J Clin Immunol (2012) 32:746–752
DOI 10.1007/s10875-012-9668-1

Inflammation-Induced Hepcidin is Associated with the Development of Anemia and Coronary Artery Lesions in Kawasaki Disease

Ho-Chang Kuo · Ya-Ling Yang · Jiin-Haur Chuang · Mao-Meng Tiao · Hong-Ren Yu · Li-Tung Huang · Kuender D. Yang · Wei-Chiao Chang · Chiu-Ping Lee · Ying-Hsien Huang

Received: 3 December 2011 / Accepted: 8 February 2012 / Published online: 6 March 2012

Abstract
Purpose Kawasaki disease (KD) is a systemic febrile vasculitis complicated by coronary artery lesions (CAL). Anemia is common in patients with KD and is associated with a prolonged duration of active inflammation. Hepcidin is a central modulator of inflammation-associated anemia, acting via control of iron absorption and a direct inhibitory effect on erythropoiesis. The aims of this study were to investigate the role of inflammation-induced hepcidin in the development of anemia, the occurrence of CAL formation, and IVIG treatment response in patients with KD.
Methods Eighty-six KD patients and 30 febrile controls were enrolled. Levels of interleukin (IL)-6 and serum hepcidin were measured in sera by enzyme-linked immunosorbent assay. Hemoglobin and serum iron levels were also measured.
Results Hemoglobin and iron levels were lower in KD patients than in controls ($p<0.001$ and $p=0.009$, respectively). Serum hepcidin and IL-6 levels were higher in KD patients than in controls (both $p<0.001$) before intravenous immunoglobulin (IVIG) treatment. After IVIG treatment, serum hepcidin, IL-6, and hemoglobin levels decreased significantly (all $p<0.001$). In addition, the serum hepcidin levels before IVIG treatment were negatively correlated with hemoglobin levels after IVIG treatment ($R=-0.188$, $p=0.046$) and positively correlated with the changes of hemoglobin levels after IVIG treatment ($R=0.269$, $p=0.015$). Furthermore, serum hepcidin levels were negatively

Ho-Chang Kuo and Ya-Ling Yang contributed equally to this work.

H.-C. Kuo
Genomic and Proteomic Core Laboratory,
Department of Medical Research and Department of Pediatrics,
Kaohsiung Chang Gung Memorial Hospital and
Chang Gung University College of Medicine,
Kaohsiung, Taiwan, Republic of China

M.-M. Tiao · H.-R. Yu · L.-T. Huang · C.-P. Lee · Y.-H. Huang (✉)
Department of Pediatrics,
Kaohsiung Chang Gung Memorial Hospital and
Chang Gung University College of Medicine,
Kaohsiung, Taiwan, Republic of China
e-mail: yhhuang123@yahoo.com.tw

Y.-L. Yang
Department of Anesthesiology,
Kaohsiung Chang Gung Memorial Hospital and
Chang Gung University College of Medicine,
Kaohsiung, Taiwan, Republic of China

J.-H. Chuang
Department of Surgery,
Kaohsiung Chang Gung Memorial Hospital and
Chang Gung University College of Medicine,
Kaohsiung, Taiwan, Republic of China

K. D. Yang
Department of Medical Research,
Show Chwan Memorial Hospital in Chang Bing,
Changhua, Taiwan, Republic of China

W.-C. Chang
Department of Medical Genetics, Kaohsiung Medical University,
Cancer Center, Kaohsiung Medical University Hospital,
Kaohsiung, Taiwan, Republic of China

correlated with serum iron levels (R=−0.412, p=0.002), which were positively correlated with hemoglobin levels (R=0.210, p=0.045). Additionally, the change of hepcidin levels was associated with IVIG treatment response and the occurrence of CAL formation.

Conclusions Inappropriately raised hepcidin levels impair iron metabolism and are associated with decreased hemoglobin levels in KD patients. Inflammation-induced hepcidin is associated with the development of anemia and disease outcomes in patients with KD.

Keywords Coronary artery lesion · hemoglobin · hepcidin · IL-6 · iron · Kawasaki disease

Kawasaki disease (KD) is an acute vasculitis syndrome of unknown etiology affecting multiple systems and is complicated by coronary artery lesions (CAL). It occurs mostly in children under the age of 5 years and is characterized by prolonged fever, conjunctivitis, diffuse mucosal inflammation, polymorphous skin rashes, indurative edema of the hands and feet associated with peeling of finger tips, and non-suppurative lymphadenopathy [1]. In addition to the diagnostic criteria, a broad range of nonspecific clinical features can also manifest including irritability, uveitis, aseptic meningitis, cough, vomiting, diarrhea, abdominal pain, gallbladder hydrops, urethritis, arthralgia, arthritis, hypoalbuminemia, liver function impairment, anemia, and heart failure [2]. Anemia is not an infrequent finding in patients with KD and is associated with a more prolonged duration of active inflammation [3–6]. Severe hemolytic anemia requiring transfusions is rare and may be related to intravenous immunoglobulin (IVIG) infusion [4, 5, 7]. However, the underlying molecular mechanism of anemia in KD is still unknown [5, 6].

Hepcidin plays an important role in iron metabolism and in the pathogenesis of anemia of inflammation. Hepcidin is induced during infections and inflammation and then acts by binding to ferroportin, which is an iron exporter present on the absorptive surface of duodenal enterocytes, macrophages, and hepatocytes [8]. After hepcidin and ferroportin interact, ferroportin is internalized and degraded, consequently leading to intracellular iron sequestration and decreased iron absorption [9]. Hepcidin not only controls iron absorption, but also has a direct inhibitory effect on erythropoiesis [10]. Immune system activation is a central feature of KD, and several studies have shown evidence of consistently elevated levels of serum and urinary interleukin (IL)-6 in patients with KD [11–13]. The pathogenesis of anemia of inflammation is thought to be cytokine-mediated due to the typical overexpression of IL-6, which is a major inducer of hepcidin production, leading to hypoferremia [14]. Although hepcidin plays a key role in anemia of inflammation, the clinical relevance of hepcidin has not been well established in patients with KD. Therefore, the first aim of this study was to investigate the role of hepcidin as an inducer of anemia of inflammation in patients with KD and the resultant alterations in iron metabolism. The second aim was to investigate the role of hepcidin in CAL formation and IVIG treatment response.

Methods

Patients Studied

Eighty-six patients with KD and 30 age-matched febrile controls were enrolled. All patients were initially treated with a single dose of IVIG (2 g/kg) during a 12-h period. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, and informed consent was obtained from the parents or guardians. Blood samples were collected both before (within 24 h before IVIG treatment, pre-IVIG) and after IVIG treatment (within 3 days after IVIG treatment, post-IVIG). Patients whose symptoms did not fit the diagnostic criteria for KD, had had an acute fever for less than 5 days, or incomplete collection of pre- and post-IVIG blood samples were excluded from the study. A CAL was defined by the internal diameter of the coronary artery being at least 3 mm (or 4 mm if the subject was over 5 years of age) or the internal diameter of a segment being at least 1.5 times that of an adjacent segment as observed in echocardiography [15, 16]. IVIG responsiveness was defined as defervescence 48 h after the completion of IVIG treatment and no fever (defined as a temperature >38°C) recurrence for at least 7 days after IVIG, with marked improvement or normalization of inflammatory signs [6, 17]. 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. Blood samples were placed in heparin-containing tubes immediately, and the remaining aliquots of serum were stored at −80°C until assay. Hemoglobin levels were determined as part of standard hospital care.

Laboratory Measurements

Measurement of Cytokines by Enzyme-Linked Immunoassay (ELISA)

We used ELISA to measure IL-6 (human IL-6, Catalog number: DY206, R&D Systems, Minneapolis, MN, USA) and serum iron levels (QuantiChrom™ Iron Assay Kit, Catalog number: DIFE-250, BioAssay Systems, Hayward, CA, USA) according to the manufacturers' instructions. The ELISA we used for hepcidin-25 amino acid is a commercially available and competitive assay using synthetic hepcidin (Catalog number: S-1337, Bachem Biosciences, St. Helens, UK, range

0–25 ng/ml) for standardization, and the methodology and performance characteristics have been previously described [18]. Briefly, the hepcidin ELISA kit is a solid-phase type based on the principle of competitive binding. The microtiter wells were coated with a monoclonal antibody directed toward the antigenic site of the bioactive hepcidin-25 molecule. The endogenous hepcidin in a patient sample then competes with the added hepcidin biotin conjugate to bind to the coated antibody. After incubation, the unbound conjugate was washed off, followed by incubation with a streptavidin–peroxidase enzyme complex and a second washing step. Substrate solution was added, resulting in color development that was stopped after a short incubation period. The color intensity that developed was reversely proportional to the hepcidin concentration in the patient samples.

Statistical Analysis

All data are presented as mean ± standard error. Quantitative data were analyzed using Student's *t* test or by one-way analysis of variance when appropriate. The least significant difference test was used for post hoc testing where appropriate. Changes in the data before and after IVIG treatment were tested by the paired sample *t* test. Correlations between quantitative variables were assessed using Pearson's coefficient or the Spearman rank test (for non-Gaussian variables). Group comparisons were calculated by χ^2 test. 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

Hemoglobin, IL-6, and Hepcidin Levels in KD Patients and Controls

As shown in Fig. 1a, KD patients had lower hemoglobin levels than the controls (11.2±0.1 and 12.1±0.2 g/dl, respectively, both $p<0.001$), which is consistent with our previous findings [6]. In addition, hemoglobin levels were significantly decreased after IVIG treatment (10.8±0.1 g/dl, $p<0.001$). We measured serum IL-6 hepcidin expressions in KD patients and controls using ELISA kits. As shown in Fig. 1b, c, we found higher IL-6 (72.4±14.2 and 9.4±2.6 pg/ml, respectively, both $p<0.001$) and hepcidin (244.1±22.1 and 144.2±16.1 ng/ml, respectively, both $p<0.001$) levels in KD patients than in the controls. The IL-6 and hepcidin levels were greatly decreased after IVIG treatment (8.3±2.2 pg/ml and 133.8±29.9 ng/ml, respectively, both $p<0.001$). Furthermore, there was no statistical significance between hepcidin levels and age ($p=0.707$).

Association Between IL-6 Levels and Hepcidin Levels in KD Patients and Controls

To explore the degree of preservation of the homeostatic control of hepcidin by IL-6, we performed a set of general linear models in the KD patients and febrile controls. Univariate analysis showed that the serum hepcidin levels were positively and significantly correlated with IL-6 levels ($R=0.381$, $p<0.001$) (Fig. 2a). Furthermore, when all patients were stratified into febrile control (blue), KD-pre-IVIG (green), and KD-post-IVIG (red) groups, there were positive and significant correlations between hepcidin and IL-6 levels in all groups ($R=0.760$, 0.477, and 0.389, respectively, all $p<0.001$) (Fig. 2b). This suggests that there was a relative preservation of control of hepcidin expression by IL-6 in the febrile controls and KD patients.

Association Between Hemoglobin Levels and Hepcidin Levels in KD Patients

To examine the ability of hepcidin to predict the hemoglobin concentration in KD patients, we investigated the association between the hemoglobin levels and hepcidin. Univariate analysis showed that the pre-IVIG hepcidin levels were negatively correlated with the post-IVIG hemoglobin levels

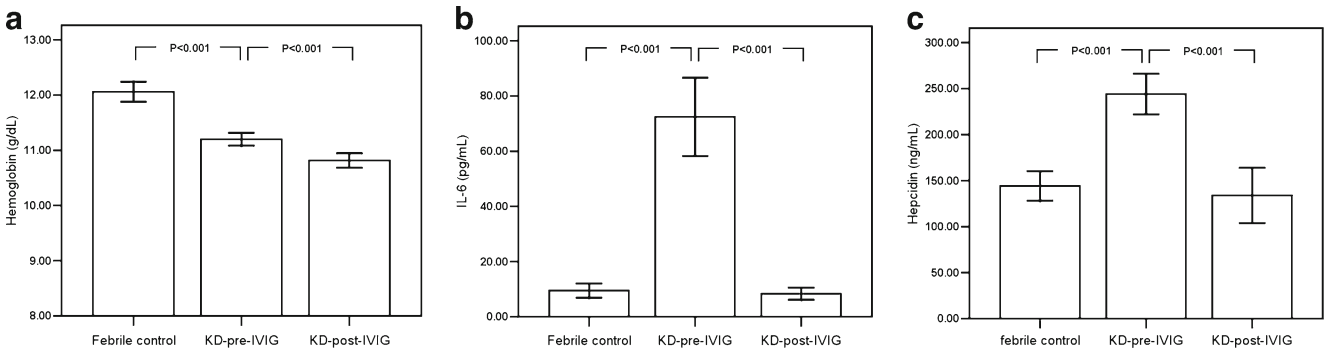

Fig. 1 Comparison of hemoglobin (**a**), IL-6 (**b**), and hepcidin levels (**c**) by ELISA between febrile controls ($N=30$) and patients with KD ($N=86$) before intravenous immunoglobulin (KD-pre-IVIG) and after IVIG treatment (KD-post-IVIG). Data are presented as mean ± standard error

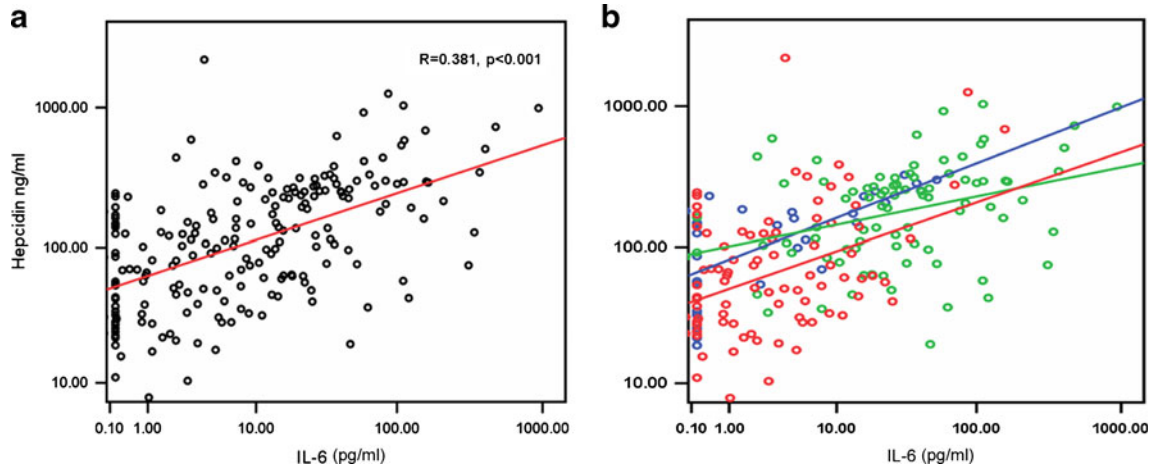

Fig. 2 Correlation plots between serum IL-6 and hepcidin levels (logarithmic scale). **a** All febrile controls (N=30) and patients with KD (N=86). **b** All patients were stratified into three groups: febrile controls (*blue*), patients with KD before intravenous immunoglobulin (KD-pre-IVIG, *green*), and patients with KD after IVIG treatment (KD-post-IVIG, *red*)

and positively correlated with the differences of hemoglobin level (pre-IVIG levels minus post-IVIG levels) after IVIG treatment (R=−0.188, p=0.046 and R=0.269, p=0.015, respectively) (Fig. 3). However, no statistically significant correlation was demonstrated between pre-IVIG hepcidin and hemoglobin levels (p=0.537) or post-IVIG hepcidin and hemoglobin levels (p=0.311).

Association Between Hepcidin Level and Serum Iron in KD Patients and Controls

The hepcidin levels were significantly higher and hemoglobin levels were significantly lower in the patients with KD compared with the febrile controls. To investigate the direct inhibitory effect of hepcidin on erythropoiesis via control of iron absorption in KD patients, we examined serum iron levels using ELISA. As shown in Fig. 4a, lower serum iron levels were observed in the pre-IVIG KD patients than in the febrile controls (p=0.009). In agreement with this, linear regression modeling confirmed that the serum iron levels were positively correlated with the hemoglobin levels and negatively correlated with the serum hepcidin levels (R=0.210, p=0.046 and R=−0.412, p=0.002, respectively) (Fig. 4b, c).

Changes in Serum Hepcidin Levels Were Associated with CAL Formation and IVIG Treatment Response in KD Patients

Extensive inflammation is related to the development of CAL in KD. Hepcidin is also a key marker of acute inflammation

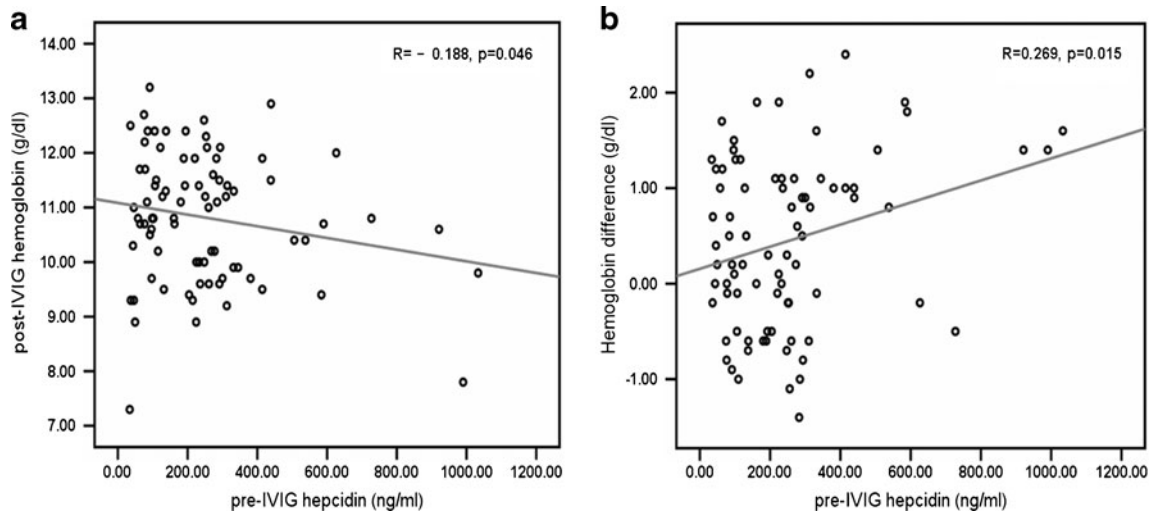

Fig. 3 Correlation between serum hepcidin and hemoglobin levels. Linear regression modeling showed that **a** hepcidin levels of KD patients (N=86) before IVIG treatment (pre-IVIG) were negatively correlated with hemoglobin levels after IVIG treatment (post-IVIG) and **b** positively correlated with the differences of hemoglobin levels (pre-IVIG levels minus post-IVIG levels) after IVIG treatment

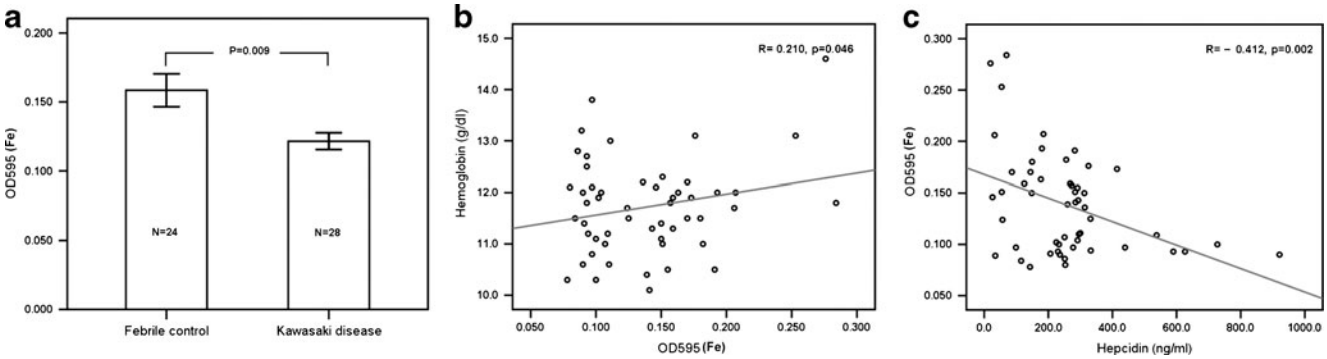

Fig. 4 Comparison of serum iron and correlation plots between serum iron, hepcidin, and hemoglobin levels in febrile controls and patients with KD. **a** Lower serum iron levels were observed in patients with KD before intravenous immunoglobulin treatment than in the febrile controls. Linear regression modeling confirmed that **b** the serum iron levels were significantly positively correlated with the hemoglobin levels and **c** negatively correlated with serum hepcidin levels. *N* indicates the sample size. Data are presented as mean ± standard error

[19–21]. To show the changes in hepcidin levels after IVIG treatment on CAL formation and IVIG treatment response, we divided the KD patients into two groups for comparison. Six (7.0%) KD patients had higher post-IVIG hepcidin levels than pre-IVIG levels, and the change of levels was less than the total average in 27 (31.3%) KD patients. Higher post-IVIG hepcidin levels were associated with resistance to IVIG treatment (Table I) (odds ratio=45.000, $p<0.001$). Additionally, we found that changes of hepcidin levels less than the total average were associated with resistance to IVIG treatment (Table I) and the occurrence of CAL formation (Table II) (odds ratio=13.182, $p=0.004$ and odds ratio=6.477, $p=0.017$, respectively).

Discussion

To the best of our knowledge, this is the first study to report that hepcidin is markedly increased in patients with KD. Anemia is a frequent finding in patients with KD and is associated with a more prolonged duration of active inflammation. However, the underlying molecular mechanism of the development of anemia in patients with KD has not previously been elucidated. The results of this study provide, for the first time, a mechanism to explain the observed anemia in patients with KD, and that this mechanism is related to markedly increased hepcidin expressions resulting in functional iron deficiency. Additionally, this study also provides a novel observation that the lower decrease in hepcidin levels after IVIG treatment is associated with the occurrence of CAL formation and resistance to IVIG treatment.

Pietrangelo et al. first demonstrated an IL-6-induced increase in hepcidin expression through a complex of the IL-6 receptor and gp130 dependence and subsequent induction of promoter binding of signal transducer and activator of transcription 3 [22] to drive hepcidin expression. In agreement with previous findings [23, 24], we also found increased IL-6 levels in the patients with KD compared to febrile controls and decreased IL-6 levels after IVIG treatment. Likewise, we also found that hepcidin levels were positively correlated with IL-6 levels in the febrile controls and KD patients.

Hepcidin plays an important role in orchestrating both iron metabolism and the pathogenesis of anemia of inflammation. High levels of hepcidin result in low serum levels of iron and a limited availability of iron for erythropoiesis. Hepcidin not only plays a key role in anemia of chronic inflammation [25], but it is also associated with anemia of acute disease [26]. It has been documented in patients with trauma that hepcidin levels rise to extremely high values and are positively correlated with injury severity and duration of anemia and negatively correlated with hypoxia [26]. Inappropriately high levels of hepcidin have also been observed in anemia associated with inflammatory disorders, such as infections [19, 27], autoimmune diseases [20, 28], critical illnesses [26, 29], and obesity [30]. Kemna et al. demonstrated temporal associations

Table I The changes in serum hepcidin levels in the 86 patients with Kawasaki disease responding or not responding to intravenous immunoglobulin (IVIG) treatment

Hepcidin	Resistant (%) (n=6)	Responsive (%) (n=80)	Odds ratio (95% CI)	p value
Post-IVIG > pre-IVIG				
Yes	5 (83.3)	8 (10.0)	45 (4.659–434.651)	<0.001
No	1 (16.7)	72 (90.0)	1	
Change of hepcidin levels less than total average				
Yes	5 (83.3)	22 (27.8)	13.182 (1.457–119.253)	0.004
No	1 (28.6)	58 (72.2)	1	

Table II The changes in serum hepcidin levels in the 86 patients with Kawasaki disease with or without coronary artery lesion (CAL) formation

Hepcidin	CAL (%) (n=7)	Without (%) (n=79)	Odds ratio (95% CI)	p value
Post-IVIG > pre-IVIG				
Yes	1 (14.3)	12 (15.1)	0.931 (0.103–8.435)	0.949
No	6 (85.7)	67 (84.9)	1	
Change of hepcidin levels less than total average				
Yes	5 (71.4)	22 (27.8)	6.477 (1.169–35.885)	0.017
No	2 (28.6)	57 (72.2)	1	

between plasma cytokines, hepcidin levels, and serum iron parameters in ten healthy individuals after lipopolysaccharide injection [21]. IL-6 was dramatically induced within 3 h after injection, and urinary hepcidin peaked within 6 h, followed by a significant decrease in serum iron. In our patients, we found that the pre-IVIG hepcidin levels were negatively correlated with the post-IVIG hemoglobin levels and positively correlated with the differences of hemoglobin levels. To the best of our knowledge, this is the first study to report such findings. Additionally, lower serum iron levels were observed in the pre-IVIG KD patients than in the febrile controls. In line with these findings, serum iron levels were positively correlated with hemoglobin levels and negatively correlated with serum hepcidin levels, reflecting a delayed effect on erythropoiesis in patients with KD. Moreover, hepcidin has also been shown to have a direct effect on erythroid precursor proliferation and survival as erythroid colony formation [31], which is in accordance with the observation of a transient erythroblastopenia in bone marrow aspiration in patients with KD [32]. In our patients, hemoglobin levels still decreased significantly after IVIG treatment, suggesting that bone marrow suppression in patients with KD does not reverse rapidly after IVIG treatment. A previous study found that urinary levels of hepcidin were strongly elevated and associated with iron maldistribution as well as antimalarial treatment results in a rapid decrease in urinary levels of hepcidin and reversal of hypoferremia [27]. In addition, a reticulocyte response within 3–5 days after the start of antimalarial treatment has been noted [33], as well as markedly increased hemoglobin levels by week 4 after treatment initiation [27]. However, it is still unknown how long anemia persists in patients with KD.

Elevated inflammatory markers and IVIG nonresponsiveness may be associated with the development of CAL in KD [34]. Our study demonstrated that a lower decrease in hepcidin levels after IVIG treatment was associated with the occurrence of CAL and resistance to IVIG treatment. Recently, it has been shown that hepcidin can be considered to be a key inducer of anemia of inflammation in patients with rheumatoid arthritis, and this inflammation has been proven to be directly linked to coronary artery atherosclerosis [20]. Additionally, pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis [35]. Therefore, the present data are consistent with the hypothesis that the inflammatory process may be involved in the development of CAL in KD.

This study has potential limitations. First, the cross-sectional nature of the present study hindered assessment of the causal relationship between hepcidin level and anemia among KD patients, and this should be ascertained through longitudinal studies to elucidate the time-dependent changes in hemoglobin, hepcidin, and cytokine levels, especially during the convalescent stage of KD. Second, we did not measure other iron biochemical parameters such as ferritin, total iron binding capacity, transferrin saturation, or soluble transferrin receptor, all of which require further investigation. Third, we cannot exclude the possibility that the higher hepcidin levels in KD patients compared to the febrile controls were due to a longer duration of fever in KD patients than in the febrile controls.

Conclusions

Hepcidin levels rose to extremely high levels in patients with KD compared to the febrile controls. High hepcidin levels were positively correlated with IL-6 levels and negatively correlated with serum iron and hemoglobin levels. Hepcidin may play a key role in the impairment of erythropoiesis and be a marker of the occurrence of CAL in KD patients. Further studies are warranted to investigate the pathophysiologic basis for these findings in KD.

Acknowledgments This study was supported by grants from the National Science Council Grant #NSC 99-2314-B-182A-032-MY2, NSC 100-2314-B-182A-048-MY3, and Chang Gung Memorial Hospital CMRPG8A021, Taiwan.

Conflicts of Interest The authors have indicated that they have no financial relationships relevant to this article to disclose.

References

1. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD. Kawasaki disease: infection, immunity and genetics. Pediatr Infect Dis J. 2005;24 (11):998–1004.
2. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation. 2004;110(17):2747–71.
3. Alves NR, Magalhaes CM, Almeida Rde F, Santos RC, Gandolfi L, Pratesi R. Prospective study of Kawasaki disease complications: review of 115 cases. Rev Assoc Med Bras. 2011;57(3):295–300.
4. Fukushige J, Takahashi N, Ueda Y, Ueda K. Incidence and clinical features of incomplete Kawasaki disease. Acta Paediatr. 1994;83 (10):1057–60.
5. Kuo HC, Wang CL, Liang CD, Yu HR, Chen HH, Wang L, et al. Persistent monocytosis after intravenous immunoglobulin therapy correlated with the development of coronary artery lesions in patients with Kawasaki disease. J Microbiol Immunol Infect. 2007;40(5):395–400.
6. Kuo HC, Yang KD, Liang CD, Bong CN, Yu HR, Wang L, et al. The relationship of eosinophilia to intravenous immunoglobulin treatment failure in Kawasaki disease. Pediatr Allergy Immunol. 2007;18(4):354–9.
7. Nakagawa M, Watanabe N, Okuno M, Kondo M, Okagawa H, Taga T. Severe hemolytic anemia following high-dose intravenous immunoglobulin administration in a patient with Kawasaki disease. Am J Hematol. 2000;63(3):160–1.
8. Le NT, Richardson DR. Ferroportin1: a new iron export molecule? Int J Biochem Cell Biol. 2002;34(2):103–8.
9. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306 (5704):2090–3.
10. Cherian S, Forbes DA, Cook AG, Sanfilippo FM, Kemna EH, Swinkels DW, et al. An insight into the relationships between hepcidin, anemia, infections and inflammatory cytokines in pediatric refugees: a cross-sectional study. PLoS One. 2008;3(12):e4030.
11. Suganami Y, Kawashima H, Hasegawa D, Sato S, Hoshika A. Clinical application of rapid assay of serum interleukin-6 in Kawasaki disease. Pediatr Int. 2008;50(2):264–6.
12. Kishimoto S, Suda K, Teramachi Y, Nishino H, Kudo Y, Ishii H, et al. Increased plasma type B natriuretic peptide in the acute phase of Kawasaki disease. Pediatr Int. 2011;53(5):736–41.
13. Wu JM, Chiou YY, Hung WP, Chiu NT, Chen MJ, Wang JN. Urinary cytokines and renal Doppler study in Kawasaki disease. J Pediatr. 2010;156(5):792–7.
14. Song SN, Tomosugi N, Kawabata H, Ishikawa T, Nishikawa T, Yoshizaki K. Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood. 2010;116(18):3627–34.
15. Shulman ST, De Inocencio J, Hirsch R. Kawasaki disease. Pediatr Clin North Am. 1995;42(5):1205–22.
16. Kuo HC, Yu HR, Juo SH, Yang KD, Wang YS, Liang CD, et al. CASP3 gene single-nucleotide polymorphism (rs72689236) and Kawasaki disease in Taiwanese children. J Hum Genet. 2011;56 (2):161–5.
17. Kuo HC, Liang CD, Wang CL, Yu HR, Hwang KP, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. Acta Paediatr. 2010;99 (10):1578–83.
18. Girelli D, Trombini P, Busti F, Campostrini N, Sandri M, Pelucchi S, et al. A time course of hepcidin response to iron challenge in patients with HFE and TFR2 hemochromatosis. Haematologica. 2011;96(4):500–6.
19. Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. Blood. 2011;118(15):4129–39.
20. Abdel-Khalek MA, El-Barbary AM, Essa SA, Ghobashi AS. Serum hepcidin: a direct link between anemia of inflammation and coronary artery atherosclerosis in patients with rheumatoid arthritis. J Rheumatol. 2011;38(10):2153–9.
21. Kemna E, Pickkers P, Nemeth E, van der Hoeven H, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood. 2005;106(5):1864–6.
22. Pietrangelo A, Dierssen U, Valli L, Garuti C, Rump A, Corradini E, et al. STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. Gastroenterology. 2007;132(1):294–300.
23. Gupta M, Noel GJ, Schaefer M, Friedman D, Bussel J, Johann-Liang R. Cytokine modulation with immune gamma-globulin in peripheral blood of normal children and its implications in Kawasaki disease treatment. J Clin Immunol. 2001;21(3):193–9.
24. Taytawat P, Viravud Y, Plakornkul V, Roongruangchai J, Manoonpol C. Identification of the external laryngeal nerve: its anatomical relations to inferior constrictor muscle, superior thyroid artery, and superior pole of the thyroid gland in Thais. J Med Assoc Thai. 2010;93(8):961–8.
25. Cullis JO. Diagnosis and management of anaemia of chronic disease: current status. Br J Haematol. 2011;154(3):289–300.
26. Sihler KC, Raghavendran K, Westerman M, Ye W, Napolitano LM. Hepcidin in trauma: linking injury, inflammation, and anemia. J Trauma. 2010;69(4):831–7.
27. de Mast Q, Nadjm B, Reyburn H, Kemna EH, Amos B, Laarakkers CM, et al. Assessment of urinary concentrations of hepcidin provides novel insight into disturbances in iron homeostasis during malarial infection. J Infect Dis. 2009;199 (2):253–62.
28. Demirag MD, Haznedaroglu S, Sancak B, Konca C, Gulbahar O, Ozturk MA, et al. Circulating hepcidin in the crossroads of anemia and inflammation associated with rheumatoid arthritis. Intern Med. 2009;48(6):421–6.
29. Isoda M, Hanawa H, Watanabe R, Yoshida T, Toba K, Yoshida K, et al. Expression of the peptide hormone hepcidin increases in cardiomyocytes under myocarditis and myocardial infarction. J Nutr Biochem. 2010;21(8):749–56.
30. del Giudice EM, Santoro N, Amato A, Brienza C, Calabro P, Wiegerinck ET, et al. Hepcidin in obese children as a potential mediator of the association between obesity and iron deficiency. J Clin Endocrinol Metab. 2009;94(12):5102–7.
31. Dallalio G, Law E, Means Jr RT. Hepcidin inhibits in vitro erythroid colony formation at reduced erythropoietin concentrations. Blood. 2006;107(7):2702–4.
32. Frank GR, Cherrick I, Karayalcin G, Valderrama E, Lanzkowsky P. Transient erythroblastopenia in a child with Kawasaki syndrome: a case report. Am J Pediatr Hematol Oncol. 1994;16(3):271–4.
33. Kurtzhals JA, Rodrigues O, Addae M, Commey JO, Nkrumah FK, Hviid L. Reversible suppression of bone marrow response to erythropoietin in *Plasmodium falciparum* malaria. Br J Haematol. 1997;97(1):169–74.
34. Kim JJ, Hong YM, Yun SW, Han MK, Lee KY, Song MS, et al. Assessment of risk factors for Korean children with Kawasaki disease. Pediatr Cardiol. 2012. doi:10.1007/s00246-011-0143-1.
35. Saeed O, Otsuka F, Polavarapu R, Karmali V, Weiss D, Davis T, et al. Pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(2):299–307.