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ORIGINAL PAPERS

Correlation Between Levels of C-Reactive Protein, D-Dimer, and Ferritin in Covid-19 Patients Infected with Different Bacterial Species

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Abstract

Introduction: The fast dissemination of SARS-CoV-2 occurred swiftly across Iraq and various nations globally, leading to a widespread viral pandemic. C-reactive protein, Ferritin, and Ddimer have emerged as pivotal molecules within the immune system, playing a central role in coordinating cellular defense mechanisms against inflammation. This study aimed to correlate the role of CRP, ferritin- and D-dimer parameters in patients of coronavirus disease - 2019 (COVID-19) associated bacterial co-infection. **Methods:** Sputum and blood samples were collected from patients who diagnosed a COVID-19 by an internal medicine specialist and positive PCR test. The collected samples of sputum were inoculated on selective culture media, the isolated colonies were further subjected to subculture and identified using cultural and biochemical tests. Antibiogram testing was performed with selected antibiotics. An automated VITEK-2 compact system using AST-N222 cards and - chroma$_{11}$ ™ D-Dimer kits was used to determine the levels or titers of these parameters using ELISA and fluorescence Immunoassay (FIA) for the quantitative determination in human whole blood/serum /plasma according to manufacturing instructions. **Results:** the results revealed a significant rise in ferritin levels among individuals with moderate and severe diseases in comparison to those with mild cases. Among severe patients, ferritin levels were significantly higher than in non-severe cases (P=0.02). The mean C-Reactive protein levels were 92.54±68.94, 97.66±65.70, and 123.83±65.80 in Covid19 patients infected with M. catarrhalis, P. aeruginosa, and K. pneumonia, respectively. The study showed levels of ferritin non-significant increase in patients infected with COVID-19 and K. pneumoniae when compared with other groups was none. **Conclusion**: In this study, the results proved the presence of an association between the elevated levels of tested biomarkers (CRP, Ferritin, D-dimer) in co-infection by bacteria of COVID-19 patients.

Keywords: CRP; Ferritin; D-dimer; bacterial co-infection; COVID-19.

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INTRODUCTION

Distinguishing between COVID-19 and classical bacterial pneumonia poses a challenge. Moreover, patients of COVID-19 enter the phase of inflammation later in their disease progression, making it challenging to differentiate from a secondary infection of bacteria. Consequently, 72% of hospitalized patients of coronavirus disease 2019 take pragmatic antibiotic treatment[1]. While most individuals testing positive for COVID-19 experience mild or no symptoms, others may develop severe conditions such as syndrome respiratory distress, clots of blood, failure of the heart, and important response of inflammation[2].

C-reactive protein (CRP) is an acute-phase inflammatory protein composed of homopentamers. In the presence of calcium, the CRP fixes to polysaccharides like phosphocholine on microorganisms. As a marker for inflammation, CRP levels rise during the infection of bacteria, responding to intermediaries of monocytic like IL_ 6 and IL_1. Synthesized mainly in hepatocytes in response to IL-6, CRP is a crucial acute-phase protein[3].

Ferritin, a universal intracellular protein responsible for storing and releasing iron, is produced by several organisms, including archaea, bacteria, algae, animals and plants[4]. While predominantly a cytosolic protein in most tissues, small amounts of ferritin secreted in serum, act as a carrier of iron. It serves as a marker for body iron and is consumed as a diagnostic test for anemia. So, ferritin changes to toxic iron called hemosiderin[5].

A hyper-inflammatory environment has been a hallmark of COVID-19 infection and is thought to be a key mediator of morbidity. Several retrospective studies have shown ferritin levels can be correlated and predictive of poor outcomes in COVID-19[6].

The present study aimed to analyze the laboratory results, including serum C-reactive protein, D- Dimer, and Ferritin in Covid-19 patients in combination with bacterial co-infections.

MATERIALS AND METHODS:

A case-control study of 110 blood samples was collected from patients who diagnosed a COVID-19 by specialist medicine and positive PCR test, who attended Ad-Diwaniyah City, Teaching Hospital, Iraq during the period from January 2020 to September 2021**.** Samples are stored in portable containers, labelled, and then transferred to the laboratory. CRP, Ferritin, and ichroma™ D-Dimer kits (Biotech- South Korea) were used to determine the levels or titers of these parameters using ELISA and fluorescence Immunoassay (FIA) for the quantitative determination in human whole blood/serum /plasma according to manufacturing instructions. On the other hand, sputum samples of those patients, including (60) samples from moderate cases of COVID-19 patients in private clinics, and (50) samples from critical cases in intensive care units were collected to isolate the bacteria associated with COVID-19. samples cultured on MacConkey agar incubated at 37˚C for 48 hours. identified using cultural and biochemical tests. "Antibiogram testing was performed with selected antibiotics that are commonly used and recommended by WHO). Antibiotic susceptibility test was assessed by an automated VITEK-2 compact system using AST-N222 cards"[6]

PCR technique was performed for molecular detection of *Klebsiella pneumonia, P. aeruginosa, and Moraxella catarrhalis* isolated from COVID-19 patients based on the amplification of *the 16S ribosomal RNA* gene. Bacterial genomic DNA was extracted bacterial isolates by using (Presto™ Mini gDNA Bacteria Kit)**.**

Ethics Consideration - According to the document at (10-1-2021)

This study is under the ethics committee of Al-Diwaniayah Teaching Hospital, Iraq. A verbal agreement was obtained from participants in the study of the relative's pre-taking samples.

STATISTICAL ANALYSIS

SPSS version 25 and Microsoft Office Excel 2020. The chi-square test was used to study the association between any two categorical variables. The odds ratio and 95% confidence interval were estimated to measure risk. The level of significance was considered at a P-value of less than 0.05 and a highly significant level of 0.01 or less than[7].

RESULTS

The present results indicated that the dominant Moraxella catarrhalis followed by K. pneumoniae and P. aeruginosa isolated microbes from the respiratory tract recorded (Table 1). The present study shows more severe frequency occurred in P. aeruginosa with 8

(66.7%) of patients with severe cases, but the difference was not significant association when compared with other bacterial species (p= 0.400).

Table 1. Comparison of the frequency distribution of severity of patients with Covid-19 infection according to the results of PCR (*16SrRNA*).

Characteristic	PCR (*16SrRNA*) results			*P*
	M. catarrhalis	*P. aeruginosa*	*K. pneumonia*	
Severity				
Severe, *n* (%)	12 (47.8 %)	7 (69.6 %)	6 (43.7 %)	0.400 ¥ NS
Moderate, *n* (%)	13 (55.0 %)	5 (43.3%)	6 (68.3%)	

SD: standard deviation; ***n***: number of cases; ¥: chi-square test; **NS**: not significant at $p > 0.05$

Table (1) shows that more severe frequency occurs in *P. aeruginosa* were show 8 (66.7) patients with severe cases, but the difference was no significant association when compared with other bacterial species (p = 0.400). *P. aeruginosa* aligned well with the peak of immune response indicators[12]. So, *P. aeruginosa* was highly associated with the bacterial co-infection induced in the patients during the critical stage of COVID-19. The results in Table (3) represented the antibiogram profile of *Moraxella catarrhalis, P. aeruginosa, and K. pneumonia*, indicating that isolates varied in their susceptibility to the antibiotics.

Table 2. Results of Antibiotics susceptibility patterns in clinical infection of *bacterial species*

bacterial species	MDR n (%)	XDR n (%)	PDR n (%)
M. catarrhalis N=25	25 (100.0%)	0 (0%)	0 (0%)
K. pneumonia N=12	8 (85.0%)	4(63%)	0 (0%)
P. aeruginosa N=12	9 (73.3%)	3 (18.7%)	0 (0%)
X2	0.140		
P value	3.889		

n: number of cases; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

The comparison of some parameters between COVID-19 patients infected with different bacterial species groups in (Table 3). Mean levels of C-Reactive protein were 92.54 ± 68.94, 97.66 ± 65.70, and 123.83 ± 65.80, in patients of COVID-19 and diseased by M. catarrhalis, patients of COVID-19 infected with P. aeruginosa and Covid-19 patients with K. pneumoniae respectively; levels were greater in Covid-19 patients infected by K. pneumoniae in contrast with other groups but the difference was non-significant (P = 0.621). D-dimer levels were 1624.16 ± 1083.07, 1013.25 ± 846.36, and 1865.75 ± 1620.24, patients of Covid-19 infected with M. catarrhalis, Covid-19 patients infected with P. aeruginosa and the patients of Covid-19 infected with K. pneumonia; the levels were increased in Covid-19 patients infected with K. pneumonia (P = 0.462). Mean levels of Ferritin showed a non-significant increase in patients (P = 0.785).

"Bacteria can infect patients with COVID-19 accidentally, and a longer hospital stay leads to a greater risk of pneumonia. Several hypotheses, such as host immune changes, mechanical damage and spread, and mucus removal within the lungs, have been proposed about the cause of secondary infection"[17].

Comparison of some parameters between COVID-19 patients infected with different bacterial species results were demonstrated in Table (4). Mean levels of C-Reactive protein were 92.54 ± 68.94, 97.66 ± 65.70, and 123.83 ± 65.80, in Covid-19 patients infected with *M. catarrhalis, P. aeruginosa* and with *K. pneumonia* respectively; the mean levels were higher in Covid-19 patients infected with *K. pneumonia* in comparison with other groups but the difference was non-significant (P = 0.621). Also, the Mean levels of D. dimer were 1624.16 ± 1083.07, 1013.25 ± 846.36, and 1865.75 ± 1620.24, Covid-19 patients infected with *K. pneumonia* in comparison with other groups and the difference was non-significant (P = 0.462).

Table 3. Levels of some parameters (CRP, D. Dimer, and Ferritin) in COVID-19 patients infected with different bacterial species

M. catarrhalis		Covid-19 patients infected with different bacterial species			
		P. aeruginosa	*K. pneumonia*	*Total P value*	
C-Reactive Protein					
Mean± SD		92.54 ± 68.94	97.66 ± 65.70	123.83 ± 65.80	P= 0.620 † NS
Range		17.00 – 200.00	20.00 – 200.00	21.00 – 200.00	
LSD	M vs P	0.196			
	M vs K	0. 831			
	P vs K	0.347			
D-Dimer					
Mean± SD		1625.16 ± 1083.07	1013.24 ± 846.36	1866.75 ± 1620.24	P= 0.460 † NS
Range		150.00 – 7420.00	350.00 – 3170.00	330.00 – 5330.00	
LSD	M vs P	0.325			
	M vs K	0.695			
	P vs K	0.235			
Ferritin					
Mean± SD		570.47 ± 344.12	578.58± 288.97	645.91± 272.43	P= 0.785 † NS
Range		81.00 - 1310.00	200.00 - 1098.00	268.00 - 1144.00	
LSD	M vs P	0.940			
	M vs K	0.500			
	P vs K	0.602			

n: number of cases; SD: standard deviation; *M: M. catarrhalis; P: P. aeruginosa; K: K. pneumonia:* †: Anova test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

DISCUSSION

The findings of the study revealed that the primary microorganism isolated from the respiratory tracts of Covid-19 patients was M. catarrhalis, followed by K. pneumoniae and P. aeruginosa. The presence secondary microbial infections in individuals with Covid-19 was associated higher mortality rates[8]. Previous documentation has highlighted the rate of mortality in infections of viruses can be influenced by many factors, including co-infection by bacteria[9].

The severe complications resulting from virus-bacterial co-infections primarily involve an ineffective immune response and pathogenic synergy[10]. SARS-CoV-2 infection has been observed to damage lung cells and infrastructure, creating conditions conducive to increased bacterial adherence and invasion[11,12]. P. aeruginosa demonstrated a strong association with immune response indicators, particularly during the critical stage of COVID-19, indicating its significant role in bacterial co-infections[13,14].

The current study reported a high rate of bacterial co-infection (100%), consistent with other investigations on severely infected COVID-19 patients admitted to hospitals. International studies also support the idea that secondary bacterial infections pose a risk for the rate of COVID-19 mortality, with bacterial infection patients in intensive care units[15,16]. In Iraq, 94% of cases displayed bacterial co-infection, emphasizing the necessity for antibiotic usage and raising concerns about problems from the bad use of antibiotics with acquired diseases of bacteria[17,18,19]. While the specific nature and source of these infections are fully travelled, the suggests that multidrug-resistant bacteria are accountable for their expansion[20,21].

Patients with COVID-19 may accidentally contract bacterial infections, with longer hospital stays increasing the risk of pneumonia. Hypotheses propose that alterations in host immune, damage and spread, and lung mucus contribute to secondary infections, especially in individuals with viral lung infections[22,23]. COVID-19 patients exhibited elevated biomarker levels with inflammatory cytokines connected to bacterial co-infection, resulting from immune system dysregulation[24,25,26]. The study indicates a non-significant increase in the mean levels of C-reactive protein in patients of COVID-19 infected by K. pneumoniae compared to other groups. While CRP is consistently elevated in bacterial infections, its predictive value for infections by bacteria in the first phase of COVID-19 may be limited. Monitoring of CRP levels over time could aid in identifying nosocomial bacterial diseases and managerial appropriate antibiotic treatment.[27- 29]

Similarly, the study found a non-significant increase in mean levels of Ferritin in COVID-19 patients infected with K. pneumoniae. Ferritin increases the response of systemic and pulmonary inflammation. COVID-19 is characterized by systemic inflammation and elevated pro-inflammatory cytokines, such as IL-6 and TNF-alpha, particularly in severe cases. Great the levels of ferritin are connected with the cytokine storm in severe hyperferritinemia.[33, 34]

In conclusion, COVID-19 represents a systemic inflammatory condition with elevated cytokines of

pro-inflammation, especially in plain cases. Cytokines, reactive oxygen species (ROS) generation, and secondary tissue damage are hypothesized mechanisms for the elevated ferritin levels of associated with COVID-19 [35]. D-dimer test measures a protein fragment produced when a blood clot dissolves, and elevated levels may indicate a blood clotting condition, though the test cannot specify the type or location of the clot.[36-39]

CONCLUSION

This study demonstrated a clear link between elevated levels of the tested biomarkers (CRP, Ferritin, D-dimer) and bacterial co-infection among patients with COVID-19 in the study area.

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Ethics Consideration - According to the document at (10-1-2021)

This study is under the ethics committee of Al-Diwaniaya teaching hospital, Iraq. A verbal agreement was obtained from participants in the study of the relative's pre-taking samples.

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