ABSTRACT

OBJECTIVES
To compare the values of the hematological and inflammatory markers in 1st and 4th waves to predict the outcome of COVID-19 in a hospital-based study.

METHODOLOGY
This comparative study was conducted in the Department of Hematology, Hayatabad Medical Complex Peshawar, from April 2020 to 20 August 2021. Tests of significance (Independent t-test/Mann Whitney U test) and Chi-square test were used. Relevant information was recorded on a pre-designed proforma prepared following the study's objectives.

RESULTS
A total of 178 patients, 71 from (the 1st wave) and 107 from (the 4th wave) with known outcomes, were studied. A statistically significant difference exists between the groups (1st vs 4th wave) regarding hematological markers; neutrophil to lymphocyte ratio (NLR) (p=0.02), Absolute Neutrophilic count (ANC) (p=0.01) and platelet count (p=0.001). Similarly, significantly higher inflammatory markers values were recorded in the 1st wave compared with the 4th wave regarding inflammatory markers; CRP (p=0.002) and D-dimer (p=0.001). During the 1st wave, Total Leukocyte Count (TLC), ANC and d-dimer were the leading prognostic indicators to predict mortality/worst outcome in COVID-19 with an Area Under Curve (AUC) of 0.74, 0.70 and 0.7 on receiver operating characteristics (ROC) respectively. In 4th, the Area under the curve (AUC) of d-dimer was 0.84 to predict mortality.

CONCLUSION
TLC, ANC, NLR, and low platelet count were the worst hematological markers in COVID-19 in the first wave, while d-dimer and CRP were the primary prognostic inflammatory markers. Unlikely in the 4th wave, the prognostic values of hematological markers were merely significant. The d-dimer values in both the waves proved to be reliable for predicting the severity and mortality of COVID-19.

KEYWORDS: COVID-19 Hematological Markers, Inflammatory Mediators, Mortality

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INTRODUCTION

Covid-19 is a respiratory disease reported to the World Health Organization in Dec 2019 from Wuhan, China. The World Health Organization (WHO) declared a global emergency due to the rapid rise in cases of COVID-19 in China and nearby countries by the mid of February 2020.1 According to the World Health Organization, globally, confirmed covid cases have reached 426,624,859 with 5,899,578 deaths. By 20 February 2022, 10,407,359,583 vaccine doses have been administered worldwide.2 By 24 February 2022, the number of laboratory-confirmed cases reached 1505,000 in Pakistan, with 30114 deaths and 62000 active cases. Khyber Pakhtunkhwa province is reported with a total number of COVID-19 cases of 215588 and 6228 deaths.3 In the first wave, laboratory investigations like hematological indices are used mainly to assess the impact of the COVID-19 on hematopoietic system and homeostasis, which significantly suffer in this deadly disease. As per our first wave experience, we observed that Neutrophilic to lymphocyte ratio (NLR) was the main prognostic factor in COVID-19 to predict mortality/worst outcome in COVID-19, with an Area Under curve on ROC of 0.68, followed by absolute neutrophilic count (ANC) with an AUC value of 0.6.4 Not a significant difference was noted for other hematological indices like TLC, Hemoglobin and platelet levels. Similarly, inflammatory markers like Ferritin levels, d-dimers, and CRP have been reported to predict mortality and severity in covid-19. A study from the metropolitan city of Wuhan, China, reported hyperferritinemia as a predictor of the disease's mortality and severity of the disease.5,6 A literature review of the first wave confirms AUC for plasma CRP levels (0.896) on the ROC curve, which was significantly higher than hematological markers like ANC (0.820) and platelet count (0.677) in outcome prediction with (Sensitivity of 90%, specificity 77%).7 Some of the authors have reportedly compared the difference in the severity of the different waves. In India, a study showed comparing the first wave with 2nd wave that they received admission to ICU during the two waves were of the similar age, but there was a significant rise in deaths in the females, and more patients had co-morbidities during the second wave. They reported a higher mortality rate in ICU patients in the second wave.8 However, the United States, with its previous experience from the first wave, with the use of steroids, remdesivir and convalescent plasma in the second wave, reported a lower mortality in the second wave compared to the first wave.9 After the emergence of the covid-19, By 24 September 2020, several vaccines (more than 200 types) were started in preclinical development. Of those, only 43 entered clinical trials. Vaccines have been widely considered the only modality to eradicate the Pandemic and reduce mortality and morbidity, thus helping resume routine working, schooling, and social activities.10 In Pakistan, we experienced the 4th wave in the third quarter of 2021. The infection spread faster than in the first and second waves, but fewer causalities were reported due to herd immunity. Since very scanty information is available to compare the severity of the 4th wave with the first wave, therefore present study was conducted to compare the values of the hematological and inflammatory markers in the 1st and 4th wave to predict the outcome of COVID-19 in a hospital-based study, to help the clinicians to identify the more clinically significant markers to predict mortality.

METHODOLOGY

This comparative study was conducted in the department of hematology, Hayatabad Medical Complex Peshawar, from April 2020 to 20 August 2021. 71 cases of wave 1st with known outcomes were compared with 107 cases in the 4th wave. This study was conducted in line with the research guidelines, followed sound medical practice, re-detected human rights, and within the principles of the declaration of Helsinki of the World Medical Association. Ethical approval was granted from the institutional ethical review board of Post-Graduate Medical Institute, Hayatabad Medical Complex under notification No (316/HEC/B & PSC/2020, Dated 15 May 2020) and notification No (346/HEC/B & PSC/2020, Dated 10 December 2020). Patients hospitalized with COVID-19 in HMC were included. Patients where all
hematological and inflammatory mediators were advised, like CBC, d-dimers, ferritin, and CRP by the consultants, were enrolled, irrespective of age and gender. Similarly those patients were further followed for disease outcomes in terms of satisfactory discharge or death due to COVID-19. COVID-19 patients where deficient hematological and inflammatory mediator readings were available on the chart of patients or those who expired before being thoroughly investigated were excluded from the study. Similarly, all patients, irrespective of symptoms/signs, that came to the emergency or outdoor patients department were also excluded. For COVID confirmation, PCR results of the nasopharyngeal swab duly reported in the Public health research laboratory of the Hayatabad Medical Complex or Khyber medical University only were considered. Data was entered in SPSS, version 25 and descriptive and correlation statistics were applied. Numerical variables like age of patients, Hb%, TLC, ANC, Platelets, serum ferritin, d-dimer, CRP, age and hospital stay were presented with Mean and SD in case of normal distribution and with median and range where the data was not normally distributed. The normality of data was assessed using Shapiro-Wilk Test. Independent t-test/ Mann Whitney U test was used to determine the difference of the numerical variables (Hb%/g/dl), TLC (x10.e3/ul), ANC (x10.e3/ul), ALC (x10.e3/ul), NLR and Platelet count, Ferritin, d-dimer and CRP in groups (discharged satisfactory vs expired). The receiver operating characteristics (ROC) curve was used to determine the relationship between clinical sensitivity and specificity of different hematological, demographic and inflammatory markers to predict the worst outcome in COVID-19. Chi-square test was used to determine the association of the age and gender and peripheral hematological & inflammatory markers with disease outcomes in COVID-19 in the 1st and 4th wave.

**RESULTS**

A total of 178 patients, 71 from (the 1st wave) and 107 from (the 4th wave) with known outcomes, were studied. The patient's mean age in the 1st wave was not significantly different from the patient admitted in the 4th wave of covid-19 ($p=0.571$). The mean age of patients with SD in the first wave was 54 +12 years, while in the 4th wave, 56+16years for hospitalized patients. A lower Median Platelet readings were recorded in the 4th wave compared to the first wave. A higher median TLC>16000/cmm3 was recorded in both waves. A statistically significant difference exists between the groups (1st vs 4th wave) regarding hematological markers; neutrophil to lymphocyte ratio (NLR) ($p=0.02$), Absolute Neutrophilic count (ANC) ($p=0.01$) and platelet count ($p=0.001$). Similarly, a significant difference exists between the groups (1st vs 4th wave) regarding inflammatory markers; CRP ($p=0.002$) and D-dimer ($p=0.001$). (Table :1) During the 1st wave, TLC and ANC were the leading prognostic hematological indicators to predict mortality/worst outcome in COVID-19, with an Area Under Curve (AUC) of 0.74 and 0.70 on ROC, respectively. Likewise, d-dimer was a matchless prognostic inflammatory indicator to predict mortality with an AUC of 0.73 in 1st wave. (Table 2), Fig 1 baseline (0.5 to 0.54) while the AUC of d-dimer was 0.84 to predict mortality. (Table 3), Fig 2 We could not find a statistically significant association with the mortality due to covid-19 in both the waves with an insignificant $p>0.05$. (Table 4).

| Parameters               | Phase         | Number of Patients | Mean/Median | Std. Deviation | Sig.     | Test Performed          |
|-------------------------|---------------|--------------------|-------------|----------------|----------|------------------------|
| Age                     | First wave    | 71                 | 54.73       | 12.32          | 0.571    | Independent T-test     |
|                         | 4th Wave      | 107                | 55.94       | 14.91          |          |                        |
| Hb%                     | First wave    | 71                 | 13.00       | 2.24           | 0.019    | Independent T-test     |
|                         | 4th Wave      | 107                | 12.17       | 2.32           |          |                        |
| TLC (Median)            | First wave    | 71                 | 16819       |                | 0.974    | Mann Whitney U test    |
|                         | 4th Wave      | 107                | 16710       |                |          |                        |
| PLT (Median)            | First wave    | 68                 | 348864      |                | 0.001    | Mann Whitney U test    |
|                         | 4th Wave      | 107                | 158572      |                |          |                        |
| NLR                     | First wave    | 71                 | 13.77       | 8.30           | 0.028    | Independent T-test     |
|                         | 4th Wave      | 107                | 5.78        | 6.37           |          |                        |
| ANC (Median)            | First wave    | 71                 | 11816       |                | 0.016    | Mann Whitney U test    |
|                         | 4th Wave      | 107                | 14679       |                |          |                        |
| ALC (Median)            | First wave    | 71                 | 1796        |                | 0.793    | Mann Whitney U test    |
|                         | 4th Wave      | 107                | 2031        |                |          |                        |
| CRP                     | First wave    | 67                 | 15.81       | 11.47          | 0.002    | Independent T-test     |
|                         | 4th Wave      | 107                | 10.54       | 10.69          |          |                        |
| Ferritin (Median)       | First wave    | 68                 | 1461        |                | 0.369    | Mann Whitney U test    |
|                         | 4th Wave      | 107                | 1302        |                |          |                        |
| D_DIMER                 | First wave    | 68                 | 28.05       | 8.40           | 0.001    | Independent T-test     |
|                         | 4th Wave      | 107                | 5.11        | 3.73           |          |                        |
DISCUSSION

Early detection of the disease and understanding the prognostic values of the simple tests available even at primary and secondary care centres can help assess the severity of the disease and predict mortality. Thus, the clinician can take remedial actions well in time to save precious lives. In the present study, we took the help of our previous experience in the first wave to compare the severity of the disease after attaining much herd immunity due to the vaccination of the citizen against the deadly disease. We observed that the mean age of patients with SD in the first wave was 54 ±12 years, and in the 4th wave, 56±16 years was not significantly different in both phases for hospitalized patients (p=0.571). As per our previous experience, we had reported that 35 (49.3%) of the patients had age >55 years and 25 (35.2%) among the ICU patients hospitalized due to COVID-19.

A study from India comparing the 1st wave with the 2nd wave reported the younger age group was affected more in the second wave. A study from Karachi reported ICU admission due to Covid-19 with increased age and hospital stay...
during the first wave. Similar findings have been reported by Asghar MS et al. comparing the first and second waves. Another study in Spain reported the predictive accuracy of severity and mortality for 6 hematological markers (NLR, CRP, LDH and ferritin, d-dimer and interleukin 6) in the first wave was 84% with an Area Under the curve of 0.84 when the signature was validated for the 2nd and third waves, the accuracy was 83%, with an AUC of 0.78. A significant difference in 1st wave TLC and ANC counts proved to be of prognostic values to predict mortality/worst outcome in COVID-19 with an Area Under Curve (AUC) of 0.74 and 0.70 on ROC, respectively, as compared to 0.5 in the 4th wave (p < 0.05). Likewise, a significant difference exists between the groups (1st vs 4th wave) regarding inflammatory markers; CRP (p=0.002) and D-dimer (p=0.001). We observed a significantly reduced inflammatory response in the 4th wave, attributed to the widespread use of immunomodulatory therapies and steroids. D-dimer was the main prognostic factor that predicted severity and mortality in both the waves and is less affected by the use of immune modulators and steroids. Ferritin levels were also higher but not statistically different in both waves. Our findings concordance with a trial reported from the United Kingdom where they compared the first wave with the second wave. Gao Y et al. have reported that the Area under curve for d-dimer was 0.840, similar to what we observed in 4th wave findings and further confirms the higher clinical impact of d-dimer as the worst outcome in COVID-19. The abnormally high serum ferritin levels predict the worst outcome and have been reported to be high and non-stoppable in hospitalized patients with COVID-19. A study reported an increase in the ferritin levels exceeding the upper limit of detection, increasing hospital stay and mortality in critically ill ICU patients. Regarding the peripheral hematological markers, as per our previous experience in AUC, the absolute neutrophilic count (ANC) was 0.6 and was significantly higher in a group with case fatality. Therefore ANC can also be used as a prognostic marker in COVID-19. Notably, a study published in the American Journal of Hematology reported a high leukocyte count in COVID-19 ICU patients with severe complications with a median peak ANC of 11600/cmm3 as compared to non-ICU patients without complications (p<0.001). D-dimer as an inflammatory marker carry higher prognostic values, and its raised levels in both the waves strongly predict mortality in covid-19. The ferritin values were higher in both waves, but the impact on outcome was less specific than d-dimer. High D-dimer values can be observed in severe disease after a long time, while the values of the ferritin and d-dimer decreased with an increase in the disease duration.

**CONCLUSION**

Most of the hematological and inflammatory markers behaved/presented similarly in both waves in 2020 vs 2021. However, using the ROC curve to determine the clinical sensitivity of these markers in both waves showed that some parameters associated with a poor prognosis (TLC, ANC, NLR, ferritin) were not found in 4th wave, which may indicate a different or stage of presentation for hospitalization with the same disease. However, few markers like NLR and d-dimer possess the same clinical significance as biomarkers for indicating the severity of the disease. These biomarkers constitute a helpful tool to classify patients' prognosis on presentation to hospital emergency.

**LIMITATION**

This study had a small sample size. Studies executed with larger sample sizes in both groups (discharged as complication-free) and patients discharged on death summary in COVID-19 will better portray the importance of different hematological and inflammatory markers from peripheral blood count.

**CONFLICT OF INTEREST:** None

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