Elevated Serum Free Carnitine Levels in Children with Kawasaki Disease and Their Relation to Unresponsiveness to Intravenous Immunoglobulin: retrospective study

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Keywords: Kawasaki disease, Carnitine, IVIG

DOI: https://doi.org/10.21203/rs.3.rs-29790/v1

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Abstract

Background

Carnitine plays an essential role in transfer of long-chain fatty acids to mitochondria for subsequent β-oxidation. No studies to date have characterized carnitine in children with KD. The objective of this study is to investigate the characteristics of serum free carnitine (FC) in hospitalized pediatric patients with Kawasaki disease (KD).

Methods

In total, 45 patients with KD measured the levels of serum FC from October 2018 to December 2019 were analyzed retrospectively. We analyzed the clinical and laboratory parameters just before the Intravenous immunoglobulin (IVIG) including serum levels of serum FC with respect to the IVIG response.

Results

The median age was 33 months. IVIG was effective in 33 children (responders) and was ineffective in 12 (non-responders). The serum FC levels were higher in non-responders than in responders [(35.3 µmol/L (range, 26.8-118.4 µmol/L) vs. 31.4 µmol/L (range, 20.9–81.2 µmol/L), p value = 0.0496]. The FC levels before intravenous immunoglobulin (IVIG) in four-fifths of responders were below the normal range. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and FC were higher in non-responders than in responders. There was a correlation between FC and AST (R2 = 0.364, P = 0.0015) and between FC and ALT (R2 = 0.423, P < 0.001) levels.

Conclusion

FC levels were upregulated in patients with KD who were refractory to IVIG. Additionally, FC levels in children with KD correlated with AST and ALT levels. The pathogenesis resulting in the elevation of FC levels remains elusive. Further studies are necessary to understand more precisely carnitine properties in patients with KD.

Background

Kawasaki disease (KD) is an acute systemic vasculitis that occurs mainly in infants. Coronary artery lesions (CAL) are a serious complication of KD, and thus, its prevention is important to improve prognostic outcomes in children with KD. Intravenous immunoglobulin (IVIG) has been known to be effective in abolishing vascular inflammations that could lead to CAL. In patients at high risks of unresponsiveness to IVIG, steroid combination therapy with IVIG is used to avoid CAL. In previous studies, several factors have been suggested as a biomarker that could predict unresponsiveness to IVIG, such as...
neutrophil counts, C-reactive protein (CRP) and procalcitonin levels [1, 2, 3, 4, 5, 6]. In Japan, Gunma, Kurume, and Osaka scores are commonly used to predict unresponsiveness to IVIG [7, 8, 9]. In these scoring systems, platelet (PLT) counts, serum sodium (Na), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), and CRP are included as parameters.

Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is a hydrophilic quaternary amine that plays an essential role in energy metabolism. The main function of carnitine is the transfer of long-chain fatty acids to mitochondria for subsequent β-oxidation [10]. Carnitine homeostasis reflects the balance among absorption from the diet, endogenous biosynthesis, and efficient renal reabsorption [11]. Our preliminary review of carnitine data (unpublished data) showed that several patients with KD had high levels of serum free carnitine (FC) and some of these patients were unresponsive to IVIG.

No studies to date have characterized carnitine in children with KD. Here, we conducted a retrospective analysis of serum FC levels to understand properties of carnitine in children with KD.

**Methods**

In this retrospective study, we collected children with KD who were hospitalized in the Department of Pediatrics of Aichi Medical University Hospital from October 2018 to December 2019. This study was approved by the ethics committee of Aichi Medical University Hospital (Approval No. 2015-H830). The diagnosis of KD was performed based on the Japanese diagnostic guidelines for KD [12]. A complete KD diagnosis reflects that a child had five or more of the six major symptoms, while an incomplete KD diagnosis reflects that a child had four or fewer major symptoms after exclusion of other illnesses with similar clinical features, such as viral and bacterial infections, cervical lymphadenitis, and toxic shock syndrome. In our hospital, children with KD were treated with IVIG and oral administration of aspirin at a dose of 30–50 mg/kg/day immediately after the diagnosis. If patients had liver dysfunction, aspirin was substituted by 3–5 mg/kg dose of oral flurbiprofen. Steroids were used for patients expected to be unresponsive to IVIG based on prediction scores, such as Gunma [7], Kurume [8], and Osaka scores [9]. Steroids were usually administered if two or more of the above scores indicated unresponsiveness to IVIG and based on the recommendation of the attending pediatricians. In this study, responders to IVIG were defined as those children who did not have pyrexia post IVIG. Non-responders to IVIG were defined as those children who had persisting or recurrent pyrexia for more than 24 hours post IVIG, thereby necessitating a second line of treatment. In this study, patients who needed steroid combination therapy were included as non-responders.

We recruited the following laboratory data before IVIG: white blood cell (WBC) count, neutrophil count, hemoglobin (Hb), PLT count, T-Bil, albumin, AST, ALT, Na, CRP, D-dimer, brain natriuretic peptide, and FC levels. Some of these factors were previously reported to be useful in predicting unresponsiveness to IVIG [1–9] and can be always measured in most hospitals. According to the 2018 Japanese guideline for the diagnosis and treatment of carnitine deficiency [13], very low, low, normal, and high FC were defined as serum FC < 20, 20 ≤ FC < 36, 36 ≤ FC ≤ 74, and FC > 74 µmol/L, respectively. Additional information
collected from medical records includes age, gender, history of taking pivalate-conjugated antibiotics within 2 weeks before hospitalization, dates of illness prior to administration of IVIG, duration of hospitalization, Ratio of diagnosis of complete KD and incomplete KD, and coronary lesions after discharge.

We used Fisher’s exact and Mann–Whitney U tests to compare categorical and numerical variables, respectively, between responders and non-responders. The rates of low, normal, and high FC between responders and non-responders were compared using the chi-square test (2×3 contingency table). The association between FC levels and other laboratory data was analyzed with Pearson's correlation test. A Poisson (p) value < 0.05 was considered statistically significant. All statistical analyses were performed with the XLSTAT (ver. 2019.1.3, Okayama, Japan) software.

Results

In total, 76 children with KD were hospitalized during the study period. We excluded 29 children in whom FC levels were not measured and 2 children who did not undergo IVIG. Following these exclusions, 45 patients were further analyzed in this study.

The demographic data of these patients are shown in Table 1. The median age was 33 months, while 20 and 25 patients were male and female, respectively. The median day of illness at the initiation of IVIG was 5 (range, 3–12 days). Ten patients were diagnosed as incomplete KD, while CAL was not observed in any patient during this study period. IVIG was effective in 33 patients (responders) and ineffective in 12 patients (non-responders). The age, gender, number of incomplete KD and usage of pivalate-conjugated antibiotics within 2 weeks before hospitalization were not different between responders and non-responders. IVIG was started earlier and the duration of hospitalization was longer in non-responders than in responders. Low FC was observed in 26 responders (79%) and 7 non-responders (59%), whereas high FC was observed in 2 responders (6%) and 3 non-responders (25%), although no significant difference between these FC levels was observed.

The relationship between laboratory data before IVIG and responsiveness to IVIG is shown in Table 2. T-Bil, AST, ALT, and FC levels were higher in non-responders than in responders. The median serum FC level was 31.4 µmol/L (range, 20.9–81.2 µmol/L) and 35.3 µmol/L (range, 26.8-118.4 µmol/L) in responders and non-responders, respectively (p = 0.0496).

We also analyzed the predictive value of FC levels that could correspond to unresponsiveness to IVIG. The area under the receiver-operating characteristic curve (ROC) or AUC of the FC level was 0.69 (95% confidence interval, 0.51–0.87) (Fig. 1). After applying the cut-off value (34.7 µmol/L) to the FC level, the sensitivity, specificity, positive predictive value, and negative predictive value were 0.667, 0.758, 0.500, and 0.862, respectively.

The correlation between the FC level and other variables is shown in Fig. 2. There was a correlation between the FC level and AST (R² = 0.364, P = 0.0015) and between the FC level and ALT (R² = 0.423, P <
0.001). However, no significant correlation was observed between FC and T-Bil levels.

**Discussion**

Our study revealed unique characteristics of carnitine in patients with KD. FC levels in the majority of responders were low before IVIG. Additionally, FC levels before IVIG were higher in non-responders than in responders. The high FC level correlated with unresponsiveness to IVIG; however, its predictive value was considered to be insufficient. Moreover, FC levels correlated with AST and ALT, but not T-Bil levels.

An elevated level of FC represents an exceptional pathological state, such as acute renal failure [14] and liver cirrhosis [15]. In contrast, various congenital and acquired conditions are known to cause carnitine deficiency [16]. Thus, it is remarkable that high FC was not prevalent in children with KD. Currently, the pathogenesis resulting in the elevation of FC levels in children with KD remains elusive. Carnitine is primarily stored in skeletal muscle, liver, myocardium, and brain. As such, injuries to these organs could lead to carnitine leakage into the bloodstream. We hypothesize that liver injury is likely to result in an elevation of FC levels in children with KD, because FC levels in our study closely correlated with AST and ALT levels, which are well known markers associated with liver injury. This hypothesis should be evaluated by further clinical and experimental studies.

It is interesting that FC levels in children with KD were related to IVIG unresponsiveness, although its predictive value may be insufficient. In this study, not only FC levels but also T-Bil, AST, and ALT level were associated with unresponsiveness to IVIG. Previous studies have shown that these values were higher in non-responders than in responders [17, 18, 19]. It is remarkable that all these factors have also been incorporated into the existing refractory prediction scores, such as Osaka, Kurume, and Gunma scores [7, 8, 9]. As mentioned above, FC levels closely correlated with AST and ALT levels. Thus, the correlation between FC and IVIG unresponsiveness indicates that an elevation of FC levels in children with KD could be a reflection of pathogenesis that could lead to IVIG unresponsiveness. IVIG unresponsiveness in children with KD has been presumed to correlate with the severity of inflammation [20, 21]. Proinflammatory cytokines such as interleukin (IL)-1 and IL-6 have been known to be related to IVIG unresponsiveness. Fury et al. reported transcript abundance of IL-1 pathway genes and MMP-8 in patients with IVIG resistant KD patients [22]. This suggests that IL-1 pathway activation could be related to IVIG unresponsiveness. IL-6 is also associated with various biological functions, such as an increase in acute-phase proteins, T cell activation, procoagulant effects and thrombocytosis, which could lead to resulting in IVIG unresponsiveness [23, 24, 25]. An elevation of FC levels as well as AST, ALT, and T-Bil levels could be attributable to tissue damage that resulted from inflammation induced by proinflammatory cytokines. Regarding the predictive value of FC levels, the sensitivity and specificity were 0.794 and 0.583, respectively, and the AUC was 0.69. While FC levels alone were not accurate enough to predict unresponsiveness to IVIG, these levels in combination with other variables such as AST, ALT, and T-Bil, could become as useful as the Gunma, Kurume, and Osaka scores.
It is noteworthy that FC levels were below the normal range in the majority of responders in this study. This could be attributable to an increased carnitine requirement caused by systemic inflammation in children with KD. It is well known that secondary carnitine deficiency could result from decreased body storage and increased requirements in patients with sepsis [26, 27]. This suggests that patients with systemic inflammation, including KD, will be at a risk of secondary carnitine deficiency. In this study, the magnitude of lower FC levels was not severe in children with KD. There were no patients with carnitine deficiency (FC levels below 20 µmol/L), or symptoms related to low FC levels such as hypoglycemia. We consider that carnitine supplementation is unnecessary in patients with KD.

This study has some limitations. First, this is a single center retrospective study with a small number of patients. Therefore, the results of this study should further be verified in other cohorts of patients. Second, serum FC levels were not measured in all children with KD during the study period. As such, prospective studies are necessary to identify carnitine properties in patients with KD more accurately. Finally, we did not examine other clinical data such as triglyceride and ammonia, which could be affected by FC levels. Thus, further studies on these clinical variables are necessary to elucidate the impact of FC levels in children with KD.

Conclusions

In conclusion, FC levels were higher in some children with KD. While FC levels were higher in non-responders than in responders, the correlation between FC levels and IVIG unresponsiveness was not significant. Additionally, FC levels in children with KD correlated with AST and ALT levels. Currently, the pathogenesis resulting in the elevation of FC levels in children with KD remains elusive. Further studies are necessary to understand more precisely carnitine properties in patients with KD.

List Of Abbreviations

Kawasaki disease (KD)
Coronary artery lesions (CAL)
Intravenous immunoglobulin (IVIG)
C-reactive protein (CRP)
platelet (PLT)
sodium (Na)
aspartate aminotransferase (AST)
alanine aminotransferase (ALT)

total bilirubin (T-Bil)

free carnitine (FC)

white blood cell (WBC)

hemoglobin (Hb)

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the ethics committee of Aichi Medical University Hospital (Approval No. 2015-H830). Waiver of informed consent was also approved, because we retrospectively analyzed the existing data with no identifiable private information and notification with opt-out was shown in the hospital.

**Consent for publication**

Not applicable

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

There was no funding about this work.

**Author's contributions**

TM and AO conceived of the study, collected data, performed the analysis, and drafted the manuscript. TM, NN, YM, SN, SK, TH, RM, KM, HM, YK, MA, HI, HK YS, TN, TH, HA and AO collected data and assisted in interpretation of data. All the authors of the study gave final approval for the published manuscript and agree to be accountable for all aspects of this work.

**References**

1. Rahbari-Manesh AA, Salamati P, Ghaforian S, Zekavat M. Relationship between ESR, CRP, platelet count and coronary artery disease in Kawasaki disease. Iran J Pediatr. 2005;15:139–44.

2. Huang M-Y, Gupta-Malhotra M, Huang JJ, Syu FK, Huang TY. Acute-phase reactants and a supplemental diagnostic aid for Kawasaki disease. Pediatr Cardiol. 2010;31:1209–13.
3. Koyanagi H, Yanagawa H, Nakamura Y, Yashiro M. Leukocyte counts in patients with Kawasaki disease: from the results of nationwide surveys of Kawasaki disease in Japan. Acta Paediatr. 1997;86:1328–32.

4. Dominguez SR, Martin B, Heizer H, Jone PN, Tong S, Davidson J, et al. Procalcitonin (PCT) and Kawasaki disease: does PCT correlate with IVIG resistant disease, admission to the intensive care unit, or development of coronary artery lesions? J Pediatr Infect Dis Soc. 2016;5:297–302.

5. Kuo HC, Liang CD, Wang CL, Hwang KP, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. Acta Paediatr. 2010;99:1578–83.

6. Lee NH, Choi HJ, Kim YH. Clinical usefulness of serum procalcitonin level in distinguishing between Kawasaki disease and other infections in febrile children. Korean J Pediatr. 2017;60:112–7.

7. 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;6:2606–12.

8. Egami K, Muta H, Ishii M, Suda K, Sugahara Y, Iemura M, et al. Prediction of resistance to intravenous immunoglobulin treatment in patients with Kawasaki disease. J Pediatr. 2006;149:237–40.

9. Sano T, Kurotobi S, Matsuzaki K, Yamamoto T, Maki I, Miki K, et al. Prediction of non-responsiveness to standard high-dose gamma-globulin therapy in patients with acute Kawasaki disease before starting initial treatment. Eur J Pediatr. 2007;166:131–7.

10. Roe C, Ding J. Mitochondrial fatty acid oxidation disorders. In: Scriver C, Beaudet A, Sly W, Valle D, editors. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill; 2001. pp. 2297–326.

11. Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. Ann N Y Acad Sci. 2004;1033:30–41.

12. Research Committee of the Japanese Society of Pediatric Cardiology; Cardiac Surgery Committee for Development of Guidelines for Medical Treatment of Acute Kawasaki Disease. Guidelines for medical treatment of acute Kawasaki disease: report of the Research Committee of the Japanese Society of Pediatric Cardiology and Cardiac Surgery (2012 revised version). Pediatr Int. 2014;56:135–58.

13. ‘Diagnosis and treatment guidelines for carnitine deficiency 2018’ revised working group of the Japanese Society of Pediatrics. The 2018 Japanese guideline for the diagnosis and treatment of carnitine deficiency. 2018. http://www.jpeds.or.jp/uploads/files/20181207_shishin.pdf.

14. Wanner C, Riegel W, Schaefer RM, Hör I. Carnitine and carnitine esters in acute renal failure. Nephrol Dial Transplant. 1989;4:951–6.

15. Amodio P, Angeli P, Merkel C, Menon F, Gatta A. Plasma carnitine levels in liver cirrhosis: relationship with nutritional status and liver damage. J Clin Chem Clin Biochem. 1990;28:619–26.

16. Magoulas PL, El-Hattab AW. Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. Orphanet J Rare Dis. 2012;18:68.

17. Eladawy M, Dominguez SR, Anderson MS, Glodé MP. Abnormal Liver Panel in Acute Kawasaki Disease. Pediatr Infect Dis J. 2011;30:141–4.
18. Tomita Y, Fukaya T, Yamaura Y, Tsujiguchi R, Muratani H, Shimaya M. Implications of hepatic dysfunction in Kawasaki disease: Time-related changes in aspartate aminotransferase, alanine aminotransferase, total bilirubin, and C-reactive protein levels. Pediatr Invest. 2019;3:19–26.

19. Tan XH, Zhang XW, Wang XY, He XQ, Fan C, Lyu TW, et al. A new model for predicting intravenous immunoglobulin-resistant Kawasaki disease in Chongqing: a retrospective study on 5277 patients. Sci Rep. 2019;9:1722.

20. Li X, Chen Y, Tang Y, Ding Y, Xu Q, Sun L, et al. Predictors of intravenous immunoglobulin-resistant Kawasaki disease in children: a meta-analysis of 4442 cases. Eur J Pediatr. 2018;177:1279–92.

21. Xie T, Wang Y, Fu S, Wang W, Xie C, Zhang Y, Gong F. Predictors for intravenous immunoglobulin resistance and coronary artery lesions in Kawasaki disease. Pediatr Rheumatol Online J. 2017;15:17.

22. Fury W, Tremoulet AH, Watson VE, Best BM, Shimizu C, Hamilton J, et al. Transcript abundance patterns in Kawasaki disease patients with intravenous immunoglobulin resistance. Hum Immunol. 2010;71:865–73.

23. Stringer E, Yeung RSM. Pathogenesis of Kawasaki disease: the central role of TNF-a. Futur Rheumatol. 2008;3:69–77.

24. Hoffman HM, Rosengren S, Boyle DL, Cho JY, Nayar J, Mueller JL, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. Lancet. 2004;364:1779–85.

25. Wu Y, Liu FF, Xu Y, Wang JJ, Samadli S, Wu YF, et al. Interleukin-6 is prone to be a candidate biomarker for predicting incomplete and IVIG nonresponsive Kawasaki disease rather than coronary artery aneurysm. Clin Exp Med. 2019;19:173–81.

26. Lheureux PE, Penaloza A, Zahir S, Gris M. Carnitine in the treatment of valproic acid-induced toxicity - what is the evidence? Crit Care. 2005;9:431–40.

27. Carlsson M, Montan P, Sundqvist K. L-carnitine enhances lymphocyte mitogenesis in depleted traumatised and infected patients. Clin Nutr. 1987;6:39–44.

Tables

Table 1. Patients characteristics
### Table 2. The relation between laboratory data before IVIG and responsiveness to IVIG.

|                              | Responders (N = 33) | Non-responders (N = 12) | P value |
|------------------------------|---------------------|-------------------------|---------|
| Age (months)                 | 31 (6-84)           | 25 (9-54)               | 0.8931  |
| Gender (M:F)                 | 15:18               | 5:7                     | 1.00    |
| PCA within 2 weeks           | 6 (18.8%)           | 2 (16.7%)               | 0.4481  |
| Incomplete KD                | 8 (24.2%)           | 2 (16.7%)               | 0.7054  |
| Days of illness at IVIG      | 5 (3-12)            | 4 (4-10)                | 0.3218  |
| Duration of hospitalization (days) | 8 (5-10)       | 10 (7-15)               | < 0.001 |
| Coronary artery lesion       | 0                   | 0                       | -       |
| Free carnitibe level         |                     |                         |         |
| Low (20 ≤ < 36 mmol/L)       | 26                  | 7                       | 0.154   |
| Normal (36 ≤ ≤ 74 mmol/L)    | 5                   | 2                       |         |
| High (> 74 mmol/L)           | 2                   | 3                       |         |

PCA: pivalate-conjugated antibiotics, KD: Kawasaki disease, IVIG: intravenous immunoglobulin

IVIG: intravenous immunoglobulin, WBC: white blood cell counts, Hb: hemoglobin, T-Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BNP: brain natriuretic peptide, CRP: C-reactive protein, FC: free carnitine
Figure 1

Receiver operating characteristic (ROC) curves comparing serum free carnitine with other predictive scores in predicting unresponsiveness to IVIG. AUC: area under the ROC.
Figure 2

Correlation between serum free calnitine (FC) and other examination values. The decision coefficient (R2) and P value analyzed with Pearson’s correlation test are shown only for correlated data. WBC: white blood cell, Hb: hemoglobin, PLT: platelet, T-Bil: total bilirubin, ALB: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, Na: serum sodium, BNP: brain natriuretic peptide, CRP: C-reactive protein.