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Original Research Article

Correlation study of CBC derivatives and biochemical prognostic marker in covid-19 patients

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ABSTRACT

Background: SARS-CoV2 infection induces inflammatory responses and acute lung injury in human beings. Infection causes certain haematological and biochemical changes in patients. This study aimed analyze the correlation between CBC derivatives and biochemical prognostic marker in COVID-19.
Materials and Methods: Total 80 individuals selected, 50 COVID-19 positive patients and 30 subjects negative for COVID-19 RT-PCR test. Blood collected was sent to NLR, RDW, assayed in Beckman Coulter DxH 800. Serum CRP estimated by Immuno-turbidimetric method, ferritin by CLIA. Plasma collected estimate for D-DIMER by CLIA. Unpaired T test & Pearson correlation (IBM SPSS 22.0) were used.
Result: Elevated levels of NLR (p value <0.001), RDW (p value <0.001), FERRITIN (p value <0.001), D-D-DIMER (p value <0.001), CRP (p value <0.001) some research parameters like LHD and RSF show significant decrease. In current study the NLR positively correlate to CRP (r value=0.5, p value <0.001), NLR positively correlate to D-DIMER (r value=0.3, p value<0.05), RDW positively correlated to CRP (r value=, 0.3, p value <0.05), RDW negatively correlated to ferritin (r value=-0.439, p value <0.001)LHD negatively correlated ferritin (r value=-0.316, p value=<0.05), MAF negatively correlated to D-dimer (r value=-0.244 <0.05), MSCV positively correlated with CRP (r value=0.336, p value= <0.05).
Conclusion: The cytokine storm produced during covid-19 infection which inturn develop neutrophilia and lymphopenia, CRP production and also cause elevation of procoagulants &D-dimer. RDW and CRP are positively correlated they are elevated during inflammatory conditions.RDW and FERRITIN show negative correlation. Retrospective study have some limitation so future studies will reveal more information.

1. Introduction

Coronavirus disease 2019 was first reported in Wuhan city, Hubei, China in last week of December 2019 and has led to a major concern of health problem in worldwide.[1] COVID-19 is caused by novel coronavirus named severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). SARS-CoV and MERS-CoV have caused outbreaks in the past. SARS-CoV-2 is classified as single-stranded and positive-sense RNA virus, which belongs to genus Betacoronavirus. SARS-CoV-2 mainly transmitted between people by aerosol and contact routes. Since 2021, variants of virus have emerged and become dominant in many countries, with the Delta Alpha, Beta and omicron variants being most virulent.[2]

The symptoms of the disease can be divided into three stages. First, the Asymptomatic stage, which lasts for 1 to 2 days after being infected. During this stage, the virus attaches to the ACE2 receptors and replicates. The virus can be detected by the swab test, nasal swabs being more effective than throat swabs. There is a limited innate immunity response. Second, the Upper Airway Infection

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stage, where the virus migrates down the respiratory tract. An innate immune response is triggered. Third and final, the Acute Respiratory Distress Syndrome (ARDS) and Hypoxia stage, in which the virus reaches, infects, and damages the alveoli in the lungs, which release interferons that signal the nearby healthy cells to release antiviral peptides. The antiviral peptides cause the breakdown of the virus. The damaged cells release danger molecules (called damage-associated molecular patterns, protein-associated molecular patterns, and cytokines) that activate the innate immune system for phagocytosis. These signals are answered by macrophages that release more inflammatory signals, resulting in the filling of fluid between the capillary and alveolus (the area responsible for gas exchange). Neutrophils also reach the site of infection during the killing of viruses and damage the healthy pneumocytes. This leads to a decrease in the surfactant present in the alveolus. These phagocytic cells also release inflammatory mediators, like IL-2, IL-6, IL-10, TNF-α, G-CSF, and MCP-1, all of which cause inflammation. This hyperactive immune response of the body is called a cytokine storm. This affects the gas exchange in the alveoli, leading to hypoxemia and ARDS. In the case of a very severe infection, the protein- rich fluid may enter the bloodstream, causing systemic inflammatory response syndrome (SIRS), which can further lead to multi-organ failure. The cytokines also lead to an increase in levels of procoagulants, leading to pulmonary embolism. In addition, the infection activates receptors on the cranial nerve and the CNS generates a cough response. Finally, the inflammatory mediators can also act in the hypothalamus, leading to fever.

The virus enters the body through the nose, eyes, or mouth. The spike protein binds specifically to the ACE2 receptors present on the type 2 pneumocytes in the alveoli in the lungs, just like the SARS-CoV1. The type 2 pneumocytes produce surfactants that reduce the collapsing pressure and also decrease the surface tension in alveoli. The binding of the ACE2 receptor allows the entry of the virus into the host cell due to host cell proteases that cleave the spike protein of the virus. The virus enters the host cell either by direct cell entry by membrane fusion or by endocytosis.

Unlike a typical flu virus that travels to the nucleus once inside the host cell, the SARS-CoV-2 releases its positive-sense RNA into the host cell cytoplasm. This RNA is translated into polyproteins, pp1a and pp1ab. These help in the replication and transcription of the viral RNA. The replication of positive-sense RNA using RNA-dependent RNA polymerase enzyme gives a negative-sense RNA. The negative-sense RNA is either replicated to give positive-sense RNAs (incorporated in the viral genome) or transcribed. The transcribed mRNAs can be translated to produce viral proteins, like the spike, membrane, envelope, and nucleocapsid proteins.[3] The host cell ER carries the proteins to the Golgi apparatus, where they are packaged into vesicles and assembled near the host cell membrane. The new viruses that are formed exit the host cell by exocytosis to infect other cells. This process results in death of the host cell.[4]

Patient with coronavirus disease experience a myriad of symptoms includes, including raised body temperature, ageusia. Symptoms my begin 1-14 days after exposure to the virus. A large proportion of infected patients reported mild symptoms of the disease and recover. Some patients progressively develop serious complication, including sepsis, acute respiratory failure, autoimmune hypersensitivity reaction, metabolic acidosis, heart failure, kidney injury, hypoxic encephalopathy, and eventually die of illness.[5,6]

Covid-19 associated coagulopathy mimicking intravascular coagulopathy. Adult older than 18 years of age are the most common patients infected with SARS-CoV-2 and there're some cases of children aged between 2 and 15 years.

The gold standard test RT-PCR (real-time reverse transcriptase polymerase chain reaction) is the corner stone of establishing the etiology with multiple other supporting modalities like laboratory investigations, that are being used in COVID-19 patients during the course of illness. HRCT (high resolution computed tomography) chest is the common radiological tool that is useful for monitoring patients. Hematological abnormalities such as thrombocytopenia, reduced number of peripheral blood lymphocytes and eosinophils with increased neutrophil lymphocyte ratio are common in covid-19 patients.[7] The main routine tests requested for COVID-19 patients include complete blood count (CBC) and its derivatives, assays investigating coagulation and fibrinolysis cascades (PT, aPTT, and D-dimers), inflammation-related parameters (ESR, CRP, ferritin, and procalcitonin) and liver function test. Due to the potential ability of the virus to severely impair several vital organs such as the heart, liver, and kidneys, analysing the biochemical factors is an appropriate way for clinicians to evaluate the functional activities of these organs.[8]

Many published studies concentrated on haematological and biochemical changes in covid-19 patients but the correlation study is limited. This study aimed to reveal the correlation of CBC derivatives and biochemical parameters in covid-19 patients who were admitted in the hospital.

2. Materials and Methods

2.1. Study design and participants

The study was double-centered hospital-based retrospective. The data was collected from the medical records of patients/subjects from the period of 3 months, i.e. August to October 2021 at Believers Church Medical College

Hospital, Thiruvalla. The study included 50 consecutive patients with COVID-19 admitted to the hospital, as well as 50 COVID-19 negative control group who were admitted in same hospital.

2.2. Exclusion criteria

Pregnant women's, Patients below 20yr,Patients above 85yr, Leukaemia patients, Patient with chronic lung disease, haematological disorders, liver disease and malignancy on treatment are excluded from the study.

2.3. Inclusion criteria

Male and female patients admitted in the hospital during covid-19 period.

2.4. Data collection

Clinical data including demographic information (age, gender), and Laboratory Parameters like biochemical prognostic markers(C-reactive protein, Ferritin, D-dimer), and CBC derivatives (NLR, RDW, LHD, MAF, RSF and MSCV) were collected from each patient during hospitalization.

2.5. Statistical analysis

Data collected from 79 individuals are processed and analysed in IBM SPSS 22.0 and Microsoft excel 2013. Differences in the levels of CRP, ferritin, D-dimer, LHD, MAF, MSCV and RSF between the RT-PCR positive and negative patients were assessed using student's unpaired t-test. Pearson's correlation coefficient was used to measure the correlation between biochemical and haematological Parameters. In this study r value=1 is perfect positive, $0.7 \leq r < 1$ highly positive, $0.3 \leq r < 0.7$ moderate positive, $0 < r < 0.3$ low positive, r value=-1 is perfect negative, $-0.7 \leq r < -1$ highly negative, $-0.3 \leq r < -0.7$ moderate negative, $0 < r < -0.3$ low negative correlation. P value <0.05 was considered to be statistically significant.

3. Result

Data collected from 79 individuals are processed and analysed in IBM SPSS 22.0 and Microsoft excel 2013.

Seventy nine subjects were included in the study, in which 50 subjects are covid-19 positive Patients and 29 subjects were healthy control. Among these the average age of patients with COVID-19 was almost 57 year old and 29(58%) patients were male and 21 (42%) patients were female. The healthy control whose age ranges from 32-85 years, of which 13 (44.8%) Males and 16 (55.2%) patients were females.

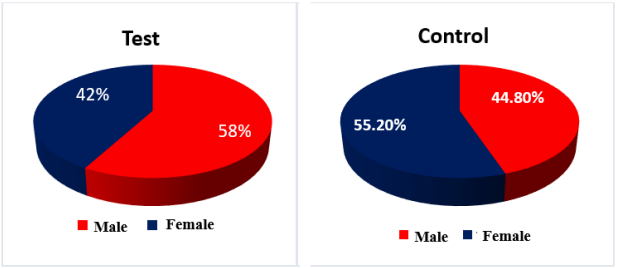

Figure 1: Gender distribution of test and control

4. Discussion

Covid-19 is an ongoing global pandemic and its effect on human body are also flashing every day. Although research are ongoing, we still have a lot to know about the effects of covid-19 on different biochemical and haematological parameter in patients. COVID-19 earlier thought as a respiratory tract infection, is now Considered a systemic disease involving respiratory, Gastrointestinal, neurological, cardiovascular, Hematopoietic and other systems. Comparison of haematological and biochemical parameters in Corona patients with healthy control group shows significant elevation in NLR, RDW, Ferritin, D-DIMER and CRP parameters. This findings are similar to earlier research shows there is a variation in biochemical, RBC and WBC parameters in covid-19 patients which is also used in risk prediction.[9,10] In addition to this certain research parameters are also tested so their LHD and RSF show significant decrease in covid patients. MAF and MSCV don't show any significant variation.

While correlation study shows that NLR-CRP, NLR-D-DIMER their is a moderate positive correlation. CRP is an acute-phase protein responsible for the clearance of pathogens through binding to pathogens and enhanced elimination by phagocytic cells. As the first line of innate host defences for clearance of viral infections, CRP might be linked to the overproduction of inflammatory cytokines. G-CSF enhances the production of granulocytes thus neutrophilia attained Neutrophil (NEU) is a major component of the leukocyte population and can kill pathogens by releasing reactive oxygen species, producing effect or molecules such as circulating vascular endothelial growth factor (VEGF), and inducing inflammatory factors as well as IL1, TNFα, and IFN-γ. However, it is not clear how lymphocyte count changes as the disease progresses, thus NLR elevated. Severe COVID-19 illness is associated with increased platelet activation as well as platelet-monocyte aggregation. Platelets from severely ill COVID-19 patients can induce monocyte TF expression (in a P-selectin and αIIb/β3 dependent manner), which may amplify inflammation and hypercoagulability in these patient, which in turn increases the specific degradation product D- dimer that produced in hydrolysis of fibrin,

Table 1: Comparison of study parameters between test and control groups

Parameters	CRP (mg/L)		D-dimer (ng/ml)		Ferritin (ng/ml)	
	r value	p value	r value	p value	r value	p value
NLR	0.529	<0.001**	0.3	<0.05*	-0.179	0.115
RDW(%)	0.3	<0.05*	0.148	0.194	-0.439	< 0.05*
LHD(%)	-0.137	0.23	-0.074	0.516	-0.316	< 0.05*
MAF	-0.089	0.438	-0.244	<0.05*	0.121	0.287
MSCV (fL)	0.336	<0.05*	-0.02	0.0889	0.246	0.085
RSF (fL)	0.213	0.138	0.032	0.828	0.216	0.131

NLR = Neutrophil to lymphocyte ratio, RDW = Red cell distribution width, LHD = Low haemoglobin density, MAF = Microcytic anaemic factor, MSCV = Mean sphered cell volume, RSF = Red cell size factor

Table 2: Correlation of study parameters in test

Parameter	Control (n=29) (mean ± SD)	Test (n=50) (mean ± SD)	T test	P Value
NLR	1.92 ± 0.914	5.90 ± 5.01	-5.46	<0.001**
RDW (%)	13.462 ± 0.419	14.25 ± 1.57	-3.35	<0.001**
LHD (%)	6.22 ± 1.38	4.71 ± 2.57	3.40	<0.001**
MAF	11.43 ± 1.59	10.84 ± 2.12	1.40	0.166 NS
MSCV (fL)	80.06 ± 3.81	78.36 ± 6.27	1.49	0.139 NS
RSF (fL)	95.63 ± 3.51	93.15 ± 6.55	2.19	<0.05*
FERRITIN (ng/ml)	154.2 ± 72.1	717 ± 644	-6.11	<0.001**
D-DIMER (ng/ml)	395.8 ± 72.8	1145 ± 1266	-4.17	<0.001**
CRP (mg/L)	4.6 ± 6.02	57.8 ± 70.5	-5.3	<0.001**

NLR = Neutrophil to lymphocyte ratio, RDW = Red cell distribution width, LHD = Low haemoglobin density, MAF = Microcytic anaemic factor, MSCV = Mean sphered cell volume, RSF = Red cell size factor

certain studies also mentioned it.[11]

RDW also positively correlated with CRP and negatively correlated with ferritin. Elevated RDW may arise from several mechanisms such as chronic inflammation, iron, vitamin B12 or folate deficiencies and oxidative stress, which may result in ineffective erythropoiesis. Among these mechanisms, oxidative stress and inflammation have been shown to be significant determinants of elevated RDW.[12–14] It has been suggested that raised levels of the cytokines interleukin 1, interleukin 6 and tumour necrosis factor-α in inflammatory diseases can shorten the lifespan of red blood cells, leading to an increased RDW.[15,16] Exact reason behind RDW elevation in covid-19 and CRP relation not know both of them elevated in inflammatory conditions. Previous study described that hyperferritinemia is associated with elevated levels of IL-6. This may confirm that hyperferritinemia is associated with inflammatory states in SARS-CoV-2 infection, and therefore, ferritin can be a useful parameter to predict disease severity and the extent of the cytokine storm.[17,18] The exact mechanism behind the correlation between RDW and ferritin is unknown.

One previous study concluded that the research parameters such as LHD, MAF, MSCV and RSF factors analysed to distinguish between iron deficiency anemia and thalassemia.[19] In present study these research parameters are also correlated with biochemical parameters. From the investigation their is a moderate negative correlation between LHD and ferritin. Low negative correlation between MAF and D-dimer. Moderate positive correlation between MSCV and CRP. RSF don't show any significant correlation.

5. Conclusion

In this retrospective study most of the heamatological and biochemical parameters are significantly higher in COVID-19 patients. The cytokine storm produced during Covid-19 infection will leads to heamatological and biochemical changes in the body and further complication such as multi organ failure. So the continuous monitoring of these biochemical and haematological parameter will predict the risk of COVID-19 infection and can prevent further complication.

The correlation analysis shows that the heamatological and biochemical parameters are connected with each other heamatological changes occur due to Covid-19 infection progressively affect the biochemical parameters. This study have some limitations. So the future study with large number of cases and parameters will helps to reveal more information's.

6. Limitations

The study was retrospective with small sample size. Only limited number of hematological and biochemical parameters are analysed, so that the correlation interpretation isn't fully understood. markers and clinical characteristics need further investigations and should be

used for risk stratification in patients with COVID-19. By studying large number of cases and more investigation marker will surely give more precise facts.

7. Source of Funding

None.

8. Conflict of Interest

None.

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