Tight junction protein ZO-1 in Kawasaki disease

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Research article

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Abstract

Background

Kawasaki disease (KD) is a form of systemic febrile vasculitis that can be complicated by coronary artery lesions (CAL). A murine model of KD vasculitis showed that the vasculitis depended on intestinal barrier dysfunction, as well as that the tight junctions maintain the intestinal barrier. In this study, we aimed to investigate the role of tight junction Zonula occludens-1 (Zo-1) in intravenous immunoglobulin (IVIG) treatment response and the occurrence of CAL formation in KD patients.

Methods

Forty KD patients, 12 healthy controls, and 12 febrile controls were enrolled in this study. Tight junction ZO-1 levels were measured in sera by enzyme-linked immunosorbent assay.

Results

The serum Zo-1 level was higher in the fever control group but did not achieve statistical significance. Patients who received a second dose of IVIG due to a failure to respond to the initial IVIG treatment had a higher serum tight junction Zo-1 level, but also without statistical significance (p value =0.0582). Patients who developed a coronary artery lesion had a lower serum tight junction Zo-1 level with statistical significance (p value =0.0275).

Conclusions

Tight junction ZO-1 levels decrease in KD patients with coronary artery lesions and are associated with the intestinal barrier dysfunction of Kawasaki disease and the occurrence of CAL in KD patients.

Background

Kawasaki disease (KD) is an acute febrile coronary vasculitis disease that primarily occurs in children under the age of five years old and was first described by Kawasaki et al. in 1974 [1]. KD patients clinically present with a prolonged fever for more than 5 days and have at least four of the following five major symptoms: diffuse mucosal inflammation, bilateral non-purulent conjunctivitis, cervical lymphadenopathy, indurative angioedema of the hands and feet, and

polymorphous skin rashes [2]. Coronary artery lesions (CAL), including myocardial infarction, coronary artery dilatation, coronary artery fistula [3] and coronary artery aneurysm (CAA) [4, 5], are major complications and a hallmark of KD. KD also affects the mucosal intestinal immune responses, and KD patients have an increased numbers of activated T cells and macrophages in the small intestine [6]. A recent multicenter study that enrolled over 300 patients revealed that abdominal and gastrointestinal symptoms at KD onset complicate KD diagnosis, cause therapeutic delay, and increase the risk for IVIG resistance and coronary aneurysms [7]. Noval Rivas et al. demonstrated that a murine model of KD

vasculitis depended on intestinal barrier dysfunction, which led to secretory IgA leakage and IgA-C3 immune complex deposition in cardiovascular lesions [8].

Epithelial tight junctions (TJs) maintain the intestinal barrier while regulating the permeability of ions, nutrients, and water. The TJ is a multi-protein complex that forms a selectively permeable seal between adjacent epithelial cells and demarcates the boundary between apical and basolateral membrane domains [9]. Zonula occludens (ZO)-1 is a multi-domain polypeptide required for the assembly of TJs [10] and links junctional membrane proteins to the cytoskeleton and signaling plaque proteins [11]. However, no studies have investigated the role of tight junction Zo-1 protein in the development of KD vasculitis. The purpose of this study was to evaluate the relationships between these Zo-1 and CAL in KD.

Methods

Patients studied

We enrolled 40 patients with KD, twelve healthy controls, and twelve febrile controls in this stud. All patients were initially treated with a single dose of intravenous immunoglobulin (IVIG) (2 g/kg) during a 12-hour period. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, and we obtained informed consent from all patients' parents or guardians. Blood samples were collected both before and after IVIG treatment. Patients whose symptoms did not fit the diagnostic criteria for KD were excluded from the study. A CAL was defined as a coronary artery whose internal diameter was at least 3 mm (or 4 mm if the subject was over 5 years of age) or a segment whose internal diameter was at least 1.5 times that of an adjacent segment as observed in echocardiography [12, 13]. IVIG responsiveness was defined as defervescence 48 hours after the completion of IVIG treatment and no recurrence of fever (defined as a temperature > 38°C) for at least seven days after IVIG, with marked improvement or normalization of inflammatory signs [14, 15]. Blood samples from the febrile control patients, who were admitted for upper and/or lower respiratory tract infections (including acute bronchiolitis, acute pharyngitis, acute bronchitis, croup, and acute tonsillitis), were used for comparison. We immediately placed blood samples in heparin-containing tubes and stored the remaining aliquots of serum at −80˚C until assay.

Measurement of ZO-1 by enzyme-linked immunoassay (ELISA)

We used enzyme linked immunoassays (ELISA) to measure ZO-1 (Aviva Systems Biology, OKDD00562) according to the manufacturer's instructions.

Statistical Analysis

All data are presented as mean ± standard error. Quantitative data were analyzed using the Student's t-test or one-way analysis of variance (ANOVA) as appropriate. The least significant difference (LSD) test was used for post-hoc testing where appropriate. Two-sided p-values less than 0.05 were considered

statistically significant. All statistical tests were performed using SPSS version 13.0 for Windows XP (SPSS, Inc., Chicago, USA).

Results

We enrolled 12 non-fever healthy control (HC) subjects, 12 fever control patients (patients with fever but not having a history of KD or diagnosed as having KD), and 40 KD patients in this study. No significant difference was observed in age or gender between the non-fever control, fever control, and KD groups. All the KD patients met the AHA 2004 diagnostic criteria [5].

To investigate Zo-1 protein expression, ELISA was used to determine the expression level. The average serum tight junction Zo-1 level of each group was 2.147 ± 0.1924 ng/mL in the HC group, 2.629 ± 0.1822 ng/mL in the fever control group, and 2.255 ± 0.1155 ng/mL in the KD group. The fever control group had a higher serum Zo-1 level, but this finding was not statistically significant. (Figure 1)

KD patients were classified into two groups: IVIG responsive (N=32) and resistant group (N=8). As shown in Figure 2, the IVIG resistant group had a higher serum Zo-1 level, but this finding did not reach statistical significance (2.146 ± 0.1169 vs. 2.691 ± 0.3097 ng/mL, p value =0.0582). In IVIG responsive group, which consisted of a total of 32 patients, 16 patient developed CAL. It is shown that the CAL group had a lower serum tight junction Zo-1 level with statistical significance (1.892 ± 0.1573 vs. 2.399 ± 0.1521 ng/mL, p value =0.0275). (Figure 3)

Discussion

The first case of KD in Taiwan was described in 1979. Although Japan has the highest incidence and the United Kingdom has the lowest, the incidence of KD has been steadily increasing all over the world [16]. However, a definitive disease pathogenesis remains uncertain. A practical biomarker may help us stratify therapy for KD according to the likelihood of developing CAL. To the best of our knowledge, our study is the first to survey the correlation of tight junction ZO-1 protein in KD patients. Notably, the ZO-1 level may be related to the development of CAL in KD.

The TJ is a complex located in epithelial cells that regulates the paracellular movement of ions, macromolecules, and immune cells [10]. The cytosolic proteins ZO-1 and ZO-2 are multi-domain polypeptides required for the formation of the TJ [10] and are essential during TJ assembly. The depletion of either ZO-1 or ZO-2 from epithelial cells results in the delayed formation of TJs and a mild increase in permeability, whereas depletion of both ZO-1 and ZO-2 disrupts the localization of the transmembrane proteins at the tight junction and causes a dramatic alteration of TJ barrier function [17-19]. TJ dysfunction can lead to the disruption of intestinal barrier integrity. Changes in pH, osmotic load, or cytoskeleton function can all affect the barrier function of TJs [20]. About 30% of KD patients presented with gastrointestinal manifestations, such as vomiting, diarrhea, abdominal distension or pain, jaundice, paralytic ileus, hepatomegaly, gallbladder hydrops, and related echographic findings [5, 21, 22]. In their

case series, Zulian et al. reported an incidence of 4.6% of atypical KD with a clinical onset characterized by acute surgical abdomen [23]. One study reviewed 33 articles reporting 48 cases of KD with intestinal involvement [24]. Small bowel obstructions may occur as a result of ischemia with stricture with adhesion formation [25]. The most frequent symptoms observed were fever, abdominal pain, and vomiting, and, in all cases, typical KD signs and symptoms appeared following the intestinal complaints.

Growing evidence has suggested that the development of KD may resemble that of an immune/autoimmune process [26]. Marked T-cell and monocyte/macrophage activation is found during the acute phase [27]. Furthermore, KD affects mucosal intestinal immune responses, and KD patients have an increased number of activated T cells and macrophages present in the small intestine [6, 8]. A recent multicenter study that enrolled over 300 patients revealed that KD patients with gastrointestinal symptoms at onset had a complicated KD diagnosis, therapeutic delay, or risk for IVIG-unresponsiveness and coronary aneurysms [7]. New evidence has shown that endothelial dysfunction caused by the vigorous development of immune responses is vital to CAL development in KD patients [28-30].

In one animal study of mice, Lactobacillus-cell wall extract (LCWE)-injected mice developed KD vasculitis and exhibited a significant increase in intestinal leakage. This intestinal barrier dysfunction was associated with increased serum levels of Zonulin. Blocking intestinal permeability by using an anti-Zonulin peptide (AT-1001) protected mice in the study from LCWE-induced KD coronary arteritis [31].

Some studies have reported that tight junction ZO-1 protein was related to some diseases and could be used as an inflammatory marker. Ram et al. reported that the systemic concentration of ZO-1 was significantly elevated in hepatocellular carcinoma patients and was positively correlated with inflammatory markers [32]. Meanwhile, Boer at el. reported that the lower epithelial α-catenin, E-cadherin, and (or) ZO-1 expression in patients with atopic asthma contributed to a defective airway epithelial barrier and a higher influx of eosinophils in the epithelium [33]. Significantly lower expressions of ZO-1 and α-catenin were detected in irritable bowel syndrome-like symptoms in quiescent inflammatory bowel disease [34]. However, we found no difference in ZO-1 expression in KD compared to the controls. Our results also showed no difference between groups of IVIG responsiveness and resistance in KD patients. Nevertheless, lower ZO-1 levels were noted in CAL patients in the IVIG responsive group of KD. We may interpret this result as CAL being potentially associated with intestinal barrier dysfunction.

Conclusion

In conclusion, tight junction ZO-1 levels decrease in KD patients with coronary artery lesions. Tight junction ZO-1 may play a role in the intestinal barrier dysfunction of KD and could be a potential marker of the occurrence of CAL in KD patients. Further studies are warranted to investigate the pathophysiologic basis for these findings in KD.

List Of Abbreviations

KD, Kawasaki disease; CAL, coronary artery lesions; CAA, coronary artery aneurysm; Zo, Zonula occludens; IVIG, intravenous immunoglobulin; TJs, tight junctions; HC, healthy control; LCWE, Lactobacillus-cell wall extract

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital under registry number 102-5947C. All informed consent will be obtained in writing from these people or in the case of children, their parent or legal guardian, prior to participation.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are not publicly available for ethical reasons, as well as privacy reasons, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Wan-Tz Lai, Ying-Hsien Huang, and Ho-Chang Kuo conceptualized and designed the study, drafted the initial manuscript, critically reviewed the manuscript, and approved the final manuscript as submitted. Mao-Hung Lo designed the data collection instruments, coordinated, supervised data collection and approved the final manuscript as submitted.

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Figures

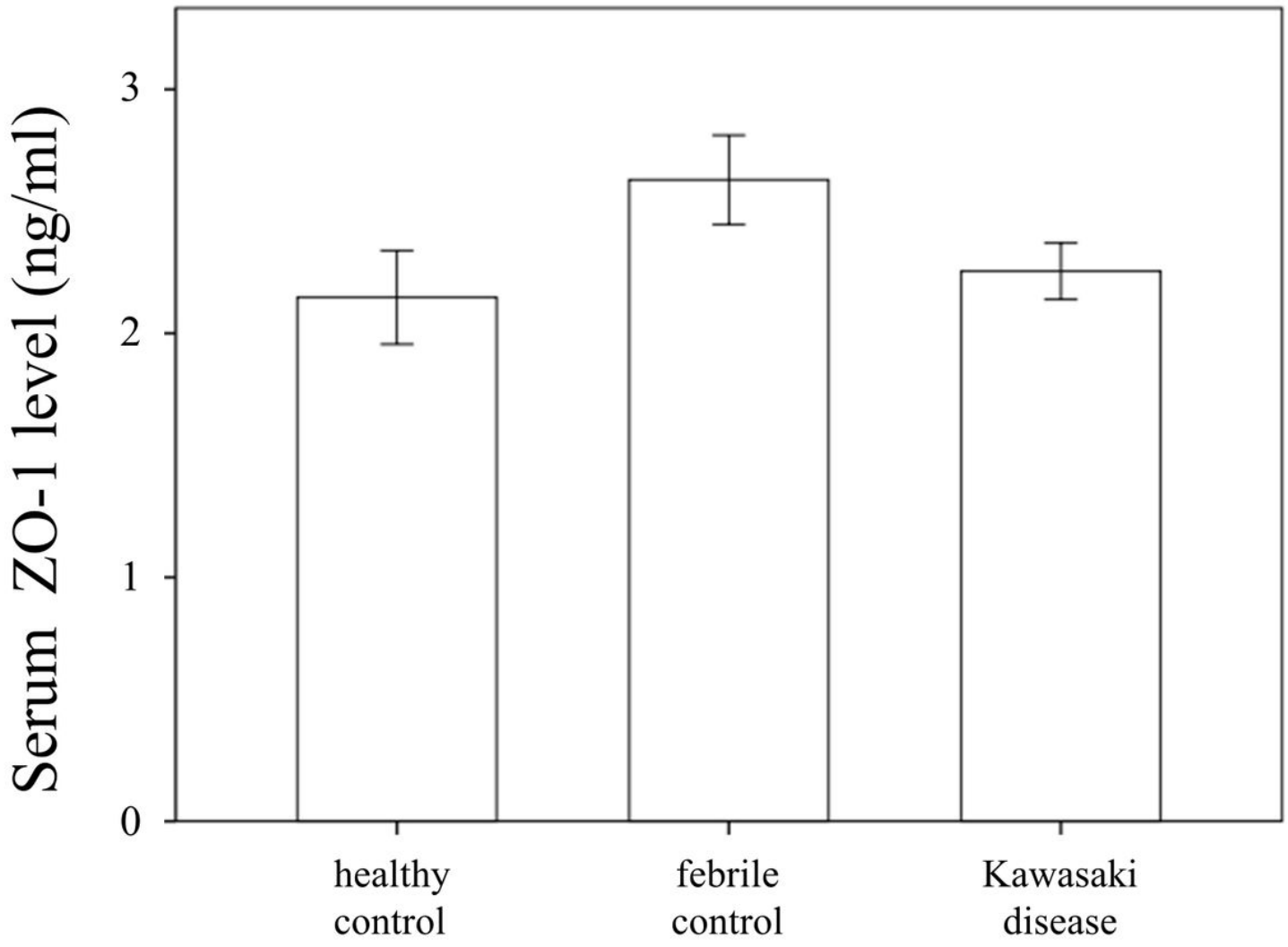

Figure 1

The Zo-1 protein expression is determined by ELISA. We enrolled 40 patients with KD, twelve healthy controls, and twelve febrile controls in this study.

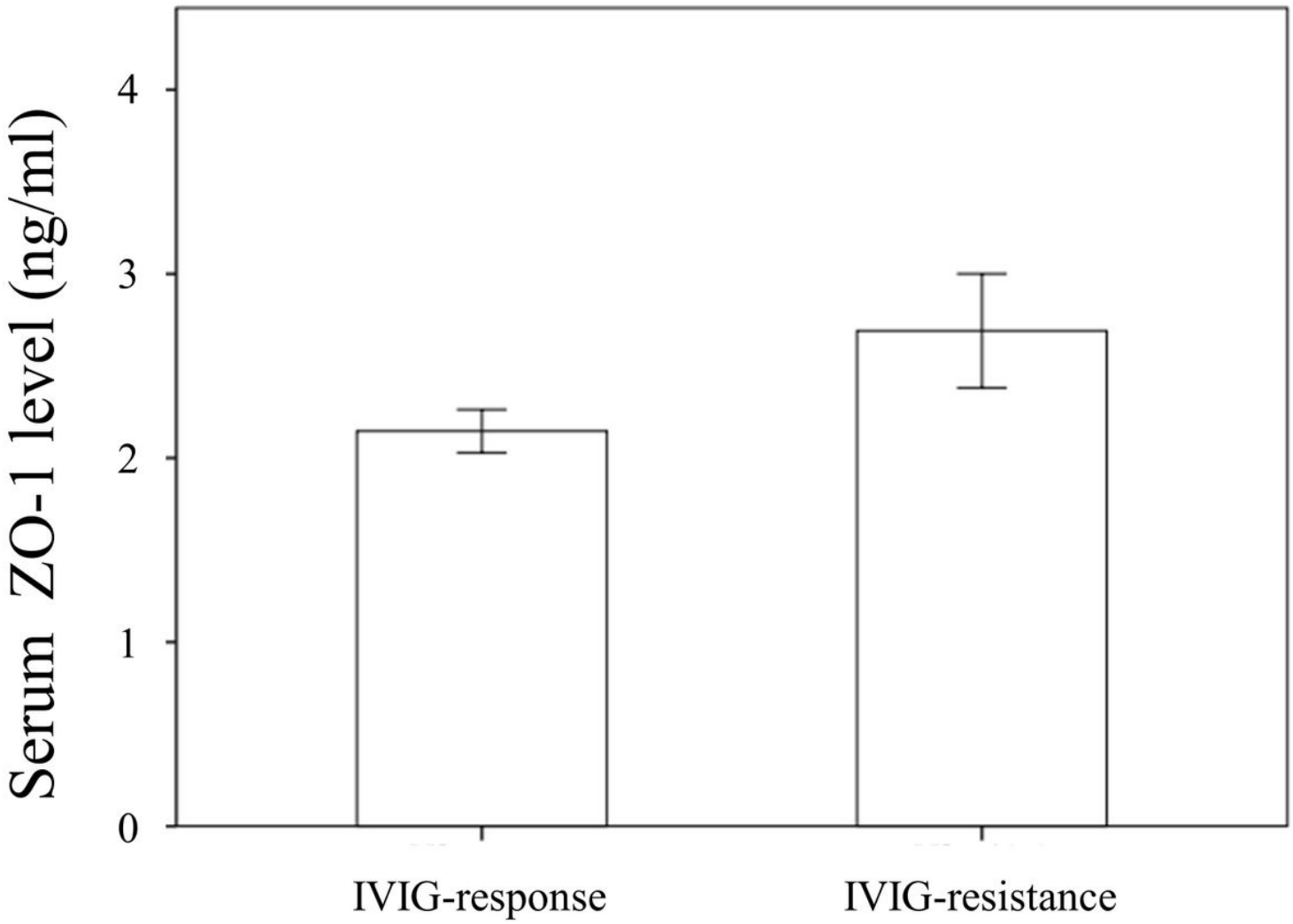

Figure 2

There is no significant difference of Zo-1 protein between intravenous immunoglobin responsive or resistance in Kawasaki disease patients.

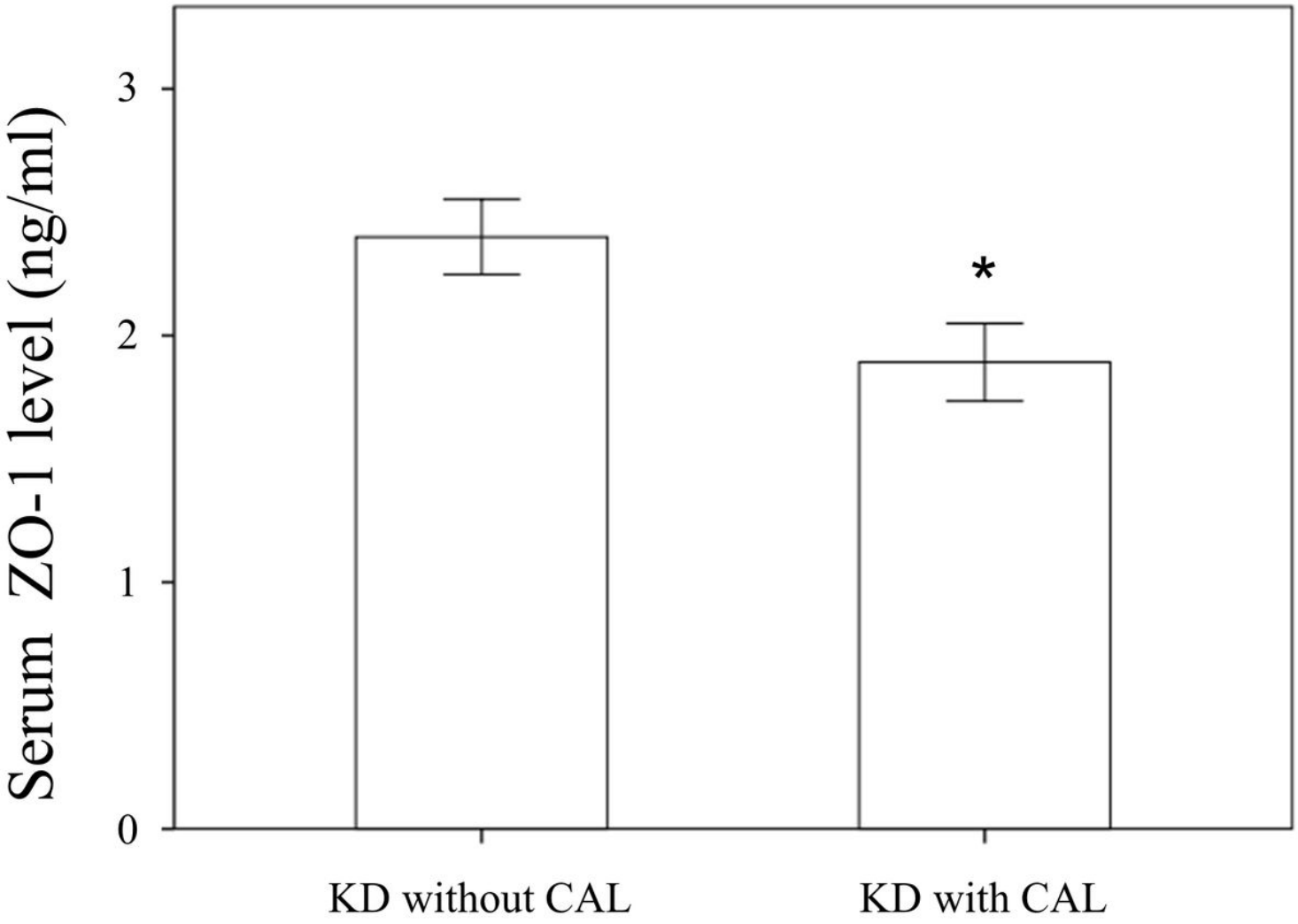

Figure 3

There is higher Zo-1 protein in Kawasaki disease with coronary arterial lesion. *p < 0.05. Data are presented as mean ± standard error