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Evaluation of Serum SARS-CoV-2 Nucleocapsid Antigen as a Diagnostic Tool in COVID-19 Patients

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Abstract

COVID-19 has caused millions of casualties and deaths around the world. Countries all over the world exert great efforts to control the fast spread of the disease. Rapid diagnosis is a key tool in controlling the infection; therefore, numerous diagnostic techniques were developed quickly and are available commercially. This study evaluated the use of nucleocapsid antigen (N-antigen) as a diagnostic tool in COVID-19 patients. A cross-sectional investigation was carried out on 164 people undergoing Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) PCR testing at various government laboratories in Alexandria. The research was carried out between March 2021 and January 2022. Data such as symptoms, lab investigations and real-time reverse transcription polymerase chain reaction (RT-PCR) cycle threshold (C_t) values were collected by interviewing participants and from medical records. A serum sample was collected from each participant for detection of N-antigen by ELISA kit. Ninety-eight (59.8%) of the 164 examined participants had positive SARS-CoV-2 RT-PCR results. Thirteen individuals (18.9%) exhibited varying quantities of the SARS-CoV-2 N-antigen. Antigen concentrations were significantly inversely connected with RT-PCR C_t values and positively correlated with CRP levels in SARS-CoV-2 N-antigen positive subjects. Furthermore, a strong correlation was found between N-antigen concentrations and hospitalization, fever, body aches, and pneumonia. SARS-CoV-2 N-antigen detection has high specificity (98.5%) but very low sensitivity (30.6%). Despite the high specificity of the SARS-CoV-2 N-antigen enzyme-linked immunosorbent assay (ELISA) evaluated in this study, its diagnostic utility is limited by its low sensitivity. The assay's poor sensitivity undermines its standalone diagnostic value, especially when compared to RT-PCR.

Keywords: SARS-COV-2 Nucleocapsid Antigen, COVID-19 Diagnosis, Serology, PCR

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INTRODUCTION

In Wuhan, China, a new, highly contagious illness that resembles viral pneumonia in its clinical manifestations initially surfaced in December 2019.[1] SARS-CoV-2 was found to be the causal agent. It belongs to the genus Beta-coronaviruses under the family Coronaviridae, which, in contrast to other coronaviruses, causes significant morbidity and mortality.[2] When it comes to diagnosing COVID-19, RT-PCR is thought to be the most precise technique available. However, it is a complicated technique that requires well trained professionals, special equipment, high costs and long time for preparing and processing samples.[3]

Testing for antiviral antibodies should not be used alone in early diagnosis of COVID-19 because they do not appear until days after the onset of the infection. Furthermore, they persist for long time even after clearance of the virus; therefore, their presence in the serum is just an indication of previous viral exposure.[4]

In contrast, the detection of viral antigens offers significant diagnostic advantages, particularly in the early phase of infection. Antigen-based assays, especially those targeting the N protein of SARS-CoV-2, can detect the presence of viral components shortly after infection and prior to seroconversion. Serum-based antigen detection methods offer additional clinical value by enabling minimally invasive sampling, facilitating early diagnosis during the viremic phase, and reducing biosafety risks associated with respiratory specimen collection. Several studies report high specificity for rapid antigen detection tests, although their sensitivity remains variable and context-dependent.[5,6]

Given the urgency of identifying infectious individuals to curb transmission chains, this study aimed to evaluate the diagnostic performance of a serum-based SARS-CoV-2 N antigen detection assay. This evaluation may provide more information regarding its potential application as a rapid and scalable diagnostic tool for early-phase COVID-19 detection, facilitating timely clinical and public health interventions.

MATERIALS AND METHODS

This cross-sectional investigation was conducted on 164 people who were suspected of having SARS-CoV-2 infection and were attending several government laboratories in Alexandria between March 2021 and January 2022.

Sample size

The bare minimum sample size needed was determined using data from an earlier investigation that looked for SARS-CoV-2 antigen in COVID-19 patients' blood.[7] By using sensitivity of 93%, specificity of 98.4% and prevalence 78.3%, estimation error 1.0, alpha 0.05, the minimal required sample size was found to be 67.[8] The number of participants was increased to 164 to compensate for potential data loss and to mitigate selection bias.

Participants were consecutively included in this study from different governmental laboratories. The study protocol was approved by the Ethics Committee of the High Institute of Public Health (HIPH). Prior to their inclusion in the study procedure, all participants were requested to voluntarily participate in this research, and their informed written consents were obtained.

An interview sheet including demographic and clinical data was filled for each participant including name, age, residence, lab investigations as C-reactive protein concentration and the C_t value from PCR result. Lymphocytic count <1000/µl was considered lymphopenia, CRP >5 mg/l was considered above normal level.

Collection and processing of samples

Sampling

A blood sample of three ml was collected from each participant using vacutainer under aseptic technique. After letting the samples clot, the sera were separated using a centrifuge running at 6000 rpm. They were divided into aliquots and kept at -80 °C for detection of the SARS-CoV-2 N antigen.

Quantitative detection of nucleocapsid antigen by ELISA assay

The quantitative detection of N-antigen was done using Human SARS-CoV-2 N ELISA Kit Ref. (EH49RB) manufactured by Invitrogen, Thermo Fisher Scientific, USA.

Test principle

The Human SARS-CoV-2 N ELISA Kit is a solid-phase sandwich ELISA designed to detect and quantify SARS-CoV-2 N-antigen in human serum. Wells are pre-coated with a monoclonal anti-SARS-CoV-2 N-antibody. Diluted serum samples are added. If N-antigen is present, it binds to the immobilized antibody. Enzyme conjugation, Streptavidin-HRP binds to the biotin on the secondary antibody. TMB substrate is added. In the presence of HRP, blue color develops. A stop solution is added, converting the blue color to yellow. Absorbance is measured at 450 nm. The intensity of the yellow color correlates with the concentration of N-antigen.

Statistical analysis

Data entry and statistical analysis were performed using MedCalc software (versions 25.0 and 20.116) and IBM SPSS Statistics (version 25.0). Qualitative variables were expressed as frequencies and percentages. Quantitative data were assessed for normality using the Kolmogorov-Smirnov test. As the data did not follow a normal distribution, they were described using range (minimum-maximum), mean ± standard deviation (SD), and median with interquartile range (IQR). A significance level of $p < 0.05$ was considered statistically significant.

The following statistical tests were applied:

Spearman's rho correlation

Used to evaluate the relationship between non-normally distributed quantitative variables.

Mann-Whitney U test

A non-parametric test used to compare two independent groups with non-normally distributed continuous data.

Chi-square test

Applied to assess associations between categorical variables in 2 × 2 contingency tables.

Table 1. Relationship between SARS-CoV-2 N-antigen results and various clinical and laboratory findings

Clinical presentations	SARS-CoV-2 N-antigen results				p-value
	Negative (n = 133)		Positive (n = 31)		
Hospitalization	N	%	N	%	
non-hospitalized (n = 108)	94	70.7	14	45.2	p = 0.007* X^2 = 7.279
hospitalized (n = 56)	39	29.3	17	54.8	
Fever	N	%	N	%	
No (n = 84)	74	55.6	10	32.3	p = 0.019* X^2 = 5.501
Yes (n = 80)	59	44.4	21	67.7	
Body aches	N	%	N	%	
No (n = 70)	63	47.4	7	22.6	p = 0.012* X^2 = 6.314
Yes (n = 94)	70	52.6	24	77.4	
Cough	N	%	N	%	
No (n = 100)	87	65.4	13	41.9	p = 0.016* X^2 = 5.824
Yes (n = 64)	46	34.6	18	58.1	
Pneumonia	N	%	N	%	
No (n = 108)	94	70.7	14	45.2	p = 0.007* X^2 = 7.279
Yes (n = 56)	39	29.3	17	54.8	
Lymphopenia	N	%	N	%	
No (n = 57)	43	32.3	14	45.2	p = 0.177 X^2 = 1.825
Yes (n = 107)	90	67.7	17	54.8	
Elevated CRP	N	%	N	%	
No (n = 26)	25	18.8	1	3.2	p^{FE} = 0.031* X^2 = 4.569
Yes (n = 138)	108	81.2	30	96.8	

p: p-value significant at level <0.05*p^{FE}: Fisher's Exact significance X^2: Chi-square test

Receiver Operating Characteristic (ROC) curve analysis

Used to determine the optimal cutoff value for the antigen concentration, as well as to assess diagnostic accuracy through area under the curve (AUC) metrics.

RESULTS

The study analyzed 164 participants with a median age of 63.5 years; 58% were male. Common clinical features included body aches (57%), fever (48.8%), cough (39%), and pneumonia (34.1%), with an equal proportion requiring hospitalization. Elevated CRP (>5 mg/l) levels were detected in 84.1% and lymphopenia (<1000 cell/µl) in 65.2% of the cohort.

N-antigen positivity and concentrations were significantly higher in females and among those presenting with fever, body aches, cough, pneumonia, hospitalization, and elevated CRP (Table 1 and 2). No significant differences were

Table 2. Relationship between SARS-CoV-2 N-antigen levels and various clinical and laboratory results

	Hospitalization		
SARS-CoV-2 N-antigen concen. (ng/ml)	Non-hospitalized (n = 108)	Hospitalized (n = 56)	p-value
Mean ± SD	0.31897 ± 1.11	0.8457 ± 1.603	p = 0.006* U = 3565.5
Median (IQR)	0.0 (0.0)	0.0 (1.096)	
Min-Max	0.0-5.82	0.0-6.786	
Fever			
	No (n = 84)	Yes (n = 80)	
Mean ± SD	0.19699 ± 0.7238	0.81576 ± 1.686	p= 0.011* U = 3885.5
Median (IQR)	0.0 (0.0)	0.0 (0.268)	
Min-Max	0.0-4.1655	0.0-6.786	
Body aches			
	No (n = 70)	Yes (n = 94)	
Mean ± SD	0.21947 ± 0.78774	0.706867 ± 1.577	p = 0.010* U = 3820.5
Median (IQR)	0.0 (0.0)	0.0 (0.1995)	
Min-Max	0.0-3.752	0.0-6.786	
Cough			
	No (n = 100)	Yes (n = 64)	
Mean ± SD	0.3387 ± 1.0763	0.74903 ± 1.6061	p = 0.016* U = 3689.0
Median (IQR)	0.0 (0.0)	0.0 (0.648)	
Min-Max	0.0-5.131	0.0-6.786	
Pneumonia			
	No (n = 108)	Yes (n = 56)	
Mean ± SD	0.3509 ± 1.2089	0.78412 ± 1.4799	p = 0.008* U = 3550.5
Median (IQR)	0.0 (0.0)	0.0 (0.924)	
Min-Max	0.0-6.786	0.0-5.131	
Lymphopenia			
	No (n = 57)	Yes (n = 107)	
Mean ± SD	0.819737 ± 1.759	0.327 ± 0.9779	p = 0.120* U = 2742.0
Median (IQR)	0.0 (0.0825)	0.0 (0.0)	
Min-Max	0.0-6.786	0.0-5.131	
Elevated CRP			
	No (n = 26)	Yes (n =138)	
Mean ± SD	0.0063 ± 0.032	0.5916 ± 1.420	p = 0.027* U = 2129.5
Median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	
Min-Max	0.0-0.1650	0.0-6.786	

p: p-value significant at level <0.05*

U: Mann-Whitney test; A non-parametric test employed to compare two groups' quantitative variables.

Table 3. Agreement between SARS-CoV-2 PCR and N-antigen tests

SARS-CoV-2 N-antigen Results	SARS-CoV-2 PCR				Total
	Negative		Positive		
	N	%	N	%	
Negative	65	98.5%	68	69.4%	133
Positive	1	1.5%	30	30.6%	31
Total	66	100%	98	100%	164
Kappa	0.250	P-value	0.047*		
Sensitivity	30.6%	Specificity	98.5%		
PPV	96.8%	NPV	48.9%		

Kappa: Measure of agreement (ranges from 0-1)·PPV: positive predictive value
p*: p-value is significant at level <0.05*; NPV: negative predictive value

Table 4. Distribution of the studied subjects according to C_t values of SARS-CoV-2 RT-PCR and N-antigen results

C_t value	SARS-CoV-2 N-antigen results				p-value
	N-antigen negative (n = 133)		N-antigen positive (n = 31)		
	N	%	N	%	
less than 25	10	26.3%	28	73.7%	$X^2 = 97.596$ p < 0.001*
from 25 to less than 30	21	91.3%	2	8.7%	
from 30 to less than 40	37	100.0%	0	0.0%	
equal or more than 40	65	98.5%	1	1.5%	

p*: p-value is significant at level <0.05*; X^2: chi-square test

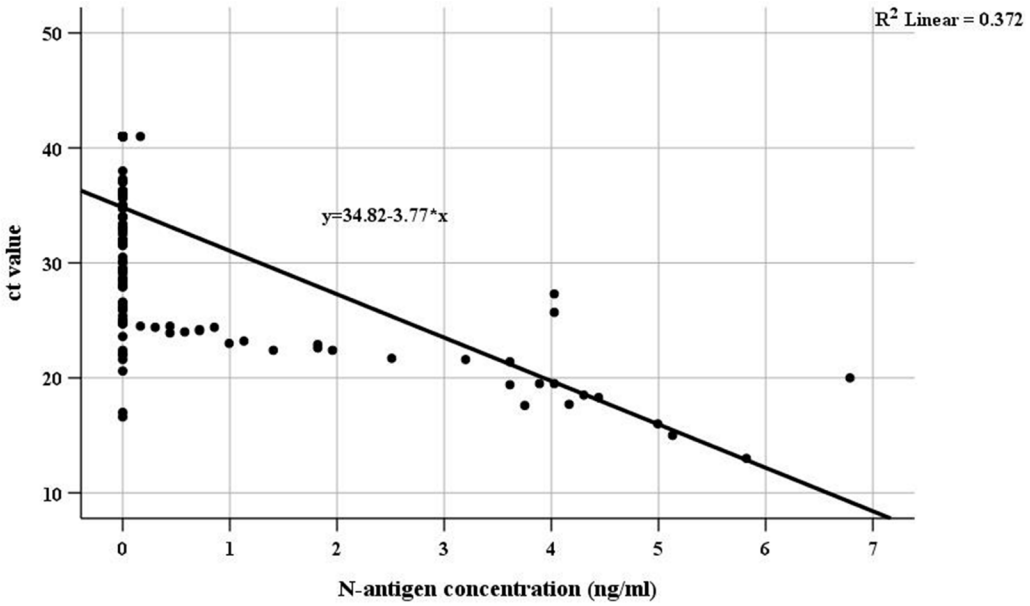

Figure 1. Scatter plot for correlation between C_t values of SARS-CoV-2 RT-PCR and N-antigen concentrations in the studied subjects

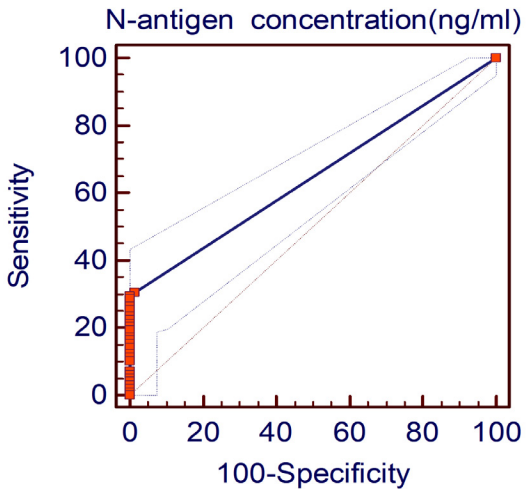

Figure 2. ROC curve of N-antigen concentration as a predictor of COVID-19 diagnosis

observed with age or lymphocyte count. A positive correlation was found between CRP and N-antigen levels, while a strong inverse correlation was noted between N-antigen levels and RT-PCR C_t values (Table 3, Table 4 and Figure 1).

The N-antigen test showed high specificity (98.5%) but low sensitivity (30.6%), with a positive predictive value of 96.8% and negative predictive value of 48.9%. ROC analysis identified a cutoff of >0.165 ng/mL, yielding an AUC of 0.648 and confirming the test's high specificity but limited sensitivity for detecting SARS-CoV-2 infection (Figure 2).

DISCUSSION

N-Antigen Levels and Disease Severity

In this investigation, hospitalized patients had significantly higher mean N-antigen concentrations than non-hospitalized individuals (0.8457 vs. 0.3190 ng/ml, p = 0.006). Prior studies have shown that elevated N-antigen levels are associated with increased severity, ICU admission, and longer hospital stays. Perna *et al*.[9] demonstrated that chemiluminescence enzyme immunoassay (CLIA)-measured N-antigen levels reflected disease progression in hospitalized patients. Ogata *et al*.[10] found a similar association using Single Molecule Array (SIMOA), while Veyrenche *et al*.[11] reported significantly higher urine N-antigen levels in ICU patients (p = 0.0077).

Likewise, patients with pneumonia in our study showed significantly greater N-antigen levels compared to non-pneumonia cases (0.7841 vs. 0.3509 ng/ml, p = 0.008), consistent with Lebedin *et al*.,[12] who found that serum N-antigen levels decreased to undetectable levels upon discharge. A statistically significant positive correlation between CRP and N-antigen levels (rs = 0.207, p = 0.012) was also observed, supporting a relationship between viral burden and inflammatory response.[11,13,14]

The diagnostic sensitivity observed in this study may have been influenced by several methodological limitations. First, the timing of sample collection was not standardized in relation to the onset of symptoms, which may have led to testing during phases of low antigenemia. Second, the use of serum as the specimen type, while practical, may offer lower sensitivity compared to respiratory tract specimens such as nasopharyngeal swabs, which typically contain higher viral loads in early infection.

Diagnostic performance vs. RT-PCR

Of the 98 RT-PCR positive individuals, only 30 (30.6%) tested positive for N-antigen, resulting in fair agreement (kappa = 0.250), high specificity (98.5%), and low sensitivity (30.6%). This is lower than other studies, such as those by Mandal *et al.*[15] (63.5%) and Mayanskiy *et al*.[16] (90.1%).

ELISA-based antigen assays

Mayanskiy *et al*.[16] using quantitative ELISA reported 90.1% N-antigen positivity in PCR-confirmed cases. Barlev-Gross *et al*.[17] showed 63.4% sensitivity and 87.0% specificity. In contrast, Ahava *et al*.[18] found no significant correlation between N-antigen levels using ELISA and C_t values.

CLIA assays

CLIA-based assays showed better diagnostic accuracy. Perna *et al*.[9] and Iqbal *et al*.[19] reported sensitivities of 62%-72% and specificities of 95-100%. Gili *et al*.[20] found 100% sensitivity, 94.8% specificity, and 95.1% agreement in nasopharyngeal swabs. Thudium *et al*.[21] reported AUC = 0.986, with sensitivity >90% when serum/plasma samples were collected within 2 weeks.

SIMOA and ultrasensitive assays

Olsen *et al*.[22] achieved 95% sensitivity and 100% specificity using SIMOA at 0.01 pg/ml. Sigal *et al*.[23] reported 89% sensitivity and 95%-97% specificity using an ultrasensitive immunoassay. These platforms consistently outperformed conventional assays in detecting low antigen levels.

Rapid antigen test (RAT) and Immunofluorescence assay (IFA) performance

RAT sensitivity varied widely. Mandal et al.[15] found 63.5% positivity, while Saveriampillai *et al*.[24] observed fair agreement (kappa = 0.242). Caruana *et al*.[25] tested four RATs, reporting specificities >99% but sensitivities of 41%-48%. Agard *et al*.[26] found weak agreement with IFA tests (sensitivity 31%-39%, specificity 100%).

C_t Value and antigen correlation

A significant inverse correlation was found between C_t values and N-antigen concentration ($r_s = -0.623$, $p < 0.001$), consistent with results from Favresse *et al*.,[27] Olsen *et al*.,[22] and Lefever *et al*.[28] Other reports noted similar relationships (e.g., r = -0.61 to -0.77) across SIMOA and CLIA platforms.[21,27,29]

However, in this study, N-antigen test sensitivity did not vary significantly across C_t strata. This contrasts with findings by Salvagno *et al*.,[30] who showed RAT sensitivity dropped from >90% at C_t <25 to <20% at C_t >30. Lefever *et al*.[28] reported CLIA sensitivity of 100% at C_t <20 and 71.3% at C_t <35.

ROC analysis

ROC analysis yielded an AUC = 0.648 and optimal cutoff >0.165 ng/ml with high specificity (98%) but low sensitivity (29.6%). By comparison, Deng *et al*.[31] using CLIA found AUCs >0.91 with 76.3% sensitivity in week 1. Li *et al*.[32] reported an AUC = 0.9756 (sensitivity = 92%, specificity = 96.8%) using quantitative ELISA.

Thudium *et al*.[21] and Verkerke *et al*.[33] reported AUCs >0.97 for plasma/serum samples with >85% sensitivity and >98% specificity. Gili *et al*.[20] showed 100% sensitivity and 94.8% specificity at a cutoff of 1.645 pg/ml.

CONCLUSION

Despite the high specificity (98.5%) of the SARS-CoV-2 N-antigen ELISA assay evaluated in this study, its diagnostic utility is significantly limited by its low sensitivity (30.6%). The assay's poor sensitivity undermines its standalone diagnostic value, especially when compared to RT-PCR. However, the significant association of higher N-antigen levels with clinical severity indicators such as hospitalization, fever, pneumonia, and elevated CRP suggests potential utility in identifying patients with higher viral loads.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

MHH conceptualized the study. AAE performed the recruitment of study participants. AKM and MMF performed laboratory work. MMF performed data analysis. AKM, MHH and MMF performed data interpretation. MHH, MMF and AAE supervised the study. MMF and AKM wrote the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the High Institute of Public Health, Alexandria University, Egypt, vide reference number 00013692.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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