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Original Research Article ***Open Access***

DETECTION OF CLINICAL BIOMARKERS ASSOCIATED WITH HEPATO AND RENAL MANIFESTATIONS IN COVID-19 PATIENTS

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Abstract *The COVID-19 pandemic has spread in many countries worldwide, surpassing one million confirmed cases and resulting in deaths globally. Developing nations such as Pakistan face heightened vulnerability to such outbreaks due to limited healthcare infrastructure and resources. This study examines clinical biomarkers linked to hepatic and renal manifestations in COVID-19 patients in North Waziristan, recognizing the vulnerability of developing countries like Pakistan to such pandemics due to limited healthcare resources. Nasopharyngeal swabs were collected from 110 suspected COVID-19 patients visiting Dr. Azim Ullah clinic and DHQ Hospital North Waziristan, Miran Shah. Hematological, hepatic, renal, C-reactive protein (CRP), and ferritin markers were assessed. Most patients were male (75%), with varying age distributions. Abnormalities were observed in hematological parameters, liver enzymes, renal function markers, CRP, and ferritin levels. Significant correlations ($p<0.001$) were found among these markers. The study concludes that inflammatory, hematological, renal, and liver markers are associated with COVID-19 infection, with higher levels indicating severe disease. Continuous monitoring of these biomarkers may enhance patient outcomes and aid in predicting disease progression.*

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Keywords: *Covid-19; hepato and renal; liver markers; CRP; ferritin*

Introduction

Throughout history, zoonotic diseases have significantly impacted human mortality, with COVID-19 being a recent example. Emerging in Wuhan, China, in late 2019, COVID-19 rapidly spread globally, underscoring the threat posed by coronaviruses (Wu et al., 2020). Coronaviruses, characterized by their distinctive crown-like morphology, possess a single-stranded positive-sense RNA genome (Su et al., 2016). SARS-CoV-2, the culprit behind COVID-19, belongs to the Beta coronavirus genus and exhibits genetic similarities with other coronaviruses, including SARS-CoVs, MERS-CoVs, and pangolin coronavirus (Naji, 2020; Njoga et al., 2021). The virus's entry into host cells involves a three-stage process: receptor binding, spike protein conformational changes, and membrane fusion (Boopathi et al., 2020). Various host species, notably bats, serve as coronavirus reservoirs, posing a persistent zoonotic transmission risk to humans. Previous outbreaks, such as SARS-CoV and MERS-CoV, have highlighted the severity of these diseases, resulting in substantial morbidity and mortality (Ho et al., 2007; Zaki et al., 2012). The COVID-19 pandemic, declared by the World Health Organization in 2020, has precipitated a global health crisis, with millions of cases and fatalities reported. Transmission primarily occurs via respiratory droplets, although other routes, including contaminated surfaces and feces, are also possible (Qasim et al., 2020). Mitigation strategies include vaccination, self-isolation, and public health measures, with particular attention to vulnerable populations, such as individuals with underlying health conditions (Lukman et al., 2020). This study investigates clinical biomarkers associated with hepatic and renal manifestations in COVID-19 patients in North Waziristan, aiming to elucidate the disease's impact on these critical organs. By evaluating hematological parameters and key biomarkers, including serum creatinine, urea, liver function tests, CRP, and serum ferritin, we seek to identify indicators of disease severity and prognosis.

This analysis aims to provide insights into COVID-19's pathophysiology in North Waziristan, informing targeted treatment strategies and interventions to improve patient outcomes and contribute to global efforts combating the pandemic.

Materials and Methods

Study Design and Sample Collection

In the study, Patients aged ≥60 years were categorized as elders, while those under 18 years were considered pediatric patients. Written consent was obtained from all participants, with parental consent secured for minors. Nasopharyngeal swabs (n=110) were collected from suspected COVID-19 patients at Dr. Azim Ullah Clinic and DHQ Hospital, North Waziristan, Miran Shah, and stored in a viral transport medium (VTM). In the study, Patients diagnosed with COVID-19 were included only, while those co-infected with other viral infections were excluded.

Detection of COVID-19 through RT-PCR

Total RNA was extracted from each group of samples using a pure virus RNA extraction Kit (Hangzhou Bigfish Biotech, China) and was reverse transcribed into cDNA. Then perform RT-qPCR assay using a novel coronavirus nucleic acid detection Kit (Genrui Biotech Inc, Shenzhen China) with magnetic induction cycler (MIC) qPCR from the bimolecular system (Genrui Biotech Inc, 2020, www.genrui-bio.com) (Liu *et al.*, 2020; Li *et al.*, 2020).

Hematological Parameters

Complete blood counts (CBC) were conducted, measuring parameters such as lymphocytes, neutrophils, and hemoglobin using a hematology machine following Cheung *et al.*, (2021).

Liver Function Tests

Liver function tests, including alkaline phosphatase (ALP), alanine transaminase (ALT), and serum bilirubin, were performed using a chemistry analyzer (Li *et al.*, (2020).

Renal Function Tests

Urea and creatinine tests were conducted using reagents and a chemistry analyzer. CRP levels were determined through dilution and analysis using a chemistry analyzer. Serum ferritin levels were measured via enzyme-linked immunosorbent assay (ELISA) (Li *et al.*, 2020).

Statistical Analysis

Data were analyzed through different statistical software like MS Excel, and SPSS. The student test (t-test) was performed for significance ($p<0.05$) between two variables. All the markers were further checked with one another by Pearson correlations (r). The mean and standard deviation were studied among different parameters.

Results

Gender-wise Distribution of COVID-19

A total of 110 COVID-19 patients were included in the current study, diagnosed via positive PCR. Among these patients, 82 (75%) were male, while 28 (25%) were female. The number of positive male individuals was higher compared to female individuals, as shown in Figure 1.

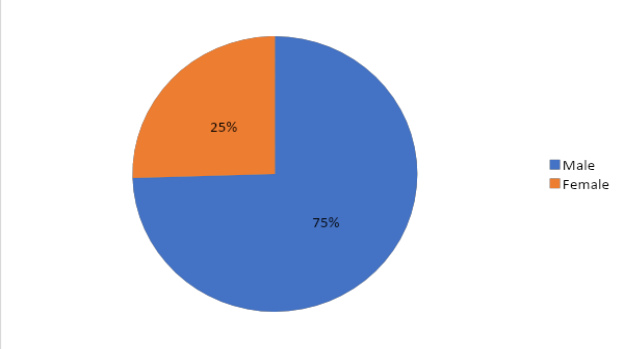

Figure 1. Gender-wise distribution of Covid-19 patients

Age-wise Distribution of COVID-19

COVID-19 positive individuals were divided into different age categories. Patients aged ≤20 were 9 (8%), those aged 21 to 40 were 44 (40%), those aged 41 to 60 were 54 (49%), and those aged ≥61 were 3 (3%). The highest incidence of COVID-19 was observed in the age group of 41 to 60 years, as shown in Figure 2.

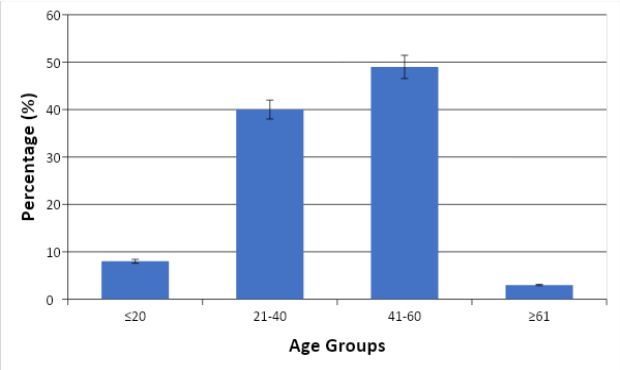

Figure 2. Age-wise distribution of Covid-19 patients

Hematological Parameters and COVID-19

Among the 110 COVID-19 positive patients, the hemoglobin level was found to be abnormal in 19 (17%) patients, while 91 (83%) had normal levels. The lymphocyte count was normal in 21 (19%) patients and abnormal in 89 (81%). The neutrophil count was abnormal in 94 (85.5%) patients and normal in 16 (14.5%), as shown in Figure 3.

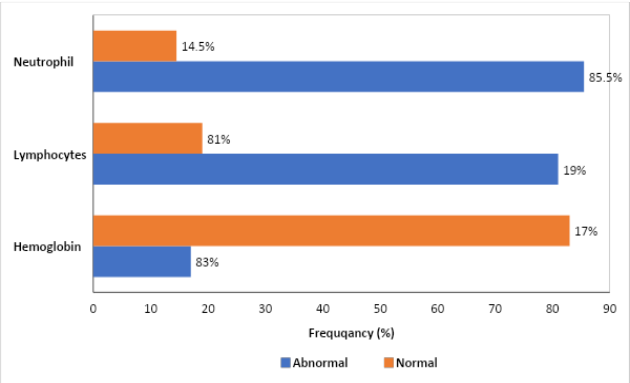

Figure 3. Relationships of hematological markers with COVID-19 patients

Liver Function Tests and COVID-19:

Among the 110 COVID-19 positive patients, the liver function parameter ALT was found to be abnormal in 10 (9%) patients, while 100 (91%) had normal levels. The ALP was abnormal in 8 (7%) patients and normal in 102 (93%). Bilirubin was abnormal in 4 (4%) patients and normal in 106 (96%), as shown in Figure 4.

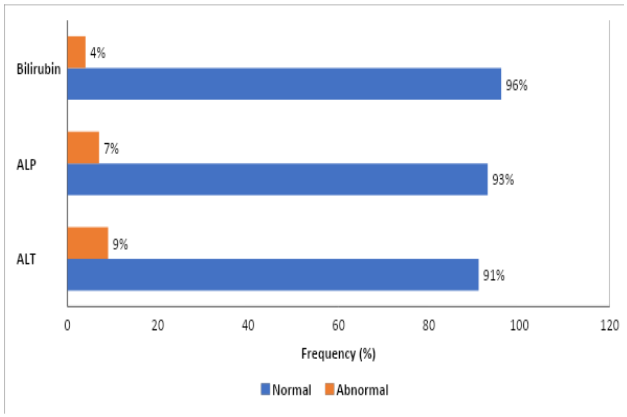

Figure 4. Relationships of liver function tests with COVID-19 patients

Renal Function Tests and COVID-19

Among the 110 COVID-19 positive patients, blood urea levels were abnormal in 68 (62%) patients and normal in 42 (38%). Creatinine levels were abnormal in 71 (64.5%) patients and normal in 39 (35.5%), as shown in Figure 5.

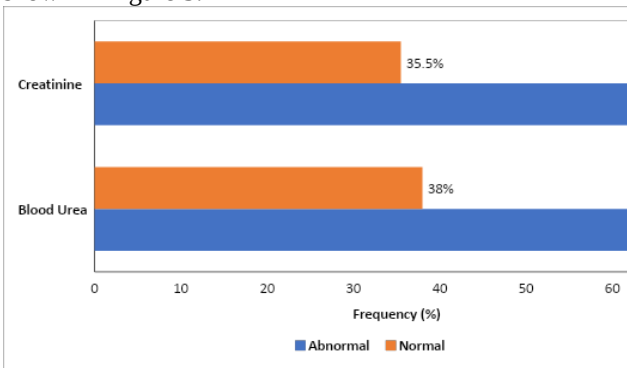

Figure 5. Relationships of renal function tests with COVID-19 patients

Inflammatory Markers and COVID-19

Among the 110 COVID-19 positive patients, the inflammatory marker CRP was found to be abnormal in 89 (81%) patients and normal in 21 (19%). Ferritin levels were abnormal in 93 (84.5%) patients and normal in 17 (15.5%), as shown in Figure 6.

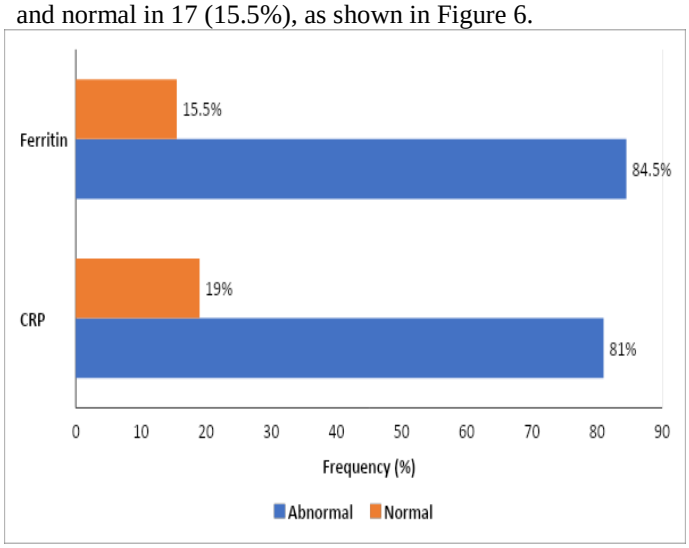

Figure 6. Relationships of inflammatory markers with COVID-19 patients

Overall Association of Markers with COVID-19 Clinical Manifestation

The overall relationships of markers with COVID-19 clinical manifestations are presented in Table 4.1. The mean ferritin level was observed to be 1196.62 ng/mL with a significant correlation ($p < 0.001$) at a 95% confidence interval. The mean blood urea level was 20.46 mg/dL, creatinine was 1.03 mg/dL, ALP was 115 IU/L, ALT was 27 U/L, bilirubin was 0.85 mg/dL, lymphocytes were 11.71%, neutrophils were 83.58%, and hemoglobin was 13.38 g/dL, all showing significant correlations ($p < 0.001$) at a 95% confidence interval.

Table 1. Statistical analysis and frequency distribution of different Parameters

Variables	Mean	Std. Deviation	t-test	p-value	Reference normal range
Ferritin	1642.73	124.41	4.610	.000	30-400 ng/mL
CRP	19.16	88.418	2.111	.037	<0.5 mg/dL
Blood Urea	20.46	2.143	-2.569	.000	06-24 mg/dL
Creatinine	1.03	22.790	3.898	.000	0.74-1.35 mg/dL
ALP	115	22.621	14.245	.000	44-147 IU/L
ALT	27	87.543	3.873	.000	04-36 U/L
Bilirubin	0.85	2.876	-9.654	.000	0.1-1.2 mg/dL
Neutrophils	83.58	11.360	7.553	.000	40--75 %
Lymphocytes	11.71	15.329	-18.456	.000	20--45 %
Hemoglobin	13.38	1.972	-3.158	.002	11.5--17.5 g/dL

ALP, Alanine phosphatase; ALT, Alanine Transaminase; CRP, C - reactive protein

Discussion

The COVID-19 pandemic has emerged as a significant public health crisis that demands

immediate intervention for control and treatment. Despite extensive efforts to develop new medications specifically for SARS-CoV-2, progress has been slow, leading to the identification of drug repurposing as a rapid solution for addressing the urgent needs posed by COVID-19 (Singh et al., 2020). Studies have consistently shown higher male mortality rates compared to female mortality rates in all age groups above 20 years in several countries, including Spain, Germany, Switzerland, Belgium, and Norway (Guan et al., 2020).

In this context, our study analyzed COVID-19 patients' coagulation markers, biochemical, and hematological profiles, with a specific focus on gender differences. Our findings revealed that 75% of the positive cases were male, while 25% were female. These results align with previous research by Guan et al. (2020), which reported a male infection rate of 58.1% compared to 41.9% for females. These findings partially contradict those of Patel et al. (2021), who reported the highest infection rate in the 51-60 age group. Biomarker analysis indicated elevated ferritin levels in 84.5% of COVID-19 patients, which is consistent with other studies that found high ferritin levels associated with increased secondary bacterial infections and exacerbation of COVID-19 symptoms. Para et al. (2022) also noted that while ferritin levels are a weak predictor of hospital outcomes in influenza infections, they are notably higher in bacterial infections compared to viral ones.

Additionally, 81% of COVID-19 patients showed abnormal levels of C-reactive protein (CRP), an inflammatory marker. During the 2002 SARS outbreak, elevated CRP levels were linked to respiratory disorders and patient mortality. In the current pandemic, CRP levels have been found to rise significantly in the early stages of COVID-19 infection, often before abnormalities are visible in CT scans (Mouliou, 2023). Research by Ayanian et al. (2020) revealed a significant correlation between elevated C-reactive protein (CRP) levels and COVID-19 severity. Neutrophils, the predominant immune cells in human blood, play a vital role in maintaining homeostasis and combating chronic inflammation. Although their role in viral infections is not fully understood, they are known to protect against fungal and bacterial infections. Neutrophilia, characterized by an elevated neutrophil count, has been linked to adverse outcomes and severe respiratory symptoms in COVID-19 patients. Our study found that 85.5% of COVID-19 patients exhibited abnormal neutrophil counts, consistent with previous findings. Lymphocytes, another crucial immune cell type, displayed abnormalities in 81% of patients. This result aligns with Yu et al.'s (2020) observation that 80% of severely ill COVID-19 patients experienced lymphopenia. Conversely, Zhou et al. (2020) reported lymphopenia in only 25% of patients with moderate COVID-19 infections, suggesting a potential link between disease severity and lymphocyte count.

Our analysis also revealed abnormal hemoglobin levels in 17% of patients, while 91% displayed normal levels. Wenzhong et al. (2020) proposed that specific viral proteins could disrupt hemoglobin function, impairing oxygen and carbon dioxide transport, and contributing to various disease symptoms (Liu et al., 2020). Liver function tests indicated abnormalities in 9% of patients for alanine transaminase (ALT), 7% for alkaline phosphatase (ALP), and 4% for bilirubin, mirroring findings by Xu et al. (2021). Furthermore, renal profiles showed abnormal blood urea levels in 62% of patients and abnormal creatinine levels in 64.5%, exceeding previously reported rates (Liu et al., 2021). Our comprehensive analysis of COVID-19 patients' clinical and biochemical profiles highlights significant gender disparities, age-related infection rates, and abnormal biomarker levels. These findings can inform more effective treatment strategies and improve patient outcomes.

Conclusion

This research examined the relationships between hematological, inflammatory, renal, and liver biomarkers and COVID-19 severity. Notably, 85.5% of patients exhibited abnormal neutrophil counts, indicating profound hematological disturbances. Conversely, liver function tests (LFTs) yielded no significant correlations with disease severity. In contrast, renal function tests (RFTs) revealed substantial abnormalities in blood urea and creatinine levels, underscoring significant renal impairment. Moreover, inflammatory markers, including C-reactive protein (CRP) and ferritin, were markedly elevated in patients, suggesting a robust association between heightened inflammation and severe COVID-19.

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Declarations

Declaration of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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AS and AW conducted the field trials and planned the experiment. AS, RA, AB, MIK, and MR analyzed the data. TR, ZT and JU assisted with data collection. All authors proofread the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for Publication
Not applicable