ICU patients [25]. Eosinopenia is a potentially more reliable laboratory predictor for SARS-COV2 infection than recommended leukocyte count and lymphopenia [26]. Sun et al, Li Q et al, Yun et al noted low eosinophil count in a major proportion of COVID-19 patients [27-29].

According to S. Bloome et al Eosinopenia is insufficiently to predict outcome of Covid-19 disease as eosinophils count are difficult to monitor due to altered low normal reference value less than 6% [25].

Thrombocytopenia is a common complication in severe ill patients with the accompanying risk of developing intravascular coagulopathies resulting in multiple organ damage [30-32]. Bloome et al [25] could not see an association between thrombocytopenia and severity/ mortality in patients with Covid-19. In our study we also found only13.2% cases of thrombocytopenia and association between thrombocytopenia and severity of disease noted. In our study we also found thrombocytosis in 6.2% cases of adult, maximum proportion of cases seen in severe category.

In our study in 35.2% females and in18.6% male cases shows anemia. No statistically significant difference in Hb % among three groups of Covid-19 patients. Increase in inflammatory factor reduce erythropoiesis and increase damage of RBCs [27].

Paediatric Covid-19 cases showed monocytopenia in 33.3%, lymphocytopenia in 16.6% and eosinopenia in 33.3%. None of case had NLR>3. Mergekar et al [14] an Indian study concluded that leukocyte indices do not appear to be reliable marker for assessing the severity and diagnosis of disease as they appear to be variance in children as compared to adults' patients. Milder form of disease is seen in children; the possible explanation

includes less frequent exposure to the main source of transmission (closed school). Chrysoula et al [33] concluded that leukocyte changes and especially lymphopenia were less commonly documented in children with Covid-19 possible because of their immature immune system and ACE2 expression. Anemia and thrombocytopenia were rarely found in paediatric patients.

Difference in mean value of various haematological parameters i.e., total leucocyte count, absolute neutrophil count, absolute eosinophil count, absolute monocyte count, NLR were statistically significant among the three groups as P-value <5 %, while no statistically significant difference noted in absolute lymphocyte count and total platelet count among three groups of covid positive patients as their P- value > 5%.

Bloome et al [25] suggest ICU patients has significantly lower Hemoglobin, ALC while TLC, ANC, NLR and PLR were significantly higher (p value <0.05). Platelet counts showed no difference among these groups. According to Pomilla Singh et al [16] ICU patients has significantly lower Hemoglobin, Absolute Lymphocytes Count while TLC, ANC, NLR and PLR were significantly higher (p value <0.05). Platelet count showed no difference among these groups.

## CONCLUSION

Baseline haematological findings in Covid -19 cases in our study was concluded in following points:

- Degree of severity of Covid-19 could be correlated with older age group.
- Most important haematological parameters noted in adults are Eosinopenia in 84%, Monocytopenia in 64%, NLR>3 in18.32 % and Leucopenia 17.5%.
- Severe category showed higher proportion of cases of NLR>3 in 40.6%, with Neutrophilia in 31.2%, Leucocytosis in 28.2% and lymphocytopenia in 12.5%.
- Difference of mean value of TLC, ANC, AEC, AMC, NLR are statistically significant among the three groups of Covid-19 cases.
- In children, more studies needed to find association of severity of Covid-19 with haematological parameters as only fewer studies documented on the role of leukocyte and other hematologic markers.

Thus, Haematological parameters in Covid- 19 positive cases could help to predict a patient risk and outcome in the Indian scenario that will provide guidance to subsequent clinical practice.

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## CONFLICT OF INTEREST

Authors have no known conflicts of interest to declare.

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