

Prediction of the risk of coronary arterial lesions in Kawasaki disease by serum 25-hydroxyvitamin D₃

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Received: 10 January 2014 / Revised: 15 May 2014 / Accepted: 23 May 2014 / Published online: 3 June 2014
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Abstract Kawasaki disease (KD) is associated with the development of coronary arterial lesions (CALs) in children. We aimed to test the hypothesis that circulating 25-hydroxyvitamin D₃ [25-(OH)D₃] could be identified as a clinical parameter for predicting CALs secondary to KD in children. We enrolled 35 children with KD in the acute phase and measured serum 25-(OH)D₃ levels in all of them, then followed up by echocardiography for CALs. Additionally, serum 25-(OH)D₃ levels were obtained in 23 febrile children with respiratory tract infections and 30 healthy children. Of the 35 KD children, nine had CALs according to echocardiography and 26 did not (NCALs). Serum 25-(OH)D₃ levels were not significantly different between NCALs and healthy children (49.2±23.8 versus 44.1±30.2 ng/ml; $P=0.49$). Serum 25-(OH)D₃ levels were significantly higher in children with CALs than those without CALs (83.9±26.3 versus 49.2±23.8 ng/ml; $P=0.001$). The cutoff value of 65 ng/ml to predict subsequent CALs had a specificity of 0.73, sensitivity of 0.78, and diagnostic accuracy of 0.74. **Conclusion:** Serum 25-(OH)D₃ levels were elevated during the acute phase in KD children who had subsequent CALs. Serum 25-(OH)D₃ levels in the acute phase of KD may be used to predict subsequent CALs.

Keywords Kawasaki disease · 25-Hydroxyvitamin D₃ · Coronary artery lesions

Abbreviations

CALs Coronary arterial lesions
KD Kawasaki disease

Introduction

Kawasaki disease (KD), first described in 1967 by Dr. Tomisaku Kawasaki as mucocutaneous lymph node syndrome [7], is an acute systemic vasculitis that primarily affects small- and medium-sized arteries. KD that predominantly affects children younger than 5 years is mostly a self-limiting disease, but sometimes gives rise to serious cardiac complications. Coronary arterial lesions (CALs), including dilatations and aneurysms, are identified as the most serious complications of KD. In China, approximately 8.5 to 20.6 % of KD patients develop CALs [4, 11, 12, 16, 28]. Even after high-dose immunoglobulin therapy, 5–10 % of KD children develop CALs [14]. CALs secondary to KD are associated with changes in certain biomarkers, such as platelet count, neutrophil count, plateletcrit, platelet distribution width, mean platelet volume, erythrocyte sedimentation rate, cardiac troponin I, endothelin-1, albumin, and hemoglobin [3]. In a retrospective study [2] involving 113 Chinese children, Kobayashi scoring system was applied to predict coronary arterial lesions in KD, and the results showed that the sensitivity of the Kobayashi scoring system was 0.56. Though the Kobayashi scoring system had the ability to predict the coronary arterial lesions in KD patients, the power was not high enough. Therefore, a laboratory marker in the initial acute

Communicated by David Nadal

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stage of KD that could predict the development of subsequent CALs is very much desired.

The exact mechanisms underlying the development of CALs secondary to KD remain poorly understood. Several markers of immune activation are increased in KD patients, suggesting their involvement in the pathogenesis [8, 19, 27]. Hyperactive immune cells, especially T cells, with excessive cytokines may be responsible for tissue injury in KD [10]. Either dietary vitamin D or that produced in the skin is transported to the liver for conversion to 25-hydroxyvitamin D₃ [25-(OH)D₃]. Circulating 25-(OH)D₃ is the precursor of the active 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃). Almost every tissue in the human body carries receptors for 1,25-(OH)₂D₃ (vitamin D receptor, VDR), which affects epithelial cell, T cell, B cell, and dendritic cell functions. In addition, VDR has been identified in the immune system [1, 5, 23, 25]. Adjunctive 1- α , 25-dihydroxyvitamin D₃ therapy exhibiting anti-inflammatory and immunomodulatory effects during KD vasculitis have been reported [9, 20]. The expression of VDR in T cells was higher in KD patients than febrile children with respiratory tract infections and healthy children [17]. Over activated T cells might promote 25-(OH)D₃ release. Therefore, more intense inflammatory response in CALs' patients may have increased VDR expression and subsequently elevated 25-(OH)D₃ levels. In addition, vitamin D deficiency as an independent risk factor for arterial disease has been reported [22].

Taken together, we hypothesized that CALs induce excessive vascular calcification; calcifications would stimulate CYP27B1 enzyme expression and in turn increase serum 25-(OH)D₃ levels. The purpose of this study was to investigate the clinical value of serum 25-(OH) D₃ levels as a predictor of CALs in children with KD.

Material and methods

Study population

We enrolled 35 children diagnosed with KD [15] between March 2011 and July 2013 at the Affiliated Hospital of Xi'an Medical College. All the children received intravenous immune globulin treatment. Once Kawasaki disease is diagnosed, immunoglobulin therapy should begin within 24 h. Echocardiography was used to detect the presence of CALs during the course of the disease. Of the 35 children diagnosed with KD, nine (six boys and three girls) developed CALs based on echocardiographic results and 26 (19 boys and 7 girls) did not develop CALs (NCALs). In addition, 23 children with respiratory tract infections were included as febrile controls. These children did not have cutaneous eruption or myocardial injury and were excluded if they had abnormal myocardial enzyme levels or an abnormal electrocardiogram. Furthermore, 30 healthy children

were selected as normal controls. Children with heart, lung, or kidney disease were excluded. The study was approved by the Ethics Committee of the Affiliated Hospital of Xi'an Medical College. Parental informed consent was obtained for each child enrolled in this study. There were no significant differences between the groups regarding baseline characteristics.

Measurement of serum 25-(OH)D₃ levels

Fasting blood samples (approximately 3 ml) were collected for each patient between 5 and 10 days of disease onset. The samples were centrifuged at 3,000 rpm/min for 10 min and stored at -70°C until analysis. Serum 25-(OH)D₃ concentrations were measured using a commercially available ELISA kit (Shanghai XinLe Biological Technology Co., Ltd.) according to the manufacturer's instructions. The same method was used to measure 25-(OH) D₃ levels in febrile children and healthy control children.

Statistical analysis

Continuous data were expressed as mean \pm standard deviation (SD) if the values were normally distributed. Statistical comparisons of continuous variables were performed by a two-tailed Student's *t* test. A χ^2 test was used to compare categorical variables. $P < 0.05$ was considered statistically significant. All analyses were performed with SPSS V.17.0 (SPSS Inc, Chicago, Illinois, USA). To compare the power of serum level of 25-(OH)D₃ levels in predicting CALs, receiver-operating characteristic curves (ROCs) were plotted and areas under the curve (AUCs) were calculated.

Results

The KD cases included 26 boys and 9 girls with an age range of 4 months to 4 years. Six boys and three girls developed CALs. The febrile children group included 11 boys and 12 girls with an age range of 5 months to 4 years. The normal controls consisted of 16 boys and 14 girls with an age range of 5 months to 4 years.

Comparison of 25-(OH)D₃ levels

The mean serum 25-(OH)D₃ levels in febrile children was significantly lower than healthy children (27.9 ± 20.6 versus 44.1 ± 30.2 ng/ml; $P = 0.025$). This level was not significantly different between NCALs and healthy children (49.2 ± 23.8 versus 44.1 ± 30.2 ng/ml; $P = 0.49$) (Table 1). In addition, the mean serum 25-(OH)D₃ levels were significantly higher in children with CALs than those without CALs (83.9 ± 26.3 versus 49.2 ± 23.8 ng/ml; $P = 0.001$).

Table 1 Comparison of serum 25-(OH)D₃ levels between different groups

Group	Number	25-(OH)D ₃ (ng/ml)
Febrile children with respiratory tract infections	23	27.9±20.2
Healthy control	30	44.1±30.2 ^a
NCALs	26	49.2±23.8 ^b
CALs	9	83.9±26.3 ^c

Values were expressed as mean±SD

CALs coronary arterial lesions, NCALs no coronary arterial lesions

^a Comparing with febrile children with respiratory tract infection group: $t=2.31$ $P=0.025$

^b Comparing with healthy control group: $t=1.16$, $P=0.49$

^c Comparing with NCALs group: $t=3.68$, $P=0.001$

ROCs

To assess the performance of serum level of 25-(OH)D₃ in predicting CALs, ROCs were plotted and the AUCs was calculated. As shown in Fig. 1, the AUC for predicting CALs of 25-(OH)D₃ was 0.831. When the cutoff value of 65 ng/ml for 25-(OH)D₃ based on the ROC was obtained for predicting CALs, it generated a sensitivity of 78 % and specificity of 73 %. Detailed characteristics of cutoff value of 65 ng/ml for CAL prediction were listed in Table 2.

Discussion

The current study showed that serum levels of 25(OH)D₃ were significantly higher in KD children who developed CALs compared with children who did not develop CALs. Higher serum 25-(OH)D₃ levels in the acute phase of KD was associated with an increased the risk of developing CALs. A cutoff value of 65 ng/ml had a specificity of 0.73 and sensitivity of 0.78 for predicting CALs. These results indicate that 25-(OH)D₃ has a potential role in the pathogenesis of CALs in children with KD.

Early identification of the risk of CALs in KD is very important to formulate a therapeutic strategy. A well-designed meta-analysis [3] summarized 16 biomarkers associated with CALs in Chinese KD children. The results indicated that the biomarkers, including platelet count, neutrophil count, plateletcrit, platelet distribution width, mean platelet volume, erythrocyte sedimentation rate, cardiac troponin I, endothelin-1, albumin, and hemoglobin, were associated with developing CALs among Chinese KD children. However, no generally accepted specific biomarkers for predicting CALs in clinical practice have been established. In addition, serum NT pro-B-type natriuretic peptide above 1,000 pg/ml [6] or serum

ROC Curve

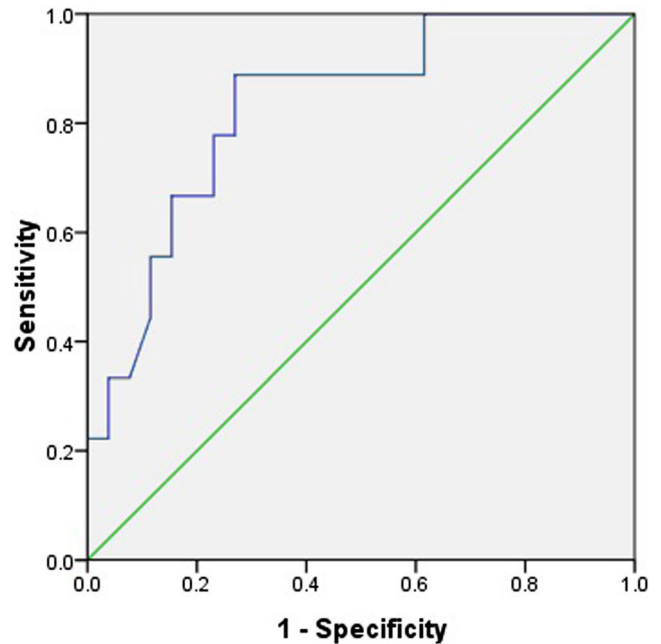


Fig. 1 Predictive performance of the serum 25-(OH)D₃. ROCs of serum 25-(OH)D₃ levels used to predict coronary arterial lesions in children diagnosed with Kawasaki disease. The area under the curve of serum 25-(OH)D₃ was 0.831

sodium less than 135 mEq/l [13] has been used to diagnose CALs secondary to KD. More recently, serum interleukin-18 was also used to identify CALs in children with KD[24].

To the best of our knowledge, the association of serum 25-(OH)D₃ levels in the diagnosis of CALs among children with KD has not been reported, and this is the first report to identify the role of 25(OH)D₃ in predicting CALs in KD. We found that serum 25-(OH)D₃ levels measured in the acute phase KD was significantly higher in children with CALs than those without CALs ($P=0.001$). A 25-(OH)D₃ cutoff point of 65 ng/ml had a sensitivity of 0.78 and specificity of 0.73 for predicting the development of subsequent CALs. Therefore, measurement of 25-(OH)D₃ levels in the acute phase KD could be beneficial in clinical practice to identify cardiac complications. However, 25-(OH)D₃ levels were not significantly different between the NCAL group and healthy children, making it difficult to be used as a parameter to exclude NCALs in KD. The mechanisms involved in the

Table 2 Performance of 25-(OH)D₃ with a cutoff level of 65 ng/ml

Parameters	Value (%)
Sensitivity	78
Specificity	73
Positive predictive value	50
Negative predictive value	90
Diagnostic accuracy	74

elevation of serum 25-(OH)D₃ levels in the acute phase of KD need to be explored further. The proposed mechanism include the higher expression of VDR in T cells in KD patients than febrile children with respiratory tract infections and healthy children. Overactivated T cells likely promote 25-(OH)D₃ release. Therefore, more intense inflammatory response in CALs' patients may have increased VDR expression and subsequently elevated 25-(OH)D₃ levels.

The newborn in China receive vitamin D supplement after day 14 of birth regularly without measurement of serum 25-(OH)D level. The dosage for under 1 year old is 500 U per day and for 1 to 2 years old is 600 U per day until 18 years old. However, due to the vitamin D preparation available in the market, almost all the children received vitamin D supplement under 2 years old and few children more than 2 years old received supplement vitamin D [26].

There were some limitations in this study. The small sample size of KD children lowered the statistical power. The high incidence of the occurrence of CALs in this study might be correlated with the small sample size. Second, the prevalence of CALs in the KD children directly influenced the predictive values. Further, 25-(OH)D₃ levels may vary according to race [18] and region [21], so caution is needed before the current results are generalized to other patients. Third, the severity of KD is classified by the severity of coronary artery dilatation. However, due to the small sample size, we did not conduct correlation analysis between the severity of CALs and 25-(OH)D₃ level; therefore, the relationships are still unclear between the severity of CALs and 25-(OH)D₃ level. Finally, since 25-(OH)D₃ levels were not elevated in KD children with normal coronary arteries in the current study, serum 25-(OH)D₃ levels may not be used to diagnose KD.

Conclusions

Serum 25-(OH)D₃ levels greater than 65 ng/ml in the acute phase of KD may be predictive of developing subsequent CALs; however, it may not be helpful in the diagnosis of KD. More studies are needed to confirm whether serum 25-(OH)D₃ levels could be used to predict CALs in KD.

Acknowledgments This work was supported by a grant from the Key Project of Social Development of Shanxi Province (2012SF2-10). We thank Medjaden Bioscience Limited for the assistance in the preparation of this manuscript.

Conflict of interest None.

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