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Association Of Biochemical Parameters Of COVID Susceptibility In Interferon Gamma Release Assay (IGRA) Positive Subjects

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Abstract
Background: COVID-19 is viral pneumonia that originated in Wuhan, China in 2019. The virus spread throughout the world in the form of a pandemic and public health emergency was declared by WHO in March 2020. People with underlying pathological conditions such as tuberculosis are more susceptible to COVID-19. This study was conducted to investigate tuberculosis as a risk factor for COVID.
Methods: IGRA testing of subjects, presenting symptoms of tuberculosis and those advised for IGRA by clinicians, was done and socio-demographic and clinical data were collected by questionnaires. The data collected during the study were organized using Excel spreadsheets and subjected to statistical analysis using SPSS version 16.
Results: The findings of our study support the fact that TB patients are more susceptible to COVID-19. We found that biochemical markers including uric acid (p=0.022), urea (p=0.035), bilirubin (p=0.001), alkaline phosphatase (p=0.046), and potassium ions (p=0.000) can be used as diagnostic markers in TB-COVID co-infectious state as these were significantly associated with COVID in IGRA positive subjects when analysed by the ROC curve.
Conclusion: Within the framework of our study, biomarkers, including urea, creatinine, uric acid, alkaline phosphatase (ALP), bilirubin, and potassium ions exhibited significant associations with COVID-19. However, further longitudinal studies should be undertaken to investigate the status of these biomarkers on an extended number of active and latent TB patients to validate and potentially employ common biomarkers in diagnostic assays for the co-infection of TB and COVID-19.

Keywords: COVID, Latent TB, IGRA, Biochemical markers, Co-infection.

Introduction

The year 2020 has been widely recognized as the pivotal period in which the global community confronted the COVID-19 pandemic [1]. The etiological agent responsible for COVID-19 pneumonia is a newly identified coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was initially identified in China in December 2019 [2]. COVID-19 has imposed a substantial health burden on a global scale, resulting in 769 million reported cases and 6.9 million fatalities, thus establishing itself as one of the most severe pandemics in history [3]. In addition to asymptomatic carriers, symptomatic patients with COVID-19 exhibit a spectrum of clinical manifestations, including dyspnea, cough, fever, emesis, diarrhea, and abdominal discomfort, affecting 2-10% of afflicted individuals. The primary target organ and the principal site of viral infection are the lungs; nevertheless, the virus may also disseminate to various other organ systems, leading to a range of pathologies affecting the cardiovascular, gastrointestinal, central nervous, hepatic, ocular, and renal systems [4]. Vulnerable populations, encompassing immunocompromised individuals, pregnant women, elderly persons, and those with pre-existing conditions such as hypertension, cardiovascular disorders, diabetes, and respiratory diseases like tuberculosis, are at an elevated risk of increased disease severity and mortality. In addition to the societal and economic ramifications of COVID-19, the full extent of its impact on public health and healthcare systems cannot be comprehensively evaluated through the mere tally of reported cases and fatalities in individual nations. According to a report from Britain, there have been around 12,000 excess deaths unrelated to the virus since the onset of the pandemic, compared to the preceding year. This underscores the likelihood that individuals with chronic and acute severe conditions faced limitations in accessing healthcare services [5]. Concurrent infection of COVID-19 alongside other diseases poses diagnostic and therapeutic challenges for medical practitioners, necessitating the identification of concurrent infections as an essential component of clinical management [6].

Paralleling the characteristics of COVID-19, tuberculosis is an infectious ailment that is communicable through respiratory transmission, with the primary affected organ being the lungs [7]. Tuberculosis has endured throughout history and, in 2019, recorded 10.0 million new tuberculosis cases and 1.4 million tuberculosis-related deaths. A significant proportion of the global

burden is attributed to eight nations, including the Philippines (60%), India (26%), China (8.4%), Pakistan (5.7%), Bangladesh (3.6%), Indonesia (8.5%), Nigeria (4.4%), and South Africa (3.6%) [8]. Presently, approximately 9 million new tuberculosis cases are diagnosed annually, with nearly 2 million fatalities, rendering Mycobacterium tuberculosis the leading cause of mortality due to bacterial pathogens [9]. Individuals afflicted with tuberculosis may exhibit lung damage, rendering them more susceptible to secondary infections, such as COVID-19, and impeding the recovery of co-infected patients [10]. Tuberculosis is principally categorized as active or latent; active tuberculosis is characterized by clinical manifestations and symptoms attributable to Mycobacterium tuberculosis infection, whereas latent tuberculosis signifies the containment of the bacteria within granulomas by the immune system, preventing its widespread dissemination in the body. An estimated 1.7 million individuals across the globe are latently infected with tuberculosis, with a lifetime risk of progression to active tuberculosis due to various factors such as advancing age, substance abuse, HIV infection, or underlying medical conditions [15]. Tuberculosis patients exhibit an elevated susceptibility to COVID-19 due to the heightened presence of myeloid subpopulations in circulation, which are similarly found in the lungs of COVID-19 patients [11]. The augmented production of immune signatures such as type I and III interferons in both diseases accelerates disease progression and increases the likelihood of fatal outcomes. Consequently, COVID-19 serves as an ominous harbinger for addressing the tuberculosis epidemic [12]. Furthermore, the use of immunosuppressive agents in COVID-19 therapy elevates the risk of converting latent tuberculosis into active tuberculosis, as latent tuberculosis can become reactivated when the immune system is compromised [13]. Both pathogens contribute to an imbalanced inflammatory response, and their shared immune response dysregulation exacerbates the disease state and progression of coinfection [14].

Notably, Pakistan ranks fifth among the countries with a high tuberculosis burden globally. The World Health Organization (WHO) reports that Pakistan records approximately 500,000 tuberculosis cases annually, with a rising incidence of drug-resistant tuberculosis [16]. In the year 2020, tuberculosis was responsible for causing the deaths of half a million individuals; while various hypotheses exist regarding the upsurge in tuberculosis-related mortality, the COVID-19 pandemic is considered a contributory factor. However, there is a paucity of conclusive evidence delineating the precise impact of COVID-19 on the tuberculosis burden [17]. The onset of the COVID-19 pandemic positioned Pakistan as the 14th most affected nation by the virus. The virus entered Pakistan through travellers and pilgrims arriving from countries experiencing a high COVID-19 burden, including Iran, with approximately 60% of these individuals harboring COVID-19 infections. The initial instances of SARS-CoV-2 infection within Pakistan were detected in Karachi and Islamabad in February 2020. As of March 2021, Pakistan reported a total of 583,916 confirmed COVID-19 cases, 13,013 deaths, and 554,255 cases of recovery. The country's point prevalence was recorded at 8.3, with a fertility rate of 2.23 [18]. Given the substantial prevalence of tuberculosis in Pakistan, individuals affected by this ailment are at an increased risk of developing COVID-19 due to their compromised immune status. To investigate the potential susceptibility of COVID-19 in individuals with tuberculosis, a study was conducted to evaluate the susceptibility of COVID-19 in interferon-gamma release assay (IGRA) positive tuberculosis patients.

Materials and Methods

1.Study Design

The study was conducted from October 2022 to June 2023 at University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi. To achieve our research objectives, a comprehensive questionnaire was designed to gather essential information, including socio-demographic characteristics (e.g., age, marital status) of patients and details about clinical tests recommended by clinicians. Questionnaires were administered to patients through face-to-face interviews after obtaining their written informed consent. Data and blood samples were collected from patients presenting symptoms such as cough, cough with blood, sputum, fever, or those scheduled for surgery, as advised by clinicians. In total, blood samples were collected from 500 patients, excluding those who had previously tested positive for COVID before the IGRA test.

2. Ethical Approval

The study was approved by the Ethics Committee of PMAS-AAUR for the use of human subjects as well as by Ethics Committee of Fauji Foundation Hospital, Rawalpindi (No:6034/2/adm-I).

3. Sample Processing

The collected blood samples were subjected to IGRA testing using the QuantiFERON TB Gold kit (kit no. 622120, Lot no. 57202948), following the manufacturer's instructions. In addition to IGRA, data regarding other tuberculosis tests such as GeneXpert MTB and acid-fast bacilli (AFB) microscopy of the patients was collected. These results were used as criteria to categorize patients into either active or latent tuberculosis groups. Individuals who tested positive for IGRA were monitored through direct contact over six months to investigate COVID-19 infections. During this duration, any clinical test reports, including liver functional tests (LFT), renal functional tests (RFT), electrolyte levels, calcium, glucose, and complete blood counts (CBP), were documented. Individuals displaying COVID symptoms underwent RT-PCR testing.

4. Statistical Analysis

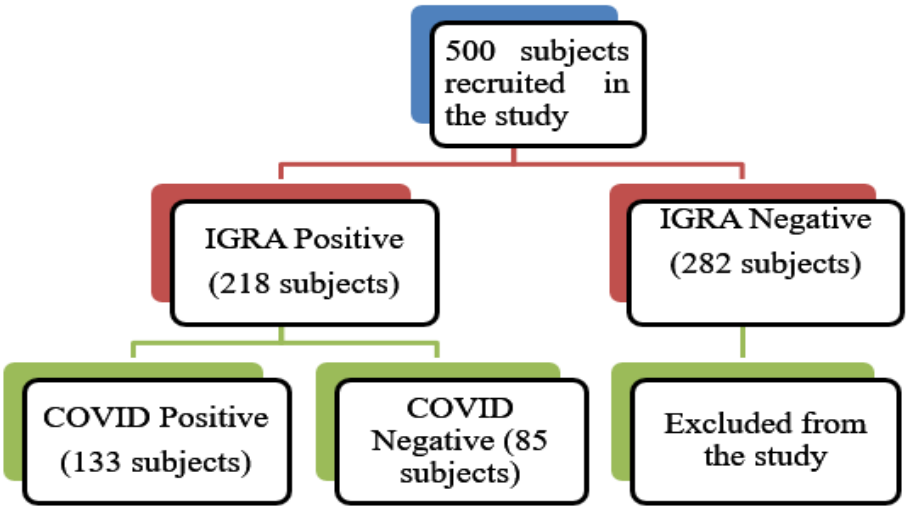
The data collected during the study were organized using Excel spreadsheets and subjected to statistical analysis using SPSS version 16. Frequency and percentage were calculated for categorical demographic and clinical parameters. The Chi-Square (χ^2) test (Pearson, 1900) was employed to assess the association of different parameters with IGRA positive COVID-19 individuals. Additionally, binary logistic regression analysis (Joseph, 1944) was conducted to identify the predictors of COVID-19. For non-categorical data, mean values and standard deviations were computed, followed by the application of the

nonparametric Kruskal-Walis test (Kruskal and Wellis, 1952) to evaluate differences in means between the two groups. Receiver operating characteristic (ROC) curves were generated for significantly associated parameters ($p<0.05$).

Results

The study recruited a total of 500 individuals who had been recommended for IGRA testing by their clinicians. The IGRA testing results indicated that 218 individuals (43.6%) tested positive for IGRA, while 282 individuals (56.4%) tested negative. The IGRA-negative individuals were subsequently excluded from further analysis (Figure 1).

Figure 1: Study Design



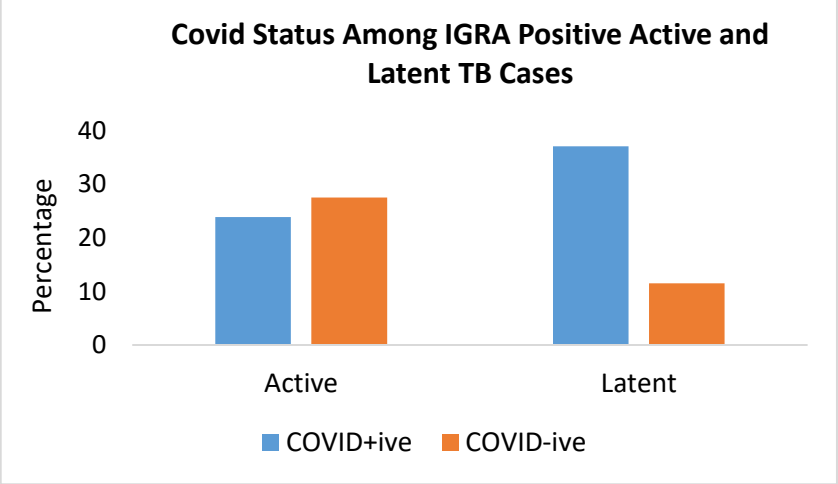
1. Socio-demographic characteristics

The sociodemographic characteristics of the IGRA-positive patients are presented in Table 1. Among the 218 IGRA-positive subjects, 45.0% were above the age of 50, and the majority (approximately 69%) were female. Approximately 40% of the subjects hailed from Punjab, while the smallest proportion (4.6%) came from Kashmir. The IGRA-positive individuals were categorized into latent and active tuberculosis groups based on routine TB diagnostic tests, including AFB sputum microscopy and GeneXpert MTB along with IGRA test. The results of these tests revealed that 62.4% of patients tested positive by AFB microscopy, while 55.5% were detected by GeneXpert MTB. Consequently, 55.5% of subjects were classified as having active tuberculosis, and 45.5% were classified as having latent tuberculosis. Among the individuals in the study, the status of BCG vaccination was unknown for 58.7% of patients, and the status of COVID-19 vaccination was unknown for 53.2% of patients.

2. Susceptibility of COVID in IGRA Positive Individuals

The susceptibility to COVID-19 was analyzed in IGRA-positive patients, irrespective of their TB status (active or latent TB). Out of these individuals, 133 (61%) developed COVID-19 infection during the follow-up period, while the remaining 85 (39%) did not exhibit any symptoms of COVID. A notably higher proportion of individuals developed COVID, suggesting a compromised immune system in IGRA-positive patients, rendering them more susceptible to secondary lung infections such as COVID-19 (Figure 2).

Figure 2: COVID Status of Study Subjects



3. Determination of Parameters Associated with COVID

The parameters were tested for possible association with COVID-19, and the results of the association are summarized in Table 2. It was observed that gender, BCG vaccination, COVID vaccination, and the type of vaccine administered for COVID immunization were significantly linked with COVID.

Table 1: Socio-demographic and Clinical Characteristics of Study Subjects

Socio-demographic and clinical Characteristics	IGRA Positive (N) 218	COVID-Positive (%) 133 (61%)	COVID-negative (%) 85 (39%)
Age			
<25	45 (20.6%)	31 (23.3%)	14 (16.5%)
25-50	75 (34.4%)	43 (32.3%)	32 (37.6%)
>50	98 (45.0%)	59 (44.4)	39 (45.9)
Gender			
Male	68 (31.2%0	29 (21.8%)	39 (45.9)
Female	150 (68.8%)	104 (78.2%)	46 (54.15)
Ethnicity			
Kashmiri	10 (4.6%)	7 (5.3%)	3 (3.5%)
Pashto	64 (29.4%)	42 (31.6%)	22 (25.9%)
Potohari	29 (13.3%)	17 (12.8%)	12 (14.1%)
Punjabi	86 (39.4%)	48 (36.1%)	38 (44.7%)
Saraiki	29 (13.3%)	19 (14.3%)	10 (11.8%)
BCG vaccination			
Nil	128 (58.7%)	100 (75.2%)	28 (32.9%)
No	39 (17.9%)	16 (12.0%)	23 (27.1%)
Yes	51 (23.4%)	17 (12.8%)	34 (40.0%)
COVID vaccination			
No	37 (17.0%)	10 (7.5%)	27 (31.8)
Yes	65 (29.8%)	27 (20.3%)	38 (44.7)
Nil	116 (53.2%)	96 (72.2%)	20 (23.5)
Vaccine name			
AstraZeneca	2 (0.9%)	2 (1.5%)	0 (0%)
Cansino	16 (7.3%)	7 (5.3%)	9 (10.6%)
No	37 (17.0%)	10 (7.5%)	27 (31.8%)
Pfizer	6 (2.8%)	2 (1.5%)	4 (4.7%)
Sino pharm	16 (7.3%)	6 (4.5%)	10 (11.8%)
Sinovac	12 (5.5%)	6 (4.5%)	6 (7.1%)
Sputnik	2 (0.9%)	1 (0.8%)	1 (1.2%)
Moderna	2 (0.9%)	0 (0%)	2 (2.4%)
Nil	125 (57.3%)	99 (74.4%)	26 (30.6%)
HIV status			
Negative	7 (3.2%)	6 (4.5%)	1 (1.2%)
Nil	211 (96.8%)	127 (95.5%)	84 (98.8%)
HCV status			
Negative	24 (11.0%)	17 (12.8%)	7 (8.2%)
Nil	190 (87.2%)	113 (85.0%)	77 (90.6%)
Positive	4 (1.8%)	3 (2.3%)	1 (1.2%)
Comorbidities			
Cancer	2 (0.9%)	0 (0%)	2 (2.4%)
Dengue	3 (1.4%)	2 (1.5%)	1 (1.2%)
Dialysis	2 (0.9%)	0 (0%)	2 (2.4%)
Heart problem	2 (0.9%)	0 (0%)	2 (2.4%)
Lung problem	1 (0.5%)	1 (0.8%)	0 (0%)
Nil	208 (95.4%)	130 (97.7%)	78 (91.8%)

COVID: corona virus disease, BCG: Bacillus Calmette-Guerin, HIV: Human Immuno Deficiency Virus, HCV: Hepatitis C Virus

Results of univariate analysis are presented in Table 2. Univariate analysis (p=0.44) revealed that age is not associated with COVID-19 infection in the present context. An association between gender and COVID status was detected (p-value=0.000), although the results of regression analysis were non-significant with a p-value of 0.065. Nevertheless, the odds ratio of 3.59 (CI= 0.925-13.953) suggested a threefold higher likelihood of COVID-19 development in IGRA-positive females compared to males. The incidence of COVID-19 was higher among Punjabi individuals, likely because this group was more represented in our study population than other ethnicities. Univariate analysis yielded a p-value of 0.698, indicating that ethnicity is not

associated with COVID status. BCG vaccination status was associated with COVID, as indicated by the chi-square analysis (p=0.000), but the association was not significant, as suggested by regression analysis, with a p-value of 0.446. Nonetheless, there was a one-fold higher chance of COVID onset in individuals who received the BCG vaccine compared to those who did not (OR=1.720, CI=0.400-7.386).

Table 2: Chi-Square and Regression Analysis for Risk Factors

Risk factor		**χ^2 test**	***p*-value**	**Regression Analysis**	
				Odds Ratio (95%CI)	***p*-value**
Age	<25	1.627	0.443		
	25-50				
	>50				
Gender	Female	14.008	0.000	3.593 (0.925-13.953)	0.065
	Male			1	
TB status	Active	79.063	0.000	0.042 (0.014-0.129)	0.000
	Latent			1	
COVID vaccination	Yes	51.388	0.000	4.888 (0.557-42.918)	0.152
	No			1	
Vaccine name	AstraZeneca	48.124	0.000	1.701 (0.557-42.918)	0.999
	Cansino			0.000	1.000
	Pfizer			0.000	1.000
	Sinopharm			0.000	1.000
	Sinovac			0.000	1.000
	Sputnik			0.000	1.000
	Moderna			0.000	1.000
	Nil			1	
HCV Status		1.491	0.474		
Ethnicity	Saraiki	2.206	0.698	0.475 (0.104-2.163)	0.336
	Pashto			0.569 (0.047-6.968)	0.659
	Potohari			1.820 (0.363-9.135)	0.467
	Punjabi			1.397 (0.226-8.636)	0.719
	Kashmiri			1	
BCG Vaccination	Yes	38.732	0.000	1.720 (0.400-7.386)	0.446
	No			0.000	1.000
	Nil			1	

CI: Confidence Interval, TB: Tuberculosis, HCV: Hepatitis C Virus, COVID: Coronavirus Disease, BCG: Bacillus Calmette-Guerin

To evaluate the potential of COVID-19 vaccination in reducing COVID-related mortality, the COVID-19 vaccination status of COVID-positive individuals was examined. COVID-19 vaccination status exhibited an association with COVID-19, with a p-value of 0.000. However, binary regression analysis indicated that the association was not significant (p=0.152). The odds ratio suggested a four-fold higher likelihood of developing COVID-19 in vaccinated individuals compared to non-vaccinated individuals (OR=4.888, CI=0.557-42.918). Various COVID-19 vaccines have been developed and administered to combat the pandemic, each with varying efficacy. Information was collected regarding the specific COVID-19 vaccine received by the subjects to assess the vaccines' effectiveness in reducing infection. A majority of the studied subjects were unaware of the vaccine's name with which they were immunized. While this parameter exhibited an association with COVID status (p=0.000), the association was non-significant, with a p-value of 0.999 and an odds ratio of 1.701 (CI=0.557-42.918) for AstraZeneca.
To comprehensively assess the impact of TB on COVID-19 incidence, the presence of other comorbidities, including HCV, was considered alongside COVID-19. However, for most of the subjects, HCV status remained unknown. As a result, a clear evaluation of the impact of this comorbid condition on COVID-19 incidence and the co-infection of TB and COVID-19 could not be made. Univariate analysis revealed no association between HCV and COVID status (p=0.474).

4. Examination of Biochemical Parameters

During the follow-up period, various biochemical parameters, including liver function tests (LFTs), renal function tests (RFTs), electrolytes, glucose, and blood cell counts, were subject to analysis. The mean and standard deviation were computed for these biochemical parameters, and the Kruskal-Wallis test was employed for evaluation. The findings revealed significant differences in levels of urea, creatinine, uric acid, alkaline phosphatase (ALP), bilirubin, and potassium ion (K^+) levels between COVID-positive and COVID-negative subjects, as indicated in Table 3.

Table 3: Biochemical parameters

Parameters	COVID Status	Total (N)	Mean± S.D	T-TEST	p-value
Glucose (mg/dl)	Negative	65	7.00 ± 2.23	0.298	0.585
	Positive	69	7.40 ± 4.27		
Urea (mg/dl)	Negative	67	8.47±6.64	15.386	0.000
	Positive	101	7.83± 9.40		
Creatinine (mg/dl)	Negative	67	1.74± 2.22	9.005	0.003
	Positive	103	1.39± 3.16		
Uric acid (mg/dl)	Negative	52	6.66±4.11	8.542	0.003
	Positive	39	5.78±3.20		
Bilirubin (mg/dl)	Negative	67	1.38±1.89	16.932	0.000
	Positive	102	1.61±3.23		
ALT (U/L)	Negative	65	49.01±79.89	0.000	0.997
	Positive	02	45.1±91.79		
ALP (U/L)	Negative	67	1.27±91.25	13.164	0.000
	Positive	101	1.55±83.42		
Sodium ions (mmol/lit)	Negative	51	1.38±5.49	0.573	0.449
	Positive	96	1.39±4.08		
Potassium ions (mmol/lit)	Negative	51	4.85±0.69	10.327	0.001
	Positive	96	4.46±0.57		
Chloride ions (mmol/lit)	Negative	51	1.02±6.40	0.16	0.901
	Positive	96	1.01±4.34		
Calcium (mmol/lit)	Negative	2	2.30±0.01	0.034	0.854
	Positive	12	2.26± 0.18		
Albumin (g/lit)	Negative	20	38.05±8.88	0.020	0.888
	Positive	73	40.13±14.7		
WBC (×10^9/lit)	Negative	77	10.80±5.49	0.346	0.556
	Positive	112	11.14±16.29		
RBC	Negative	25	4.47±0.77	0.867	0.352

ALT: alanine transaminase, ALP: alkaline phosphatase, WBC: white blood cells, RBC: red blood cells, S.D: standard deviation
Parameters having significant associations in the Kruskal-Wallis test were further analyzed by the ROC curve. ROC curve analysis showed that urea, uric acid, ALP, Bilirubin, and potassium ions levels have a good diagnostic capability for COVID in IGRA-positive subjects as indicated by AUC values in Table 4.

Table 4: ROC Curve Analysis

Factor	Area under curve	P value	Cut-off value
Urea	0.709	0.035	5-20 (mg/dl)
Creatinine	0.664	0.100	0.6-1.3(mg/dl)
Uric acid	0.727	0.022	3.5-7.2(mg/dl)
ALP	0.698	0.046	44-147 U/L
Bilirubin	0.695	0.001	0.2-1.2 mg/dl
K	0.705	0.000	3.7-5.1mmol/lit

Discussion

The present study was undertaken to assess the susceptibility of COVID-19 in individuals who tested positive for Interferon-Gamma Release Assay (IGRA). Notably, Pakistan ranks among the top eight countries with a considerable number of incident tuberculosis (TB) cases, particularly in 2021, along with a high incidence of multidrug-resistant tuberculosis (MDR-TB) [19]. Consequently, the likelihood of developing latent TB infection increases, especially among certain high-risk groups such as children, household contacts of TB patients, and healthcare workers. In Pakistan, a significant portion of healthcare workers, approximately 40%, have been diagnosed with latent TB due to occupational exposure and contact with active TB patients [20]. These undiagnosed individuals with latent TB may experience heightened susceptibility to acquiring additional infections. Given that both TB and COVID affect the respiratory system, individuals already grappling with TB exhibit compromised lung function, rendering them more susceptible to secondary infections such as COVID, thereby exacerbating the situation. Consequently, it is imperative to allocate medical resources effectively, particularly for higher-risk groups. Given the small number of prior studies on the co-infection of COVID and TB in Pakistan, this study was conducted to provide preliminary evidence to clinicians for the improved management of co-infection cases.

The prevalence of COVID was 61% in IGRA-positive individuals signifying an elevated susceptibility to COVID among IGRA-positive individuals. Furthermore, gender, COVID vaccination status, the specific COVID-19 vaccine used for vaccination, and BCG vaccination status were identified as factors associated with COVID among IGRA-positive individuals. We did not find association of age with COVID in IGRA- positive subjects but a study states that coronavirus infection demonstrates a higher mortality rate among patients aged 18 years and older compared to those younger than 18 years,

necessitating more vigilant treatment protocols for adults [21]. Our study revealed a higher frequency of COVID-19 among IGRA-positive females. A study conducted in Wuhan, China in 2020, focusing on gender differences among COVID patients, reported a higher prevalence of COVID among males and older age groups [22]. The discrepancy in results may be attributed to our study's exclusive focus on IGRA-positive subjects. It is worth noting that IGRA is not routinely used as a TB diagnostic test in Pakistan; instead, it is typically recommended for patients undergoing various surgeries as a precautionary measure, similar to blood pressure and diabetes tests. Typically, there is a higher rate of female hospital visits and surgical procedures compared to males [23, 24]. The higher prevalence of IGRA positivity in females aligns with a previous study conducted in Brazil in 2022, which reported a higher prevalence of latent tuberculosis in females [25]. The higher prevalence of primary infection in females leads to an increased chance of developing secondary infection in the same gender i.e., females. Despite COVID vaccination, individuals in our study still developed COVID, potentially due to differences in vaccine efficacy. 29.8% IGRA IGRA-positive subjects in our study got COVID vaccination out of which 20.3% of subjects developed COVID-19 infection and 44.7% did not. To further validate our findings, additional research is needed to investigate COVID-19 reinfections following vaccination, particularly concerning the specific vaccine administered. The effectiveness and efficacy of these vaccines remain incompletely understood [26].

Some studies suggested that BCG induces innate immunity, which could play a protective role against COVID-19 [27]. Consistent with these studies we also found that in our study population, 23.5% of subjects had BCG vaccination with 12.8% COVID-positive and 40% COVID-negative subjects reflecting a passive effect of BCG vaccination for COVID infection. These results require further investigations on an extended number of individuals by considering other factors such as individual behaviors, regional differences, and healthcare access. COVID-19 infection can lead to renal dysfunction due to systemic inflammatory responses and the impairment of multiple organ functions. Literature reported renal injury in COVID patients [28]. Notably, renal damage has also been reported in TB patients, often associated with anti-tuberculosis treatments. Rifampicin, commonly used in TB treatment, forms complexes with rifampicin antibodies, which accumulate in blood vessels and the renal interstitium, ultimately affecting glomerular membrane function and renal function [29]. In the co-infectious state, a significant association was observed between biomarkers such as urea, creatinine, and uric acid and a decrease in their levels from the normal range in COVID-positive individuals compared to COVID-negative subjects. The normal range of urea, creatinine, and uric acid in the blood is 5-20mg/dl, 0.2-1.2mg/dl and 3.7-7.2mg/dl respectively. In IGRA-positive subjects in our study, we found a normal range of urea in COVID-positive (7.83) and COVID-negative (8.47) subjects. However, a decreased level of urea was found in COVID-positive subjects than in COVID-negative. Urea is a by-product originating in the liver which assists in nitrogen elimination and fluid balance regulation. Decreased levels of urea in COVID-positive subjects can be indicative of impaired liver or kidney function in TB-COVID co-infected subjects. Similarly, decreased uric acid level was found in COVID-positive subjects (5.78) than in COVID-negative (6.66). Uric acid is a by-product of protein metabolism. A portion of uric acid undergoes metabolism in the liver. Liver diseases or conditions that influence liver function can affect both the synthesis and metabolism of uric acid, potentially resulting in diminished levels in COVID-positive subjects. While creatinine is a chemical waste product produced from energy-generation processes within the muscles, the level of creatinine was elevated but decreased in COVID-positive individuals (1.39) than in COVID-negative (1.74) which might be caused by impaired muscle function.

Clinical manifestations of COVID often involve alterations in electrolyte levels and potassium imbalances. Our study revealed decreased potassium levels (hypokalemia) in COVID-positive individuals. The virus disrupts potassium ion balance by affecting the activity of epithelial sodium channels (ENaC). As the virus enters host cells through angiotensin-converting enzyme 2 (ACE2), it can stimulate increased renin-angiotensin-aldosterone system activity, leading to elevated aldosterone production. Aldosterone, in turn, influences the activity of epithelial sodium channels and results in potassium loss from epithelial cells [30]. Electrolyte imbalances are also observed in TB, contributing to both hyponatremia and hypocalcemia. Hypokalemia, in particular, can be caused by anti-tuberculosis medications administered to active TB patients, and it can lead to complications in individuals with multi-drug-resistant TB [31]. Hypokalemia was also recorded in our study in COVID-positive subjects. A normal range of potassium ions (3.5-5.1 mmol/L) was recorded in both groups, but it decreased in COVID-positive (4.46) individuals than in COVID-negative (4.85). A decrease in potassium ions level can have numerous effects on the body, due to the critical role potassium plays in several physiological functions, including muscle weakness and fatigue, cardiac arrhythmias, impaired nerve function, impaired respiratory function and digestive issues in co-infected individuals. Since our study included both active and latent TB patients, further investigations on electrolyte levels in active versus latent TB are required to validate the findings in the context of COVID-latent and COVID-active TB co-infections.

Markers of liver injury, such as elevated alkaline phosphatase (ALP), have been previously reported in COVID-19 patients. The virus can damage the liver by injuring bile duct cells [32]. Similarly, altered liver function test (LFT) levels have been reported in TB patients, often due to hepatotoxicity induced by anti-tuberculosis therapy. Notably, the combination of isoniazid and rifampicin leads to more hepatotoxicity than either drug alone [33]. Consistent with the previous research, an increased level of bilirubin is observed in our study in COVID-positive (1.61) as compared to COVID-negative (1.38) subjects indicating liver damage in a COVID-TB co-infectious state provided the normal range of bilirubin is 0.2-1.2mg/dl. Bilirubin is generated through the breakdown of old or impaired red blood cells in both the spleen and liver, constituting a by-product of heme molecule degradation. Elevated levels of bilirubin might indicate that the body is breaking down red blood cells faster than the liver can keep up with. This might indicate hemolytic disease or an adverse reaction to a blood transfusion. Like bilirubin, an elevated level of ALP was observed in COVID-positive individuals (1.55) than in COVID-negative (1.27) given that the reference range of ALP was 0.73-2.45µkat/L. ALP is an enzyme found in the bloodstream. It helps break down

proteins in the body and exists in different forms, depending on where it originates. The liver is one of the main sources of ALP. Higher-than-normal levels of ALP in the blood may indicate a health concern with liver or gallbladder in IGRA-positive COVID positive individuals. However, distinguishing between latent and active TB and then examining COVID-latent and COVID-active TB states would provide a clearer understanding of co-infectious states. It is important to acknowledge the limitations of our study, including a relatively small sample size, gender bias in the data, and a limited number of true respondents. Additionally, inaccurate self-reporting of COVID status by patients may hinder a comprehensive understanding of co-infections. Furthermore, the lack of a national health database makes it challenging to obtain comprehensive data regarding the health status of TB patients. The prevalence of COVID also decreased in 2022, which contributed to the relatively small number of COVID-positive patients during the follow-up period. Additionally, the biochemical markers were only validated for IGRA-positive individuals. To gain a comprehensive perspective, these markers should also be validated for IGRA-negative subjects. Another limitation is the failure to differentiate between COVID-positive active and COVID-positive latent TB patients among IGRA-positive individuals. Despite these limitations, our study offers valuable insights into the susceptibility of COVID in TB patients, especially in a setting with limited data availability and a higher likelihood of follow-up loss. It reflects the co-infection scenario in the Rawalpindi and Islamabad cities and serves as a foundation for future research endeavors. Future studies with extended durations, larger sample sizes, longer follow-up periods, comprehensive data collection, and rigorous inclusion criteria will aid in elucidating the co-infection dynamics of respiratory diseases with diverse etiologies.

Conclusion

In conclusion, the emergence of the COVID-19 pandemic has inflicted significant disruption on the tuberculosis (TB) eradication initiatives in Pakistan. Individuals afflicted with TB often present with compromised lung function, rendering them more susceptible to secondary infections, such as COVID-19, resulting in a challenging co-infection scenario that demands specialized attention and treatment. To effectively combat the co-occurrence of COVID-19 and TB and to strive for their complete eradication in Pakistan, it is imperative to identify high-risk groups and allocate resources accordingly to prioritize those at elevated risk. Within the framework of our study, we conducted an assessment of COVID-19 incidence in individuals who tested positive for Interferon-Gamma Release Assay (IGRA) and performed an analysis of biomarkers linked to the severity of COVID-19. Our investigation has pinpointed several biomarkers, including urea, creatinine, uric acid, alkaline phosphatase (ALP), bilirubin, and potassium ions, which exhibit significant associations with COVID-19. However, further research should be undertaken to investigate the status of these biomarkers in TB patients to validate and potentially employ common biomarkers in diagnostic assays for the co-infection of TB and COVID-19.

Conflict of Interest

The authors have no conflicts of interest to declare for this study.

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