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Original Article

Impact of seasonality on COVID-19 screening in vulnerable areas: Comparison between antigen tests and RT-PCR in the extreme west of Algeria

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ABSTRACT

Understanding the dynamics of coronavirus disease 2019 (COVID-19) emergence requires investigation with regard to the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the accuracy of detection tests. This research aims to evaluate the impact of some demographic factors and seasonal fluctuations on the positivity and efficacy of diagnostic tests. The study included a total of 14,642 participants from extreme west of Algeria, comprising 4,799 individuals positively diagnosed for COVID-19 and 9,843 negative controls. Our results demonstrated that women were more likely to be infected with COVID-19 (OR=8.32; 95% CI: 7.43-9.33; p=0.0001), indicating a significantly higher probability of reverse transcription and real-time polymerase chain amplification (RT-PCR) positivity compared with men. Our analysis shows a noticeable seasonal tendency, with an increase of RT-PCR positivity in summer, when positive cases are almost twice as frequent as in other seasons (OR=1.98; 95% CI = 1.77-2.22; p=0.0001). However, autumn appears to influence antigen test sensitivity, with a positive antigen test in autumn around ten times higher than in other seasons of the year (OR=9.90; 95% CI = 1.16-84.66; p=0.036). The logistic regression models revealed strong discriminatory power, with area under the curve values of 0.83 and 0.81 for the RT-PCR and antigen test, respectively. These values indicate an excellent ability to distinguish positive from negative individuals for both testing methods. Our study showed that seasonality strongly influences the spread of COVID-19 and the performance of diagnostic tests.

KEYWORDS: COVID-19, seasonal variability, RT-PCR, antigen test, extreme west of Algeria.

INTRODUCTION

A thorough understanding of the impact of environmental factors on the spread of SARS-CoV-2, and a comparison of the efficacy of

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antigenic tests versus RT-PCR, is essential [1]. Such an evaluation not only enables a rigorous assessment of the diagnostic sensitivity and specificity of antigen-based tests, as supported by several recent studies [2], but also informs the optimization of screening strategies in response to varying environmental conditions. [3]. Seasonal variations and environmental conditions may significantly impact diagnostic performance, thereby underscoring the necessity of comparative testing to ensure both accurate and timely identification of COVID-19 cases, particularly in resource-limited settings typical of many developing countries [4].

Climatic conditions influence virus stability and dispersion, which can modify the risk of transmission. Several studies have shown that certain seasons, such as summer, are associated with increased positivity rates, potentially due to favorable conditions for SARS-CoV-2 survival in the environment [5].

This seasonal variability may also impact the reliability of antigenic screening tests. Indeed, even though these rapid tests offer the advantage of easy implementation and low cost, their sensitivity and specificity seem to fluctuate according to environmental conditions, in contrast to RT-PCR, which is considered the gold standard method for SARS-CoV-2 detection [6].

Numerous studies are also examining the individual determinants that may influence the risk of infection [7]. Age, comorbidities, immune system status and socio-economic factors significantly influence disease dynamics. Among these determinants, sex is considered to be an important risk factor. While early research predominantly reported increased COVID-19 severity in men, more recent studies also highlight a notable vulnerability in women [8, 9].

The fight against COVID-19 has been challenging in the far west of Algeria due to high population density, inadequate healthcare infrastructures, and significant socio-economic disparities [10].

This contrasts with certain regions of Europe, where, health systems typically have more resources and a larger capacity to set up RT-PCR tests, even in the face of a high urban population [11].

Furthermore, the economic status of populations, characterized by significant income disparities and unequal access to healthcare, intensifies vulnerabilities to COVID-19.

In an Algerian context, these economic disparities lead to additional constraints for the implementation of effective screening strategies, notably by limiting access to testing and influencing adherence to preventive measures.

This study therefore investigates the influence of seasonal variations on the risk of COVID-19, considering individual determinants such as age and gender, while comparing the efficacy and reliability of antigen tests to those of RT-PCR, the gold standard for diagnosis.

We also need to provide answers to questions regarding the choice of screening tools, which should not be limited solely to their technical performance. It is essential to consider constraints related to seasonal variations and local economic realities, in order to develop optimized screening strategies that guarantee a prompt and effective response during epidemics.

MATERIALS AND METHODS

Patients and data collection

This study was a retrospective case-control study conducted in the Wilaya of Tlemcen (northwest Algeria), with a sample of 14642 subjects, including 4799 individuals with a confirmed diagnosis of COVID-19 and 9843 without a confirmed diagnosis of COVID-19. Written informed consent from all patients, as well as ethics committee approval from university of Tlemcen, was obtained prior to the start of this study.

Some COVID-19 patients were determined by the RT-PCR whereas other patients were confirmed with antigen test.

Data collection related to COVID-19 took place between March 2020 and May 2021 at the COVID-19 service of the University Hospital Center Tlemcen.

Molecular diagnostics

The qualitative detection of SARS-CoV-2 with RT-PCR was based on viral RNA extraction from

nasopharyngeal samples. A reverse transcription was performed to convert viral RNA into cDNA and two regions of the SARS-CoV-2 nucleocapsid gene (called N1 and N2) and a constitutively expressed human RP gene were amplified, all in a single reaction using the Bio-Rad Reliance SARS-CoV-2 RT-PCR kit [12].

Antigen test

The antigenic test kit used was the SARS-CoV-2 Rapid Antigen Test Roche, a diagnostic test based on rapid immunochromatography, designed for the qualitative detection of SARS-CoV-2 antigens in nasopharyngeal samples of suspected patients. Clinical sample was collected and then twisted into a container filled with extraction buffer. Three drops of the sample material were added to the reagent tray after removing the swab. The presence of viral antigens in high enough concentration allows them to bind to particular mouse monoclonal anti-SARS-CoV-2 antibodies, which is then reflected by the appearance of colored line in the lower section of the test strip's result window and another control colored line in the top section of the result window [13].

Statistical analysis

Statistical analysis includes describing qualitative variables in percentage (%) and quantitative variables by their means and standard deviations. We performed a chi-square test to study the association between qualitative variables and a Student t-test for comparison between quantitative variables. Binary logistics regressions were performed to determine the impact of the variables studied on the positivity of the COVID-19 diagnostic test. Receiving operating characteristics (ROC) curve was plotted to estimate the predictive capacity of logistics models. The significance of the differences was determined at a threshold of 0.05. Statistical processing of the data was carried out by Minitab 18 and Microsoft Excel 2010.

RESULTS

Table 1 shows that the average age of cases and controls is 44.2±18.2 years, with confirmed cases at 44.7±17.6 years and controls at 43.6±18.8 years. The age of confirmed cases is significantly higher than that of subjects not affected (p=0.001).

Women represent more than three-quarters of the population studied (76.27%). There is virtually the same distribution by sex between subjects tested positive for COVID-19 and those tested negative (p=0.277). Notably, younger women were more frequently affected, while in men, the older age group was more affected.

More than half of those surveyed are over the age of 40, with more than 33% aged between 40 and 60 years and almost 23% aged over 60 years. However, 44.10% of unconfirmed cases are less than 40 years old. We noted a similar distribution according to the positivity of the COVID-19 test (p=0.452), with 44.53% of patients aged less than 40 years, 32.39% aged between 40 and 60 years, and 23.07% aged over 60 years, while the percentages of subjects without COVID-19 are 43.88% subjects under 40 years, 33.44% individuals between 40 and 60 years, and 22.68% subjects over 60 years.

The percentage of subjects tested in summer is 38.29%, while the rest of the tests were carried out during winter (28.86%), autumn (19.13%), and spring (13.72%). In summer, there are more positive cases for COVID-19 (47.18%) than negative cases (33.72%), while negative tests for COVID-19 are more frequent during the rest of the seasons: 31.36% in winter, 19.84% in autumn, and 15.08% in spring compared to positive tests with 23.99%, 17.75%, and 11.07% respectively (p=0.0001).

For individuals tested using RT-PCR, sex plays a role in the prevalence of COVID-19, with p-value=0.017. The chi-square test shows that COVID-19 and age groups are independent (p= 0.233). The chi-square analysis showed a very strong association (p=0.0001) between COVID-19 and the seasons (Table 2).

In the population diagnosed with the antigen test (Table 2), the average age of patients with COVID-19 is 44.6±17.4 years and of negative subjects is 44.8±19.1 years, with no significant difference (p=0.954). The chi-square test shows that sex (p=0.137) and age groups (p=0.440) are not related to COVID-19. However, the study of the association between COVID-19 and the

Table 1. Description of the population studied.

Parameters	Participants (N=14642)	Positive (N=4799)	Negative (N=9843)	*p*-value
Age (years)	44.2±18.2	44.7±17.6	43.6±18.8	0.001
Sex				
Men	3475 (23.73%)	1113 (23.19%)	2363 (24.01%)	0.277
Women	11167 (76.27%)	3686 (76.81%)	7480 (75.99%)	
Age groups				
<40 years	6457 (44.10%)	2137 (44.53%)	4319 (43.88%)	0.452
40-60 years	4844 (33.08%)	1555 (32.39%)	3292 (33.44%)	
>60 years	3341 (22.82%)	1107 (23.07%)	2232 (22.68%)	
Seasons				
Winter	4226 (28.86%)	1152 (23.99%)	3087 (31.36%)	0.0001
Spring	2009 (13.72%)	531 (11.07%)	1484 (15.08%)	
Summer	5606 (38.29%)	2264 (47.18%)	3319 (33.72%)	
Autumn	2801 (19.13%)	852 (17.75%)	2953 (19.84%)	

seasons reveals a potential influence of autumn on the sensitivity of the antigenic diagnostic test (p=0.0001).

The logistic model showed that in individuals diagnosed with the RT-PCR test, infection was significantly associated with female sex and the summer season ($p<0.05$) (Table 3). Female subjects are eight times more affected than male subjects (OR=8.32; 95% CI=7.43-9.33; p=0.0001).

It is noted that COVID-19 infection, in individuals diagnosed with the RT-PCR test, has a seasonal tendency. The increase is observed in summer, twice as large as for the other seasons (OR=1.98; 95% CI=1.77-2.22; p=0.0001).

However, in individuals diagnosed with the antigen test there is an increase in autumn (Table 4).

The risk of positive antigen testing in the fall is about ten times (9.90) higher than antigenic testing in other seasons of the year (OR=9.90; 95% CI=1.16-84.66; p=0.036).

We kept the sex variable although it is not significant in the predictive model because the adequacy tests are more favorable to its restraint than to its withdrawal.

The true positive ratio (sensitivity) and the false positive ratio (1-specificity) were included in ROC curve. Typically, we compare a p (w) at the S=0.5 level to predict y (w). This allows us to construct the confusion matrix and extract the two predicted indicators, sensitivity and 1-specificity. The ROC curve expands upon this concept by altering the complete continuum of all potential values for levels 0 and 1. We construct the confusion matrix and compute sensitivity and 1-specificity for each configuration.

The models including RT-PCR and antigen testing showed areas under curve (AUC) of 0.83 (Figure 1A) and 0.81 (Figure 1B), indicating a strong ability to differentiate between positive and negative test results.

DISCUSSION

Given the lack of data on the use of antigenic tests compared to gold standard method (RT-PCR) in

Table 2. Association between the data studied and the diagnostic test.

Parameters	RT-PCR test			Antigenic test		
	Positive (N=4718)	**Negative (N=9765)**	***p*-value**	**Positive (N=81)**	**Negative (N=78)**	***p*-value**
Age (years)	45.1±18.4	44.6±20.7	0.158	44.6±17.4	44.8±19.1	0.954
Sex						
Men	987 (20.92%)	2215 (22.68%)	0.017	30 (37%)	38 (48.72%)	0.137
Women	3731 (79.08%)	7550 (77.32%)		51 (63%)	40 (51.28%)	
Age groups						
<40 years	2094 (44.38%)	4249 (43.51%)	0.233	37 (46%)	36 (46%)	0.440
40-60 years	1521 (32.24%)	3287 (33.66%)		27 (33%)	20 (26%)	
>60 years	1103 (23.38%)	2229 (22.83%)		17 (21%)	22 (28%)	
Seasons						
Winter	980 (20.77%)	3042 (31.15%)	0.0001	58 (72%)	60 (77%)	0.0001
Spring	543 (11.51%)	1491 (15.27%)		6 (7%)	17 (22%)	
Summer	2415 (51.19%)	3380 (34.61%)		0 (0%)	0 (0%)	
Autumn	780 (16.53%)	1852 (18.96%)		17 (21%)	1 (1%)	

Table 3. RT-PCR logistic model.

Predictor	Coefficients	Z (Wald)	*p*-value	OR	95% CI
Constant	-2.05755	-39.97	0.0001		
Women	2.11911	36.45	0.0001	8.32	7.43-9.33
Summer	0.683343	11.77	0.0001	1.98	1.77-2.22

OR: odds ratio, CI: confidence interval.

Table 4. Logistic models of antigen test.

Predictor	Coefficients	Z (Wald)	*p*-value	OR	95% CI
Constant	-0.517120	-1.41	0.158		
Women	0.611621	1.28	0.201	1.84	0.72-4.71
Autumn	2.29289	2.09	0.036	9.90	1.16-84.66

OR: odds ratio, CI: confidence interval.

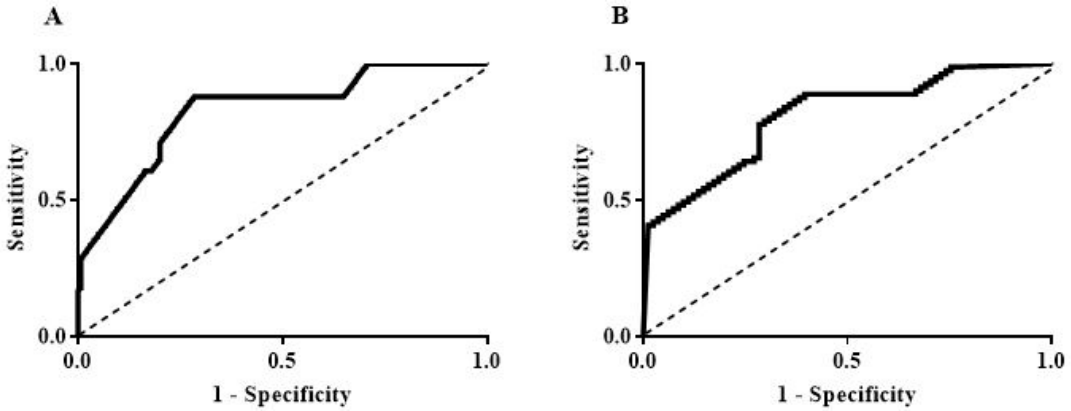

Figure 1. ROC curves of diagnostic tests. A: RT-PCR, B: antigenic test.

affected patients and others with warning signs (the unaffected) in screening logics, the present work presents data not yet recorded in the extreme west of Algeria. This work also proposes to study the potential influence of seasons on the sensitivity of antigenic diagnostic tests in comparison with the reference test which is RT-PCR. The results of our study reveal the first information on COVID-19 in the Tlemcen region. We studied and evaluated the performance of the antigen test in both sexes and over the four seasons, and we compared the capacity of these tests with the reference diagnosis which is RT-PCR. Based on association chi-square, antigenic test results were sex-independent ($p>0.05$). However, the logistic regression concerning the reference test shows that women are twice as affected by COVID-19 than men. Previous studies reveal significant diversity in relation to the susceptibility of both sexes to COVID-19 infection. But no research interested the effect of sex on the sensitivity of the test. Investigations have shown male predominance [9] while other studies have shown female predominance [8], however, previous results have concluded that both men and women have the same risk of exposure to COVID-19 [14]. This heterogeneity of results can be explained by the diversity in the expression and activity of ACE2 in response to sex hormones including estrogen [15, 16]. In addition, women have a more effective immune response against COVID-19 compared to men; estrogen can regulate certain immune cells that express estrogen receptors, namely macrophages, B and T lymphocytes [17], as it stimulates immunoglobulin G (IgG) and immunoglobulin M (IgM) [18]. In our population, the average age of patients with COVID-19 tested by the antigen test is not significantly different compared to negative subjects. Nevertheless, it is noted that the most affected women are the least aged, 46% are under 40 years. Unlike men, 47% are between 40 and 60 years old. The age group of 25 to 49 years represents 42.1% of the Algerian population affected by COVID-19. However, the percentage of subjects over 49 years is 52.6% [19]. Nevertheless, the influence of age on COVID-19 infection and diagnostic outcomes appears to vary across previously published studies, reflecting heterogeneity in study population. The median age was 49 years in patients with COVID-19 associated with diabetes and cardiovascular disease [20], while the median age was 63 in a population of Lombardy [21]. Our results showed significant variability in virus detection over time (p=0.0001). The seasonal dependence clearly visible in this study raises the question of the influence of the seasons on the transmission of COVID-19. However, it is controversial according to the diagnostic test. If we consider the reference test which is RT-PCR, the trend is in favor of winter, unlike the antigenic test which is in favor of an autumn trend. However, the results of this study indicate that autumn may probably cause more contamination than summer and winter in the far west of Algeria. This seasonal disparity may confirm the positive or negative effect of some climatic factors on the spread of viruses,

including temperature [22, 23], humidity [23, 24], ultraviolet radiation, and wind speed [25]. Indeed, the evolution curve of the daily number of cases recorded in Algeria shows a peak that reaches about 700 cases per day in late June and late August [19]. The second peak was between October and December with more than 800 cases per day; the number of summer cases decreased and stabilized between 200 and 100 cases per day during the winter season [26]. Globally, the number of cases increased from one million to two million between June and August, from two to four million between September and November, and cases with COVID-19 reached five million between the end of December and the beginning of January. This figure declined to 3 million in February [27]. For RT-PCR and antigen testing, our logistic models had a very strong ability to identify whether a test result was positive or negative (AUC value was 0.83 and 0.81, respectively). In comparison with RT-PCR, the antigenic test shows very similar sensitivity and specificity in test result classification to that reported in our results with AUC equal to 0.85; moreover, the levels of antigens are correlated with the viral load, which shows that the sensitivity of the antigen test is almost similar to that of RT-PCR [28]. A Spanish study concluded that the rapid antigenic test PanBioRT offers good performance in the detection of COVID-19. RT-PCR was positive in 37.5% of the tested subjects, while the rapid antigenic test PanBioRT was positive in 34.7% of the studied population [29]. A meta-analysis reports a very high sensitivity of the enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) tests; their sensitivity is comparable to that of RT-PCR [30]. Similarly, the Ansh-based anti-SARS-CoV-2 ELISA assay was highly effective in the identification of SARS-CoV-2 patients [31].

Although no direct data on participants' economic status is available in our study, the existing literature highlights that income inequality and unequal access to healthcare resources significantly influence the COVID-19 transmission and the effectiveness of screening strategies [10].

In the Algerian context, characterized by significant socioeconomic disparities, we can legitimately assume that socioeconomic status contributes to the observed inequalities in the diagnostic tests used and results.

Consequently, our results need to be considered in the light of these societal issues, emphasizing on the need to integrate socio-economic indicators into future research to better analyze vulnerability and the effectiveness of screening interventions.

CONCLUSION

Our analytical study proclaims that seasonality strongly influences the spread of COVID-19 as well as the performance of the antigen test, and that, among those tested via RT-PCR, the spread of the virus is particularly intense during summer, with a significantly higher positivity rate observed in women. In addition, the analysis revealed that the sensitivity of antigenic tests was optimal in the autumn. The robustness of the predictive models, with AUCs of 0.83 for RT-PCR and 0.81 for antigen testing, confirms these associations.

These results provide previously unrecorded data on the importance of reconsidering screening strategies, considering seasonal fluctuations, individual factors and the use of antigen testing as an alternative, to enhance the effectiveness of epidemic control measures, particularly in restrained resource regions.

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CONFLICT OF INTEREST STATEMENT

The authors do not have a direct or indirect interest (financial or non-financial) with any private, industrial or commercial organization in relation with the subject presented.

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