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Evaluation of Bio-markers in Vaccinated and Unvaccinated COVID-19 Patients with Comorbidities

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Abstract

Background: SARS-CoV-2 typically causes pneumonia and ARDS has created massive disruptions and loss of human lives around the world and continues to pose several diagnostic and therapeutic challenges. Measurement of different biomarker levels help to assess the severity and progression of the disease, as many studies shown. Besides vaccination helps to reduce both infection rate and severity as recent studies report. So, study on biomarkers on COVID-19 would create great public health impact to control the pandemic. This study investigates the impact of vaccination on biomarker profiles and its role in providing protection against severe outcomes in high-risk populations.

Materials and methods: A cross-sectional study was conducted comprising fifty (50) vaccinated and fifty (50) unvaccinated COVID patients. Different biomarkers levels were measured and compared in various groups of COVID patients based on their vaccination status.

Results: In total, 100 patients were included in the study (One-dose vaccinated patients: 25, two-dose vaccinated patients: 25, unvaccinated: 50), of whom 46 were male and 54 were female. There was no statistical difference in mean between vaccinated and unvaccinated patients in terms of age and gender. The most common comorbidities were hypertension (n=49) diabetes mellitus (n=28) and obesity (n=30). Ferritin levels were significantly lower in two-dose vaccinated patients compared to one-dose and unvaccinated groups (p=0.018). D-dimer and procalcitonin levels were significantly lower in two-dose vaccinated patients compared to one-dose and unvaccinated groups (p = 0.002, 0.004) respectively (p=0.004). No significant differences were observed in hemogram or CRP levels among unvaccinated, one-dose, and two-dose vaccinated patients.

Conclusions: Unvaccinated COVID-19 patients with comorbidities had higher serum ferritin, D-dimer, and procalcitonin levels than vaccinated patients.

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Introduction

The persistent pandemic of Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has resulted in significant disruptions and loss of life globally, while continuing to present numerous diagnostic and treatment hurdles. In December 2019, the initial case was reported in Wuhan, China. On February 11, 2020, the World Health Organization (WHO) formally designated this infection as coronavirus disease 2019 (COVID-19) and the virus as SARS-CoV-2.[1] This virus spread from its origin to other countries across the world rapidly due to its high transmissibility and lack of preexisting immunity to this virus in the population. On March 11, 2020 it was declared as pandemic by the WHO (World Health Organization).[1] SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) typically causes pneumonia and Acute Respiratory Distress Syndrome (ARDS) but it may progress to severe, life threatening systemic diseases.[2,3] Patients are classified as mild, moderate or severe based on clinical symptoms and lab results.[2] Many COVID-19 patients are asymptomatic. Severe symptoms affect 14% of patients and up to 5% are critical, with a 2.3% case fatality rate.[4,6] Virtually 20% of hospitalized patients require ICU support and nearly 61.5% die for various reasons.[3-5] Studies also reported that a significantly higher proportion of patients with comorbidities died compared to those with none.[6] Critical laboratory biomarkers can monitor and prevent disease progression to severe form due to the lack of a directed therapy and expanding vaccine service. A biomarker is a "Characteristic that can be objectively measured and evaluated as an indicator of normal biological and pathological processes, or pharmacological responses to a therapeutic intervention." COVID-19 biomarkers can help in these areas:

i) Early suspicion of disease
ii) Confirmation and classification of disease severity

iii) Framing hospital admission criteria
iv) Identifying high-risk cohort
v) Framing ICU admission criteria
vi) Rationalizing therapies
vii) Assessing response to therapies
viii) Predicting outcome
ix) Framing ICU and hospital discharge criteria.

Clinical, laboratory and radiologic markers have been identified, but their results vary due to disease behavior and geography. Various laboratory markers indicate disease severity and progression.[7] Hematological biomarker studies showed that severe patients had lower lymphocyte, monocyte, eosinophil, hemoglobin and platelet counts but higher neutrophil counts. Severe patients had worse liver and kidney outcomes than non-severe patients, with ALT (Alanine aminotransferase) AST(Aspartate aminotransferase) Total Bilirubin, BUN (Blood urea nitrogen) and Creatinine levels rising after albumin levels fell. Inflammatory/infection markers—ESR (Erythrocyte Sedimentation Rate) CRP (C-Reactive Protein) LDH (Lactate Dehydrogenase) and PCT (Procalcitonin) were positively associated with COVID-19 severity in various studies.[8] In severe and critical patients, fibrinogen, PT (Prothrombin Time) and D-dimer were positively associated.[8] Electrolyte imbalance, both hypo and hyper status, were reported for sodium, potassium and calcium levels among patients with severe disease and worse outcome.[9-11] The world accelerated vaccine development as losses continued. By 2020, over 200 vaccine candidates on various platforms were in development. Three have received EUA/EUL from maturity level 4 regulatory authorities and are being rolled out in multiple countries. Additional vaccines have received national regulatory approval and are used in some countries before efficacy trials. Vaccine efficacy, effectiveness and impact are usually assessed in a population. Vaccine efficacy is the reduced risk of infection or disease in carefully controlled vaccinations, estimated from randomized clinical trials. Observational (Nonrandomized) studies estimate vaccine effectiveness as reduced risk of infection or disease in real-world conditions. Vaccines reduce infection and disease in vaccinated populations. Vaccine impact depends on vaccine coverage and includes direct and indirect effects in the vaccinated and unvaccinated case due to herd protection.[12] Impact can also be measured by health system capacity and economic indicators. Recent studies show that controlling the pandemic by reducing disease severity in vaccinated people is effective.[13] COVID-19 hospitalizations are 29 times higher in unvaccinated people. People who weren't vaccinated were nearly five times more likely to contact COVID-19.[13] Therefore, vaccination coverage on a greater scale would help the countries to fight the burden. In this context, massive awareness is required to make people willingly take the vaccine. Vaccine impact studies would help in this regard to make vaccination programs effective and successful.

Materials and methods

This cross-sectional observational study was conducted in the Outpatient Department of Medicine, Chittagong Medical College Hospital. The study ran from January 2022 to January 2023. The Chittagong Medical College Ethical Review Committee approved the study.Informed consent was obtained from each patient.

After conducting non-probability consecutive sampling methods one hundred (100) Hospitalized COVID patients with comorbidities comprising (50 vaccinated and 50 unvaccinated) were selected. Biomarkers such as C-Reactive Protein (CRP) D-dimer, ferritin, procalcitonin were compared among the vaccinated (One dose, two dose) and unvaccinated group.

Inclusion criteria

- Vaccinated and unvaccinated COVID patients.
- Had comorbidity.
- Age >18 years.

Exclusion criteria

- COVID patients without comorbidity.
- Refuse to participate in the study.

Data were entered into Microsoft Excel data sheet to generate a master sheet. After completion of the data entry, the master sheet was fed into Statistical package for social science (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) for processing and analysis. Data were expressed as frequencies and percentages of categorical variables: mean, median standard deviation and interquartile range values of numerical

variables were calculated. One-way ANOVA test, Independent sample Kruskal Wallis test, post-hoc test was performed for quantitative data. Categorical variables were tested with the chi-square test. A p-value of <0.05 was considered statistically significant.

Results

Table I Demographic characteristics of patients with COVID-19 (n=100)

Variables		2-dose vaccinated patients (n=25)	1-dose vaccinated patients (n=25)	Unvaccinated patients (n=50)	p-value
Age, Years (Mean±SD)		61.2±9.41	58.7±8.14	60.8±8.64	>0.05
Sex(n%)	Male	14(56.0)	12(48.0)	20(40.0)	
	Female	11(44.0)	13(52.0)	30(60.0)	

Table 1 shows the Demographic characteristics of patients with COVID-19. In total, 100 patients were included in the study (One-dose vaccinated patients: 25, two-dose vaccinated patients: 25, unvaccinated: 50) of whom 46 were male and 54 were female. The mean age was 60.14±9.5 years (vaccinated: 50.7±8.14, unvaccinated: 60.8±8.64). There was no statistical difference between vaccinated and unvaccinated patients in terms of age and gender ratio (p>0.05, Chi-squaretest).

Table II Comorbidity pattern of the patients with COVID-19. (*Multiple response table. Data were expressed as n (%)

Comorbidities	2-dose vaccinated patients (n=25)	1-dose vaccinated patients (n=25)	Unvaccinated patients (n=50)
Hypertension	9(36.0)	10(40.0)	20(40.0)
Diabetes mellitus	4(16.0)	5(20.0)	19(38.0)
Asthma	1(4.0)	2(8.0)	2(4.0)
COPD	3(12.0)	2(8.0)	2(4.0)
Heartfailure	0(0)	1(4.0)	1(2.0)
Cerebrovascular events	0(0)	1(4.0)	0(0)
Chronicrenal failure	7(28.0)	3(12.0)	2(4.0)
Malignancy	2(8.0)	0(0)	1(2.0)
Obesity	13(53.0)	7(28.0)	10(20.0)

Table II shows that the most common comorbidities were hypertension (n=39, 67.53%) diabetes mellitus (n=28) and obesity (n=30).

Table III Comparison of hematological and biochemical parameters in vaccinated and unvaccinated COVID-19 patients

Variables	2-dose vaccinated patients (n=25)	1-dose vaccinated patients (n=25)	Unvaccinated patients (n=50)	p value
Lukocyte ($\times10^3$/µl)	8.23±4.00	8.73±5.33	9.06±4.60	0.606*
Neutrophil ($\times10^3$/µl)	6.04±3.56	7.07±4.94	7.24±4.52	0.302*
Lymphocyte ($\times10^3$/µl)	1.46±0.90	1.01±0.53	1.20±0.83	0.051*
Monocyte ($\times10^3$/µl)	0.60±0.36	0.59±0.41	0.56±0.45	0.838*
CRP(mg/L)	82(33–145)	112(32–172)	71(29–135)	0.203†
Ferritin (ng/ml)	385(179–809)	512(319–1033)	612 (216–1331)	0.018†
D-dimer (µg/L)	0.67±0.79	0.86±0.89	1.62±1.93	0.002*
Procalcitonin (ng/ml)	0.13(0.70–0.32)	0.25(0.09–0.68)	0.21(0.08–0.58)	0.004†

(Data were expressed as either mean±SD or median (Interquartile range). *One-WayANOVA test. †Independent sample Kruskal Wallis test).

When comparing laboratory parameters between one-dose vaccinated, two-dose vaccinated and unvaccinated patients, no statistical significance was found in leukocyte, neutrophil, lymphocyte, monocyte and CRP levels. In comparison to the two-dose vaccinatedgroup, the unvaccinated and one dose vaccinated groups had considerably higher median serum procalcitonin, ferritin and higher mean D-dimer levels (With statistically significant p-value<0.05)(Table III).

Discussion

The study comprised 100 COVID-19 patients, categorised into one-dose vaccinated (n=25) two-dose vaccinated (n=25) and unvaccinated (n=50) groups, exhibiting a nearly balanced gender distribution (46 males, 54 females). The mean age of participants was 60.14 ± 9.5 years, with vaccinated individuals being marginally younger (50.7 ± 8.14) than their unvaccinated counterparts (60.8 ± 8.64). Statistical analysis indicated no significant differences in age or gender ratio between vaccinated and unvaccinated groups, implying comparable baseline demographics. This suggests that any differences in biomarker profiles or clinical outcomes between the groups are unlikely to result from age or sex differences (Table I). According to the Shirin Heidari and Tracey Goodman, W.H.O. there is substantial documentation regarding the differences in immune responses to pathogens, including SARS-CoV-2, and vaccines based on gender.[14] Women typically exhibit more robust humoral and cell-mediated immune responses to antigenic stimulation, vaccination and infections compared

to men. Research suggests that adverse reactions to vaccines may occur more frequently in women than in men.

Females develop higher antibody response post-vaccination compared to males and vaccine responses are diminished in older adults due to immune senescence.[15]

The study found no significant differences in leukocyte, neutrophil, lymphocyte, monocyte, or CRP levels among one-dose vaccinated, two-dose vaccinated, and unvaccinated COVID-19 patients (Table III). However, compared to the two-dose vaccinated group, both unvaccinated and one-dose vaccinated patients exhibited significantly higher median levels of procalcitonin and ferritin, as well as higher mean D-dimer levels ($p < 0.05$). These findings suggest that two-dose vaccination may help mitigate hyper inflammatory and prothrombotic responses, as reflected by lower procalcitonin, ferritin and D-dimer levels-markers associated with severe disease. The lack of difference in other inflammatory markers (CRP, leukocytes) implies that vaccination status may selectively influence specific pathways linked to disease severity. These results highlight the potential benefit of full vaccination in reducing biomarkers of severe COVID-19 complications.

The study highlights that key laboratory markers of disease severity-such as ferritin and D-dimer-are significantly elevated in unvaccinated COVID-19 patients compared to those fully vaccinated (Two doses), correlating with worse clinical outcomes. However, the dynamics of acute-phase proteins (CRP, ferritin, ESR, fibrinogen, haptoglobin, serum amyloid A and procalcitonin) remain unclear in breakthrough infections, as their levels post-vaccination were not well characterized at disease onset. The findings reinforce that full vaccination reduces biomarkers linked to severe disease, supporting its role in blunting harmful inflammatory and thrombotic cascades. Chrostek, L. et al. demonstrates a clear association between disease severity and elevated levels of acute-phase proteins (CRP, ferritin and pro-calcitonin) in COVID-19 patients.[16] Critical cases exhibited significantly higher concentrations of these biomarkers compared to moderate cases, while severe cases also showed markedly increased levels relative to moderate cases. This progressive rise in CRP, pro-calcitonin and ferritin aligns with worsening clinical status, reinforcing their role as indicators of hyper inflammation and disease severity. The findings suggest that these biomarkers may help stratify patient risk, with higher levels signaling a greater likelihood of critical illness.

Docherty A.B. et al. identifies higher-risk groups for severe COVID-19 outcomes, including males, elderly individuals, smokers, Black/Asian populations, obese patients, and those with comorbidities like diabetes or heart disease.[17] CRP levels greater than 41.8 mg/L were more likely to be related to severe disease, with respective hazard ratios of 4.4 (95% confidence interval : 1.9-10.3). Yu, B. et al. found that COVID-19 pneumonia had higher D-dimer levels and inflammation.[18,19] In our study, the D-dimer level in unvaccinated individuals was approximately twice that in two-dose vaccinated individuals. Fatima et al. found that unvaccinated people had higher mean D-dimer values (1.2) than vaccinated people (0.8).[20] According to Sree et al. the unvaccinated COVID-19 infected group had a higher mean D-dimer value (4.71±25.71) than the vaccinated group (0.67±3.41).[21] Ersan et al. found no significant D-dimer differences between vaccinated and unvaccinated patients.[22]

Limitations

- Convenience sampling was used from asingle center.
- Sample size was small
- Relation between biomarkers levels and disease severity or outcomes was not assessed in this study.

Conclusions

In conclusion, even when unvaccinated patients were vaccinated and became ill, the COVID-19 disease was milder and ferritin D-dimer and procalcitonin levels related to disease severity were higher. Although the vaccine is insufficient to prevent the disease completely,it may lead to amilder course.

Recommendations

Inflammatory indicators like ferritin, D-dimer and procalcitonin have been shown to predict the disease severity. Vaccinated groups had a lower

risk of serious illness. The findings will increase public vaccine acceptability. More clinical investigations are needed to confirm the findings. More clinical trials with different COVID-19 vaccines are needed to determine the markers of mortality and morbidity variables.

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Contribution of authors
MHI-Conception, design, acquisition of data, data analysis, drafting and final approval.
SMK-Interpretation of data, critical revision and final approval.

MRMI-Data analysis, drafting and final approval.

Disclosure
The authors declared no conflict of interest.

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