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Research Article

Comparison of Biochemical Parameters in Vaccinated and Non-vaccinated SARS-COV-2 Patients in Erbil-City

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ABSTRACT

COVID-19 is one of the major pandemic diseases caused by SARS-CoV-2. Vaccination is one of the methods used that reduce the spread of an infectious disease. Consequently, we aimed to measure liver parameters (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total serum bilirubin (TSB), and lactate dehydrogenase (LDH) and albumin), as well as bone markers (Calcium, phosphorous, parathyroid hormone (PTH) with thyroid stimulating hormone (TSH) as marker for the severity of the disease in non-vaccinated and vaccinated SARS-COV-2 patients. Between November 2021 and May 2022, 130 samples of both genders (healthy and infected with COVID-19) were obtained and divided into three groups: Healthy individuals (50), vaccinated (30) and non-vaccinated (50) group" (ages; 20–65). The findings of the current study showed that ALT, ALP, and LDH significantly elevated ($P < 0.01$), and despite comparing patients who were not vaccinated to those who were, albumin considerably dropped. Moreover, AST and TSB moderately elevated in unvaccinated group in comparison to unvaccinated patients. Calcium and phosphorous slightly decreased in non-vaccinated and vaccinated patients as compare to control. Whereas PTH and TSH significantly less in both patient groups than in control. "The optimal cutoff values for parameters were determined by ROC curve analysis. In conclusion, vaccine plays a major role in minimizing liver biomarkers in COVID-19 patients. Moreover, low concentrations of bone markers were associated with both vaccinated and non-vaccinated COVID-19 patients. Therefore, the bone health status of COVID-19 patients and liver markers are helpful for the diagnosis and monitoring of disease severity in COVID-19 patient.

Keywords: Vaccine, bone markers, liver enzymes, biochemical, SARS-CoV-2

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an extremely infectious and pathogenic infectious disease carried on by the recently identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In March 2020, the World Health Organization (WHO) classified the COVID-19 outbreak as a pandemic disease.[1] "One of the biggest RNA viruses, with a genomic size of 27–32 kb, is the coronavirus. SARS-CoV-2 infects cells through binding to ACE2, a cell-surface receptor found in the kidney, blood vessels, heart, and crucially in alveolar epithelial Type II cells (AT2) which line the lungs".[2] The clinical manifestations of COVID-19 vary from mild to severe difficulty breathing, exhibiting symptoms such as fever, exhaustion, taste and smell loss, coughing, nose congestion, dyspnea, and so on.[3] Droplets from the respiratory system are the main method of transmission; SARS-CoV-2 can always spread through the air if an uninfected individual comes into contact with an infected or any of his possessions, such as clothing or doorknobs.[4,2]

Vaccination can be used to control the pandemic of SARS-COV-2. The risk of developing severe illnesses, requiring hospitalization, and dying from COVID-19 is decreased with COVID-19 vaccines in a safe and efficient manner.[5] Unprecedented international attempts to create vaccines to stave off infection and serious illness, reverse the pandemic's course, and lessen the terrible economic and societal effects of SARS-CoV-2 were rapidly initiated in the wake of the global SARS-COV-2 epidemic and the discovery of it.[6] The FDA approved a widespread immunization program to stop the spread of SARS-CoV-2 and lessen the severity of the disease in infected individuals. Two authorized vaccines based on messenger RNA (mRNA) are mRNA-1273 (Moderna Therapeutics Inc.) and BNT162b2 (Pfizer Inc/BioNTech

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SE).[7] The vaccination demonstrated great efficiency against numerous fresh forms and provided protection against reinfection. With the help of immunization programs, hope was raised for lowering the incidence of coronavirus sickness. The benefits of COVID-19 vaccination must be understood in the context of sickness attenuation. To do this, research should be done to see if those who get COVID-19 despite immunization have fewer symptoms than those who are unvaccinated.[8] A systematic review by Fatima *et al*.[9] of 51 research from 14 different countries found that vaccinations were effective against 89.1%, 97.2%, 97.4%, and 99% of illnesses, hospitalizations, and ICU admissions, respectively.

For evaluation, clinical care, and the prevention of severe consequences, efficient biomarkers would be helpful.[10] A significant predictor of COVID-19 patient death has also been found to be liver function. According to a research report, the virus may directly attach to cholangiocytes that are ACE2-positive, and as a result, COVID-19 patients' liver problems might be caused by cholangiocyte malfunction.[11] Early in the pandemic's development, liver involvement determined by abnormal liver parameters in infected individuals was regarded as one of the clinical symptoms. Major liver damage is rare. SARS-COV-2 could impact the liver directly or indirectly. Liver abnormalities can result from a variety of pathogenic mechanisms, including immune-mediated injury brought on by the intense inflammatory response, direct cytotoxicity brought on by viral replication in the hepatocytes, anoxia brought on by COVID-19-related respiratory failure, pharmacological liver damage primarily caused by hepatotoxic antivirals, reactivation of pre-existing liver disease, and vascular injury brought on by hypercoagulation.[12] According to the research that is now available on COVID-19, patients who are male, fat, and elderly are more likely to suffer liver dysfunction. Liver injury is caused by multiple causes, such as a direct cytopathic effect, a strong inflammatory response, the unique effects of treatment regimens, and cytokine storm.[13] Based on additional studies, COVID-19 frequently manifests as liver disease, which is connected to a higher mortality rate.[14] "Patients with COVID-19 have been found to frequently have increased levels of the serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TBIL), and gamma-glutamyl transpeptidase (GGT)", along with decreased levels of albumin (ALB).[15,16] Hence, these parameters can be useful marker to evaluate liver functions in patient groups.

Reduced bone mineral density and other musculoskeletal symptoms have been linked to SARS-COV-2 infection. These symptoms could be brought on by a number of disease-related variables, including medication, mobility restrictions, or diet.[17] To infect host cells, SARSCoV-2 interacts with ACE2 receptors that exist on cell targets, including bone cells.[18] Bone fragility may result from ACE2 downregulation's impact on bone homeostasis.[19] "The balance of P and Ca in the body, which is essential for many physiological functions, including bone health, muscular function, nerve transmission, and cellular signaling, is maintained by phosphocalcium metabolism".[20] Another research investigation[21] demonstrated that COVID-19 and its therapy had negative impacts on COVID-19 survivors' bone health. These effects tend to be stronger in older and weak patients, thus it is important to regularly monitor their risk of bone loss and falls. Furthermore,[22] discovered that greater phosphate concentration might be related to improved lung CT imaging consequences in SARS-COV-2, whereas hypophosphatemia is related to serious lung damage. In earlier research by Yang *et al*.,[23] hypophosphatemia was seen in patients with chronic obstructive pulmonary disease (COPD) and acute lung failure. It has also been noted that the virus-induced illness has abnormal serum Ca concentration. The parathyroid hormone produced by parathyroid glands, is vital in controlling phosphorus and calcium concentration in bone, kidneys, and digestive system.[22] In addition,[24] investigated that COVID-19 might influence parathyroid gland function in a pair of manners: Directly through COVID-19 infection of parathyroid glands, and indirectly as a result of chronic respiratory alkalosis and lung failure, it could be interpreted as extra explanations for why hypocalcemia is common in COVID-19 individuals.

The hormones thyroid stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) control the hypothalamus-pituitary-thyroid axis.[25] Medically, COVID-19 individuals who have low TSH and FT3 concentration seem to have a greater rate of mortality, which is not exactly in line with earlier investigations of the non-thyroidal illness syndrome (NTI).[26] Also, Zaidi *et al*.[27] reported that lowered TSH signaling is a factor in hyperthyroid osteoporosis. Moreover, Deng *et al*.[28] indicated that through controlling osteoblast proliferation and differentiation, TSH lowers the incidence of osteoporosis. In addition, Yanachkova *et al*.[29] made the suggestion that COVID-19 might have negative long-term effects on thyroid function. TFT should therefore be included in the follow-up algorithm even for individuals presenting with mild-to-moderate COVID-19. These variables which are thought to be COVID-19 indicators will help with patient care and epidemic containment. The present study aimed to assess the value liver parameters including (AST, ALT, ALP, LDH, TSB, and Albumin), as well as bone markers such as (Ca, P, PTH, and TSH) to diagnosis and severity of disease in non-vaccinated and vaccinated SARS-COV-2 Patients.

MATERIAL AND METHODS

Participants

This study recruited 130 participants in total between November 2021 and May 2022. The patients (aged from 20 to 65 years) diagnosed in two hospitals in Erbil city, Erbil emergency hospital, and Lalav emergency that were confirmed through PCR using sample from pharyngeal or nasal swabs. The patients were split into two groups: the unvaccinated group, comprising 26 male and 24 female patients, who complained of mild to severe manifestations; and the vaccinated group, consisting of 26 male and four female patients, who had received two doses of the BNT162b2 ("Pfizer-BioNTech") vaccine before becoming ill and being admitted to the hospital. In addition, there were a total of 50 non-infected people (23 men and 27 women).

Blood Samples

For every participant, a vein-based blood sample (approximate 3 ml) was taken from all, and it was immediately put into

a serum-separating gel tube. Whole blood was allowed to coagulate in gel tubes for 30 min. To obtain serum for serological and biochemical testing, the specimens were centrifuged for 5 min at 4000 RPM. Cobas E411-Roche (Germany) was used to measure PTH and TSH levels. Cobas C111-Roche (Germany) was used to estimate the liver parameters as well as the remaining bone markers.

Statistical Analysis

Graph Pad Prism 9.0 was used for analyzing the data. The standard error of the mean (SE) and mean for non-parametric variables were accurately stated. The One-way ANOVA (Kruskal–Wallis Test) was utilized to assess between-group comparisons for categorical variables using the liver variables and bone markers data provided in this study. The study's predictive value determined severity through receiver operating characteristic (ROC) curve in which Area under the curve (AUC), cutoff value, specificity, and sensitivity were utilized to show the results. To evaluate statistical significance, a $P = 0.05$ utilized as significant. Non-significant group differences are shown by the sign of (ns), whereas highly significant group differences between patients and healthy control groups are indicated by the sign of (*).

RESULTS

Statistical analysis of the current study summarized in [Table 1 and Figures 1-5]. The values of liver function tests such as (ALT, AST, ALP, and LDH) were significantly increased in unvaccinated COVID-19 patients when compared with vaccinated patients and the control healthy group. The mean ± SD values of ALT were (83.73 ± 127.8) in unvaccinated individuals, (29.06 ± 17.91) in vaccinated patients, and (20.45 ± 9.05) in the control group. ROC evaluation identified that the best cutoff value of ALT for determining severity in unvaccinated COVID-19 patients was > 29.70 U/L (sensitivity = 77.78 %; specificity = 91.67%; AUC= 0.879) with $P < 0.0001$, while in vaccinated patients was 20.85 U/L (sensitivity = 78.89 %; specificity = 56.25%; AUC = 0.697) with P value = 0.0027. The AST values were (56.15 ± 65.86 in unvaccinated patients, 28.57 ± 23.47 in vaccinated and 21.63 ± 12.08 in control group). By using the ROC evaluation recognized that the best cutoff value of AST for determining severity in unvaccinated COV-2 patients was > 21.65 U/L (sensitivity= 77.27 %; specificity = 62.50 %; AUC= 0.741) with $P < 0.0001$, in vaccinated patients was 22.65 U/L (sensitivity, specificity and AUC were 54.55 %, 64.58 % and 0.597, respectively) with $P = 0.137$. In addition, the mean concentration of ALP in unvaccinated, vaccinated, and control were (190.9 ± 138.0, 104.9 ± 87.52, and 64.99 ± 20.49), respectively. Based on ROC curve studies, in the vaccinated group, the AUC was 0.635 and the cutoff value serum of ALP level was > 65.43 U/L with a sensitivity of 66.67 % and specificity of 52.08 %. While the non-vaccinated group occupied a significant AUC, which was 0.850 ($P < 0.0001$); the cutoff value was > 85.65 U/L of serum TSH level,

Table 1: Comparison of liver parameters between control and patient groups

Liver Markers	Control	Patients		*P* value
		Vaccinated	Non-vaccinated	
ALT (IU/l)	20.45±9.05	29.06±17.91	83.73±127.8	0.0001
AST (IU/l)	21.63±12.08	28.57±23.47	56.15±65.86	0.0001
ALP (IU/l)	64.99±20.49	104.9±87.52	190.9±138.0	0.0001
TSB (mg/dl)	0.72±0.33	0.58±0.32	0.78±0.43	0.0873
LDH (IU/l)	271.1±103.9	290.8±82.19	621.6±277.6	0.0001
Albumin (mg/dl)	4.80±0.29	4.65±0.57	3.79±0.86	0.0001

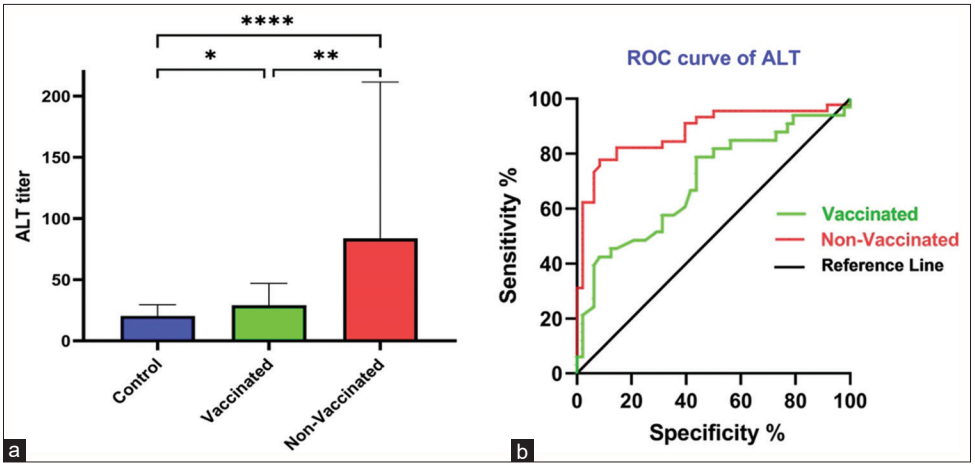

Figure 1: (a) Comparison of ALT concentration in control and patient groups. (b) The area under the curve value shows ALT as a biomarker of the patients' group

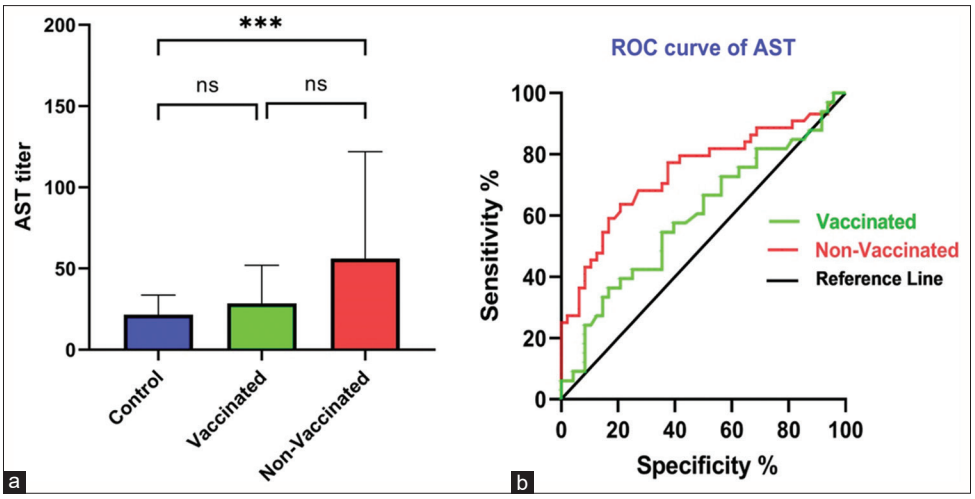

Figure 2: (a) Comparison of AST concentration in control and patient groups. (b) The area under the curve value shows AST as a biomarker of the patients' group

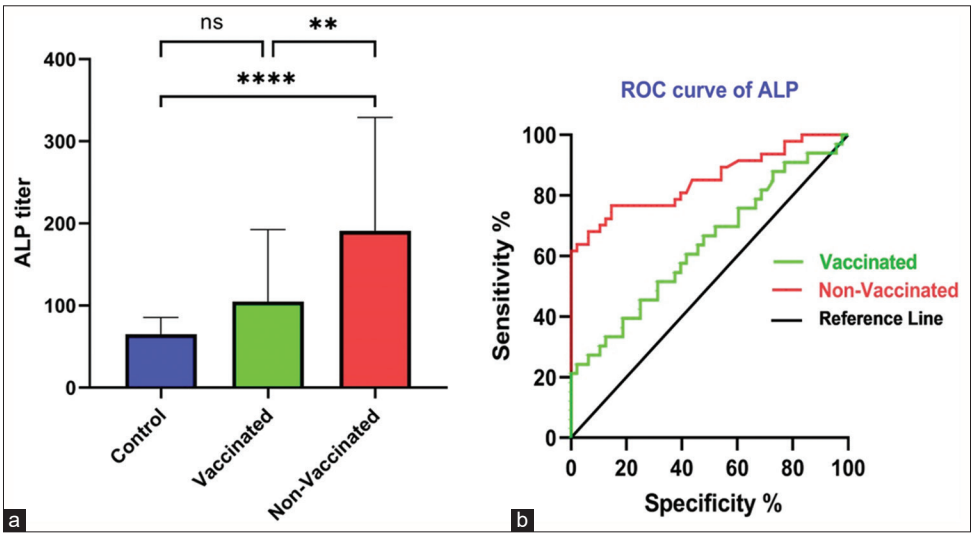

Figure 3: (a) Comparison of ALP concentration in control and patient groups. (b) The area under the curve value shows ALP as a biomarker of the patients' group

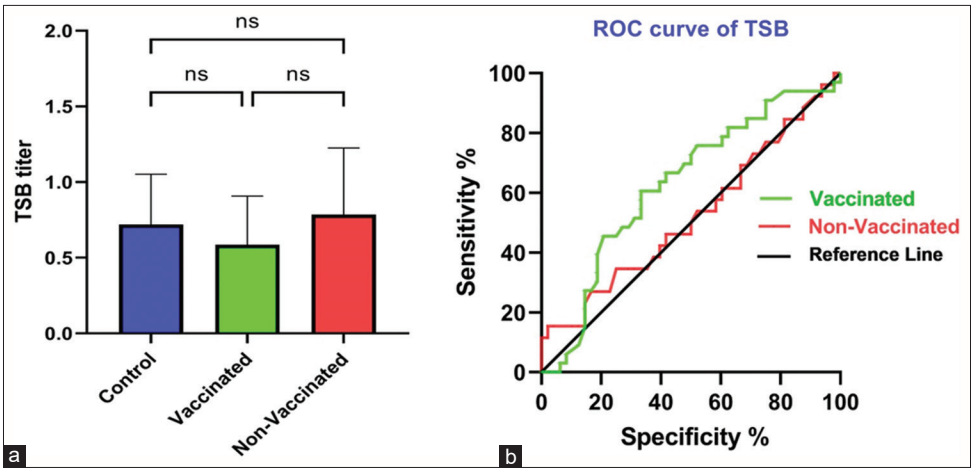

Figure 4: (a) Comparison of TSB concentration in control and patient groups. (b) The area under the curve value shows TSB as a biomarker of the patients' group

the sensitivity and specificity were 76.60 % and 85.42 %, respectively [Table 2 and Figure 3b].

Moreover, the mean of LDH value significantly rose to (621.6 ± 277.6) in unvaccinated patients and (290.8 ± 82.19) in vaccinated patients as compared to the control group (271.1 ± 103.9) with p value of < 0.0001. "The cutoff values of LDH for predicting severity in COVID-19 patients were determined by (ROC) curve analysis for unvaccinated 337.0 U/L (sensitivity= 83.33%; specificity=81.25 %; (AUC) = 0.898), vaccinated 278.0 U/L (sensitivity, specificity, and AUC were 66.67 %, 68.75 % and 0.606, respectively)". As shown in [Table 1 and Figure 4], the mean ± SD of total bilirubin slightly increased from (0.72 ± 0.33) in the control group to (0.78 ± 0.43) in unvaccinated patients, while this value declined to (0.58 ± 0.32) in the vaccinated group but did not reach at a significant level. The results of the (ROC) curve indicated that the cutoff values of TSB for predicting severity in COVID-19 patients were <0.565 mg/ml (sensitivity, specificity, and AUC were = 60.61%, 66.67%, and 0.627, respectively) in the vaccinated group and <0.985 mg/ml (sensitivity = 34.62%; specificity = 75.00%; (AUC) = 0.523) in unvaccinated groups.

On the other hand, serum albumin level is crucial biomarkers in SARS-COV-2 patients. The mean value of albumin significantly ($P < 0.0001$) decreased from (4.80 ± 0.29 mg/dl) in control group to (3.79 ± 0.86 mg/dl and 4.65 ± 0.57 mg/dl in unvaccinated and vaccinated patients, respectively. As shown in Table 1 and Figures 6a and b, cutoff values of albumin to identify severity in COVID-19 patients were determined

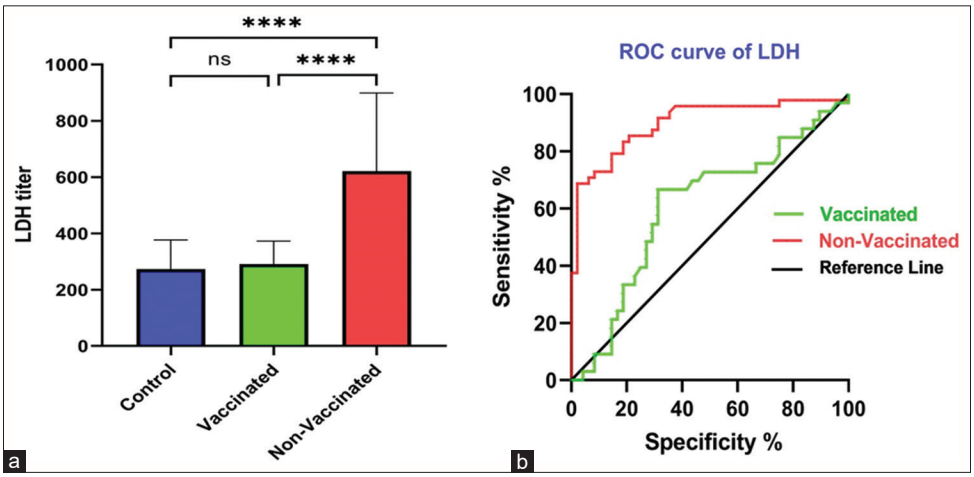

Figure 5: (a) Comparison of LDH concentration in control and patient groups. (b) The area under the curve value shows LDH as a biomarker of the patients' group

Table 2: ROC curve analysis of Liver biomarkers in SARS-COV-2 patients

Liver markers	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	*P* value
ALT (IU/l)					
Vaccinated	0.697	20.85	78.79	56.25	0.0027
Non-Vaccinated	0.879	29.70	77.78	91.67	0.0001
AST (IU/l)					
Vaccinated	0.597	22.65	54.55	64.58	0.1375
Non-Vaccinated	0.741	21.65	77.27	62.50	0.0001
ALP (IU/l)					
Vaccinated	0.635	65.43	66.67	52.08	0.0392
Non-Vaccinated	0.850	85.65	76.60	85.42	0.0001
TSB (mg/dl)					
Vaccinated	0.627	0.565	60.61	66.67	0.0516
Non-Vaccinated	0.523	0.985	34.62	75.00	0.7426
LDH (IU/l)					
Vaccinated	0.606	278.0	66.67	68.75	0.1053
Non-Vaccinated	0.898	337.0	83.33	81.25	0.0001
Albumin (mg/dl)					
Vaccinated	0.618	4.605	51.52	76.19	0.0800
Non-Vaccinated	0.821	4.535	76.00	85.71	0.0001

through ROC curve analysis. For the unvaccinated group, these values were < 4.535 mg/dl, (sensitivity = 76.00%; specificity = 85.71%; AUC = 0.821); for the vaccine-receiving group, they were < 4.605 mg/dl, (sensitivity = 51.52%; specificity = 76.19%; AUC = 0.618).

The level of bone markers shown in (Table 3), in which the mean ± standard deviation of Ca level values decreased somewhat, from 9.40 ± 1.19 mg/dl in the control group to 8.88 ± 1.28 mg/dl and 9.27 ± 1.21 mg/dl in the vaccinated and unvaccinated patient categories, respectively, but not to a significant degree. Table 4 and Figures 7a and 7b demonstrate the optimal cut-off values of calcium for predicting severity in COVID-19 patients. Vaccinated patients had cutoff values of less than 9.635 mg/ml, with sensitivity, specificity, and AUC of 75.76%, 47.92%, and 0.616, respectively. Non-vaccinated patients had cut-off values of less than 9.635 mg/ml, with sensitivity, specificity, and AUC of 66.67%, 47.92 %, and 0.540, respectively. Regarding the concentration of the serum phosphate, we found that value of phosphate significantly decreased from (3.85 ± 0.48 mg/dl) in healthy individuals to (3.35 ± 0.74 mg/dl) in vaccinated patients and somewhat reduced to (3.65 ± 1.02 mg/dl) in patients who did not take

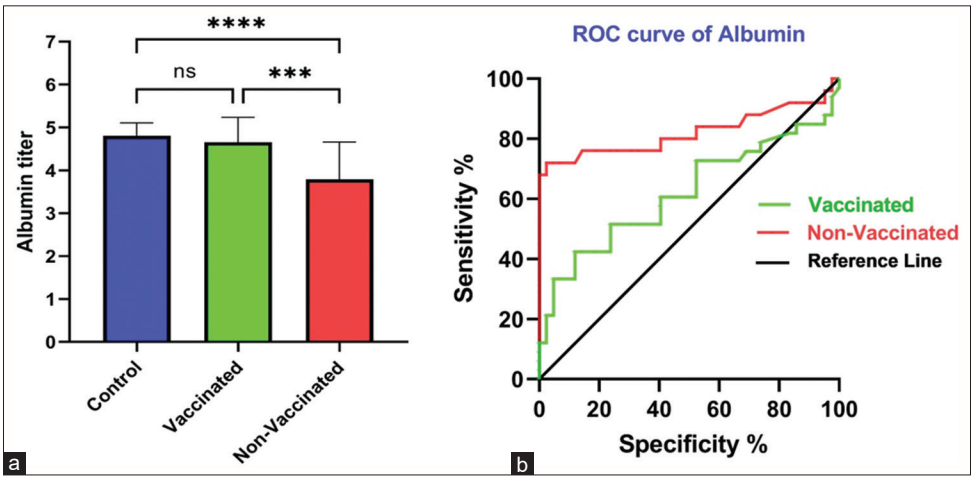

Figure 6: (a) Comparison of Albumin concentration in control and patient groups. (b) The area under the curve value shows Albumin as a biomarker of the patients' group

Table 3: Comparison of bone markers between control and patient groups

Bone Markers	Control	COVID-19 Patients		*P* value
		Vaccinated	Non-Vaccinated	
PTH (pg/ml)	45.53±21.37	25.96±16.81	21.06±15.22	0.0001
TSH (uIU/ml)	2.79±2.70	1.68±0.95	1.52±1.28	0.0295
Calcium (mg/ml)	9.40±1.19	8.88±1.28	9.27±1.21	0.2188
Phosphate (mg/ml)	3.85±0.48	3.35±0.74	3.65±1.02	0.0022

Table 4: ROC curve analysis of Bone markers in SARS-COV-2 patients

	AUC	Cut off value	Sensitivity (%)	Specificity (%)	*P* value
PTH (pg/ml)					
Vaccinated	0.814	27.76	73.53	88.64	0.0001
Non-vaccinated	0.875	27.34	82.35	88.64	0.0001
TSH (uIU/ml)					
Vaccinated	0.594	3.705	100.0	23.40	0.1588
Non-vaccinated	0.679	1.025	46.43	91.49	0.0096
Calcium (mg/ml)					
Vaccinated	0.616	9.635	75.76	47.92	0.0754
Non-vaccinated	0.540	9.635	66.67	47.92	0.5046
Phosphate (mg/ml)					
Vaccinated	0.762	3.66	74.07	70.45	0.0002
Non-vaccinated	0.605	3.43	46.67	90.91	0.1259

vaccine against SARS-COV-2; however, it did not reach at a significant level as demonstrated in [Figure 8 and Table 2]. The ROC was used to estimate the values of cut-off for phosphate that determine severity in SARS-COV-2 patients in vaccinated were < 3.66 mg/dl, sensitivity = 74.07 %; specificity = 70.45%; AUC= 0.762 with $P = 0.0002$ and non-vaccinated < 3.43 mg/ml, sensitivity = 46.67 %; specificity = 90.91 %; AUC = 0.605 [Table 4].

Following the statistical analysis, as Table 2 and Figure 9a show, it was found that the level of the serum PTH in the vaccinated and unvaccinated COVID-19 groups considerably less than what the healthy group's serum PTH levels showed ($P < 0.0001$), while the serum levels of PTH between COVID-19 patient groups were non-significant. In healthy group, the mean ± SD of parathyroid hormone was 45.53 ± 21.37 pg/ml, whereas in a vaccinated group of patients, it was 25.96 ±16.81 pg/ml, and in unvaccinated group of patients was 21.06 ± 15.22 pg/ml. ROC analysis was carried out to assess the diagnostic significance of PTH levels among Covid-19 patients. ROC curve results show that the AUC in vaccinated and non-vaccinated patients respectively were 0.814 and 0.875 with a *P*-value < 0.0001. Thus, the cutoff value of PTH to predict disease severity, sensitivity and specificity for vaccinated were (27.76 pg/ml, 73.53 % and 88.64 %), unvaccinated group was (27.34 pg/ml, 82.35, and 88.64%) respectively, [Figure 9b and Table 4]. When comparing the non-vaccinated group patients to the control category, a significant drop in TSH was seen (P = 0.0295), whereas the serum concentration of TSH between vaccinated and non-vaccinated COVID-19 patients was non-significant [Figure 10a]. The mean value of healthy individuals significantly diminished from (2.79 ±2.70 uIU/ml) to (1.68 ± 0.95 uIU/ml) and (1.52 ± 1.28 uIU/ml) in vaccinated and unvaccinated groups, respectively. To determine the diagnostic value of TSH, ROC analysis was performed. In vaccinated group, the AUC was 0.594 and the cutoff value serum of TSH level was 3.705 uIU/ml with a sensitivity of 100.0 % and specificity of 23.40%. While the non-vaccinated group occupied a significant AUC, which was 0.679 ($P = 0.0096$); the cutoff value was 1.025 uIU/ml of serum TSH level, the sensitivity and specificity were 46.43 % and 91.49%, respectively [Figure 10b and Table 4].

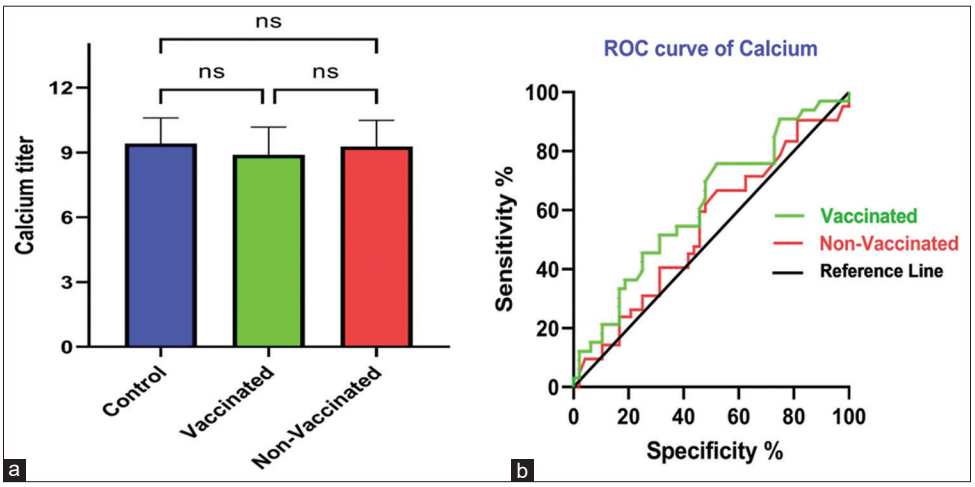

Figure 7: (a) Comparison of Calcium concentration in control and patient groups. (b) The area under the curve value shows Calcium as a biomarker of the patients' group

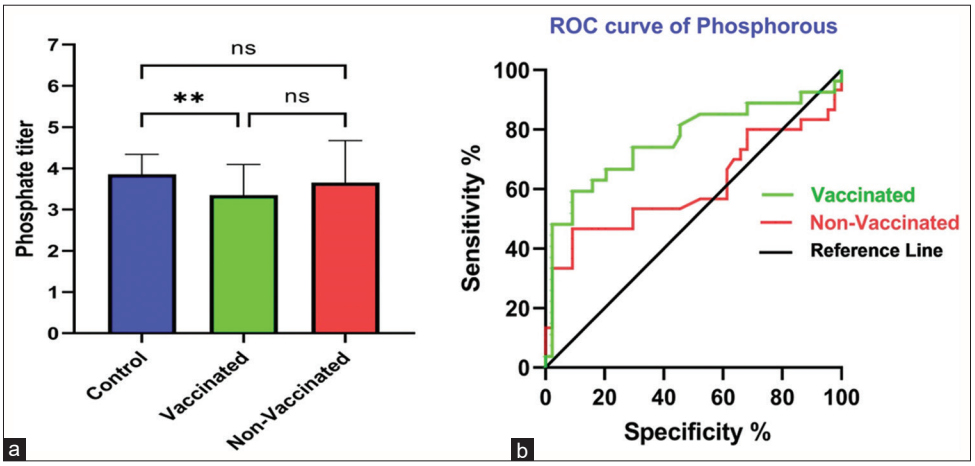

Figure 8: (a) Comparison of phosphate concentration in control and patient groups. (b) The area under the curve value shows phosphate as a biomarker of the patients' group

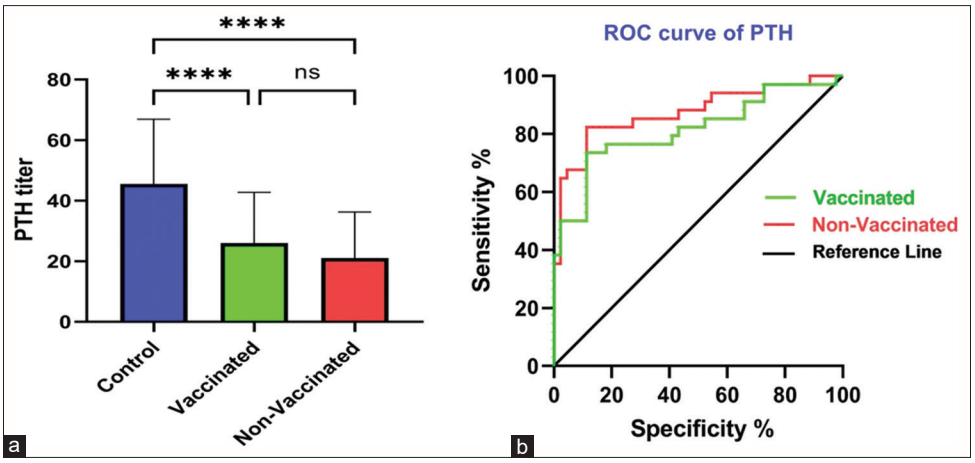

Figure 9: (a) Comparison of PTH concentration in control and patient groups. (b) The area under the curve value shows PTH as a biomarker of the patients' group

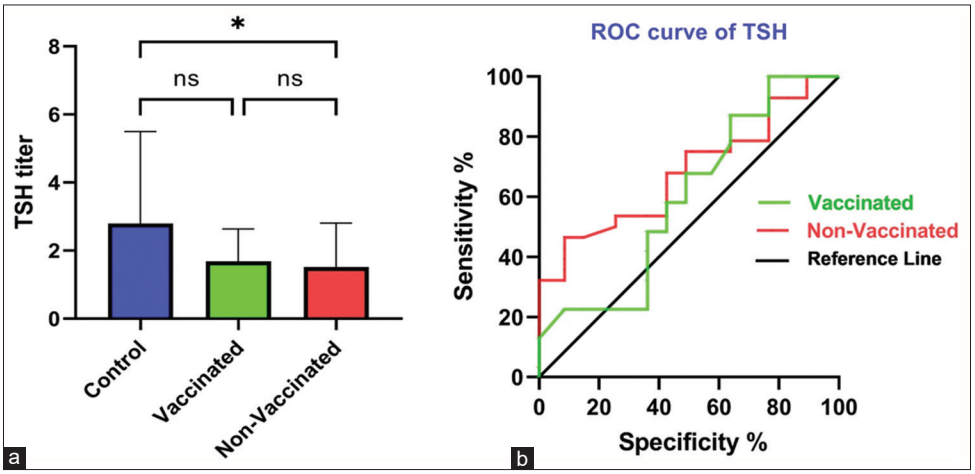

Figure 10: (a) Comparison of TSH concentration in control and patient groups. (b) The area under the curve value shows TSH as a biomarker of the patients' group

DISCUSSION

"COVID-19 represents a wide spectrum of clinical presentations, from asymptomatic to critical pneumonia, acute respiratory distress syndrome (ARDS), and even death.[30] In this study, vaccinated and non-vaccinated COVID-19 patients were admitted. Laboratory tests provide critical support for the proper clinical management of COVID-19 from screening to diagnosis, prognosis, and monitoring.[31] Liver damage is prevalent in COVID-19 patients, and it can be brought on by an antiviral medication's functional impairment or a viral infection of the bile duct cells. Changes in the primary liver disease should be monitored in patients with liver illnesses, and individuals with severe diseases should have more frequent monitoring and evaluation of their liver function during medication.[32] According to the results of the present study, the alterations were identified in the average levels of ALT, AST, ALP, LDH, TSB, and Albumin." This result supported by Wu *et al*.[33] who stated that decreased albumin levels and increased GGT, AST, and ALT levels are present in individuals with severe COVID-19. Liver tests including the liver enzymes should be monitored for diagnosing liver damage.[34] While some research revealed no correlation between abnormal LFTs and death, disease progression, ICU admission, or length of hospital stay, other studies identified a relationship between abnormal liver function tests (LFTs) and increased disease severity and death rates, particularly raised AST and ALT.[35] The higher expression levels of ACE2 in the cholangiocytes and the changes in liver parameters in individuals with acute and prolonged COVID-19, without concurrent increase of TSB levels, point to a persistent systemic inflammatory response in these patients.[36] Patients with severe COVID-19 had higher levels of AST and ALT, according to findings from China.[37-39] Numerous investigations have revealed varying degrees of elevated liver enzymes in COVID-19 individuals, primarily manifested by abnormal amounts of AST and ALT, along with modestly raised levels of GGT, ALP, and TSB.[40] "In addition, Piano *et al*.[41] showed that LFTs abnormality is commonly observed on admission in patients with COVID-19 and it is associated with systemic inflammation, organ dysfunction and is an independent predictor of transfer to the ICU or death during the hospitalization. In COVID-19 disease a similar trend was found; analysis of 11 studies Aziz *et al*.[42] which showed that the mean serum of albumin on admission was 3.50 g/dl and 4.05 g/dl in severe and non-severe COVID-19,

respectively". Our results also are consistent with a recent study by Petimani *et al*.[43] who showed that total and direct bilirubin, ALT, AST, and ALP levels were highly increased in unvaccinated group. As well as fewer levels serum albumins and total protein were noted in the unvaccinated patients compared to vaccinated individuals. In addition, in their retrospective analysis,[44] found that the concentration of serum ALT and AST in unvaccinated patients with COV-2 significantly higher (P<0.01) than those in vaccinated group patients. There is strong evidence that the onset of COVID-19 illness is correlated with LDH levels.[45] According to a study, intensive care unit (ICU) patients had far higher levels of LDH than non-ICU patients (248 U/L vs. 151 U/L, $P = 0.002$). LDH might be a severe disease indicator that can be predicted.[46] Furthermore, Malik *et al*.[47] found a significant association of COVID-19 severity with different biomarkers among them elevated levels of ALT and LDH. likeness to our study, the findings of a prior study conducted by Fatima *et al*.[9] who documented that LDH value significantly decreased from (419 IU/L) in non-vaccinated patients to (367 IU/L) in vaccinated individuals. This finding is in line with research conducted by Raza *et al*.[8] who explained that the mean blood LDH concentration was much higher ($P < 0.01$) in unvaccinated patients than in vaccinated group. Elevated quantities of LDH are indicative of cellular damage brought on by plasma membrane destruction, and pulmonary damage can be well predicted by measuring LDH levels in the early stages of severe COVID-19 patients.[48] Another research that in line with our findings[49] demonstrated that LDH levels were much lower in patients who received vaccines than those who did not receive vaccines. Thus, given that altered hepatic enzymes have been linked to an increased risk of ICU support, death rates, ARDS, and comorbidities, the effect of vaccination status on liver markers might be considerable.

The process of bone mineralization requires the minerals calcium and phosphorus. These two elements can be dissolved in serum or located intracellularly in soft tissues; they are present in bones.[20] Three hormones mainly regulate phosphorus and calcium levels in the body: parathyroid hormone (PTH), fibroblast growth factor and Vitamin D 25 (OH).[50] According to our result, we found that the concentration of PTH in vaccinated and unvaccinated COVID-19 patients was significantly lower than that in the serum PTH of the healthy group, additionally, calcium and phosphate decreased in COVID-19 patients when compared to the control group. "The first report of a severely hypocalcemic COVID-19 patient was made in April 2020. Since then, several studies have reported that hypocalcemia is correlated with inflammation, biomarkers of thrombosis, disease severity, and mortality in COVID-19 patients".[21] Our findings were consistent with the study of Yang *et al*. and Cappellini *et al*.[23,51] they arrived at the conclusion that deficiencies of calcium and phosphorus could serve as promising clinical biomarkers for discriminative assessment, suggesting the severity of COVID-19 patients. Studies have found that patients with osteoporosis are more likely to be infected with SARS-COV-2 and have more severe osteoporosis after SARS-COV-2, and some patients have osteoporosis as a complication.[18] The research results of Elham *et al*.[52] demonstrated that COVID-19 patients' serum concentration of calcium, Vitamin D, and zinc were lower than those of the healthy groups. Furthermore, Cappellini *et al*.[51] documented that Serum calcium modifications may be due to alterations in intestinal absorption, imbalance in the regulatory mechanism involving PTH and D-Vitamin, or a direct effect caused by SARS-COV-2. Similarity, Bajpai *et al*.[53] compared the severity of COVID-19 Pneumonia in Vaccinated with Non-vaccinated Patients from a Tertiary Care Center in India, they demonstrated that serum calcium slightly fewer in non-vaccinated group than in patients who received vaccine.

Serum phosphate dysregulation in COVID-19 patients may be linked to many disorders. Low serum phosphorus is associated with a number of clinical problems, especially in critically ill and ICU patients.[54] A study of Javdani *et al*.[22] demonstrated how hypophosphatemia is linked to serious lung damage, while greater phosphate levels may be associated with improved lung outcomes from COVID-19 computed tomography (CT) scans. This may establish a connection between the parathyroid gland and COVID-19 and aid physicians in the management of hospitalized patients. Hypophosphatemia in COVID-19 individuals can be caused by renal loss, large bowel dysfunction, acute kidney injury (AKI), insufficient intake, and pulmonary alkalosis.[54,55] Severe and critically ill COVID-19 patients have disrupted Ca and P homeostasis. For individuals with COVID-19 at a severe or critical stage, it is crucial to increase the monitoring of serum Ca and P levels and to start treatment as soon as possible to improve the prognosis.[23] The findings of Abobaker *et al*.[24] stated that there are two potential ways in which COVID-19 may impact parathyroid gland function: directly, through the SARS-CoV-2 virus invading parathyroid gland tissues, or indirectly, through lung damage and chronic alkalosis of the lungs. These latter two mechanisms may be further explanations for the high incidence of hypocalcemia in COVID-19 patients. Inflammatory cytokines and immune system activation can lead to alterations in the concentrations of parathyroid hormone, phosphorus, and calcium.[50]

In COVID-19 patients, thyroid function problems are common, particularly in severe cases. The non-thyroidal disease syndrome could help to partially explain this.[56] On the basis of our study, the serum level of TSH significantly decreased in unvaccinated COVID-19 patients and somewhat depletion in vaccinated individuals as compared to the control group. Omit it that results are in line with previous study of Gong *et al*.[26] who indicated that decreased TSH levels were a separate risk factor for death in these patients, and that reduced FT4 and TSH levels were linked to mortality in individuals with both COVID-19 and non-thyroidal disease syndrome. Furthermore, Chen *et al*.[25] reported that after admission, the TSH and FT4 levels of verified COVID-19-positive individuals were low, but on their recovery, the levels rebounded to normal. Similarity, Khoo *et al*.[57] conducted a research on 456 individuals in London and found modest decreases in TSH and FT4, consistent with a non-thyroidal disease condition. On the other hand, in a study of Chen *et al*.[58] in China of 50 patients with COVID-19, they noted a generalized reduction in TSH, T4, and T3 more consistent with a non-thyroidal illness pattern. The studies indicated that TSH inhibits osteoclastogenesis, which protects bone.[59] It' is been suggested that TSH might directly inhibit bone turnover by acting on both osteoblasts and osteoclasts'

TSH receptors.[60] Hence, these clinical parameters are helpful for the diagnosis and monitoring of COVID-19 patients

Vaccinated persons are now generally acknowledged to be less risky than unvaccinated individuals. An Israeli study found that those who received the Pfizer-BioNTech vaccination had a higher level of long-term safety from COVID-19 syndrome than those who did not receive the vaccination.[61] An additional study conducted in Israel using data from 5526 unvaccinated COVID-19 cases revealed that 4.6 out of 100,000 infected persons required hospitalization, compared to 0.3 out of 100,000 in the vaccinated community. Only 596 cases were available for the vaccinated population, though. Comparably, the statistics showed that for the unvaccinated group, the death rate was 0.6 per 100,000, while for the vaccinated population, it was 0.1 per 100,000.[62] In additions, Havers *et al*.[5] According to the results of their descriptive investigation, unvaccinated adults' hospitalization rates for COVID-19 were more than ten times greater than those of vaccinated individuals during the 1st year the vaccine was made available in the US.

CONCLUSION

This study demonstrates that COVID-19 vaccination can be helpful at minimizing the disease's severity. Vaccinated patients had milder disease with less abnormality of liver parameters and bone markers compared to the unvaccinated group. Increased serum level of liver enzymes including ALT, AST, ALP, TSB, and LDH, as well as decreased serum albumin were correlated with non-vaccinated COVID-19 patients when compared to the vaccinated group. In addition, according to bone markers, we found that SARS-COV-2 either directly or indirectly affects bone health. Low level of serum Ca, P, PTH, and TSH concentrations were associated with both vaccinated and non-vaccinated COVID-19 patients. Therefore, the bone health status of COVID-19 patients and liver biomarkers are important for the monitoring and diagnosis of disease severity in SARS-COV-2 patients. COVID-19 vaccines cannot prevent infection with SARS-COV-2, but they decrease hospitalization rate and disease severity in most patients.

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