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Serum Ferritin as a Diagnostic Biomarker for Kawasaki Disease

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Diagnosis of Kawasaki disease (KD) is occasionally delayed because it is solely based on clinical symptoms. Previous studies have attempted to identify diagnostic biomarkers for KD. Recently, patients with KD were reported to have elevated serum ferritin levels. We investigated the usefulness of the serum ferritin level as a diagnostic biomarker for distinguishing KD from other acute febrile illnesses. Blood samples were obtained from pediatric patients with KD (N=77) and those with other acute febrile illnesses (N=32) between December 2007 and June 2011 for measuring various laboratory parameters, including serum ferritin levels. In patients with KD, laboratory tests were performed at diagnosis and repeated at 2, 14, and 56 days after intravenous immunoglobulin treatment. At the time of diagnosis, serum ferritin levels in patients with KD (188.8 μg/L) were significantly higher than those in patients with other acute febrile illnesses (106.8 μg/L, P=0.003). The serum ferritin cut-off value of 120.8 μg/L effectively distinguished patients with KD from those with other acute febrile illnesses, with a sensitivity and specificity of 74.5% and 83.3%, respectively. Serum ferritin may be a useful biomarker to distinguish KD from other acute febrile illnesses.

Key Words: Kawasaki disease, Diagnosis, Ferritin, Biomarker

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Kawasaki disease (KD) is an acute febrile systemic vasculitis of unknown etiology [1, 2]. Among all patients diagnosed as having KD, 15%–20% showed incomplete presentation [2]. KD diagnosis depends on the use of adjuvant diagnostic markers, such as echocardiographic findings and laboratory biomarkers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), white blood cell count (WBC), hemoglobin, platelet count, and pyuria [2]. Although the laboratory findings observed in KD are not specific enough to conclusively establish a diagnosis, they can be useful, particularly in patients with a high suspicion for KD [2]. Tumor necrosis factor alpha (TNF-α), which is released from activated macrophages under certain inflammatory conditions, induces ferritin synthesis. A serum ferritin level ≥200 μg/L has been associated with an increased risk of myocardial infarction and, reportedly, plays an important role in inflammation [3-5]. Like in patients with systemic juvenile idiopathic arthritis, elevated serum ferritin levels have been observed in patients with KD [6-8]. Serum ferritin level is being considered a useful predictor of non-responsiveness to initial intravenous immunoglobulin (IVIG) therapy in KD and a useful biomarker to distinguish between KD and systemic juvenile idiopathic arthritis [7, 8]. However, the utility of serum ferritin levels as a diagnostic biomarker to distinguish between KD

and other acute febrile illnesses has not been investigated. We investigated this utility by measuring serum ferritin levels in pediatric patients with acute- and/or convalescent-phase KD.

All procedures involving human participants were carried out in accordance with the ethical standards of the Institutional and/or National research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Institutional Review Board of Chonnam National University Hospital, Gwangju, Korea, approved this study (protocol number: I-2009-09-103).

We enrolled 77 pediatric patients (age range, 3 months–6.9 years) who were admitted to Chonnam National University Hospital between December 2007 and June 2011 with a diagnosis of KD. Informed written consent was obtained from the parents of all patients. All patients with KD were administered IVIG at a dose of 2 g/kg, concomitantly with medium-dose aspirin (30–50 mg/kg), during the acute phase with fever. Among the 77 patients with KD who received the initial IVIG infusion, 11 (14.3%) received a second dose. At the time of admission, among the 77 patients with KD, 73 (94.8%), 69 (89.6%), 70 (90.9%), 55 (71.4%), and 45 (58.4%) showed bilateral bulbar conjunctival injection, polymorphous rash, oral mucosal changes, cervical adenopathy with lymph nodes measuring >1.5 cm in diameter, and changes in their extremities, respectively.

In addition, we enrolled 32 patients with other acute febrile diseases presenting with fever lasting more than three days. Exclusion criteria were the presence of hemophagocytic lymphohistiocytosis, systemic juvenile idiopathic arthritis, and viral infections, such as hepatitis C, avian influenza A (H5N1), dengue fever, and *Mycoplasma pneumoniae* pneumonia with increased serum ferritin levels [9-13].

We measured the complete blood cell count, ESR, CRP, total protein, albumin, electrolytes, blood urea nitrogen, creatinine, AST, ALT, creatine kinase (CK), CK-cardiac isoenzyme (CK-MB), myoglobin, troponin-I, N-terminal pro-brain natriuretic peptide (NT-proBNP), and ferritin in patients with KD at the time of diagnosis and in those with other acute febrile illnesses. Blood samples (5-8 mL) was analyzed within two hours after collection [14]. In addition, we measured serum ESR, CRP, NT-proBNP, and ferritin in these patients at 2, 14, and 56 days after IVIG treatment. A microparticle enzyme immunoassay (MEIA; AxSYM System, Abbot Diagnostics, IL, USA) was used to quantitatively assess serum ferritin levels.

Chi-square test was applied to assess the statistical significance of differences between independent variables and Student's *t*-test or Mann–Whitney U-test was used, depending on normality of the collected data, as indicated by the Shapiro–Wilk test, to assess differences between the patients with KD and those with other acute febrile illnesses. Only white blood cell count, hemoglobin, ESR, total protein, potassium, and chloride were normally distributed. The serial changes in CRP, ESR, NT-proBNP, and ferritin, at the time of diagnosis and at 2, 14, and 56 days after IVIG treatment were analyzed by Kruskal–Wallis test, followed by Bonferroni correction. We used receiver operating characteristic (ROC) curves to determine a cut-off value to distinguish between patients with KD and patients with other acute febrile illnesses. Continuous variables with normal distribution are expressed as the mean±standard deviation, while non-normal variables are expressed as median and range. $P<0.05$ was considered statistically significant. The SPSS software (version 20.0, IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) was used for all data analyses.

Table 1 shows the demographic features and the laboratory findings of the patients with KD and those with other acute febrile illnesses at the time of diagnosis. The clinical diagnoses for patients with other acute febrile illnesses (N=32) were as follows: viral pneumonia (N=18), acute tonsillitis (N=3), acute bronchiolitis (N=2), acute obstructive laryngitis (N=2), bacteremia (N=2), cervical lymphadenitis (N=1), acute gastroenteritis (N=1), bacterial keratoconjunctivitis (N=1), acute otitis media (N=1), and acute gastroenteritis (N=1) (Table 1). There were no significant sex differences.

Table 2 shows changes in CRP, ESR, NT-proBNP, and ferritin, at the time of diagnosis and at 2, 14, and 56 days after IVIG treatment. In patients with KD, the increased ferritin levels showed a gradual drop after IVIG treatment (Table 2). There was no significant sex-based difference in ferritin levels in patients with KD (male: 196.0±120.7 μg/L, vs. female: 197.0±88.1 μg/L, $P=0.601$).

Table 3 shows the diagnostic serum ferritin cut-off value in patients with KD and in those with other acute febrile illnesses. We also evaluated the platelet count, ESR, CRP, and NT-proBNP cut off values, which have been identified as potential diagnostic markers in previous reports (Table 3) [15-17]. The serum ferritin cut-off value of 120.8 μg/L effectively distinguished patients with KD from those with other acute febrile illnesses, with a sensitivity and specificity of 74.5% and 83.3%, respectively (AUC=0.830, 95% confidence interval: 0.704-0.955) (Table 3).

In our study, serum ferritin levels were significantly higher in patients with KD than in those with other acute febrile illnesses, indicating that the serum ferritin level can serve as a useful diagnostic biomarker for KD. We determined the optimal serum ferritin cut-off value to distinguish between KD and other acute

Table 1. Demographic features and laboratory findings in patients with KD and patients with other acute febrile illnesses at the time of diagnosis

	Patients with KD		Patients with other acute febrile illnesses		*P*
	N	Value	N	Value	
Age (yr), median (range)	77	1.9 (0.03–6.9)	32	2.1 (0.1–8.1)	0.670
Sex (male/female)	77	50/27	32	23/9	0.513
Total duration of fever (days), median (range)	77	6 (1–14)	32	6 (1–14)	0.337
Duration of fever before admission (days), median (range)	77	4 (1–13)	32	4 (1–8)	0.668
WBCs ($\times 10^9$/L), (mean ± SD)	76	14.5 ± 4.6	32	11.9 ± 5.8	0.014
Neutrophils (Proportion of 1.0), median (range)	76	0.66 (0.26–0.95)	32	0.66 (0.21–0.86)	0.336
Eosinophils (Proportion of 1.0), median (range)	76	0.02 (0.00–0.14)	32	0.01 (0.00–0.06)	**< 0.001**
Hemoglobin (g/L), (mean ± SD)	76	111 ± 12	32	116 ± 13	0.099
Platelets ($\times 10^9$/L), median (range)	76	349 (132–888)	32	259 (162–510)	**< 0.001**
Erythrocyte sedimentation rate (mm/hr), (mean ± SD)	48	74 ± 27	10	30 ± 20	**< 0.001**
CRP (mg/L), median (range)	77	84 (8–232)	32	45 (5–118)	**< 0.001**
AST (μkat/L), median (range)	74	0.53 (0.27–7.10)	32	0.58 (0.32–1.79)	0.373
ALT (μkat/L), median (range)	74	0.61 (0.08–9.32)	32	0.28 (0.13–2.57)	**0.003**
Total bilirubin (μmol/L), median (range)	36	12.0 (3.4–68.4)	9	10.6 (5.1–65.0)	0.425
Total protein (g/L), (mean ± SD)	70	65 ± 7	30	66 ± 5	0.217
Albumin (g/L), median (range)	72	37 (26–45)	31	40 (20–44)	0.050
Sodium (mmol/L), median (range)	73	136 (127–141)	31	137 (130–143)	0.064
Potassium (mmol/L), (mean ± SD)	73	4.4 ± 0.5	31	4.2 ± 0.5	0.075
Chloride (mmol/L), (mean ± SD)	73	101.0 ± 12.3	31	103.9 ± 3.2	**0.017**
Creatine kinase (μkat/L), median (range)	50	0.8 (0.2–11.2)	19	1.4 (0.7–4.2)	0.053
Creatine kinase-MB (μg/L), median (range)	64	14.2 (2.2–35.9)	28	15.0 (4.0–33.6)	0.391
NT-proBNP (ng/L), median (range)	75	788 (56–3,500)	20	204 (21–2,069)	**< 0.001**
Ferritin (μg/L), median (range)	77	188.8 (25.5–750.5)	32	106.8 (11.1–632.2)	**0.003**

The statistical significance of differences between males and females was assessed by the Chi-square test. White blood cell count, hemoglobin, ESR, total protein, potassium, and chloride were analyzed using Student's *t*-test. Results of the other laboratory tests were analyzed using the Mann–Whitney U-test.
Abbreviations: KD, Kawasaki disease; WBC, white blood cell count; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; creatine kinase-MB, cardiac isoenzyme of creatine kinase; NT-proBNP, N-terminal pro-brain natriuretic peptide; SD, standard deviation.

Table 2. Comparison of laboratory findings between patients with KD at the time of diagnosis and at 2, 14, and 56 days after IVIG treatment and patients with other acute febrile illnesses

Laboratory Findings	Patients with KD								Patients with other acute febrile illnesses	
	At the time of diagnosis		2 days after treatment		14 days after treatment		56 days after treatment			
	N	Value	N	Value	N	Value	N	Value	N	Value
CRP (mg/L), median (range)	77	84 (8–232)	77	22 (2–164)	74	2 (0–31)	65	0 (0–1)	32	45 (5–118)
ESR (mm/hr), (mean ± SD)	48	74 ± 27	61	78 ± 30	74	44 ± 27	65	11 ± 12	10	30 ± 20
NT-proBNP (ng/L), median (range)	75	788 (56–3,500)	76	569 (14–2,3367)	72	91 (16–648)	7	133 (22–348)	20	204 (21–2,069)
Ferritin (μg/L), median (range)	77	188.8 (25.5–750.5	71	159.5 (4.8–483.0)	68	78.2 (12.4–271.1)	14	26.1 (11.0–133.5)	32	106.8 (11.1–632.2)

Abbreviations: KD, Kawasaki disease; IVIG, intravenous immunoglobulin; CRP, C-reactive protein, ESR, erythrocyte sedimentation rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; SD, standard deviation.

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Table 3. Comparison of diagnostic serum ferritin, platelet count, ESR, CRP, and NT-proBNP cut-off values between patients with KD and those with other acute febrile illnesses

Diagnostic marker	Cut-off value	Area under curve	95% Confidence Interval	Sensitivity (%)	Specificity (%)	*P*
Ferritin (μg/L)	120.8	0.830	0.704–0.955	74.5	83.3	0.009
Platelets ($\times 10^9$/L)	279.5	0.750	0.526–0.974	76.6	83.3	0.048
ESR (mm/hr)	37	0.897	0.741–1.000	93.6	83.3	0.002
CRP (mg/L)	44	0.812	0.013–0.988	78.7	83.3	0.013
NT-proBNP (ng/L)	571.3	0.848	0.711–0.984	66.0	83.3	0.006

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; KD, Kawasaki disease. Receiver operating characteristic (ROC) curves were used to determine a cut-off value to distinguish between patients with KD and patients with other acute febrile illnesses.

febrile illnesses to be 120.8 μg/L.

Ferritin is an acute-phase reactant that is utilized in clinical practice as a serum biomarker [3]. Ferritin synthesis is markedly induced by TNF-α and IL-1α; thus, serum ferritin levels are elevated in patients with certain inflammatory conditions [7]. Elevated serum ferritin levels have also been implicated in the development of diabetic microvascular disease through interaction with vascular endothelial growth factor (VEGF), which is involved in the pathogenesis of vasculitis [7, 18]. VEGF is also associated with the formation of coronary artery lesions (CALs) in patients with KD; however, an association between VEGF and the serum ferritin level in KD has not been established [7, 19].

While the serum ferritin level is elevated in patients with KD, markedly elevated serum ferritin level is not a common feature in KD, although it has been observed in KD patients with concomitant hemophagocytic lymphohistiocytosis and in adult patients with KD [3, 9, 20]. Nasir, *et al.* [9] reported three neonates who presented with clinical features suggestive of KD, with high serum ferritin levels. In their series, the patients demonstrated prompt and complete resolution of symptoms within 48 hours of IVIG treatment, without recurrence or CALs at the six-month follow-up [9]. They suggested that children and neonates with KD might have high serum ferritin levels and that serum ferritin could be a useful diagnostic biomarker for KD [9]. Mizuta, *et al.* [8] reported higher mean ferritin levels in patients with systemic juvenile idiopathic arthritis (1,189 μg/L [range, 63–68,310]) than in patients with KD (147.5 μg/L [range, 14–2,376]).

In our study, WBC, CRP, and NT-proBNP levels were also significantly higher in patients with KD than in patients with other acute febrile illnesses, which was in line with previous studies [2, 15-17].

There were some limitations in our study. (i) Some data pertaining to patient follow-up were missing, perhaps because parents refused to permit blood draws as their children began to recover and appear healthy. (ii) The patients with acute febrile illnesses other than KD had heterogeneous febrile illnesses, which may have affected the measurement of serum ferritin levels owing to differences in inflammation severity. (iii) The number of patients with acute febrile illnesses other than KD was significantly smaller than that of patients with KD. Therefore, large prospective investigations are required for obtaining a more accurate cutoff for ferritin as a diagnostic biomarker.

In conclusion, this was the first study to compare serial serum ferritin levels between patients with KD and those with other acute febrile illnesses. The serum ferritin level may be a useful biomarker to distinguish KD from other acute febrile illnesses.

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AUTHOR CONTRIBUTIONS

Kim SH, Song ES, Kang G, and Cho YK made substantial contributions to study planning and design and writing of the manuscript; Yoon S and Eom GH performed data collection, statistical analysis, and provided valuable feedback. Kim SH and Song ES contributed equally to all aspects of this paper. All authors approved the final manuscript submitted and agreed to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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