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An Evaluation of Laboratory Tests for COVID-19 Infection in Patients Residing in the Baghdad - Iraq

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Abstract

The COVID-19 pandemic has had global health and socio-economic impacts, highlighting the need for effective detection, treatment, and prevention methods. Real-time PCR and serological tests are commonly used to diagnose COVID-19 infections. To evaluate the performance of these tests in different patient populations, we conducted a study on 63 human subjects from Baghdad, Iraq between December 2021, and August 2023. Our findings suggest that real-time PCR is an effective method for detecting COVID-19 in the Iraqi population with high sensitivity and specificity. Specifically, we found that 77.8% of patients tested positive for COVID-19 using real-time PCR with a P-value <0.05*. Additionally, our study revealed that serological testing using Fin Care™ can be useful in detecting IgG and IgM antibodies against COVID-19. Among those tested with Fin Care™ for IgG antibodies, 50.8% had valid positive results while among those tested with Fin Care™ for IgM antibodies, 73% had valid positive results with a P-value <0.05*. Furthermore, biochemical tests on CRP, Ferritin, and D-dimer levels in blood samples taken from all participants showed statistically significantly higher than normal ratios among those infected with COVID-19. In this study, we evaluated the performance of real-time PCR and serological tests in detecting COVID-19 infection in 63 human subjects from Baghdad, Iraq. Our results suggest that real-time PCR is a highly sensitive and specific method for detecting COVID-19 in the Iraqi population. Additionally, serological testing using Fin Care™ can be useful in detecting IgG and IgM antibodies against COVID-19. These findings underscore the importance of accurate diagnostic methods to manage and control the spread of COVID-19.

Keywords: Covid-19 virus, fluorescence immunoassay, IgG, IgM, real-time-Polymerase chain reaction, PCR COVID-19.

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1. Introduction

In December 2019, the emergence of SARS-CoV-2 in Wuhan, China, resulted in the highly contagious respiratory illness known as COVID-19 [1-2]. Since then, infections have rapidly spread worldwide and were declared a pandemic by the WHO on November 1st, 2021. As of September 29th, 2022, Iraq has reported over 2.1 million confirmed cases and over 24,000 deaths from COVID-19 [3]. In the Baghdad government alone, there have been nearly 195,000 reported cases and almost 3,000 deaths. Although many aspects of this virus remain unknown, human-to-human transmission has been identified as a cause for its rapid spread [4]. Clinical laboratory testing provides valuable information to direct patient care in both inpatients. and outpatient settings. Since the emergence of COVID-19 in December 2019, several molecular and serological-based tests have been developed for point-of-care testing capabilities. These include ELISA tests for detecting IgG and IgM antibodies levels among patients infected with SARS-CoV-2 [5]. In this study, we evaluated samples collected from suspected COVID-19 patients in different hospitals across Baghdad government between December/2021 to August/2023 using a fluorescent serological assay kit called FinCare™ validated for the detection of SARS-CoV-2 status. As delineated in the preceding research, this product exhibits exceptional quality [6]. Accurate diagnosis of COVID-19 virus infection requires a combination of immunological and/or molecular methods such as Enzyme-Linked

Immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR), respectively.

2. Materials and methods

Venous blood samples were collected from all patients via vein puncture using a 10-5 ml disposable syringe, dispensed into sodium citrate tubes and gel tubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes to assess D-dimer, CRP, and serum ferritin quantity using an immunofluorescence assay kit and the Getein device 1100 (Almubdaa). A total of 63 subjects were tested for IgG and IgM antibodies level using an immunofluorescence assay kit Fin Care™. Additionally, PCR swabs were performed on all patients as it is considered the gold standard.

2.1. Statistical analysis

This study was designed by Completely randomized design (CRD) that used in the analysis of variance for data of NBT and MTT values by using one-way test and independent *t*-test at a 5% level of significance. Moreover, the correlation between IgG and IgM values in patient's serum sample groups was statistically analysed by Pearson correlation coefficient. In addition, several statistical tests such as Chi-Square, Fisher's exact test, Binomial test, and Likelihood Ratio test at a 5% level of significance were used to analyse microbial isolation results. Data were processed and analysed by using statistical program social science (SPSS 26) and the results were expressed as Mean ± SD (Table-1, 2, 3, 4, 5, 6, 7 and 8).

3. Results and Discussions

Essential scalable solutions are needed for effective pandemic management. It is also crucial to design assays that can predict the protection status of previously infected or immunized individuals against emerging SARS-CoV-2 variants in order to truly control the pandemic. Rapid diagnostic assays that identify immunoglobulin G (IgG) and immunoglobulin M (IgM) offer an alternative and instant modality to conventional real-time reverse transcription polymerase chain reaction (RRT-PCR) assays for diagnosing COVID-19. Accurate and timely diagnosis of symptomatic and asymptomatic SARS-CoV-2 infections remains essential in limiting human-to-human transmission. The highly immunogenic structural proteins of SARS-CoV-2 lead to the generation of IgM and IgG antibodies [7]. Our study showed that the sensitivity values of IgM tests were more sensitive compared to the IgG tests, while in another study, the sensitivity values of the IgG test were more sensitive compared to the IgM tests. This is because samples that tested positive for IgM indicated recent infection [8-9]. These results are consistent with previous research, which demonstrated the potential significance of quantitatively detecting IgM and IgG antibodies against SARS-CoV-2 for evaluating the severity and prognosis of COVID-19. Some studies showed great detection consistency among samples from finger stick blood, serum, and plasma of venous blood. The combined IgM-IgG assay has better sensitivity and utility compared to a single IgM or IgG test. It can be rapidly used for screening symptomatic or asymptomatic SARS-CoV-2 carriers in hospitals, clinics, and test laboratories [10]. Additional findings profiled the serological responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid (N) protein and spike (S) glycoprotein. It was observed that many patients developed robust antibody responses between 17 and 23 days after illness onset, while critical patients exhibited delayed but stronger antibody responses [11]. In the current COVID-19 pandemic caused by SARS-CoV-2, rapid and simple serological assays for characterizing antibody responses are important. Therefore, multiplex immunoblot (IB) assays named COVID-19 IB assays were developed to detect IgG and IgM antibodies to SARS-CoV-2 virus proteins in COVID-19 patients [12].

Interestingly, S-IgG had a parallel or similar dynamic pattern as S-IgM in the first two weeks, but S-IgG continued to increase in the third week while S-IgM in some patients showed plateau or decline in some patients. A similar pattern also occurred in non-ICU patients, but not in ICU patients. This result suggested that the early class switching of IgM to IgG may help predict a better outcome of COVID-19 disease [13]. Also, from the results of the 1014 patients, 601 of 1014 (59%) had positive RT-PCR results and 888 of 1014 (88%) had positive chest CT scans. The sensitivity of chest CT in suggesting COVID-19 was 97% (95% confidence interval: 95%, 98%; 580 of 601 patients) based on positive RT-PCR results [14]. Some patients with positive chest CT findings may present with negative results of real-time reverse-transcription polymerase chain reaction (RT-PCR) tests for coronavirus disease 2019 (COVID-19) [15]. The SARS-CoV-2 seroconversion is important for epidemiological studies as well as contact tracing. The Eliza and the VIDAS methods with a combination of IgG/IgM measurements demonstrated a high sensitivity with no false positive results [16].

Finally, serological assays for the detection of infection with SARS-CoV-2 were compared to RRT-PCR. These assays yielded lower sensitivities than RRT-PCR-based assays. However, given that these immunoassays are more affordable, faster, and easier to execute, they could be recommended for epidemiological research or characterizing the immune status of post-infection or post-vaccination subjects [17]. The result was the agreement with patients critically ill with coronavirus disease-2019 (COVID-19) feature hyper inflammation and the associated biomarkers may be beneficial for risk stratification. Investigate the association between several biomarkers, including serum C-reactive protein (CRP), procalcitonin (PCT), D-dimer, serum ferritin, and COVID-19 severity. Results of a total of 5350 patients were pooled from 25 studies by meta-analysis showed that an elevated serum CRP, PCT, D-dimer, and ferritin were associated with a poor outcome in COVID-19 [18]. The study of biochemical tests in represented by CRP, Ferritin, and D-dimer, where the results showed that the CRP which is the inflammatory index as shown its reached 155, this is considered high and statistically affected. As for Ferritin, the results also showed that it is very high compared to the normal ratio and this is statistically affected, finally for the D-dimer, the index of clots, the results showed that it is also high, and this is statistically affected.

Table 1. Descriptive statistics of *gender*.

		Frequency	Percent	Valid Percent	*P*- Value
Valid	Male	31	49.2	49.2	>0.05
	Female	32	50.8	50.8	
	Total	63	100.0	100.0	

Table 2. Descriptive statistics of *Age.*

	N	Minimum	Maximum	Mean	Std. Deviation
Age	63	24.00	42.00	31.2857	5.14446

Table 3. Descriptive statistics of *patients examined by PCR.*

		Frequency	Percent	Valid Percent	*P*- Value
Valid	Positive	49	77.8	77.8	<0.05*
	Negative	14	22.2	22.2	
	Total	63	100.0	100.0	

Table 4. Descriptive statistics of *COVID IgG.*

		Frequency	Percent	Valid Percent	*P*- Value
Valid	Positive	32	50.8	50.8	>0.05
	Negative	31	49.2	49.2	
	Total	63	100.0	100.0	

Table 5. Descriptive statistics of *COVID IgM*

		Frequency	Percent	Valid Percent	*P*- Value
Valid	Positive	46	73.0	73.0	<0.05*
	Negative	17	27.0	27.0	
	Total	63	100.0	100.0	

Table 6. Descriptive statistics of *patients examined by PCR with IgG.*

Positive PCR		Positive IgG	
No.	%	No.	%
49	77.8	24	38.1

Table 7. Descriptive statistics of *patients examined by PCR with IgM.*

Positive PCR		Positive IgM	
No.	%	No.	%
49	77.8	36	57.1

Table 8: Laboratory Test Results

Lab Test	**N**	**Mean ± SD**	**Min**	**Max**	**Normal Range**	**P-Value**
CRP (mg/L)	63	84.25 ± 46.43	11.00	155.0	Up to 10 mg/L	<0.05*
S. Ferritin (ng/mL)	63	596.4 ± 156.5	288.0	856.0	Men:15 -400 ng/mL Women:12-150 ng/mL	<0.05*
D-dimer (ng/mL)	63	648.1 ± 149.1	388.0	873	Up to .5 mg/L	<0.05*

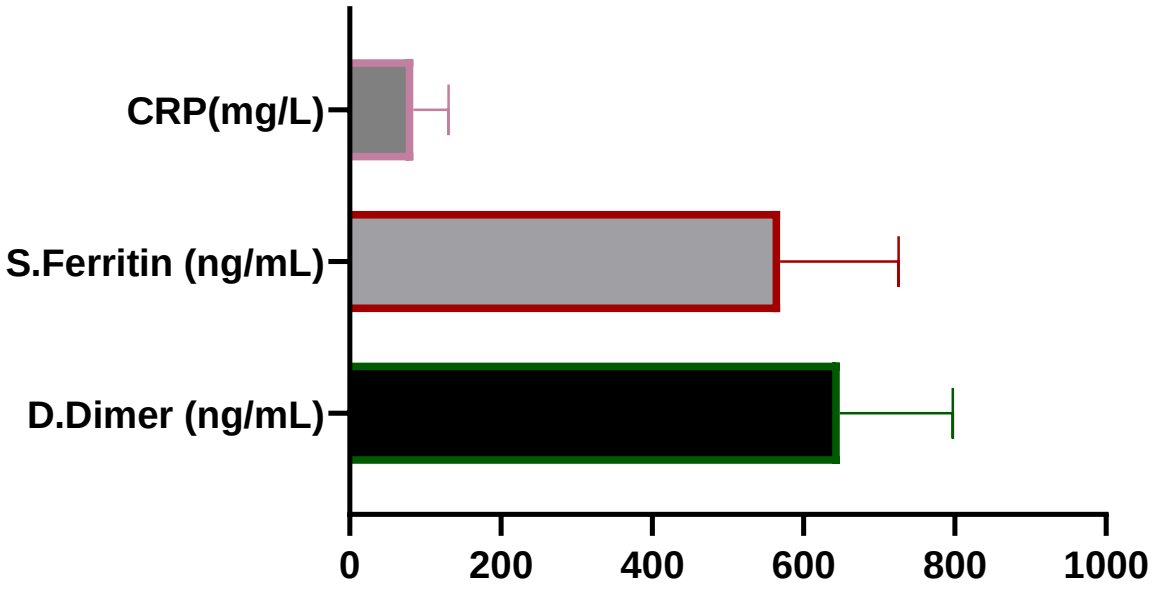

Figure 1. This figure represents the biochemical tests concentrations.

During the study period, sixty-three 63 patient sample was collected from the different lab in Baghdad city, among these patients, 31 (49.2%) were males and 32 (50.8%) were females, P-value was significantly not affected (>0.05) between males and females. as mentioned in Table 1. Among the results obtained, it was found that the percentage of infected females more than males as shown in Table 1, maybe due to several reasons, which may be due to weak immunity during pregnancy or breastfeeding, or because she bears the responsibility of the home and the burdens of shopping, which forces her to go out for shopping, and these places are naturally crowded. Among the results that emerged during our study, it became clear that the economic conditions had a significant impact on the number of infections with Covid-19, and it may be the reason for not adhering to prevention and caution. Urban health, in addition to their lack of cultural and health awareness. The data in Table 2 demonstrated the descriptive statistics of age, the minimum range of age was 24, where the maximum range was 42, also the mean was 31.2 and the standard deviation was 5.14 as shown in Table 2. After doing this questionnaire for people infected with COVID-19 and through the results obtained, we conclude the following: It was found that the age groups 24 to 40 is the age group most affected by Covid-19, as shown in the Table 2 and this may be attributed to its frequent mixing with society because it is the most active age group in Iraqi society.

Additionally, in Table 3 and Figure 2 we can see the descriptive statistics of PCR; the number of positive PCR 49 (77.8%) and the negative 14 (22.2%) the *P-value* was Significantly affected (<0.05) between Infected and uninfected people. PCR is the gold standard for diagnosing active COVID-19 infections, offering high sensitivity and specificity. While not 100% perfect, it remains the most reliable diagnostic tool for detecting the virus. Also, Table 4 and 5 we've done immunological tests IgG and IgM, and as we see it in the case of IgG the number of positive results 32 (50.8%) and the number of negative results 31 (49.2%) the *P-value* was Significantly not affected (>0.05), and in the case of IgM the number of positive results 46 (73%) and the negative results 17 (27%) the *P-value* was Significantly affected (<0.05). A COVID IgG Positive result indicates the presence of IgG antibodies specific to SARS-CoV-2, suggesting a past infection. However, these antibodies don't necessarily guarantee immunity against future infections.

Likewise, we made a relationship for those who had PCR positive and IgG positive as in Table 6 and the results showed 49 (77.8%) and 24 (38.1%) respectively, and those who had PCR positive and had IgM positive as in Table 7 and the results showed 49 (77.8) and 36 (57.1) respectively. Finally, in Table 8 and figure 1 we did some biochemical tests represented by CRP, Ferritin, and D-dimer, where the results showed that the CRP which is the inflammatory index as shown its reached 155, and this is considered high and statistically affected. As for Ferritin, the results also showed that it is very high compared to the normal ratio and this is statistically affected, finally for the D-dimer, the index of clots, as the results showed that it is also high, and this is statistically affected.

CRP (C-reactive protein) levels may be elevated in COVID-19 patients due to the body's inflammatory response to the infection, indicating inflammation or tissue damage. Ferritin levels may be elevated in COVID-19 patients as a result of the body's immune response to the infection, and it can also indicate inflammation or tissue damage [19]. Another cause of elevated ferritin levels in COVID-19 patients could be secondary hemophagocytic lymphohistiocytosis (sHLH), a hyperinflammatory syndrome characterized by excessive immune activation that can lead to multiorgan failure. Elevated ferritin has been associated with sHLH in the context of severe COVID-19 cases [20].

4. Conclusions

Compared to rRT-PCR, serological assays provide a faster way to detect SARS-CoV-19 infection. Sensitivity values for the IgM test were found to be lower than those of IgG. However, these assays have lower sensitivity compared to standard rRT-PCR based assays. Additionally, they may not detect the presence of the virus early in the course of infection, and a positive result does not necessarily indicate immunity to the virus. A recent study aimed to improve COVID-19 diagnosis sensitivity through standard rRT-PCR testing. Patients with COVID-19 experience hyperinflammation and associated biomarkers such as serum C-reactive protein (CRP), procalcitonin (PCT), D-dimer, and serum ferritin exhibit significantly elevated levels compared to normal ratios, which is statistically significant.

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The authors stated that they do not have any conflicting interests.

Author contributions

Al-Karawi A. performed the statistical analysis and drafted the manuscript., Al-Jandeel T. contributed to the conception and design of the study, Abdulrazzaq O. was involved in clinical evaluation, Aljandeel S. interpreted the results and Rasool K. Contributed to research modifications. The entire manuscript was read and approved by all authors.

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