Effect of Hepcidin and Iron Deficiency Anemia on Clinical Outcome in patients with Kawasaki Disease

Hyun Jung Kim
Eulji University College of Medicine
Eun Hye Choi
Eulji University College of Medicine
Hong Ryang Kil (gilhong@cnu.ac.kr)
Chungnam National University School of Medicine https://orcid.org/0000-0003-4925-8240

Research article

Keywords: Anemia, Iron deficiency, Hepcidin, Leptin, Kawasaki disease

Posted Date: December 18th, 2019

DOI: https://doi.org/10.21203/rs.2.19208/v1

Abstract

Background: The role of iron in children with Kawasaki disease (KD) and the importance of iron deficiency anemia (IDA) in children with KD are not fully known. We aimed to evaluate the effects of IDA on clinical outcomes of KD patients and the role of inflammation-induced hepcidin and leptin in the development of anemia in patients with KD.

Methods: A total of 50 children with KD and 47 age-matched children with controls were enrolled. Hepcidin, leptin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were measured by an enzyme-linked immunosorbent assay and IDA work-up was performed before and after IVIG therapy, and in the convalescent phase in patients with KD and control patients.

Results: (1) Pre-IVIG iron and transferrin saturations in KD children were significantly lower than those in controls (P =0.001). (2) Pre-IVIG hemoglobin and iron levels were positively correlated with leptin levels (P =0.018 and P =0.021, respectively). (3) Serum hepcidin levels were significantly elevated after IVIG treatment in patients with KD (1.53 ± 1.36 ng/ml in the acute stage vs 3.09 ± 4.22 ng/mL in the subacute phase, P = 0.001). (4) There was no difference in parameters of IDA, leptin, and hepcidin levels between KD patients with and without coronary artery lesion (CAL).

Conclusion: Serum iron and transferrin saturations were lowest in the acute phase, while hepcidin levels were highest in the subacute phase of KD. Although the presence of iron deficiency in the acute stage of KD did not predict CAL, further studies are necessary to clarify the association of hepcidin on the pathogenesis of anemia in KD.

Background

Kawasaki disease (KD) is an acute febrile disease that occurs mostly in infancy and early childhood with coronary vasculitis as its main complication. Although the development of vasculitis in KD has not been fully explained, serum levels of proinflammatory cytokines—such as interleukin (IL)−6, IL−10, and tumor necrosis factor (TNF)-α, are increased during the acute phase of KD, indicating that they are involved in the pathogenesis of KD [1]. The standard treatment of acute KD is a combination of high-dose (2 g/kg) intravenous immunoglobulin (IVIG) and high- or medium dose acetylsalicylic acid (ASA).

There is no diagnostic test for KD, but patients usually have characteristic laboratory findings in the acute phase such as leukocytosis with neutrophilia, hypoalbuminemia, hyponatremia, abnormal plasma lipids and anemia. Normocytic, normochromic anemia is a common laboratory finding in patients with KD, and related to a more prolonged fever duration [2]. However, the prevalence and clinical significance of iron deficiency anemia (IDA) in patients with KD has not yet been clarified.

Iron is necessary for normal development of the immune system, especially for immune cell proliferation [3]. Iron deficiency is the most prevalent micronutrient deficiency in children, so IDA in KD can be missed

or regarded as a mere coexisting finding. A recent study has suggested that iron deficiency was associated with the chronic mild inflammatory state in obese children and adolescents [4].

Hepcidin is an inflammation-related peptide hormone that influences on the immune system. The liver and the adipose tissue produce hepcidin in response to inflammatory stimuli and iron overload [5].A previous study found that hepcidin inhibits absorption of dietary iron in the intestine and placental iron transport [6]. Raised concentrations of hepcidin were observed in anemia of inflammatory disorders, autoimmune diseases such as rheumatoid arthritis, critical illnesses occurring after major surgery, severe trauma or sepsis and obesity [6, 7]. Although hepcidin plays an important role in anemia of inflammation, the clinical significance of hepcidin on anemia of KD has not been fully explained.

There are currently contradictory reports on the potential role of leptin in the pathogenesis of KD. Leptin is an adipocytokines which is mainly produced by the adipose tissue [8].A previous study found that there was association between leptin and C-reactive protein (CRP) levels in healthy adults, suggesting that leptin was involved in the systemic inflammatory response [9].In a prospective case-control study, an increased levels of leptin was the independent risk factor for coronary heart disease [10].On the contrary, a clinical studysuggested that leptin was not involved in the inflammatory state in KD, considering lower serum levels of leptin in patients with KD compared with children with controls [11].

Taken together, we hypothesized that hepcidin and leptin might exert direct or indirect effects on the progression of KD, and that they may be predictive factors for IVIG resistance and development of coronary artery lesion (CAL). The aim of this study was to evaluate the changing levels of hepcidin, leptin and parameters of IDA in KD patients and control groups and to investigate the association between IDA parameters and clinical outcomes in patients with KD.

Materials And Methods

Patients and data collection

This prospective study included 50 children with KD and 47 age-matched children recruited between November 2017 and April 2019. The 50 patients with KD were enrolled within 10 days of the onset of illness, with day 1 defined as the first day of fever. Complete KD was diagnosed in the presence of fever lasting for at least 5 days together with at least 4 of the principal clinical features based on 2017 American Heart Association criteria [12].Patients with refractory KD were defined as those who had a persistent fever (≥38.0℃) that lasted for more than 24 to 36 hours after completion of the initial IVIG infusion.

The control group (n = 47) had two types of subjects: 38 febrile patients and 9 afebrile patients. The febrile control patients had either viral pneumonia, acute tonsillitis, or cervical lymphadenitis. The afebrile control patients had acute gastroenteritis or acute urticaria.

Blood sampling

Two milliliters of blood were obtained and put in a serum separate tube. The samples were centrifuged at 1,000 ×g to for 10 minutes and were stored in a deep freezer (−80°C)until analysis. Hepcidin levels were measured by enzyme-linked immunosorbent assay (ELISA) kit (MyBiosource, San Diego, CA, USA). The serum levels of leptin, interleukin−6 (IL−6), and tumor necrosis factor-α (TNF-α) were assayed with an ELISA kit (abcam^{R}, R&D Systems, Cambridge, United Kingdom). The sensitivity of the assay was 0.1 ng/mL and the intraassay coefficient of variation (CV) was less than 9%.

Echocardiography

Echocardiographic examination was performed within 10 days of the onset of fever or before IVIG administration and 2 months after treatment. GE echocardiography system Vivid 7 (GE healthcare Korea, Seoul, Korea) was used. CALs were diagnosed on the basis of the Z scores of the left main coronary artery, proximal left anterior descending coronary artery, and proximal right coronary artery, and were defined as the Z scores of 2.0 or more. The value of Z scores from a standardized coronary artery dimension was based on the new guidelines of 2017 [12].

Laboratory analysis

Since the lower limit of normal hemoglobin value varies by age, anemia was defined using the following cut-off value according to age: 11.0 g/dL for under 4 years of age, 11.5 g/dL for ages 57, and 12.0 g/dL for ages 811 [13]. Iron deficiency was defined as presenting with low serum iron (50 μg/dL) and low transferrin saturation (16%). Iron deficiency anemia (IDA) was diagnosed only when the above mentioned definitions of both anemia and iron deficiency were met.

Levels of hepcidin, leptin, IL−6 and TNF- were measured in 97 cases including patients with KD (n = 50), the acute febrile control group (n = 38), and the afebrile control group (n = 9). White blood cells counts (WBC), hemoglobin, mean corpuscular volume (MCV), platelet count, sodium, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), iron, total iron binding capacity (TIBC), transferrin saturation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were obtained for all study subjects.

Serial blood samples were obtained from all KD patients both before (before IVIG treatment, pre-IVIG) and after IVIG treatment (within 3 days after IVIG infusion, post-IVIG), and in the convalescent phase, when ESR was normal. Samples were prepared at the appropriate dilutions and paired samples were assayed together according to the manufacturer's instructions. In control patients, hepcidin, leptin and other laboratory parameters were measured once at either admission or at outpatient clinic.

Statistical analysis

Normally distributed continuous data were expressed as mean ± standard deviation. Comparisons of the frequencies between groups were analyzed using the χ^2 test. Differences among groups were assessed using the 2-sample t-test and analysis of variance (ANOVA). The correlation of serum hepcidin with hemoglobin, ferritin, iron, iron binding capacity and CRP levels were tested with Pearson correlation analysis. The significance of difference was calculated by Scheffe's test, and a *P* value less than 0.05 was considered to be statistically significant.

Results

Baseline patient characteristics and laboratory findings

The KD group included 24 boys and 26 girls with a mean age at diagnosis of 32.62 ± 18.64 months with a range from 2 months to 8 years. Of the 50 patients with KD, 30 patients (60.0%) were diagnosed with complete KD. High-dose ASA (50 mg/kg/day) was given for a mean duration of 4.06 ± 1.34 days (range, 2–6 days).

In the febrile control group, Epstein-Barr virus infection was confirmed in 3 patients and adenovirus was identified in 1 patient by using a nasopharyngeal swab. In addition, bocavirus and rhinovirus were identified in 2 patients and respiratory syncytial virus B and influenza B were confirmed in 2 patients in the febrile control group.

Children in these KD groups and febrile controls did not differ in gender distribution, age at presentation and body mass index (BMI). Of the 50 patients with KD, 19 patients (34.0%) were diagnosed with IDA in the acute stage. Of the 38 patients with febrile controls, 8 patients (21.1%) were diagnosed as IDA. Only 1 patient (11.1%) was diagnosed with IDA in the afebrile control groups. The pre-IVIG iron levels were 19.40 ± 16.89 μg/dL in the KD groups and 27.51 ± 23.84 μg/dL in the febrile control groups. The pre-IVIG TIBC and ferritin levels in patients with KD were significantly higher compared with the febrile controls (Table 1).

Initial levels of hepcidin, leptin and IDA parameters in KD patients and control groups

There were significant differences between the KD and control groups in hemoglobin, WBC, albumin, ESR, CRP, HDL-C, NT-proBNP and hepcidin levels. The levels of WBC, ESR, CRP and NT-proBNP were significantly elevated in patients of KD compared to control groups. The serum iron levels and transferrin saturations were significantly lower in the KD groups compared to the control groups ($P < 0.001$ and $P = 0.001$, respectively). In the acute phase, high serum ferritin levels were observed in the KD group compared with those in the febrile control group ($P = 0.001$). Initial serum hepcidin levels were significantly lower in patients with KD compared with febrile controls ($P = 0.005$). However, there is no significant difference in serum leptin levels between the KD and control groups (Table 2).

Response to initial intravenous immunoglobulin

Of a total 50 patients with KD, 35 (70.0%) patients completely responded to the initial IVIG treatment. Fifteen (30.0%) patients resisted the initial IVIG infusion, but 8 patients responded to the second IVIG treatment without requiring corticosteroid treatment. Seven patients were crossed over methylprednisolone pulse therapy with a second IVIG treatment. All the patients responded to the second line treatments.

Laboratory data of IDA in acute, subacute and convalescent phase in patients with KD

The serum hemoglobin levels significantly decreased immediately after IVIG treatment but were increased to above baseline levels in the convalescent phase ($P < 0.001$). Both the iron levels and TIBC were significantly lower in pre-IVIG compared to the convalescent phase ($P < 0.001$). The transferrin saturation (iron/TIBC) and MCV levels were lowest prior IVIG treatment and increased significantly in the convalescent phase (Table 3).

After IVIG treatment, hepcidin levels were significantly increased compared to the pre-IVIG level (1.53 ± 1.36 ng/mL vs. 3.09 ± 4.22 ng/mL, $P = 0.001$). Subsequently, post-IVIG hepcidin levels were decreased in the convalescent phase, which did not reach statistical significance (3.09 ± 4.22 ng/mL vs. 2.94 ± 4.65 ng/mL, $P = 0.755$). There was no significant difference in leptin levels between each period.

Comparison of laboratory data of KD patients with IVIG resistance and responsiveness

The initial serum HDL cholesterol levels were significantly lower in the IVIG resistance group compared with the IVIG responsive group (26.8 ± 6.3 mg/dL vs. 31.4 ± 6.3 mg/dL, $P = 0.034$). However, there were no differences in leptin, hepcidin, hemoglobin, NT-proBNP and CRP levels between these two groups of KD (Table 4). Serum hepcidin did not have good diagnostic accuracy for predicting IVIG resistance. The area under the receiver operating characteristics (ROC) curve of serum hepcidin for predicting IVIG resistance was 0.44 (95% confidence interval, 0.260.52).

Relationship between clinical parameters in KD patients and development of CAL

The gender distributions were different between groups of KD with and without CALs. The percentage of boys were 81.8% and 62.5% in CAL-positive group and CAL-negative group, respectively ($P = 0.011$). In addition, BMI was higher in the CAL-positive group (17.94 ± 1.71 kg/m^2 vs. 16.61 ± 1.89 kg/m^2, $P = 0.043$). There were no significant differences in total fever duration, parameters of IDA, leptin, and hepcidin levels between KD patients with and without CALs (Table 5).

Echocardiographic findings

Eleven of the 50 patients (22.0 %) showed coronary artery dilation in the acute stage of KD. In the convalescent phase, echocardiography revealed the presence of coronary ectasia in 2 patients (4.0 %) with KD. None of patients with KD developed coronary artery aneurysms before or after IVIG therapy. There was no difference between these 2 periods in the degree of left ventricular fractional shortening. The early transmitral flow velocity (E) / the early diastolic mitral annular velocity(e') ratio measured by tissue Doppler imaging (TDI) was significantly higher in the acute phase of KD compared to the convalescent phase (Table 6).

Correlations among hepcidin, leptin and other clinical parameters

Age in patients with KD was positively associated with leptin levels ($r = 0.583$, $P = 0.001$). In addition, weight and BMI were positively associated with leptin levels in KD ($r = 0.075$, $P = 0.001$ and $r = 0.465$, $P = 0.001$, respectively). Serum HDL cholesterol levels were positively associated with leptin levels ($r = 0.403$, $P = 0.008$). Furthermore, the pre-IVIG hemoglobin and iron levels were positively correlated with leptin levels (Table 7).

Serial changes of iron, hepcidin and hemoglobin levels in KD patients

Fig. 1 shows the changes in serum leptin, hepcidin and hemoglobin levels between the acute stage (before IVIG therapy), subacute (2—3 days after IVIG treatment), and the convalescent phase..Serum iron levels was lowest in the acute phase of KD and increased significantly in the subacute phase (18.13 ± 11.14 μg/dL in the acute phase vs. 36.88 ± 32.34 μg/dL in the subacute phase, $P < 0.001$). Subsequently, serum iron levels significantly increased to the levels of 83.38 ± 39.02 μg/dL in the convalescent phase.

Serum hepcidin levels were significantly elevated after IVIG treatment in KD patients (1.53 ± 1.36 ng/ml in the acute stage vs 3.09 ± 4.22 ng/mL in the subacute phase, $P = 0.001$). In addition, serum hemoglobin levels were lowest immediately after IVIG treatment (11.37 ± 0.94 g/dL in the acute phase vs. 10.81 ± 0.84 ng/mL in the subacute phase, $P < 0.001$). In the convalescent phase, post-IVIG hemoglobin was significantly increased to the mean levels of 12.04 ±0.71 g/dL in all KD patients without iron supplementation.

Discussion

This study was conducted to investigate the iron status in patients with KD and to determine the role of inflammation-induced hepcidin and leptin on the clinical outcome and IVIG resistance. In the present study, serum iron levels were significantly lower in acute KD patients compared with the control groups. However, the presence of iron deficiency in the acute phase of KD did not indicate the poor outcomes such as coronary artery abnormalities and IVIG resistance.

Hepcidin is a small, cysteine-rich peptide which can affect both anemia of inflammation and red blood cell kinetics in health and disease [5]. It is an iron-regulatory hormone and well-known biomarker for IDA. However, a recent study showed that the predictive accuracy of serum hepcidin was poor and urinary hepcidin was good diagnostic tool for IDA [14]. They found that urinary hepcidin levels decreased as the severity of anemia increased. In this study, serum hepcidin was not useful for predicting coronary artery complications in patients with KD. Moreover, the initial serum hepcidin levels were significantly lower in patients with KD compared with febrile control groups.

KD is an acute febrile vasculitis of unknown origin. During the acute phase of KD, certain unidentified agents activate monocytes, T cell and B cell, and up-regulate proinflammatory cytokines such as IL–1, IL–2, IL–6, and TNF-α. Intravenous immunoglobulin (IVIG) has been shown to reduce both the duration of fever and the incidence of CAL [15]. 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 development of anemia in patients with KD has not been clearly elucidated up to now.

Anemia of inflammation is a common disease that exhibits normocytic and normochromic anemia, and sometimes presents with microcytic and hypochromic anemia. In KD patients, normochromic, normocytic anemia occurs commonly and resolves with decrease of inflammation in KD [11]. In particular, iron deficiency anemia is the most common cause of anemia in infants aged 9—24 months. A recent study found that IDA significantly impaired cell-mediated immunity in children although it did not influence humoral immunity [16].In this study, IDA was diagnosed in 34% of patients with acute phase of KD. Meanwhile, IDA was diagnosed in 21.1% of febrile controls and 11.1% of afebrile controls. Although IDA did not influence coronary artery complications, iron levels were positively associated with leptin levels in the acute phase of KD.

Hepcidin plays an important role in iron metabolism and the pathogenesis of anemia of acute by inducing ferroportin degradation. It is expressed in the liver and the adipose tissue at both mRNA and the protein levels [17].Pietrangelo et al. found that IL–6 increased hepcidin expression through a complex of the IL–6 receptor and gp130 dependence [18].However, we didn’t find a correlation between hepcidin and IL–6 levels in patients with KD, most likely due to the small sample size.

In animal study, hepcidin inhibits the absorption of iron from the intestine resulting in hypoferremia [19].However, the level of serum ferritin elevates in the presence of infection, chronic inflammation, malignancy, or liver disease, because it is an acute-phase reactant, so simultaneous measurement of CRP is necessary. Hyperferritinemia with decreased transferrin saturation indicates the presence of functional IDA or anemia of inflammation [20].In this study, ferritin levels in patients with acute KD were significantly higher than those of the febrile controls. We cannot exclude the possibility that higher ferritin levels in KD patients were due to a longer fever duration in KD patients compared with the febrile controls. Measurement of serum ferritin with a combination of other disease markers such as CRP and NT-proBNP levels could be useful for differentiating KD with other febrile conditions.

Iron deficiency can occur as absolute or functional deficiency or combined [20]. A previous study found that anemia in KD patients is related to increased hepcidin levels resulting in functional iron deficiency [21].However, above-mentioned study did not check the time-dependent changes in hemoglobin, hepcidin and cytokine levels especially during the convalescent stage of KD. We demonstrated that high hepcidin and low hemoglobin levels are present in subacute phase of KD while high iron, hemoglobin and transferrin saturation in the convalescent phase.

In addition, a recent study showed that anemia in KD occurs in patients exhibiting more severe inflammation [22].Up to now, it is still unknown how long anemia persists in patients with KD. In our patients, hemoglobin levels decreased significantly after IVIG treatment, but increased above baseline in the convalescent phase without an iron supply. This indicates that IDA in the acute phase of KD is the functional IDA as a redistribution of iron from key sites of its utilization to storage sites rather than absolute IDA, a true decrease in the body's iron content.

Leptin has a dual role as both a hormone and a cytokine. It influences multiple endocrine functions and bone metabolism as a hormone and promotes inflammatory responses as a cytokine. Inflammatory cells produce leptin, and leptin mRNA expression and circulatory leptin levels are increased by a number of inflammatory stimuli, including IL–1, IL–6 and lipopolysaccharide [23].Conversely, reduced levels of leptin in malnourished patients was associated with the increased infection rates and reduced cell-mediated immunity secondary to insufficient immune cell effector activity [24].Our studyfound that the initial serum leptin levels in patients with KD were lower than that of afebrile control groups, although statistical significance was not reached. It is possible that a low concentration of serum leptin might increase susceptibility to KD. Moreover, there is a strong correlation between leptin, weight and BMI in patients with KD. We speculate that leptin could play an important role by stimulating secretion of inflammatory cytokines especially in obese patients with KD. Furthermore, we found a positive correlation of leptin with age in patients with KD.

This study has some limitations. First, the present study was performed in a single center and had the relatively small number of patients. Therefore, we could not determine whether our results are also applicable to different geographic populations. Second, there were no coronary aneurysms as a complication in patients with KD. Third, serum iron is labile and prone to diurnal variations [25].Morning levels of iron generally assumed to be higher than afternoon or evening levels. However, it is hard to control the time of blood sampling in patients with KD and control subjects. Lastly, the cross-sectional nature of the present study hindered assessment of the causal relationship between hepcidin and anemia in KD patients.

Conclusions

In this prospective study, we demonstrated that high hepcidin levels are present in subacute KD patients with concurrent the lowest hemoglobin levels, indicating that hepcidin is involved in the anemia of acute KD. Furthermore, pre-IVIG iron and transferrin saturations in KD children were significantly lower than

those in controls, probably caused by functional iron deficiency of acute inflammation with spontaneous recovery of anemia in the convalescent phase. Although this study did not establish a direct causal link between IDA and CAL, we found that initial hemoglobin and iron levels were positively correlated with leptin levels. However, the parameters of IDA, leptin and hepcidin levels did not predict IVIG resistance or coronary artery complications. A large multicenter, randomized clinical research is therefore needed to determine the role of hepcidin in the pathogenesis of anemia in KD vasculitis.

List Of Abbreviations

KD: Kawasaki disease; BMI: body mass index; MCV: mean corpuscular volume; TIBC: total iron binding capacity; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein, ESR: erythrocyte sedimentation rate; HDL-C: high density lipoprotein-cholesterol; IVIG: intravenous immunoglobulin; NT-proBNP: N-terminal pro-brain natriuretic peptide; CAL: coronary artery lesion; IL–6: Interleukin–6; LV: left ventricular; E: base peak early diastolic E-wave velocity (cm/sec); A: base peak early diastolic A-wave velocity (cm/sec); E': peak early diastolic myocardial velocity (cm/sec); A': late diastolic myocardial velocity (cm/sec); LDL-C: low density lipoprotein-cholesterol; TNF-α: tumor necrosis factor-α

Declarations

Ethics approval and consent to participate

Informed consent was obtained from the parents of all the children, and this study protocol was reviewed and approved by Institutional Review Board of Eulji University Hospital (No. 2017–07–018).

Consent for publiciation

Written consent was obtained.

Availability of data and material

All data are included in this article.

Competing interests

Authors have no financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subjects of this article. No potential conflicts of interest to this article was reported.

Funding

No funding resource

Author Contribution

Conceptualization: Kil HR. Data curation: Kim HJ. Investigation: Choi EH. Writing-original draft: Kim HJ. All authors read and approved the final manuscript.

Acknowledgements

This research was supported by EMBRI Grants (2017 EMBRIDJ) from the Eulji University.

References

1. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. Clin Exp Immunol 2016;186(2):134-43.
2. Nemeth E, Ganz T. Anemia of inflammation. Hematol Oncol Clin North Am 2014;28(4):671-81.
3. Mascotti DP, Rup D, Thach RE. Regulation of iron metabolism: translational effects mediated by iron, heme, and cytokines. Annu Rev Nutr 1995;15:239-61.
4. 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.
5. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001;276(11):7806-10.
6. Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brisson P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001;276(11):7811-9.
7. 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.
8. Cava AL. Leptin in inflammation and autoimmunity. Cytokine 2017;98(1):51-8.
9. Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. Circulation 2004;109(18):2181-5.
10. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104(25):3052-6.
11. Liu R, He B, Gao F, Liu Q, Yi Q. Relationship between adipokines and coronary artery aneurysm in children with Kawasaki disease. Transl Res 2012;160(2):131-6.

12. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. Circulation 2017;136(17):e927-99.
13. Stuart H, et al. Nathan and Oski's Hematology and Oncology of Infancy and childhood, 7th, Philadelphia, 2009.
14. Dewan P, Dixit A, Gomber S, Kotru M, Banerjee BD, Tyagi V, et al. Serum and urinary hepcidin for diagnosing iron-deficiency anemia in under-5 children. J Pediatr Hematol Oncol 2019;41(4):216-20.
15. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. Circulation 2006;113(22):2606-12.
16. Das I, Saha K, Mukhopadhyay D, Roy S, Raychaudhurl G, Chatterjee M, et al. Impact of iron deficiency anemia on cell-mediated and humoral immunity in children: A case control study. J. Nat. Sci. Biol. Med 2014;5(1):158-63..
17. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113(9):1271-6.
18. Pietrangelo A, Dierssen U, Valli K, 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.
19. Mena NP, Esparza A, Tapia V, Valdes P, Nunez MT. Hepcidin inhibits apical iron uptake in intestinal cells. Am J Physiol Gatrointest Liver Physiol 2008;294(1):192-8.
20. Nairz M, Theurl I, Wolf D, Weiss G. Iron deficiency or anemia of inflammation? : Differential diagnosis and mechanism of anemia of inflammation. Wien Med Wochenschr 2016;166(13):411-23.
21. Kuo HC, Yang YL, Chuang JH, Tiao MM, Yu HR, Huang LT, et al. Inflammation-induced hepcidin is associated with the development of anemia and coronary artery lesions in Kawasaki disease. J Clin Immunol 2012;32(4):746-52.
22. Ha KS, Jang GY, Lee JH, Lee KC, Son CS. Laboratory markers in incomplete Kawasaki disease according to coronary artery outcome. Korean Circ J 2018;48(4):287-95.
23. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell response. J Clin Invest 2003;111(2):241-50.
24. Matarese G, Cava L, Sanna V, Lord GM, Lechler RI, Fontana S, et al. Balancing susceptibility to infection and autoimmunity: a role for leptin? Trends Immunol 2002;23(4):182-7.
25. Dale JC, Burrritt MF, Zinsmeister AR. Diurnal variation of serum iron, iron-binding capacity, transferrin saturation, and ferritin levels. Am J Clin Pathol 2002;117(5):802-8.

Tables

Table 1. Baseline characteristics of patients with KD and febrile controls

Parameters	Kawasaki disease (n = 50)	Febrile controls (n = 38)	*P*
Age, mon	32.62 ± 18.64	35.97 ± 26.44	0.508
Gender (male/female)	24/26	16/22	0.582
Weight, kg	13.90 ± 4.79	14.42 ± 6.66	0.668
BMI, kg/m^2	16.91 ± 1.92	16.14 ± 2.20	0.085
Iron deficiency	25 (50.0%)	23 (60.5%)	0.326
Iron deficiency anemia	19 (34.0%)	8 (21.1%)	0.088
Hemoglobin, g/dL	11.37 ± 0.94	11.6 ± 0.91	0.153
MCV, fL	78.38 ± 3.47	77.67 ± 5.04	0.436
Iron, μg/dL	19.40 ± 16.89	27.51 ± 23.84	0.086
TIBC, μg/dL	247.0 ± 36.33	282.49 ± 50.36	0.001
Ferritin, μg/L	179.63 ± 93.34	101.36 ± 58.06	0.001
Transferrin saturation, %	7.78 ± 5.97	10.03 ± 8.38	0.175

Table 2. Laboratory data before IVIG treatment in KD patients and controls

Parameters	KD (n = 50)	Febrile controls (n = 38)	Afebrile controls (n = 9)	*P*
Hemoglobin, g/dL	11.37 ± 0.94	11.66 ± 0.91	12.46 ± 1.34	0.009
White blood cell, 10^3/μL	12.17 ± 4.18	10.80 ± 4.13	7.76 ± 3.29	0.011
Neutrophil, %	64.26 ± 13.73	53.20 ± 16.19	45.49 ± 9.07	0.011
AST, U/L	59.06 ± 87.75	38.20 ± 23.71	37.89 ± 15.84	0.294
ALT, U/L	72.56 ± 100.55	29.76 ± 43.78	22.56 ± 21.27	0.023
ESR, mm/hr	65.13 ± 31.36	38.32 ± 32.70	23.00 ± 16.23	< 0.001
CRP, mg/dL	7.12 ± 4.49	3.64 ± 4.95	0.73 ± 0.91	< 0.001
HDL-C, mg/dL	30.0 ± 7.03	36.06 ± 10.47	43.42 ± 12.41	< 0.001
Sodium, mEq/L	135.48 ± 2.20	136.87 ± 1.98	138.33 ± 1.41	< 0.001
NT-proBNP, pg/mL	733.72 ± 785.41	165.37 ± 203.53	94.24 ± 81.53	< 0.001
Albumin, g/dL	4.09 ± 0.32	4.33 ± 0.26	4.47 ± 0.32	< 0.001
D-dimer, μg/mL	1.56 ± 1.12	0.88 ± 0.93	0.79 ± 0.64	0.006
Iron, μg/dL	19.40 ± 16.89	27.51 ± 23.84	60.56 ± 34.83	< 0.001
TIBC, μg/dL	247.0 ± 36.33	282.49 ± 50.36	320.44 ± 44.57	< 0.001
Ferritin, μg/L	179.63 ± 93.35	101.36 ± 58.06	60.76 ± 39.80	< 0.001
Transferrin saturation, %	7.78 ± 5.97	10.03 ± 8.38	18.39 ± 9.33	0.001
Hepcidin, ng/mL	1.51 ± 1.36	3.12 ± 1.6	2.19 ± 1.80	0.005
Leptin, pg/mL	11.25 ± 38.60	9.40 ± 29.2	14.2 ± 24.7	0.478

Table 3. Laboratory data of IDA, hepcidin and leptin in acute, subacute and convalescent phase in patients with KD

	Acute phase	Subacute phase	Convalescent phase	*P*
Hemoglobin, g/dL	11.34 ± 0.95	10.84 ± 0.86	12.05 ± 0.71	< 0.001
MCV, fL	78.33 ± 3.48	77.95 ± 3.56	80.15 ± 3.81	< 0.001
Iron, μg/dL	18.13 ± 11.14	36.88 ± 32.34	83.38 ± 39.02	0.011
TIBC, μg/dL	253.0 ± 41.01	255.25 ± 49.49	331.00 ± 23.31	0.034
Ferritin, μg/L	194.16 ± 53.68	225.86 ± 57.41	73.40 ± 50.39	0.001
Transferrin saturation, %	6.77 ± 3.40	26.69 ± 5.46	20.97 ± 9.83	0.019
Hepcidin, ng/mL	1.53 ± 1.36	3.09 ± 4.22	2.94 ± 4.65	0.001
Leptin, pg/mL	13.35 ± 38.99	12.85 ± 38.94	21.24 ± 84.25	0.278

Table 4. Laboratory data in KD patients according to IVIG responsiveness

Parameters	IVIG resistance (n = 15)	IVIG responsiveness (n = 35)	*P*
Hemoglobin, g/dL	11.2 ± 0.8	11.4 ± 1.0	0.550
MCV, fL	78.9 ± 3.3	78.2 ± 3.6	0.475
White blood cell, 10^3/μL	12.4 ± 4.87	12.0 ± 3.91	0.758
Neutrophil, %	69.0 ± 17.6	62.2 ± 11.4	0.109
Platelet count, 10^4/μL	34.75 ± 11.34	36.52 ± 13.91	0.667
CRP, mg/dL	7.9 ± 4.2	6.8 ± 4.6	0.450
HDL-C, mg/dL	26.8 ± 6.3	31.4 ± 7.0	0.034
Sodium, mEq/L	135.0 ± 1.8	135.7 ± 2.3	0.317
NT-proBNP, pg/mL	754.2 ± 838.7	725.0 ± 774.0	0.906
Albumin, g/dL	4.1 ± 0.3	4.1 ± 0.3	0.992
D-dimer, μg/mL	1.7 ± 1.2	1.4 ± 1.1	0.526
Iron, μg/dL	23.1 ± 26.8	23.6 ± 21.3	0.951
Transferrin saturation, %	9.4 ± 9.1	9.8 ± 9.8	0.894
Hepcidin, ng/mL	1.2 ± 0.2	1.3 ± 0.5	0.392
Leptin, pg/mL	2.0 ± 1.3	14.3 ± 48.6	0.521
TIBC, μg/Dl	239.7 ± 41.3	247.3 ± 36.1	0.521
Ferritin, μg/L	208.5 ± 120.5	163.6 ± 71.6	0.196

Table 5. Relationship between clinical parameters in KD patients and development of coronary artery lesion in acute stage

	CAL(+) (n = 11)	CAL(-) (n = 39)	*P*
Age, month	34.91 ± 21.56	31.97 ± 18.00	0.650
Gender (male/female)	9/2	15/24	0.011
BMI, kg/m^2	17.94 ± 1.71	16.61 ± 1.89	0.043
Hemoglobin, g/dL	11.33 ± 1.25	11.3 8± 0.86	0.867
MCV, fL	80.0 ± 3.04	77.93 ± 3.48	0.080
Neutrophil, %	67.05 ± 13.09	63.47 ± 13.96	0.451
CRP, mg/dL	7.62 ± 4.47	6.99 ± 4.54	0.685
HDL-C, mg/dL	31.09 ± 5.84	29.69 ± 7.37	0.566
NT-proBNP, pg/mL	833.94 ± 770.48	705.45 ± 797.17	0.637
Albumin, g/dL	4.00 ± 0.40	4.11 ± 0.30	0.318
Leptin, pg/mL	10.80 ± 30.19	11.38 ± 41.0	0.966
Iron, μg/dL	16.82 ± 7.51	20.24 ± 18.98	0.566
Transferrin saturation, %	6.56 ± 2.43	8.18 ± 6.71	0.443
TIBC, μg/dL	252.36 ± 39.72	245.26 ± 35.62	0.579
Ferritin, μg/L	152.35 ± 71.75	188.46 ± 98.64	0.270
Hepcidin, ng/mL	1.17 ± 0.13	1.60 ± 1.53	0.363
IL-6, pg/mL	693.61 ± 1856.18	200.13 ± 577.56	0.482

Table 6. Cardiovascular findings on echocardiographic examination in 50 KD patients

	Acute phase	Convalescent phase	*P*
CAL			
CA dilatation	11 (20.0 %)	2 (4.0%)	0.004
CA aneurysm	0	0	NA
LV fractional shortening (%)	33.64 ± 4.98	35.27 ± 3.80	0.064
E/A ratio	1.36 ± 0.24	1.40 ± 0.16	0.252
E/e’ ratio	10.51 ± 2.03	9.27 ± 1.55	0.001

Table 7. Correlation of pre-IVIG hepcidin, leptin and IL-6 with clinical and other laboratory variables in patients with Kawasaki disease

	Hepcidin		Leptin		IL-6	
	r	*P*	*r*	*P*	*r*	*P*
Age, mon	-0.293	0.060	0.583	0.001	-0.093	0.612
Weight, kg	-0.286	0.067	0.705	0.001	-0.075	0.683
BMI, kg/m^2	0.062	0.695	0.465	0.001	-0.093	0.611
Pre-IVIG hemoglobin, g/dL	0.094	0.552	0.365	0.018	-0.087	0.638
Post-IVIG hemoglobin, g/dL	0.008	0.962	0.098	0.536	0.002	0.989
White blood cell, $10^3/\mu L$	0.054	0.734	0.294	0.059	-0.256	0.158
CRP, mg/ dL	-0.271	0.083	0.013	0.936	0.304	0.090
Iron, µg/dL	-0.149	0.348	0.355	0.021	-0.165	0.368
TIBC, µg/dL	-0.021	0.897	-0.051	0.746	-0.249	0.170
Ferritin, µg/L	-0.268	0.087	0.047	0.766	0.001	0.998
HDL-C, mg/dL	-0.030	0.849	0.403	0.008	-0.202	0.268
NT-proBNP, pg/mL	-0.084	0.597	-0.186	0.238	0.265	0.143
Hepcidin, ng//mL	—	—	-0.155	0.328	-0.185	0.312
Leptin, pg/mL	-0.155	0.328	—	—	-0.075	0.683
TNF-α, pg/mL	0.140	0.445	0.077	0.677	-0.024	0.897
IL-6, pg/mL	-0.185	0.312	-0.075	0.683	—	—

Figures

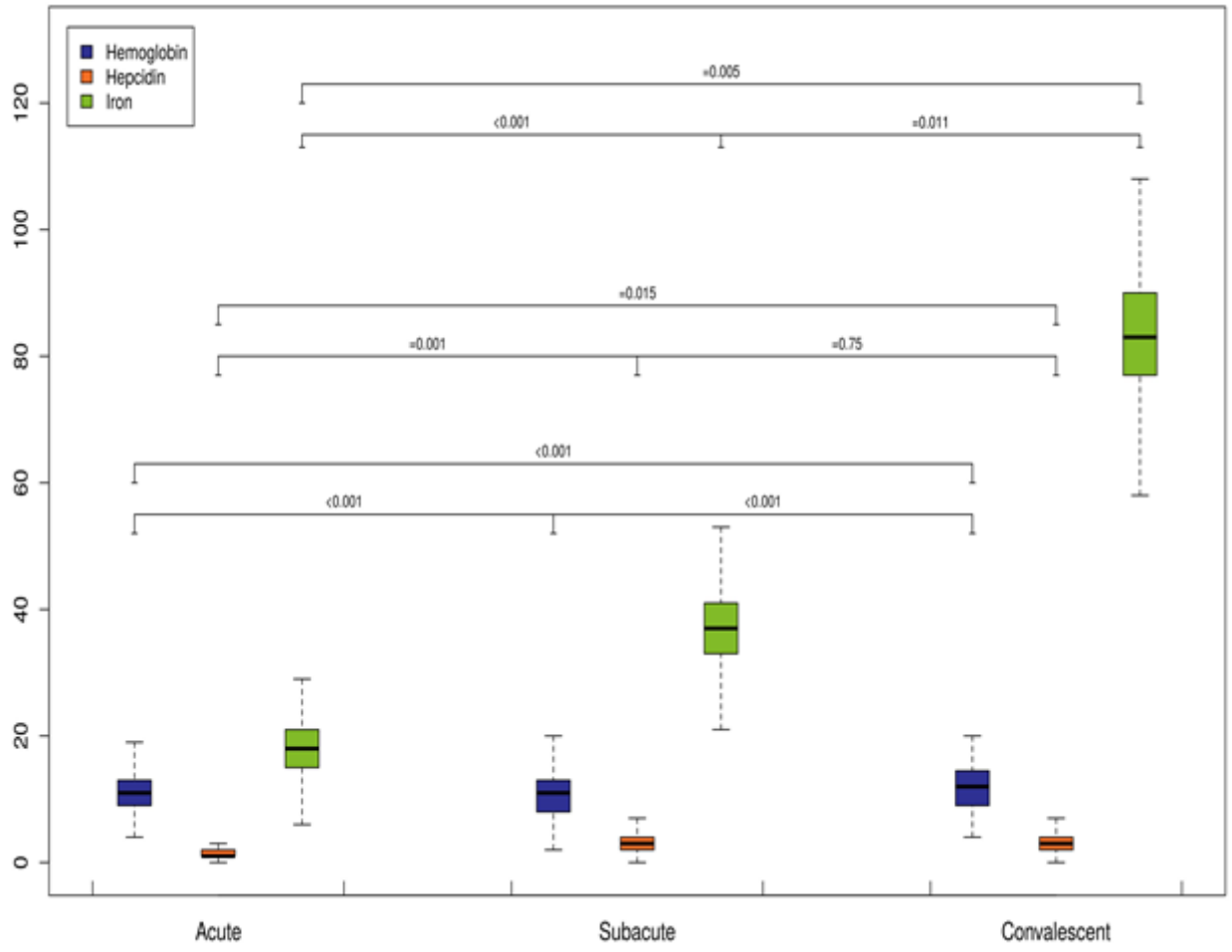

Figure 1

Changes in iron, hepcidin and hemoglobin levels before intravenous immunoglobulin (IVIG) infusion, at 48 hours after IVIG infusion and in the convalescent phase in patients with KD