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Original Research Article

TO EVALUATE THE CORRELATION BETWEEN INFLAMMATORY INDICATORS AND THE SEVERITY OF COVID-19

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Abstract

Background: The global COVID-19 pandemic has impacted the whole global human population. It is a global epidemic that is resulting in the deaths of millions of individuals worldwide. Statistical investigations have shown a strong correlation between the intensity of inflammation in COVID-19 patients and the levels of several inflammatory markers such as CRP, ferritin, LDH, TLC, D-dimer, Procalcitonin, and IL 6. An abnormal level of these markers suggests a negative prognosis. It is necessary to assess the correlation between the severity of COVID-19 and key inflammatory markers such as CRP, ferritin, LDH, TLC, D-dimer, Procalcitonin, and IL-6. **Aim:** To evaluate the correlation between inflammatory indicators and the severity of Covid-19. **Materials and Methods:** The research was carried out at the Department of Biochemistry, Rajshree Medical Research Institute. There were 110 individuals that tested positive for RT PCR and were included in the study. The serum samples were collected and analysed using the MAGLUMI series for the following parameters: C-reactive protein (CRP), ferritin, LDH, Procalcitonin, D-dimer, total leukocyte count (TLC), and interleukin 6 (IL-6). The MAGLUMI series fully automated chemiluminescence immunoassay analyzer was used to quantitatively determine PCT, D-Dimer, and IL-6 in human blood and plasma. A total of 110 patients who tested positive for RT PCR were included in this research. Only patients who had CRP, FERRITIN, LDH, Procalcitonin, D-dimer, TLC, and IL 6 levels in their blood at the time of admission to the hospital, and who provided their informed written permission, were included. **Results:** The total number of patients was 110, consisting of 39 female and 71 males. Every metric was assessed for each subject. The average age of men was 47.17±8.25 years, whilst the average age of females was 41.96±7.25 years. Out of the seven markers examined in the study, only four - CRP, D-Dimer, Ferritin, and IL6 - showed statistical significance, with a p value of less than 0.05. The three remaining markers, namely TLC, LDH, and procalcitonin, did not show any significant results. The statistical significance of LDH was not seen, since the p value was 0.14. The p-value for TLC was 0.08, whereas the p-value for procalcitonin was 0.15. Consequently, these assessments did not show substantial levels of inflammation in people with COVID-19 in our study. **Conclusions:** The biomarkers CRP, D-Dimer, Ferritin, and IL 6 showed a significant association with the severity of COVID-19. However, the other three parameters, TLC, LDH, and Procalcitonin, did not show a significant correlation with the severity of COVID-19.

INTRODUCTION

COVID-19 is a zoonotic illness caused by a new coronavirus, leading to a worldwide epidemic. One The mortality rate associated with COVID-19 is lower compared to the previous two coronaviruses, namely severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). However, the extended period of time before symptoms appear and the lack of effective antiviral treatment have resulted in numerous fatalities, causing widespread devastation globally. While the clinical features of COVID-19 have been established, there is currently little knowledge on the laboratory abnormalities seen in individuals with COVID-19.[1,2] The global COVID-19 pandemic has impacted the whole global human population. It is a global epidemic that is resulting in the deaths of millions of

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individuals worldwide. Most individuals afflicted with Covid-19 will encounter mild to severe symptoms and recuperate without requiring hospitalisation. The following symptoms are the most widespread: The symptoms that may manifest include fever, dry cough, fatigue, body aches, sore throat, conjunctivitis, headache, loss of smell and taste, skin rash or discoloration of fingers or toes, trouble breathing or shortness of breath, chest discomfort, and loss of speech or movement. Statistical investigations have shown that the levels of several inflammatory indicators, such as CRP, ferritin, LDH, TLC, D-dimer, Procalcitonin, and IL 6, are strongly linked to the degree of inflammation in COVID-19 patients. An abnormal level of these markers suggests a negative prognosis.[3]

C-reactive protein (CRP) is an acute phase protein that is generated by cytokines and increases in concentration as a reaction to inflammation. This makes it a valuable early marker for detecting infection and inflammation. CRP has a higher degree of sensitivity in its test compared to ESR or leukocyte count, and its levels demonstrate a more rapid increase and subsequent return to normalcy after the resolution of illness. Statistical analysis have shown a strong correlation between CRP levels and the degree of pulmonary inflammation in COVID-19 patients. An increased CRP level is indicative of a worse prognosis. Upon the entry of a virus into the body, an excessive production of inflammatory cytokines occurs in order to battle it. However, when these cytokines become too active, they may cause injury to the lungs and other internal organs. Silent hypoxia, characterised by inadequate oxygen supply to physiological tissues, leads to trauma, shock, heart failure, myocardial infarction, and multi-organ dysfunction, hence causing an elevation in CRP levels.[4] Upon dissolution of a blood clot inside the body, a protein fragment known as a D-dimer is generated. It is not often present in human blood plasma. D-dimer tests are used to exclude the existence of an unhealthy blood clot (thrombus). Patients diagnosed with Covid-19 have regular testing for D Dimer levels. Recent autopsies conducted on 12 people diagnosed with Covid-19 revealed the presence of deep vein thrombosis in the most severe instances, with two cases showing a prominent pathological evidence of pulmonary embolism. The clinical cause of death was plainly stated. Studies have shown that there is a correlation between D dimer levels and the severity of sickness in patients with SARS Cov 2. Additionally, D dimer levels are a good predictor for in-hospital mortality in patients with COVID-19.[5]

The human body lacks the ability to endogenously produce iron and instead obtains it via dietary absorption. Most of the iron that is consumed is used for the production of red blood cells, while the rest is stored as ferritin. inside all living creatures, this protein serves as a crucial reservoir for storing iron inside cells. Upon the entry of the Corona virus into the body, an overabundance of cytokines is generated by the body as a means to counteract the infection. This cytokine storm induces internal infection and inflammation, resulting in the impairment of internal organs. Cell destruction leads to the release of ferritin into the circulation, causing hyperferritinemia. Recently published recommendations suggest that continuously monitoring a patient's written records throughout hospitalisation might help identify severe cases and forecast the severity of Covid-19, therefore aiding in clinical prognosis.[6] LDH is a ubiquitous enzyme found in almost all living cells. LDH facilitates the interconversion of lactate and pyruvate by converting NAD+ to NADH and vice versa. A dehydrogenase is an enzyme that facilitates the passage of hydride ions between molecules. LDH is extensively distributed in many human tissues, including blood cells and heart muscle, due to its release after tissue injury. Consequently, LDH is used as a diagnostic indicator for prevalent injuries and conditions such as heart failure. Due to the presence of LDH in lung tissue (specifically isoenzyme 3), individuals with severe Covid-19 infections are likely to experience increased release of LDH into the circulation. This leads to the development of a severe kind of interstitial pneumonia that often advances to acute respiratory distress syndrome. The specific role of different LDH isoenzymes in the increase of LDH levels seen in COVID-19 has not been established yet.[7] The thyroid parafollicular C cells typically produce and secrete PCT, a 116-amino-acid precursor of the hormone calcitonin. During bacterial infection, the production of this substance may occur in several organs outside of the thyroid, and this is caused by increased levels of tumour necrosis factor alpha (TNF alpha) and interleukin 6.[6] IL-6 is a significant pro-inflammatory cytokine that has been linked to faster disease advancement and a greater likelihood of complications in COVID-19 instances. The existing research has shown that cytokine storm syndrome is linked to more severe illness and consequences.[8-10] Conversely, patients of anosmia caused by COVID-19 have less severe symptoms of the condition. Our objective was to examine the correlation between IL-6 levels and the occurrence of COVID-19 associated anosmia, based on these data. Our objective was to provide a comprehensive analysis of the correlation between inflammatory markers and the severity of Covid-19.

MATERIALS AND METHODS

The research was carried out at the Department of Biochemistry, Rajshree Medical Research Institute. Approval was granted by the institutional ethics committee after obtaining ethical clearance. There were 110 individuals that tested positive for RT PCR and were included in the study. Each patient provided written informed consent. The serum samples were collected and analysed using the MAGLUMI series for the following parameters: C-reactive protein (CRP), ferritin, LDH, procalcitonin, D-dimer, total

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leukocyte count (TLC), and interleukin 6 (IL-6). The MAGLUMI series fully automated chemiluminescence immunoassay analyzer was used to quantitatively determine PCT, D-Dimer, and IL-6 in human blood and plasma. The evaluation of C reactive protein (CRP) in human serum or plasma was conducted using a latex-enhanced turbidimetric immunoassay. A total of 110 patients who tested positive for RT PCR were included in this research. Only patients who had CRP, FERRITIN, LDH, Procalcitonin, D-dimer, TLC, and IL 6 levels in their blood at the time of admission to the hospital, and who provided their informed written permission, were included. Exclusions were made for paediatric and pregnant patients, patients with a history of head trauma, individuals with preexisting abnormalities in smell and taste for any other cause, and patients with allergic rhinitis and chronic rhinosinusitis based on clinical history and CT results.

Statistical Analysis

The statistical analysis was conducted using NCSS (Number Cruncher Statistical System, 2007, Kaysville, Utah, USA). The descriptive statistics were presented as the count and percentage for categorical variables, and as the mean, standard deviation, median, minimum, and maximum for numerical variables. The Shapiro-Wilk test was used to ascertain the presence of a normal distribution in the data. The Student's t-test and Mann-Whitney U test were used to analyse data with parametric and nonparametric characteristics, respectively. The chi-square test, Fisher's exact test, and Fisher-Freeman-Halton exact test were used for categorical variables. The Spearman's rank correlation test was used to assess the correlation. A P value of less than 0.05 was deemed to be statistically significant.

RESULTS

The total number of patients was 110, consisting of 39 female and 71 males. Every metric was assessed for each subject. The average age of men was 47.17±8.25 years, whilst the average age of females was 41.96±7.25 years. Out of the seven markers examined in the study, only four - CRP, D-Dimer, Ferritin, and IL6 - showed statistical significance, with a p value of less than 0.05. The three remaining markers, namely TLC, LDH, and procalcitonin, did not show any significant results. The statistical significance of LDH was not seen, since the p value was 0.14. The p-value for TLC was 0.08, whereas the p-value for procalcitonin was 0.15. Consequently, these assessments did not show substantial levels of inflammation in people with COVID-19 in our study

Table 1: Gender and age of the participants

	Number=110	Percentage	P value
Gender			0.12
Male	71	64.55	
Female	39	35.45	
Age in year			0.14
below 25	10	9.09	
25-35	32	29.09	
35-45	45	40.91	
45-55	15	13.64	
Above 55	8	7.27	
Mean age	45.25±3.64		

Table 2: Male and Female regarding inflammatory markers

	Male	Female		
Parameter	**Mean±SD**	**Mean±SD**	**T value**	**P value**
D-dimer	2301.19±74.25	2011.06±69.33	1.03	0.03
Procalcitonin	0.42±0.08	0.84±0.09	2.33	0.15
TLC	8999.97±39.85	8007.05±40.22	2.41	0.08
CRP	35.03±3.69	26.85±3.33	3.63	0.03
LDH	369.96±38.81	319.31±41.52	3.74	0.14
Ferritin	649.85±45.52	265.58±39.85	3.98	0.0001
IL 6	45.52±5.28	26.63±5.28	3.74	0.03

Table 3: Raised and Unraised value in male and female regarding inflammatory markers

	Raised	Unraised	T Value	P Value
CRP				
Male	35.14±4.33	5.52±1.31	12.65	0.0001
Female	3.41±0.41	3.01±0.25	0.21	0.74
D- Dimer				
Male	4525.24±28.63	2385.41±27.33	6.66	0.52
Female	3341.52±21.36	2211.28±20.37	12.74	0.14
Ferritin				
Male	1311.05±35.85	241.52±15.52	8.22	0.001
Female	371.25±13.74	54.85±4.31	9.74	0.0001
IL 6				
Male	65.85 ± 4.05	32.87±3.74	6.37	0.001

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Female	57.41±4.08	26.42±2.55	11.74	0.0001
LDH				
Male	421.05±12.22	232.52±11.74	8.74	0.21
Female	371.52±15.24	179.55±8.85	10.28	0.34
Procalcitonin				
Male	0.52±0.06	0.24±0.02	6.88	0.41
Female	0.41±0.07	0.19±0.01	10.96	0.32
TLC				
Male	10625.54±87.96	9199.74±39.74	6.37	0.22
Female	9425.74±69.74	8252.47±74.55	10.95	0.11

DISCUSSION

The current COVID-19 epidemic has created a pressing need to investigate efficient predictors of illness severity. The current investigation identifies biomarkers that may precisely forecast the severity of COVID-19 patients. Covid-19 is believed to be caused by cytokine release syndrome (CRS), an inflammatory immune response that results in organ dysfunction. Heightened concentrations of interleukin (IL) 6, a cytokine that stimulates the liver to produce C-reactive protein (CRP) and fibrogen, have been associated with severe cases of Covid-19 and cytokine release syndrome (CRS). LDH and ferritin, together with CRP and fibrinogen, are associated with plasma IL-6 levels.[11] Empirical research has shown a correlation between alterations in the concentrations of several haematological markers and the severity and mortality of individuals afflicted with Covid-19. Out of these clinical markers, serum CRP has been recognised as a crucial indicator that significantly fluctuates in severe Covid-19 patients. C-reactive protein (CRP) is a hepatically synthesised protein that serves as an early biomarker for infection and inflammation. The normal blood value of CRP is less than 10 mg/L. It increases rapidly within 6-8 hours and reaches its highest point 48 hours after the commencement of the illness. The substance has a half-life of 19 hours, and its concentration decreases as the inflammatory stage concludes and the patient recovers. CRP has a preference for binding to phosphocholine, a molecule that is highly synthesised on the outer layer of damaged cells. The binding of this substance triggers the classical complement pathway of the immune system, leading to the regulation of phagocytic activity. This process helps to remove harmful infections and damaged cells from the body. CRP levels decrease as inflammation diminishes, making it a good indicator.[12,13] Individuals undergoing treatment for Covid-19 had a significant elevation in C-reactive protein (CRP) levels, with an average range of 30-50 mg/L. According to a research, those with more severe symptoms had an average concentration of C-reactive protein (CRP) of 39.4 mg/L, while patients with minimal symptoms had an average CRP concentration of 18.8 mg/L. A separate research discovered that the average concentration of CRP in patients with severe symptoms was 46 mg/L, whereas it was 23 mg/L in patients without severe symptoms.[14] In 1988, Zaman et al. conducted a study to investigate the effectiveness of serum LDH in viral infections, marking the first instance of such research. It was shown that blood LDH levels might serve as an indicator for P. Jirovecii pneumonia in persons with HIV.[15] Ede et colleagues found a correlation between the degree of cellular damage in the nasopharynx during viral upper respiratory tract infection and the occurrence of acute otitis media complications. This correlation was assessed by measuring LDH levels in nasopharyngeal discharges. They asserted that there is a positive correlation between LDH levels and all cytokines, including interleukin-1, IL-6, and tumour necrosis factor. Elevated serum LDH levels have been seen in many studies on severe acute respiratory syndrome (SARS).[16] An analysis conducted in 2003 on SARS data revealed that severe cases were characterised by the presence of lymphopenia, elevated levels of LDH, AST, and creatinine kinase. Liu et al reported that 58 percent of SARS patients had elevated LDH levels.[17] Ferritin has a crucial role in regulating immune system dysfunction, particularly in cases of very high levels of ferritin in the blood. It contributes to the development of a cytokine storm by directly suppressing the immune system and promoting inflammation. Covid 19 has found that fatal outcomes are linked to cytokine storm syndrome, indicating that the severity of the sickness is contingent upon cytokine storm syndrome. A considerable proportion of individuals with diabetes exhibit elevated blood ferritin levels, which is well acknowledged to increase their susceptibility to experiencing severe complications from covid-19. A study involving 20 patients with Covid-19 found that those with severe and extremely severe cases of the disease had elevated levels of serum ferritin.[18] In our investigation, the importance of ferritin as a biomarker for monitoring and predicting illness severity was much lower when compared to CRP and LDH. This result contradicts the findings of several other research, the majority of which identified serum ferritin as a key indicator for predicting the severity of the disease.[19,20] Nevertheless, our research had a few limitations, with the most significant one being the confounding aspects of different co-morbidities that might potentially be the underlying cause of severe immunological dysregulation in certain individuals. In addition, the immunological dysfunction might be further exacerbated by secondary bacterial infections and multi-organ failure, which cannot be primarily linked to the specific viral agent under investigation.

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CONCLUSION

The biomarkers CRP, D-Dimer, Ferritin, and IL 6 showed a significant association with the severity of COVID-19. However, the other three parameters, TLC, LDH, and Procalcitonin, did not show a significant correlation with the severity of COVID-19.

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