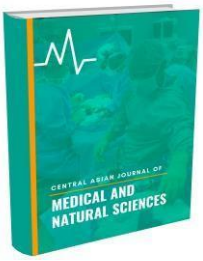

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Evaluation of Serum Zonulin Levels in Iraqi Patients Infected With the Emerging Coronavirus (CoV-19)

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Abstract: CoV-19 is a novel infectious disease resulting from the SARS-CoV-2 coronavirus, which initially surfaced in Wuhan and swiftly expanded globally. Cross-species transmission of CoVs can be extremely deadly, as seen by the 2003 and more recent outbreaks of severe acute respiratory syndrome (SARS) and Middle-East respiratory disease. The globe has been threatened by the novel severe acute respiratory syndrome coronavirus 2 in a number of ways (SARS-CoV-2). Zonulin belongs to a family of proteins, the first of which was identified about a decade ago and is called pre-haptoglobin 2. Serum samples from 120 CoV-19 patients in total were gathered and apparently healthy group (n=60) with age range (20-70) years, admitted from AL-Hussien Teaching Hospital" in Al-muthana – Iraq. Zonulin levels were measured by enzyme-linked immunosorbent assay (kit. Metabolic parameters were measured by enzymatic spectrophotometer methods. The correlation coefficients between serum Zonulin levels and age, BMI, Elements and electrolytes were also evaluated. Serum Zonulin, CRP, D-dimer, and ferritin levels were substantially higher in CoV-19 patients (319.2±15.05) compared to control subjects (79.69±11.77), in CoV-19 patients (426.21±198.73) compared to control subjects (289.43±251), and in CoV-19 patients (738±20.09) compared to control subjects (130.66±9.2) (P <0.001). In COVID-19 patients, there was a significant negative correlation with iron, calcium, and sodium levels, but a positive correlation with age, CRP, D-dimer, and ferritin levels. When comparing the moderate COVID-19 patient group to the critical and severe patient group, the moderate patients' serum Zonulin levels were considerably higher. In CoV-19 patients, serum Zonulin levels were elevated, particularly in severe cases. As a result, Zonulin levels show promise in prognosticating CoV-19 severity. Throughout the trial period, Zonulin findings were consistently higher in the severe group than in the non-severe group.

Keywords: Coronavirus, Zonulin and respiratory syndrome

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Introduction

The first new coronavirus to be identified was the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was found in Wuhan, China in December 2019. (1). Because SARS-CoV-2 is so contagious, even asymptomatic individuals could spread the virus (2). The World Health Organization (WHO) designated the illness caused by SARS-CoV-2 as (CoV-19) on Feb., 2020. Patients with (CoV-19) experience a variety of clinical symptoms, such as dyspnea, fever, coughing, fatigue, anorexia, headaches, diarrhea, nausea, or vomiting, and even acute respiratory distress syndrome (ARDS). Numerous critically ill or severe patients needed to be admitted to the intensive care unit (ICU). The total death rate for CoV-19 individuals ranges from 2 to 5%, and it may even be greater among the elderly, according to the described clinical characteristics of these patients (3,4). In the early stages of the epidemic, mortality in Wuhan City peaked at about 7% (5). Coronaviruses (CoVs) are enclosed viruses having a positive sense, single-stranded RNA genome. They belong to the order Nidovirales and the families Coronaviridae and Coronavirinae. The largest RNA viruses are CoVs, whose genomes can be as long as 26 or 32 kilobases (kb). Based on genetic and antigenic criteria, CoVs have been classified into three classes: -CoVs, -CoVs, and -CoVs. (6,7). Mostly affecting birds and mammals, coronaviruses can cause a range of deadly illnesses that are especially detrimental to the farming sector (8, 9). The main roles of the intestinal epithelium are to control the exchange of solutes and fluids and to absorb nutrients. (10). An increasing number of research suggests that the gut epithelium and its associated components, together with gut-associated lymphoid tissue and the neuroendocrine network, may also function as regulators in the passage of environmental antigens from the intestinal lumen into the submucosa. (11). The hypothesized paradigm states that the mucosal barrier is dysregulated, which increases the amount of antigens and other macromolecules that infiltrate the host and initiate immunological activation and/or localized or systemic inflammation. The World Health Organization has declared CoV-19 to be a pandemic and linked it to SARS-CoV-2 infection. The SARS-CoV-2 virus can interact with the stomach, despite the fact that it has mostly been demonstrated to affect the respiratory system. Intestinal homeostasis requires the gut barrier to be maintained, and disease can occur when it is compromised. This so-called "gut leakiness" process affects immunity and tolerance. 12). Using indicators of disease severity and clinical progression in this situation would make it easier to identify individuals who need aggressive management and surveillance early on and would help ensure that healthcare resources are used wisely.

Because it can disassemble tight junctions reversibly, the protein zonulin has been connected to the control of mucosal permeability (13, 14). Zonulin was first shown to be an endogenous human equivalent of zonula occludens toxin (Zot), a bacterial enterotoxin produced by the intestinal bacteria Vibrio cholera (14, 15). It has been suggested that zonulin transactivates EGFR to start the dissolution of tight junctions via activating EGFR through proteinase activated receptor 2 (PAR2) and G proteincoupled receptor PAR2. The activation of these two receptors lowers transepithelial electrical resistance and may increase intestinal permeability. (13). The finding that Zot activates intracellular pathways that result in actin polymerization driven by protein kinase C shows that cytoskeleton modification contributes to increasing intestinal permeability (17). Zot and zonulin are analogues, and it thought that zonulin has a similar activation mechanism.

Materials and Methods

The case-control study comprised 90 patients (min.-max.ages:20-70 years) infected individuals with COVID-19 who were admitted to AL-Hussien Teaching Hospital,in the province of AL- Al-muthana in Iraq. Prior to the study's commencement, all subjects gave their informed consent from January 2024 – April 2024, once the Iraqi Ministry of Health and the Environment's ethics commission gave its approval. These patients confirmed the diagnoses by a chest X-ray, CT scan, and quantitative RT-PCR performed 7 to 12 days following the onset of symptoms to make the diagnosis. The patients were split into three smaller groups: If they had a fever, respiratory symptoms and radiological pneumonia evidence, (50) mild/moderate cases of COVID-19, (41) severe case and the (29) critically patients after then dead. Patients with COVID-19 were collected at admission and the disease severity was determined using Murray scores (19). If a patient satisfied any of the following criteria, they were judged to have severe COVID-19.

1. (≥ 30 /min) repertory diversion.
2. A saturation level of 90% or less for resting oxygen
3. Arterial oxygen (PaO2) / percentages of inspired oxygen less than 300 mmHg.
4. A repertory failure that necessitates mechanical breathing and an intensive care unit (ICU).

Critical patients who pass away are also regarded as having not survived.

The patients' names, ages, sexes, weights, and heights were entered into a file along with their registration information. Ninety supposedly healthy persons were chosen as the control group. They were similar to the patients in terms of age and sex distribution.

Those who meet the exclusion criteria are smokers, pregnant women, those with systemic immunological illnesses, and volunteers who have thyroid gland disease or any other chronic conditions, such as diabetes, cardiovascular disease, or who are taking long-term oral corticosteroids. Medical syringes that used to collect five milliliters of venous blood from each patients and controls group. Two milliliters were placed in EDTA tubes for complete blood count analysis, and the remaining blood was placed in gel tubes sand left at room temperature for 15 minutes to coagulation before being centrifuged for 10 minutes at (3000 Xg) to provide the serum. The sera were isolated and kept at (-20 C0) in Eppendorf tubes until a biochemical assay could be done. complete blood count levels were assessed using an auto hematological analyzer (linear, Spain). Enzyme linked immune sorbent assay (ELISA) (Melsin, Chain) was used to measure the levels of Zonulin assays in serum samples. These measurements were typically made within the first 24 to 72 hours following admission. The levels of D-dimer and serum ferritin were assessed by fluorescence immunoassay (ichromaTM). Elements (Zn, Ca and Fe) were measured by using colorimetric methods kits (Agappe).

Statistical Analysis

We conducted the statistical analysis using SPSS 26 (Statistical Package of Social Science). Constant variables (SD) were expressed using the mean and standard deviation. The significance of the differences was assessed using the independent t-test and the paired t-test for variables with equal and unequal frequencies, respectively. We assessed bivariate correlations using standardized Pearson coefficients. Significant and highly significant P values were determined to be less than 0.05 and 0.01 respectively. Recipient operating characteristic (ROC) analysis was employed to determine Zonulin's cutoff value. By utilizing the ROC curve, the area under the curve (AUC) value was computed.

Results

Table (1) were shown, 120 confirmed CoV-19 cases and healthy group (60 males and 60 females).

Table 1. Comparison of general characteristic between CoV-19 patient's and the healthy group

Parameters	COVID-19 patients' group Mean ± SD			Healthy (60) Mean ± SD	P-value
	Critical (18)	Sever (22)	Mild/moderate (20)		

Sex, F/M	10/8	9/13	9/11	32/28	
Age (years)	67.31±3.59	60.79±2.89	46.12±6.41	58.26 ± 5.76	a= 0.10 b= 0.20 c =0.10 d =0.06
BMI (kg/m²)	29.11±5.31	29.13.04	25.51±1.99	24.81 ± 3.43	a =0.61 b =0.85 c =0.03 d =0.85
SBP (mmHg)	169.0±5.91	149.17±4.83	153.01±2.16	132.83±6.52	a=0.06 b= 0.00 c= 0.08 d= 0.00
DBP (mmHg)	82.93±0.6	80.47±0.4	77.93±0.8	80.76 ± 4.89	a= 0.00 b= 0.01 c= 0.07 d= 0.60

The data is displayed as follows: mean ± standard deviation, body, ,mass, ,index ,,(BMI), systolic,, blood,, pressure (SBP), and diastolic, , blood , , pressure (DBS). M: men, F: women P.values for A and B are respectively critical + severe and moderate. P.value (moderate + severe) D = (healthy + covid))

The overall features of the research groups are presented in table (1) which consists the data of the 120 patients of Covid-19, this group was divided in to three cases (Mid/moderate, severe and critical) compared with group of (60) apparently healthy subject. The baseline characteristics are non-significant in age between healthy and total covid.19 group. Systolic Blood pressure are significant between the between healthy and covid.19 group. In the current study, severe covid-19 patient group has higher age than critical and mild groups.

The patients' mean age according to severity of CoV-19 (67.31±3.59 years, 60.79±2.89 years and 46.12±6.41) was none significantly when compared with control group's age (58.26 ± 5.76 years). CoV-19 patients with severe infection have considerably higher mean BMI (29.13.04) than patients with critical and mild/moderate disease (29.11±5.31and 25.51±1.99 kg/m2, respectively). In actuality, severe and critical cases were recorded as being above 50, while more than half of mild/moderate cases were reported as being under 50. Except in the moderate category, sex distributions in the two illness severity groups of critical and severe (males more than females) (females more than males).

Table 2: Comparison of serum level of biochemical and laboratory test results of patients with CoV-19 parameters in groups with healthy group.

Parameters	Critical Group (18) Mean ± SD	Sever Group (22) Mean ± SD	Moderate group (20) Mean ± SD	healthy group (60) Mean ± SD	P-value
Iron conc (µg/dL)	40.95±3.63	41±3.57	48.15±3.95	100.19±5.99	a-0.00 b-0.00 c-0.00
zincconc (µg/mL)	127.3±7.55	111.05±10.55	122.75±10.03	84.22±3.32	a-0.00 b-0.00 c-0.00
Ca (mg/dl)	9.11±0.135	8.51±0.23	8.7±0.134	9.56±0.133	a-0.02 b-0.00 c-0.00
Hb %	12.66±0.29	12.5±0.27	13.07±0.273	12.78±0.22	a- 0.74 b- 0.423 c- 0.408
T-WBC %	13.6±0.25	11.61±0.29	10.65±0.37	9.05±0.194	a- 0.00 b- 0.00 c- 0.00
Neut %	9.8±0.47	8.67±0.4	6.15±0.43	5.86±0.3	a- 0.00 b- 0.00 c- 0.00
Lymph. %	2.49±0.13	2.8±0.181	4.22±0.192	4.2±0.08	a- 0.00 b- 0.00 c- 0.911
Plt. (10^9/L)	309.07±8.59	251.6±11.25	221.82±13.9	294.4±5.35	a- 0.133 b- 0.00 c- 0.00
CRP (mg/L)	42.67±1.84	30.53±2.13	29.06±1.763	3.36±0.25	a- 0.00 b- 0.00 c- 0.00
D-Dimer (ng/ml)	4188.2±198.7	2796.15±14862	1438.33±80.10	289.43±251	a- 0.00 b- 0.00 c- 0.00
Ferritin (ng/ml)	738±20.09	503.75±19.46	453.8±13.87	130.66±9.2	a- 0.00 b- 0.00 c- 0.00
Zonulin (ng/mL)	319.2±15.05	313.46±11.16	424.18±89.25	79.69±11.77	a- 0.00 b- 0.00 c- 0.00

The data is displayed as follows: mean ± standard deviation, hemoglobin (Hb), white blood cells (WBC), lymphocytes (LYM), neutrophils (NEUT), neutrophil/lymphocytes (N/L), platelets (PLT); p-values (critical + control), severe + control (b), and mild + control (c).

The data of Iron conc, zinc conc and Ca conc were significantly in compare with healthy group. The data of serum ferritin, D-Dimer levels were significantly higher (738±20.09) and (4188.21±198.73) respectively.

The level of serum Zonulin compared with healthy group was significantly higher. In the severe patients, serum Zonulin was higher than that of critical and moderate patients groups (110.25±3.99), (96.8±3.27) and (82.82±11.57) respectively according to laboratory data from 120 patients.

Additionally, major coagulation indicators, including D-dimer, were markedly increased in CoV-19 patients., especially in circumstances when the condition was severe.

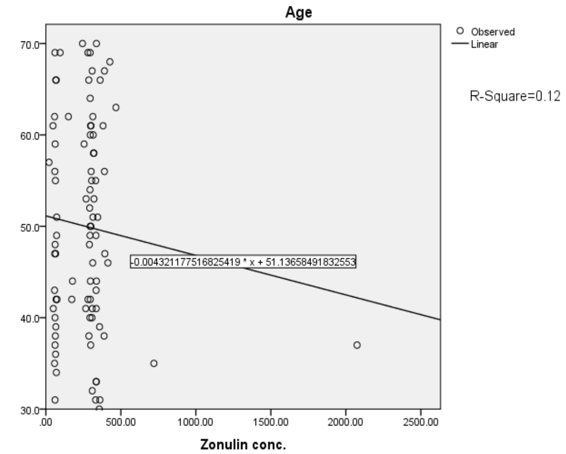
Table 4: Comparison of serum Zonulin level between males and females groups of patients and healthy

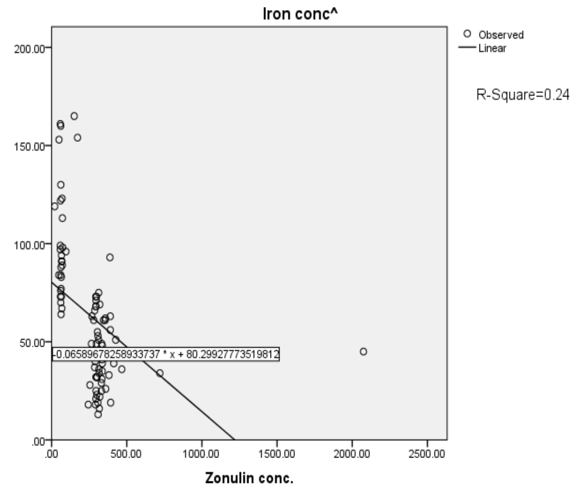
Parameters	Group	Mean±S.D	T-test Value	P-Value
Zonulin conc. (ng/mL)	**Male (c)**	81.36±85.77	-8.603	0.000
	Male (p)	314.56±73.74		
	Female (c)	78.02±35.47	-16.251	0.000
	Female (p)	333.85±41.81		

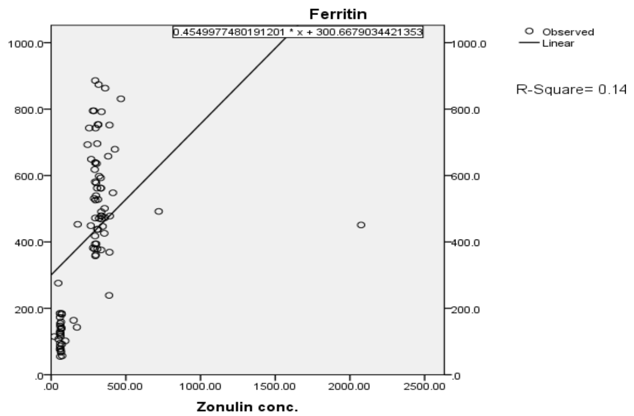
Demonstrated there is a high significant difference in serums Zonulin level when compare between male, female patients and control (314.56±73.74, 333.85±41.81and 81.36±85.77, 78.02±35.47) respectively (p> o.o5).

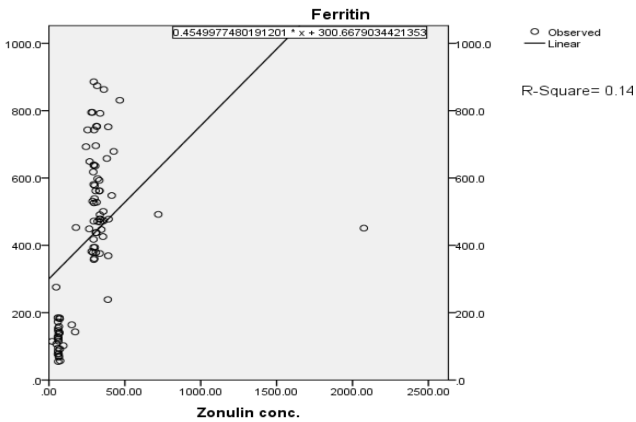
Table 5: Correlation between serums Zonulin Level with clinical Parameters in CoV-19 patients group.

Parameters	r	P. value
Age	0.293	0.050
BMI (kg/m2)	0.034	0.001
SBP (mmHg)	0.681	0.011
DBP (mmHg)	0.269	0.002
SpO2	0.877	0.000
Iron (µg/dL)	-0.431	0.000
Zinc (µg/mL)	0.304	0.004
Ca (mg/dl)	-0.211	0.046
Hb % (g/dL)	-0.099	0.353
T-WBC %	0.312	0.003
Neut. %	0.206	0.052
Lym. %	-0.250	0.018
Plt (10^9/L)	0.122	0.250
CRP (mg/L)	0.228	0.031
D-Dimer (ng/ml)	0.031	0.000
Ferritin (ng/ml)	0.374	0.000


Age
Observed
Linear
R-Square=0.12
-0.004321177516825419 * x + 51.13658491832553
Zonulin conc.


Iron conc^
Observed
Linear
R-Square=0.24
-0.06589678258933737 * x + 80.29927773519812
Zonulin conc.


Ferritin
0.4549977480191201 * x + 300.6679034421353
Observed
Linear
R-Square= 0.14
Zonulin conc.


Ferritin
0.4549977480191201 * x + 300.6679034421353
Observed
Linear
R-Square= 0.14
Zonulin conc.

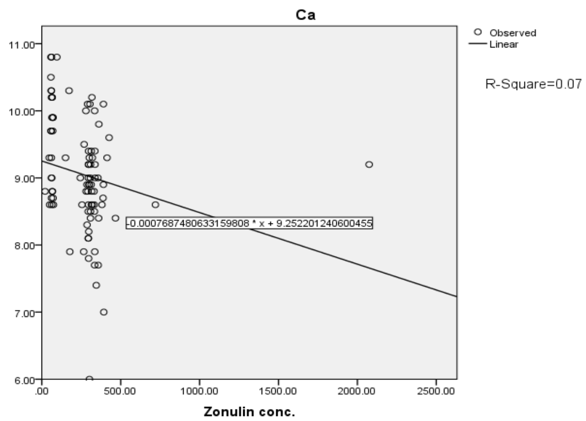

Ca
Observed
Linear
R-Square=0.07
-0.0007687480633159808 * x + 9.252201240600455
Zonulin conc.

Figure 1: Correlation between Zonulin levels with Age, ferritin, Zinc, Ca, and Iron in CoV-19 patients.

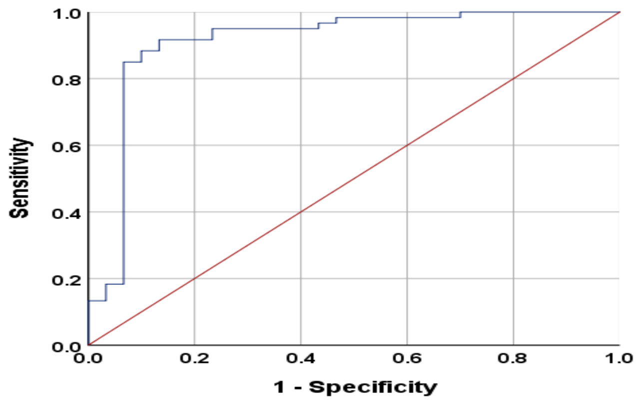
Figure 2: ROC curve of Zonulin levels

Table 6: sensitivity and specificity of serum Zonulin levels in CoV-19 patients

Variable	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	p-value
Zonulin (ng/mL)	270.812	91.7	89.2	0.912	0.833-0.991	<0.001

Discussion

A family of viruses known as the Coronaviridae includes the coronaviruses. There are four types of coronaviruses: delta, gamma, beta,



and alpha.are the four genera that make up this viral family (19). The alpha-coronavirus and beta-coronavirus genera are where human coronaviruses (HCoVs) originate. Both upper and lower respiratory tract infections are brought on by CoV-19. The infection progresses from asymptomatic or mild sickness to severe systemic symptoms primarily affecting the lung and digestive system, and lastly to critical symptoms that result in multi-organ failure (20).

Growing research indicates that CoV-19 severity and mortality are correlated with poor immune response and hyper-inflammatory response (21). Severe CoV-19 is frequently associated with metabolic problems such sepsis and systemic inflammation (22).

Gastrointestinal symptoms (fever, lethargy, dry cough, dyspnea, , abdominal discomfort, and diarrhea) are also seen by CoV-19 patients. While respiratory symptoms are second most common in CoV-19 patients, gastrointestinal symptoms are among the most prevalent. (23) Viral interactions with cells are intricate. Recent research indicates that cellular lipids are crucial for viral replication, fusion of the viral membrane with the host cell membrane, and endocytosis and exocytosis (24). According to Van Lenten et al. (1995) and Khovidhunkit et al. (2004), hdl can cause either an inflammatory or an anti-inflammatory profile. (25) Patients with infections and sepsis had lower hdl plasma levels (Cirstea et al., 2017). (26) A change in lipid profile can serve as a good early warning sign for CoV-19 disease severity (moderate or severe) (Nie et al., 2020). (27)

One of the aberrant laboratory values reported in patients with CoV-19 infection is elevated D-dimer and ferritin. An elevated D-dimer level indicates hyperfibrinolysis and excessive coagulation activation. As a result, D-dimer exhibits a low specificity but a high sensitivity for the detection of active thrombus (26).

According to our findings, CoV-19 patients with elevated levels of D-dimer and ferritin had a higher probability of contracting a serious infection and dying from any cause than those with normal levels. Patients with a severe COVID-19 infection and those who passed away had greater levels of D-dimer than patients with a less severe infection and those who lived. The present study find elevated D-dimer, ferritin, CRB and neutrophil in severe CoV-19 patients as compare with non-severe. It is frequently used to diagnose disseminated intravascular coagulation in people with low and moderate pretest probabilities for deep vein thrombosis (DVT) and pulmonary embolism (PE). D-dimer is the fibrin breakdown product generated upon cleavage of cross-linked fibrin by plasmin (33). As is consistent with our findings, earlier research shown that increasing d-dimer levels during hospitalization were linked to lower long-term outcomes. (34) D-dimer has recently been researched to find people who are expected to acquire severe CoV-19 infection earlier in the course of their illness. (35) The CoV-19 severity was positively correlated with coagulation function tests (PT, D-dimer, fibrinogen), hyperglycemia, and indicators of inflammation and infection (ESR, CRP, LDH, and PCT, but not IL-6). According to other investigations, SARS-CoV-2 infection causes lymphocytopenia. (36). Additionally, recent research found that in severe cases, neutrophil counts were slightly elevated but lymphocyte, eosinophil, and monocyte counts were all lowered. (37) Lymphoma is mapped as an immune response dysregulation in severe CoV-19 patients by a greater loss in T cells, particularly T helper cells (38).

The current study highlighted on the role of Zonulin in the severity of CoV-19 infection as predictor for severity. These mechanisms were somewhat clarified by the discovery of zonulin Wang et al., a molecule that controls epithelial and endothelial permeability in various areas, including the airways. (15)

Zonulin has been mostly found in the GI tract and has been associated with GI disorders such as celiac disease. (39). Our results shown that high significant increase in serum levels of Zonulin in critical group than in other (severe and moderate) also significant increase in CoV-19 patients as compere with control group. Numerous indicators suggest that zonulin may be involved in the neurological effects of "SARS-CoV-2" infection: zonulin may function in the brain despite BBB damage. A new study employing the zonulin agonist peptide AT-1002 demonstrates that zonulin is linked to enhanced BBB permeability, providing evidence in favor of this theory (40).

The BBB's permeability being made more permeable is a typical virus damaging mechanism (41, 42). CoV-19 patients with neurological abnormalities have lower rates of discharge home and a higher chance of dying in hospital compared to those without neurological difficulties. (43).

In a different study, Simone Di et al. found that zonulin is involved in the pathogenesis of acute lung injury (ALI) in animal models and that by lowering mucosal permeability to fluid and neutrophil extravasation into the lungs, its peptide inhibitor, larazotide acetate (commonly known as AT1001), decreased the severity of ALI and the ensuing mortality.(44). There are few treatments available for acute lung injury (ALI) and acute respiratory disease syndrome (ARDS) in this patient population, and there is no established and effective treatment for CoV-19 infection at this time. According to a recent review research of 109 CoV-19 patients in China who had ARDS (Zhou et al., 2020), The death rate was higher in patients with moderate and severe, and antiviral, glucocorticoid, or immunoglobulin therapy had no appreciable effect on survival. (35)

It is important to consider the limitations of our study when evaluating the results. First of all, the sample size was relatively modest. Second, because this was a study with a single measurement and no patient follow-up over time or observation of how Zonulin behaviour evolved, we are planning a cohort study in which Zonulin will serve as the baseline measurement and include following-up on the variables of interest, giving the findings of this study more significance.

Conclusion

Biomarkers have an important impact on the prognosis, issue management, and patient discharge from hospital settings. In addition to clinical assessment, biomarkers need to be heavily considered in therapeutic decision-making and clinical processes. A factor that should be closely monitored for CoV-19 development to the critical stage and perhaps prevented is serum zinc levels more than 326.4 ng/ml. Ferritin, serum potassium, high-density lipoprotein cholesterol, lymphocyte count, and serum potassium are additional prognostic indicators

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