



## ORIGINAL ARTICLE

# Zonulin and claudin-5 levels in multisystem inflammatory syndrome and SARS-CoV-2 infection in children

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**Aim:** SARS-CoV-2 infection in children is generally asymptomatic or mild; however, it can lead to a life-threatening clinical condition, multisystem inflammatory syndrome in children (MIS-C), days or weeks after the infection. Increased intestinal permeability is a possible triggering factor at the onset of the hyperinflammation associated with MIS-C. Zonulin and claudin-5 are involved in intestinal permeability. In this study, we aimed to investigate serum zonulin and claudin-5 levels in SARS-CoV-2 infection and MIS-C disease.

**Methods:** The study group consisted of children diagnosed with MIS-C or SARS-CoV-2 infection who presented to a university hospital paediatric emergency or infectious diseases departments. The control group included well patients seen at the General Pediatrics units for routine follow-up. Serum zonulin and claudin-5 levels were measured at the time of diagnosis.

**Results:** Fifteen patients were included in the MIS-C group, 19 in the SARS-CoV-2 infection group and 21 in the control group. The mean zonulin level in the MIS-C group was significantly higher than in the control group ( $P < 0.001$ ). Mean Claudin-5 levels were significantly lower in the SARS-CoV-2 infection group than in the control group ( $P < 0.001$ ).

**Conclusion:** These results indicate that increased intestinal permeability may be involved in the pathogenesis of SARS-CoV-2 infection and MIS-C disease. Larger clinical trials are needed to clarify the role of serum zonulin and claudin-5 on intestinal permeability in MIS-C and SARS-CoV-2 infection in children.

**Key words:** children; claudin-5; multisystem inflammatory syndrome (MIS-C); SARS-CoV-2 infection; zonulin.

## What is already known on this topic

- 1 It has been reported that the increase in intestinal permeability is involved in the pathogenesis of many diseases.
- 2 The increase in intestinal permeability can be detected by measuring the serum level of zonulin and claudin-5 molecules.

## What this paper adds

- 1 In our study, it was determined that intestinal permeability increased in MIS-C disease and SARS-Cov-2 infection.

SARS-CoV-2 infection has a milder course in children than adults.<sup>1</sup> Infection can manifest itself with symptoms such as cough, fever and chills, or it can be asymptomatic.<sup>2,3</sup> Mortality rate in children has been reported as 1.7/1 000 000.<sup>4</sup> Even if SARS-CoV-2 infection in children is generally asymptomatic or mild, it can lead to a life-threatening clinical condition called as multisystem inflammatory syndrome in children (MIS-C), days or weeks after the acute infection.<sup>5</sup>

The incidence of MIS-C has been reported as 2/100 000.<sup>6</sup> Clinical findings including persistent fever, gastrointestinal system symptoms—such as nausea, vomiting, abdominal pain—myocardial dysfunction, shock and Kawasaki disease-like symptoms.<sup>7</sup>

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Several studies have reported that hyperinflammation takes part in the pathogenesis of MIS-C.<sup>8,9</sup> It has been shown that hyperinflammation in MIS-C occurs due to factors including activation of monocytes and T cells, hyperphagocytosis and cytokine storm.<sup>9–11</sup> It has also been reported that an excess of SARS-CoV-2 antigenic particles in the circulating blood stream plays a role in the pathogenesis of MIS-C.<sup>12</sup> While it is known that a Real-Time Polymerase Chain Reaction (RT-PCR) test of nasopharyngeal swab for SARS-CoV-2 found to be negative in a significant proportion of patients with MIS-C,<sup>13,14</sup> the origin of SARS-CoV-2 antigenic load is being discussed.

The gastrointestinal tract and its mucosal barriers are important gateway for antigens to enter the body.<sup>15</sup> The micro-skeleton formed by tight junctions provides the task of selective permeability during the passage of antigens from the intestinal mucosa into the blood stream.<sup>16</sup> It has been reported that increased intestinal permeability activates inflammatory processes, forms the starting point of the uncontrolled inflammation cascade, and acts as a biological gate for inflammation.<sup>17</sup>

Zonulin and the family of claudin proteins play roles in intestinal permeability due to its effects on intercellular tight junctions. Increased serum zonulin levels after the release of zonulin from epithelial cells indicate increased intestinal permeability.<sup>15</sup> These proteins provide barrier function by controlling pore permeability.<sup>18</sup> The relationship between serum levels of these molecules and intestinal permeability has been described in various diseases including inflammatory bowel diseases, coeliac disease, allergic asthma, obsessive-compulsive disorder and attention-deficit/hyperactivity disorder.<sup>19–23</sup>

While there are not enough studies in the literature about intestinal permeability in the pathogenesis of SARS-CoV-2 infection and MIS-C, here we aimed to evaluate intestinal permeability in these diseases.

## Materials and Methods

### Study population

The study included patients with MIS-C and SARS-CoV-2 infection who applied to pediatric emergency and pediatric infectious diseases departments of Necmettin Erbakan University Meram Medical Faculty between 6 and 10 January 2021. The control group consisted of healthy children who applied to the general pediatric department for routine health check-up during the same time period. Children with any gastrointestinal system disease (i.e. inflammatory bowel disease, coeliac disease and chronic gastrointestinal system diseases) were not included in the study. While the diagnosis of MIS-C was determined according to the diagnostic criteria of Centers for Disease Control and Prevention (CDC),<sup>24</sup> the diagnosis of SARS-CoV-2 infection was made with a positive SARS-CoV-2 RT-PCR test via nasopharyngeal swab.

### Procedure

When the diagnosis of MIS-C and SARS-CoV-2 infection was made, a 5 mL venous blood sample was obtained after taking informed consent from the patients and their parents. This study was conducted with the approval of Necmettin Erbakan University Clinical Research Ethics Committee (2021/3301). All procedures were carried out in accordance with the Declaration of Helsinki.

### Biochemical analysis

Samples were analyzed at the biochemistry laboratory of Necmettin Erbakan University Meram Medical Faculty. Serums obtained by centrifugation of blood samples were stored at  $-80^{\circ}\text{C}$ , until the day of analysis as suggested in the literature. Zonulin and claudin-5 levels were measured by the enzyme-linked immunosorbent assay (ELISA) technique, by using commercial ELISA kits in line with the procedures of the manufacturer (serum zonulin: Elabscience catalogue no: EL-H5560, Lot: J14F61QRC5; serum claudin-5: USCN Life science catalogue no: SEF295Hu, Lot: L210602371, Wuhan, China).

### Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The variables were investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov–Smirnov) to determine whether they were normally distributed. Numerical measurements were presented with mean and standard deviation or median with interquartile range (IQR) based on distribution; qualitative data with numbers and percentages. Since the age and BMI were normally distributed parametric tests (one-way ANOVA test) were used to compare these parameters. As zonulin and claudin-5 measurements were not normally distributed, Kruskal–Wallis tests were conducted to compare these parameters. Mann–Whitney *U*-test was performed to test the significance of pairwise differences, using Bonferroni correction to adjust multiple comparisons. An overall 5% type-1 error level was used to infer statistical significance. For categorical variables, a Chi-square test or Fisher exact test was performed. One way ANOVA was used for power analysis. *P* value  $< 0.05$  was considered to be statistically significant.

## Results

### Demographic data

Serums were received from 55 children. Fifteen children were clinically diagnosed with MIS-C; 19 were infected with SARS-CoV-2 and 21 presented as healthy controls (Table 1). The mean age of children with MIS-C was  $10.7 \pm 3.4$  years, while the mean age of the patients with SARS-CoV-2 infection was  $9.3 \pm 6.8$ . Female gender was more dominant in patients presenting with

**Table 1** Demographic and clinical features of MIS-C, SARS-CoV-2 infection and control groups

	MIS-C* ( <i>n</i> = 15)	SARS-CoV-2 infection ( <i>n</i> = 19)	Control ( <i>n</i> = 21)	Total ( <i>n</i> = 55)
Age, years, mean (SD)	10.7 (3.4)	9.3 (6.8)	8.3 (4.7)	9.3 (5.2)
Sex, male, <i>n</i> (%)	6 (40)	10 (52.6)	13 (61.9)	26 (47.2)
BMI, mean (SD)	66.2 (25.6)	33.2 (22.4)	38.1 (28.3)	44.1 (28.7)
Hospitalisation need, <i>n</i> (%)	15 (100)	4 (21)	0	19 (34.5)
Intensive care need, <i>n</i> (%)	4 (26.6)	1 (5.2)	0	5 (9)
Mortality, <i>n</i> (%)	2 (13.3)	1 (5.2)	0	3 (5.4)

\* Multisystem inflammatory syndrome in children.

MIS-C (60%) and male gender was more common in patients with SARS-CoV-2 infection.

### Clinical courses

Body mass index was highest in the MIS-C group ( $P < 0.001$ ). All patients with MIS-C were hospitalized (100%), and four of these patients were followed up in the pediatric intensive care unit. Four patients presenting with SARS-CoV-2 infection were hospitalized (21%) and only one needed intensive care. Two patients with MIS-C and one with SARS-CoV-2 infection died in the study period.

### Serum zonulin and claudin-5 levels

A statistically significant difference was found when zonulin levels were compared between the groups ( $P < 0.001$ ). Median levels of zonulin were higher in the MIS-C group than in the SARS-CoV-2 infection and control groups (35.9, 25.7 and 13.8 ng/mL, respectively). When paired comparisons were made with Bonferroni correction, it was found that the zonulin level in the MIS-C group was statistically significantly higher than in the control group (Mann–Whitney  $U$ -test; MIS-C and control groups,  $P < 0.001$ ; Table 2).

Claudin-5 levels also showed statistically significant difference ( $P < 0.001$ ). The highest median level was found to be 2.2 ng/mL in the control group. It was found to be 1.1 in the MIS-C group and 0.76 ng/mL in the SARS-CoV-2 infection group. When paired comparisons were made with Mann–Whitney  $U$ -test, it was seen that there was a statistically significant difference between SARS-CoV-2 infection and the control group ( $P < 0.001$ ).

At the end of our study, power analysis was performed with one-way ANOVA, since the Kruskal–Wallis test was used. It was found to be 0.91 for the zonulin level.

### Discussion

The relationship between increased intestinal permeability and MIS-C disease has not been fully clarified yet. In our study, serum zonulin levels, which is the marker of increased intestinal permeability, were found to be significantly higher in the MIS-C group than in the control group.

In many studies, it has been reported that serum zonulin level is high in diseases with increased intestinal permeability.<sup>20,21,25</sup> In the study of Yonker et al.,<sup>12</sup> in which only zonulin levels were evaluated, similar to our study, serum zonulin levels were found to be significantly higher in the MIS-C group when compared with the controls.

A decrease in the number of T cells, the activation of CD8<sup>+</sup>T cells and the activation of monocytes by Ig-G type antibodies that persist for a long time in the blood after SARS-CoV-2 infection have been suggested to take role in the pathogenesis of MIS-C.<sup>9,10,26,27</sup> However, these hypotheses are insufficient to explain entities such as gastrointestinal system findings, myocardial damage, and multiple organ failures that occur in the clinical course of the disease. In the study of Yonker et al.,<sup>12</sup> SARS-CoV-2 antigenemia was reported in children with MIS-C. In the same study, it was also reported that this antigenemia resulted due to the increased intestinal permeability.<sup>12</sup> Our study also supports this data, with additional findings of lower claudin-5 levels in patients with SARS-CoV-2 infection. The increase in intestinal permeability may activate the inflammation cascade by causing SARS-CoV-2 antigens to pass into the systemic circulation. Also, the gastrointestinal system complaints in MIS-C disease may be related to the chain of reactions that lead to hyperinflammation, which starts at the intestines. Inflammation that starts in the intestinal cells may spread to the submucosa, mucosa and serosa layers, causing irritation in the visceral peritoneum, and increased peristalsis of intestines may cause gastrointestinal system complaints.

In our study, serum claudin-5 level was found to be significantly lower in the SARS-CoV-2 infection group than in the control group. To the best of our knowledge, there is no study with serum claudin-5 levels in children with SARS-CoV-2 infection. In a study conducted on rats with sepsis, serum claudin-5 level was found to be lower than the control group.<sup>28</sup> There are many studies reporting decreased claudin-5 gene expression in the intestine in the presence of various stressors.<sup>29–31</sup> Although the lack of claudin-5 gene expression in the intestines does not exactly indicate the decrease of claudin-5 levels in serum, it can be thought that there is an inverse relationship between low serum claudin-5 level and intestinal permeability. Lower levels of serum claudin-5 in the SARS-CoV-2 infection group in our study may be due to the stress of the disease on the intestines. The scenario of increased intestinal permeability may be that multi-organ damage due to hyperinflammation caused by this increase in permeability also affects the intestines, resulting in a further increase in intestinal permeability. While increased intestinal permeability leads to hyperinflammation and further development of destruction in the intestines, in the context of multiple organ damage, and this destruction may also cause increased intestinal permeability. The result of low serum claudin-5 levels leading to increased intestinal permeability in our study may be both a result and a cause of SARS-CoV-2 infection, which suggests a vicious circle mentioned above.

In our study, no significant difference was found between the group with SARS-CoV-2 infection and the MIS-C group in terms

**Table 2** Zonulin and claudin-5 levels in MIS-C, SARS-CoV-2 infection and control groups

	MIS-C† (n = 15)	SARS-CoV-2 infection (n = 19)	Controls (n = 21)	P value
Zonulin, median (IQR)‡	35.9 (26.7–50.0)	25.7 (11.4–39.7)	13.8 (7.4–19.4)	0.000
Claudin-5, median (IQR)‡	1.1 (0.7–1.7)	0.76 (0.66–1.30)	2.2 (1.4–6.2)	0.001

† Multisystem inflammatory syndrome in children. ‡ Interquartile range.

of claudin-5 levels. However, claudin-5 levels were significantly lower in the SARS-CoV-2 infection group compared to the control group. In various studies with adults, it has been reported that increased intestinal permeability causes clinical conditions such as endothelial damage, cytokine storm, microbial translocation, endotoxemia and thrombosis which are seen in the course of SARS-CoV-2 infection.<sup>32–35</sup> Increased intestinal permeability may be occurred due to local damage to the intestines by SARS-CoV-2 virus. Indeed, in our study, serum zonulin levels were found to be higher in SARS-CoV-2 infection group compared to the controls. The usage of the intestines as a reservoir by the SARS-CoV-2 virus may play a role in the pathogenesis of both acute infection and MIS-C disease. To our knowledge, there is no sufficient data about intestinal permeability in children with SARS-CoV-2 infection. In this respect, our study contributes to the literature.

There are limitations in our study, such as the low sample size and the measurement of zonulin and claudin-5 only from serum.

## Conclusion

In our study, serum zonulin levels were found to be significantly higher in the MIS-C group than in the control group. Serum claudin-5 levels were significantly lower in the SARS-CoV-2 infection group than in the control group, while there was no significant difference between the SARS-CoV-2 infection and MIS-C groups. These findings indicate that increased intestinal permeability may be involved in the pathogenesis of SARS-CoV-2 infection and MIS-C disease. Larger clinical trials are needed to clarify the role of zonulin and claudin-5 on intestinal permeability in MIS-C disease and SARS-CoV-2 infection in children.

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