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Original Article

Assessing the Dynamic Interplay of Omicron Spike Protein Receptor-Binding Domain (S-RBD) IgG Antibody Responses in Hospitalized COVID-19 Patients in Egypt

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ABSTRACT

Background: The SARS-CoV-2 Omicron variant (B.1.1.529) has raised concerns the reason behind them being having numerous spike protein mutations and potential impact on disease severity and immune response. This research set out to determine the level of anti-SARS-CoV-2 IgG antibodies targeting the receptor-binding domain (S-RBD) and correlate the antibody levels with COVID-19 infection severity and outcomes.

Methods: This prospective cohort study included 61 Sinopharm/Sinovac-vaccinated individuals who came down with SARS-CoV-2 infection. Patients were categorized as moderate, severe, or critically ill. Omicron,B.1.1.529 IgG (anti-S-RBD) antibody concentrations were measured using ELISA at admission and 3 weeks later, correlations with clinical parameters were analyzed.

Results: The median antibody concentration at ICU admission for all patients (n=61) was 30.2 ng/ml . The median antibody concentration after 3 weeks for survivor patients (n=43) was 58 ng/ml. A statistically significant negative correlation has been determined between antibody concentration and age. Antibody levels significantly correlated with COVID-19 severity and patient outcomes. The optimal cutoff for predicting mortality was ≤19.49 ng/ml (sensitivity 50%, specificity 72.1%), while the cutoff for critically ill disease was ≤18.43 ng/ml (sensitivity 100%, specificity 87.8%).

Conclusion: Serial (S-RBD) IgG antibody levels could function as a crucial prognostic marker for recognizing patients at risk of developing critical illness. The study highlights the future potential utility of antibody measurements in clinical risk stratification and patient management particularly when considered alongside vaccination history and previous infections. Further long term follow up studies with genetic verification are needed to establish causal relationships.
Keywords: SARS-CoV-2 variants; Spike Glycoprotein; Antibodies, Viral; COVID-19.

INTRODUCTION

COVID-19's causative agent, SARS-CoV-2, continually mutates, leading to the showing up of novel variants (1). These variants are categorized as variants of concern (VOC), variants of interest, or variants of high consequence based on their potential risk to public health (1,2). Four VOCs have been

identified: Alpha, Beta, Gamma, and Delta, with Omicron later added to this list (3). These variants share mutations that can increase transmissibility, severity of illness, and risk of reinfection while potentially reducing vaccine efficacy and protection from neutralizing antibodies (3,4).

South Africa has reported the discovery of a new strain of SARS-CoV-2. In the year 2021′11, the World Health Organization (WHO) designated this mutant as Omicron (B.1.1.529), a variation that raises concerns (5). In their study, Ismail et al. found the first case of SARS-CoV-2 Omicron in an Egyptian patient. Omicron variants found in South Africa had the highest level of similarity according to Genome BLAST (6).

The S glycoprotein is a class I viral fusion protein that is accountable for adherence of cells and viral fusion. It is a metastable prefusion homotrimer that consists of separate polypeptide chains ranging in length from 1,100 to 1,600 residues (7,8). Two separate sections, known as S1 and S2, make up each protomer of a S protein. The S1 subunit is a V-shaped polypeptide with four separate domains, namely A, B, C, and D (9). Domain B serves as the receptor-binding domain (RBD) for the majority of coronaviruses, including the pathogenic β-coronaviruses like Middle East respiratory syndrome (MERS) and SARS-CoV-2, severe acute respiratory syndrome (SARS). According to recent research, the SARS-CoV-2 RBD attaches to cells by interacting with the ACE2 receptor (10).

Regrettably, additional changes in spike proteins will probably certainly occur throughout 13 SARS-CoV-2 strains, possibly with higher pathogenicity (11).

With around 30 different mutations, the spike protein have been found in the newly emerged Omicron variant B.1.1.529, which has set off worries about the possibility of escape from vaccinations and therapeutic antibodies (12).

The greatest severity of B.1.1.529 infections is associated with the fact that the spike protein and human ACE2 interact more strongly than expected (5). Structure-based cryo-electron microscopy of the Omicron variant spike protein bound to human ACE2 has revealed the creation of novel salt bridges and hydrogen bonds through mutations in the RBD involving residues R493, S496 and R498. Similar biochemical ACE2 affinities of binding for Delta and Omicron variants are likely explained by these interactions, which seem to compensate for further mutations in Omicron like K417N, which are known to lower ACE2 binding affinity (7,8).

The degree to which the body reacts to an infection in nature is influenced, to some extent, by what severity the disease is (13,14).

To fight the Omicron variety, it is crucial to create neutralizing antibodies (nAbs). Omicron has a strong ability to evade the immune system because of mutations in its N-terminal domain (NTD), which change its antigenic structure, and in its spike receptor binding domain (RBD), which increase its affinity for angiotensin-converting enzyme 2 (ACE2), than to mutations like N501Y, R346K, and T478K (15).

The anti-receptor-binding domain (RBD) antibodies against SARS-CoV-2's spike (S) protein are a reliable, inexpensive, and accurate way to measure the effectiveness of the host defense mechanism against the virus because of their high degree of favorable association with the neutralizing antibodies (NAbs) (16).

Few studies focused on the role of S-RBD IggG Antibody responses and in the severity and outcome of Omicron subvariant infection though it could lead us to investigate novel clinical antiviral reagents more quickly and a new class of wide NnAbs as potential treatments for SARS-CoV-2 (15).

Our research set out to is to determine the level of anti-SARS-CoV-2 Omicron B.1.1.529 IgG antibodies against RBD as markers of the humoral response in Egyptians infected with SARS-CoV-2 and correlate level with severity and consequences of infection. Our secondary objectives are to evaluate the relationship between S-RBD IgG levels and clinical outcomes as well as to determine the influence of demographic factors on antibody response.

Methods

Calculation of the sample size:

Assuming that the rate of admission of adult COVID patients fulfilling the inclusion criteria at chest ICUs at Zagazig University hospitals is 10 cases per month so a comprehensive sample of 60 patients (6months period study) were enrolled in the study.

Subjects

This prospctive cohort comprised 61 vaccinated patients with with two doses Sinopharm or Sinovac SARS CoV-2 vaccines 2 years ago and infected with COVID 19. Al patients were admitted to Chest ICUs of Zagazig University Hospitals. They were collected from patients diagnosed , admitted at chest ICUs at Zagazig University Hospitals , Egypt, between December 2003 to June 2024.

Forty three patients survived throughout the study while eighteen patients died within the first three weeks of ICU admission.

The study included patients aged ≥18 years of both sexes with confirmed COVID 19 infection (by real time reverse transcriptase-PCR of nasopharyngeal swab and oropharyngeal swab samples) (17) (20) admitted to chest ICUs. Disease severity was classified as moderate (clinical symptoms with oxygen saturation ≥93% in room air), severe (clinical pneumonia with oxygen saturation <93% in room air, respiratory rate >30/min (19,20), or severe respiratory distress), or critical (ICU admission requiring mechanical ventilation or Fraction of inspired oxygen (FIO2) ≥60%) (21). Chronically ill individuals (those suffering from cancer and cardiovascular disease) were excluded as chronic health conditions (CHCs) can significantly impact antibody responses to SARS-CoV-2 infection and vaccination (22). Patients get vaccinated within the last 2 years were excluded.

Ethical consideration:

Patients or their legal guardians gave their informed written permission before taking any data and the study protocol was approved by Zagazig Faculty of Medicine Research Ethics Committee (IRB number: 11326-29-11-2023).

Clinical and Laboratory Assessment

Clinical assessment included collection of demographic data, symptom onset, course and duration, prior COVID-19 infection history, vaccination details, and physical examination findings including chest examination.

Diagnostic workup comprised chest computed tomography and laboratory investigations including complete blood count, C-reactive protein, serum ferritin, renal and liver function tests, SARS-CoV-2 PCR testing and assessment of SARS-CoV-2 S-RBD Omicron IgG Antibody.

We have carried out RT-qPCR testing for SARS-CoV-2 at the laboratory of Microbiology Department of Zagazig faculty of medicine. All other laboratory tests were performed at the Clinical and Chemical Pathology Department at Zagazig University Hospitals.

The methodology of real time reverse transcriptase-PCR involved collecting nasopharyngeal (NP) and oropharyngeal (OP) swabs by trained healthcare workers at ICU, following CDC recommendations. Both swabs from each participant were combined in a 3-ml tube containing viral transport medium (VTM, Ismailia free zone, Egypt, Ref: 1/V T01.001.0001) and stored at -80°C until further analysis. RNA extraction and SARS-CoV-2 RNA detection were performed using standard RT-qPCR RNA extraction under BSL-2 conditions on 410 µl of the VTM from both swabs using the QIAamp Viral RNA mini kit (cat. no. 52906, Qiagen) according to manufacturer's recommendations. During extraction, RNase-free DNase set (cat. no. 79254, Qiagen) was used to treat the RNA samples to eliminate genomic DNA contamination. RNA quality and quantity were determined using the Nanodrop S1000 spectrophotometer (Thermo Fisher Scientific). One-step RT-qPCR was performed on extracted RNA using a real-time PCR kit (Primerdesign Ltd, Ref: Z-Path-COVID-19CE, UK) in Stratagene Mx3000P qPCR System (Agilent). This assay targets the RNA-dependent RNA polymerase (RdRP) gene within SARS-CoV-2, with the kit detecting 0.58 copies/l of SARS-CoV2 viral RNA with ≥95% confidence with forward and reverse primer sequences identified as ACCGTAGCTGGTGTCTCTAT and GTGCCAACCACCATAGAATTTG, respectively (23,24). The 20 µl reaction mixture consisted of 10 µl 2X RT-qPCR Master Mix, 2 µl of COVID-19 Primer & Probe, and 8 µl sample extract. Each run included a positive control template and negative amplification control with nuclease-free water. The one-step protocol involved reverse transcription (DNA/cDNA formation) at 55°C for 10 min, followed by initial denaturation at 95°C for 2min, and 45 cycles of denaturation at 95°C for 10 seconds, annealing, and extension at 60° for 1 min. The cycle threshold (Ct) values were recorded for each sample, with samples considered negative if they had a Ct value ≥40 or when no Ct values were reported.

Assessment of SARS-CoV-2 S-RBD IgG Antibody using mouse anti SARS COV-2 was carried out using (S-RBD Omicron B.1.1.529)IgG ELISA kit through the following steps:

A volume of 5.0 milliliters of blood was obtained from the antecubital vein using a syringe and a wide-bore needle (5ml). Samples underwent centrifugation at 1500× g rpm for 15 minutes at room temperature, after which the serum will be stored at 4 °C and utilized within five days.

The serum samples collected from each individual were tested for quantification of anti–SARS-CoV-2 S-RBD IgG antibodies using the using mouse anti SARS COV-2 (S-RBD Omicron B.1.1.529) IgG ELISA kit (ELK Biotechnology Co, Ltd, USA) https://cymitquimica.com/products/EK-

ELK0963/mouse-anti-sars-cov2s-rbd-omicronb11529-igg-elisa-kit/.

The ELISA procedure involved preparing reagents at room temperature, adding standard solutions and samples to designated wells, and proceeding through a multi-step process of incubation and washing. After initial setup, the protocol requires adding biotinylated antibody and streptavidin-HRP solutions with specific incubation times at 37°C, interspersed with multiple wash cycles. The final stage included adding TMP substrate, preheating the microplate, introducing a stop reagent to turn the liquid yellow, and measuring at 450 nanometers optical density immediately by the use of a microplate reader, on a spectrophotometer (TECO-DIAGNOSTIC ELISA Reader Instruments., HospiMedica, UK). The concentration of anti-SARS-CoV2 (Omicron,B.1.1.529) IgG (anti-S-RBD) in the samples is subsequently established by contrasting the optical density of the samples with the standard curve.

III. Statistical methods:

Data analysis

SPSS (Statistical Package for the Social Sciences) version 28 was utilized for the purpose of analysis of data. The absolute frequencies of categorical variables were used for description. Assumptions utilized in parametric testing were checked using the Shapiro-Wilk test. Depending on the data type, quantitative variables were characterized by means and standard deviations, median, or interquartile range. We utilized the Mann Whitney test (for data that is not regularly distributed) to compare two sets of quantitative data. The Kruskal-Wallis test was employed to compare quantitative data across multiple groups, even when the data was not regularly distributed. Pairwise comparison was employed to identify differences between each pair of groups where the difference was substantial. In order to determine the degree and direction of the correlation between two variables, the Spearman rank correlation coefficient was utilized. To establish a robust cutoff for a certain quantitative parameter in the diagnosis of a particular health concern, the ROC curve was employed. To find the independent risk factors linked to certain health problems, binary logistic regression was employed. The threshold for statistical significance was set below 0. 05. If $p \leq 0.001$, a highly significant difference was found.

Results

This study included 61 patients with an age range from 42 to 81 years with mean age 60.84 years. Males constituted 54.1% of them. Smokers represented 24.6% and 49.2% had severe disease. About 30% of them died within the first 3 weeks of ICU admission. Median WBCs, neutrophil and lymphocytes were 15.6, 12.6 and 1.9 (103/mm3). Median CRP at ICU admission was 100 mg/L and median Omicron, B.1.1.529 IgG (anti-S-RBD) concentration at ICU admission and 3 weeks later were 30.2 and 58 ng/ml respectively(Table 1).

There is statistically significant negative correlation between Omicron,B.1.1.529 IgG (anti-S-RBD) concentration and age at ICU admission ($r=-0.478$, $p<0.001$), suggesting older patients had lower antibody responses. However, this correlation weakened after 3 weeks ($r=-0.225$, $p=0.148$). Other clinical parameters (hospital stay duration, WBC count, neutrophils, lymphocytes, and CRP) showed weak correlations that weren't statistically significant, either at admission or after 3 weeks (Table 2).

Table 3 shows serological data comparing Anti-RBD (Receptor Binding Domain) antibody levels across different variables in COVID-19 patients, measured at ICU admission and 3 weeks later for survivors. While males showed slightly higher median Anti-RBD levels at ICU admission (30.2 vs 19.6 ng/ml), this difference wasn't statistically significant ($p=0.528$). The levels after 3 weeks were also comparable between sexes ($p=0.862$). Smokers had significantly higher Anti-RBD levels both at admission (43.3 vs 19.7 ng/ml, $p=0.005$) and after 3 weeks ($p=0.030$). There was a strong inverse relationship between disease severity and Anti-RBD levels ($p<0.001$). Moderate cases had the highest levels (52.33 ng/ml at admission, 94.0 ng/ml at 3 weeks), while critically ill patients had the lowest levels (14.69 ng/ml at admission, 30.9 ng/ml at 3 weeks).

These findings suggest that age, smoking status, and disease severity are important factors influencing antibody responses in COVID-19 patients, with disease severity showing a particularly strong inverse relationship with antibody levels.

There is a statistically significant relation between Omicron, B.1.1.529 IgG (anti-S-RBD) Abs concentration and degree of COVID. On doing pairwise comparison, differences are significant between each two individual groups (Figure 1).

A statistically significant relationship exists between Omicron, B.1.1.529 IgG (anti-S-RBD) Abs concentration and outcome. Lower level is associated with mortality (Table 3 4 and figure 2 and 3).

The best cutoff of Omicron,B.1.1.529 IgG (anti-S-RBD) Abs concentration in prediction of mortality is ≤19.49 ng/ml at ICU admission with area under curve 0.727 (95% CI; 0.592 – 0.861) with sensitivity 50% and specificity 72.1%. Positive and negative predictive values were 42.9%, 77.5% and overall accuracy was 65.6% (p=0.006) (Table 5 in the supplementary file).

The best cutoff of Omicron, B.1.1.529 IgG (anti-S-RBD) Abs concentration in prediction of critically ill is ≤18.43 ng/ml with area under curve 0.954 (95% CI; 0.906 – 1) with sensitivity 100% and specificity 87.8%. Positive and negative predictive values were 66.7%, 100% and overall accuracy was 90.2% (p<0.001) (Table 6 and figure 4 in the supplementary file).

Table (1) : Characterization of the research participants based on initial data

	Number	**% (range)**
Gender (n=61)		
Female	28	45.9%
Male	33	54.1%
Age (year) [mean ± SD]	60.84 ± 11.18	42 – 81
Smoking (n=61)		
Non-smokers	46	75.4%
Smokers	15	24.6%
Degree of COVID-19 (n=61)		
Moderate	19	31.1%
Severe	30	49.2%
Critically ill	12	19.7%
Survival percentage (n=61)		
Survivors	43	70.5%
Non-survivors	18	29.5%
	Median (IQR)	**Range**
ICU stay (day) (n=61)	14(10 – 20)	3 – 25
WBCs (n=61)	15.6(9.8 – 20.05)	1 – 29
Neutrophil (n=61)	12.6(7.8 – 17.9)	0.7 – 25.6
Lymphocytes (n=61)	1.9(1.3 – 2.85)	0.5 – 3.9
CRP (mg/L) (n=61)	100(56 – 140)	3 – 300
(Omicron,B.1.1.529) Spike Receptor-Binding Domain (S-RBD) IgG Abs concentration (ng/ml) at time of admission for all patients (n=61)	30.2(16.65 – 46.38)	13.77 – 55.52
Omicron,B.1.1.529)Spike Receptor-Binding Domain (S-RBD) IgG Abs concentration (ng/ml) 3 weeks later for survivors (n=43)	58 (38-87)	27.8-109

Table (2): Correlation between Omicron,B.1.1.529 Spike Receptor-Binding Domain (S-RBD) IgG Abs concentration of all patients confirmed COVID19 at the ICU admission and after 3 weeks and the variables that were considered

	Correlation at ICU admission for all patients (n=61)		**Correlation 3 weeks after ICU for survivors (n=43)**	
	r	***p*-value**	**r**	***p*-value**
Age (year)	-0.478	<0.001**	-0.225	0.148
Hospital stay (day)	0.169	0.192	-0.120	0.445
WBCs	0.163	0.209	-0.056	0.719
Neutrophil	0.228	0.078	-0.007	0.962
Lymphocytes	0.041	0.756	-0.032	0.837
CRP (mg/L)	-0.169	0.193	-0.292	0.057

r Spearman rank correlation coefficient **p≤0.001 is statistically highly significant

Table (3): Serologic Data of Serum Samples Obtained From Confirmed COVID-19 patients: Among Different varibles, and Stratified by Time From Baselinea

Variable		Anti-RBD, median (IQR), Anti-RBD, median (IQR), ng/ml			
		Level at time of ICU admission for all patients (N= 61)	P value	3 weeks from ICU admission level for survivors (N=43)	P valueb
Sex	Male	30.2 (14.9-52.3)	0.528	49.7 (28.5-88.5)	0.862
	Female	19.6 (17.4-36)		39.1 (34.9-75)	
Smoking	Yes	43.3 (19.6-52.3)	0.005	39.4 (32-85)	0.030
	No	19.7(16.3-43.6)		39.4(32-85)	
Degree of COVID-19	Moderate	52.33 (46.38 – 55.21)	< .001	94.0(87.0-102)	< .001
	Severe	19.62 (19.42 – 30.34)		39.1 (34.9-60.2)	
	Critically ill	14.69 (13.8 – 16.95)		30.9 (27.8-34.9)	

Table (4): Relation between Omicron,B.1.1.529 Spike Receptor-Binding Domain (S-RBD) IgG Abs concentration of all patients confirmed COVID19 at the admission and outcome and degree of COVID-19:

	Median (IQR)	KW	p
Degree of COVID-19			
Moderate	52.33 (46.38 – 55.21)		
Severe	19.62 (19.42 – 30.34)	47.41	<0.001**
Critically ill	14.69 (13.8 – 16.95)		
Outcome		Z	
Survivors	30.34 (19.42 – 52.33)	-2.779	0.005*
Non-survivors	18.5 (13.9 – 33.52)		

KW Kruskal Wallis test Z Mann Whitney test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

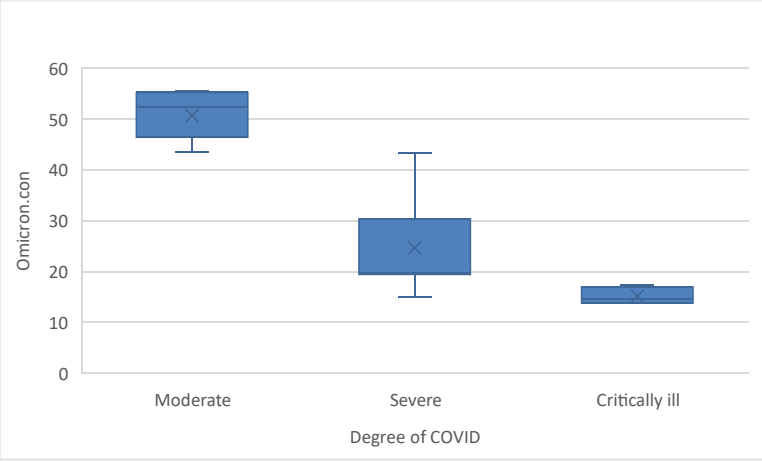

Figure (1) Boxplot showing relation between Omicron,B.1.1.529 IgG Abs concentration and degree of COVID

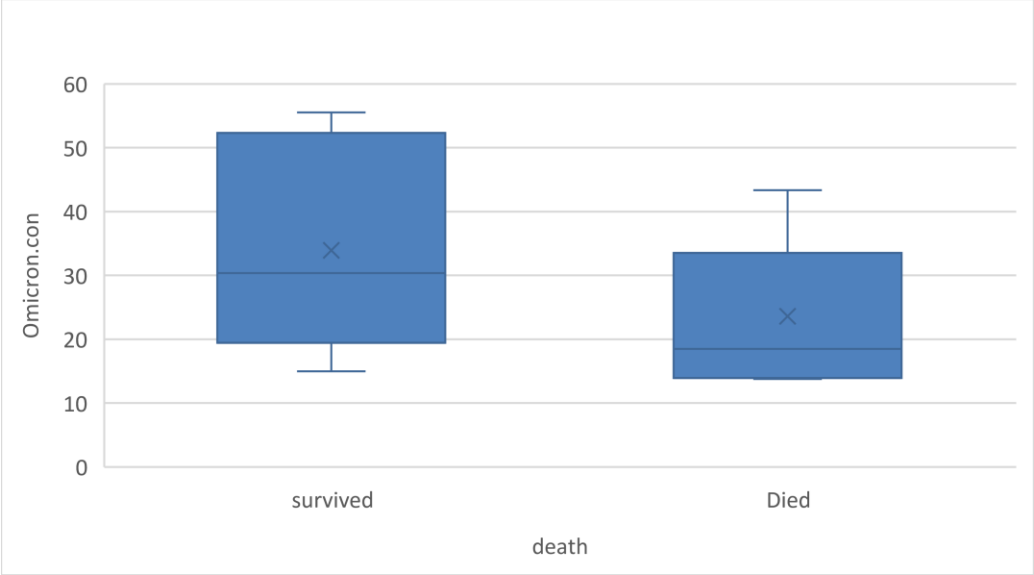

Figure (2) : Boxplot showing relation between Omicron, B.1.1.529 IgG Abs concentration and outcome

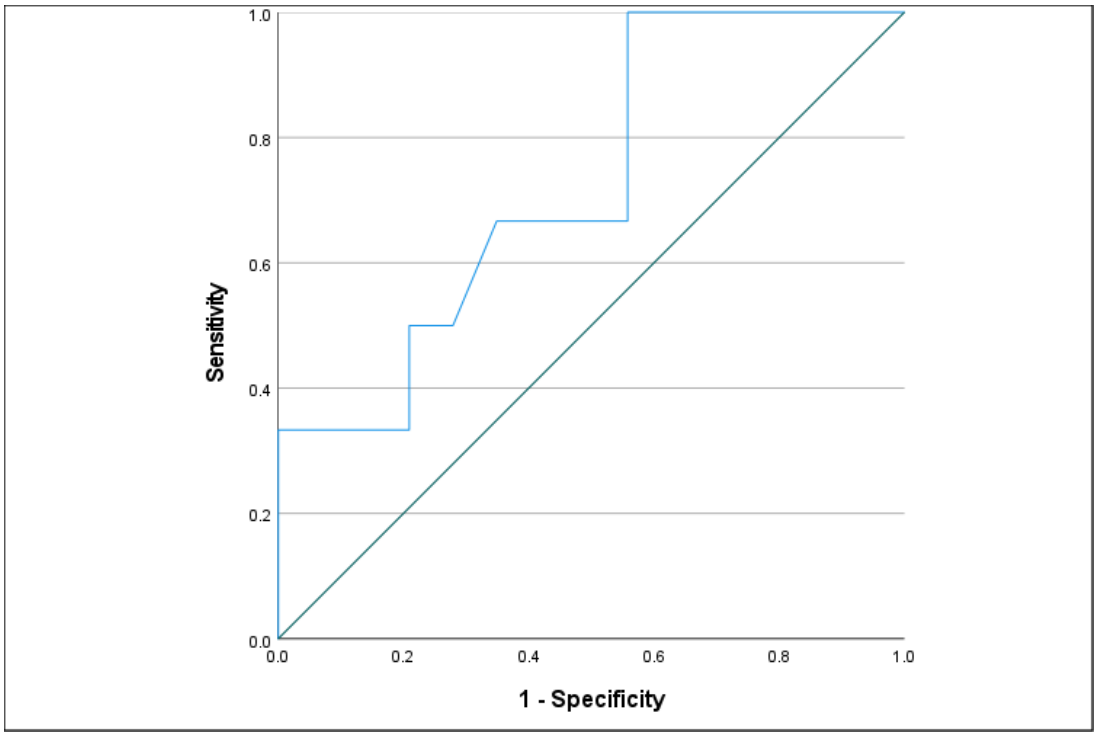

Figure (3) ROC curve showing sensitivity of Omicron,B.1.1.529 IgG Abs in prediction of mortality

DISCUSSION

In this study we found that Omicron,B.1.1.529 anti-RBD IgG Abs concentration correlated significantly with the outcome of COVID 19 infection particularly in the context of age and smoking status. Lower level was associated with mortality. The best cutoff of Omicron,B.1.1.529 IgG Abs concentration in prediction of mortality is ≤19.49 ng/ml at ICU admission. There is statistically significant relation between Omicron,B.1.1.529 IgG Abs concentration and degree of COVID.

Recent studies have explored the development and evaluation of ELISA-based methods for detecting strain-specific anti-SARS-CoV-2 antibodies, particularly against the Omicron variant with ability to discriminate and quantify strain-specific anti-Spike IgG antibodies (22). The sensitivity and specificity of the anti-SARS-CoV2(S-RBD) (Omicron, B.1.1.529) IgG ELISA Kit have been

evaluated in various studies, revealing promising diagnostic capabilities. The sensitivity of the RBD-ELISA for detecting SARS-CoV-2 IgG antibodies is reported to be as high as 91% with a specificity of 99.25% (25). The performance of the assay improves significantly when samples are collected 15-21 days post-symptom onset (26).

In fact, this is the first study to investigate SARS-CoV-2 (Omicron, B.1.1.529)IgG (anti-S-RBD) antibodies as markers of the humoral response in patients infected with SARS-CoV2 Omicron B.1.1.529 Variant in Egypt and correlate level with severity and outcome of infection.

Anti-RBD IgG levels peak around 14-28 days post-vaccination or infection and then gradually decrease (27). So, for acute assessments, measuring RBD antibodies between 15 to 28 days post-infection is effective, as this period shows high sensitivity for detecting antibodies in infected individuals (28).So, we serum samples were obtained From Confirmed COVID-19 patients at admission and 3 weeks later.

The rise in RBD IgG levels post-hospitalization may suggest a recent infection, especially if accompanied by clinical symptoms (29). This is supported by the fact that IgG antibodies against RBD are detected within 1-7 days post-infection, indicating recent exposure. A significant increase over time is more indicative of a recent infection rather than past infection (30).

In terms of age, there was a statistically significant negative correlation between Omicron,B.1.1.529 IgG anti-S-RBD) antibodies concentration and age. Our results are in accordance with Chiara et al (31). This finding was in disagreement with Mongkolsucharitkul et al who reported that older people had higher levels of anti-RBD IgG compared to younger people (32). This discrepancy would be explained by the fact that antibody responses to infections vary with age due to factors such as vaccine effectiveness, immune system maturity, and exposure history (33).

Smokers represented 24.6% of the study population and 49.2% had severe diseases. These findings are consistent with Zheng et al who revealed that smoking was significantly linked to more severe COVID-19 outcomes and increased mortality rates among patients hospitalized with the virus (34).

This study demonstrated that Omicron,B.1.1.529 IgG anti-S-RBD) antibodies concentration correlated significantly with the outcome of infection. This is attributed to the fact that how strong the immune response gets in response to a natural infection is influenced, in part, by how bad the illness is. (13,14). Increased interactions between B.1.1.529 spike protein and human ACE2 are associated with the most severe B.1.1.529 infections (35). These results are in accordance with Javier García-Abellán et al who denoted that the severity of SARS-CoV-2 disease has actually been associated with the magnitude and duration of the antibody response (33).

Smokers had significantly higher Anti-RBD levels both at admission and after 3 weeks. Our results are in agreement with Harrache et al. study which highlighted that smoking could lead to altered immune responses, potentially resulting in higher anti-RBD levels in certain contexts (36)

These results shown that there is statistically non-significant correlation between omicron B.1.1.529 IgG anti-S-RBD) antibodies concentration and either hospital stay, WBCs, neutrophil, lymphocytes or CRP. Our results are in disagreement with Kim and colleagues who denoted that increased antibody concentrations were linked to CRP elevation, and lymphopenia (37). This discrepancy may be explained by the fact that in more severe cases of SARS-CoV-2 infection, lymphopenia is observed. However, in the weeks after infection, there is viral increase in the frequency of T cells that respond to several antigens, including Spike, Nucleocapsid, membrane, and accessory (functional) protein (e.g., ORF 1ab) fragments (38).

Implications for future research:

The results of this study may help us speed up the search for new clinical antiviral reagents and discover a new class of neutralizing antibodies that can combat SARS-CoV-2. In addition to highlighting the potential utility of Omicron, B.1.1.529 IgG anti-S-RBD) antibodies in clinical risk stratification and patient management.

Strengths of this study are the thorough investigations conducted in patients and being a cohort prospective study allowed monitoring the changes in antibody levels. Moreover, specificity of new ELISA-based methods for detecting strain-specific anti-SARS-CoV-2 antibodies, particularly against the Omicron variant is a strength point of our study.

Limitations

The current study has several limitations. We did not perform sequencing or genetic study to verify VOC by name (B.1.1.529). We have just confirmed COVID 19 infection by real time- verse transcriptase PCR. . Being a single-centered study limits the generalizability of our findings. The characteristics of hospitalized patients will vary across countries

and contexts due to differences in healthcare infrastructure and the resources that are accessible. Moreover, the limited number of participants in the research represents a potential constraint on the study's findings and generalizability.

Genetic verification by sequencing is mandatory to confirm that the elevation in Omicron, B.1.1.529 IgG (anti-S-RBD) antibodies are attributed to SARS-CoV-2 mutant Omicron (B.1.1.529) variant. Well-designed longitudinal studies with genetic verification, longer follow up period and larger sample size are needed to address questions about the risk of hospitalization and mortality with SARS-CoV-2 mutant Omicron (B.1.1.529) variant.

Conclusion

Mutant SARS-CoV-2 Variant Omicron B.1.1.529 IgG antibody levels could serve as a valuable prognostic marker in COVID 19 infected patients, particularly in the context of ae and smoking and for recognizing patients at risk of progression to critical illness. While the antibody levels did not correlate significantly with inflammatory markers or length of ICU stay, their strong association with disease severity and outcomes highlights their future potential utility in clinical risk stratification and patient management.

Abbreviations

Abs: Antibodies.
nAbs: neutralizing antibodies
PCR: Polymerase Chain Reaction
TMP substrate: Tetramethylbenzidine substrate.

Authors' contributions

H.S contributed to the interpretation of results, assisted in data collection and provided technical support. L.G.Z and R.M.E were responsible for recruiting patients and drafted the manuscript. S.A.M was responsible for the overall study design and statistical analysis. L.A.E conducted the literature review and played a role in the interpretation of results. M.H.M was responsible for conceptualization, laboratory analysis and critical revisions. The complete manuscript was reviewed and endorsed by all contributing authors.

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