Can fibroblast growth factor (FGF)-23 circulating levels suggest coronary artery abnormalities in children with Kawasaki disease?

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Abstract

Objective

Kawasaki disease (KD) is an acute self-limited panvasculitis, primarily affecting young children, with an outstanding risk of cardiovascular complications. Fibroblast Growth Factor-23 (FGF23) is the latest member of the FGF family, acting on phosphate metabolism, which has been shown to display a potential role in the vascular remodelling. The aim of our study was to test the hypothesis that circulating serum levels of FGF23 might be related to the occurrence of coronary artery abnormalities (CAA) in children with KD.

Methods

Serum of 109 consecutive KD patients (median age 30.5 months) were collected for the evaluation of intact FGF23 by ELISA test. Sixty sex/age-matched healthy children were studied as controls, after having excluded rheumatic, endocrinological and chronic renal diseases. In all these subjects a familiar predisposition to atherosclerosis was excluded.

Results

FGF23 levels resulted significantly higher in patients with KD than in controls (72±40 pg/ml vs. 12.3±3.2 pg/ml; p=0.01). Twenty-eight/109 KD patients having developed CAA (aneurysms or dilatations) presented significantly higher FGF23 levels than those without any coronary artery damage (120±40 pg/ml vs. 38.2±5 pg/ml; p<0.0001). Multiple logistic regression analysis showed that only serum FGF23 levels, among different general clinical and biochemical variables, were suggestive of coronary artery damage (OR=4.86).

Conclusions

Based on this preliminary investigation, high serum FGF23 levels would seem suggestive of the potential occurrence of cardiac vascular complications in children with KD.

Key words

Kawasaki disease, fibroblast growth factor-23, coronary artery disease

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Introduction

Kawasaki disease (KD) is an acute systemic panvasculitis of unknown origin, mainly occurring in infants and young children, which affects all medium-small sized vessels, with a relevant risk of generating coronary artery abnormalities (CAA). The etiology of the disease continues to be elusive, though an infectious agent entering through the respiratory tract is the suspected trigger in genetically predisposed individuals (1). Following a complex immune response through T lymphocytes, a significant overproduction of different cytokines occurs in the acute phase of KD (2). The progression from systemic immunological activation to local inflammation in the coronary arteries is shown by endothelial cell activation through different chemokines and upregulation of adhesion molecules, though the molecular changes that contribute to these changes are still unclear (3, 4). CAA, in terms of dilatations, aneurysms, stenosis and even occlusions, occur in 15–25% of untreated cases during the subacute phase of KD with subsequent increased risk of myocardial ischaemia and sudden death (5). Moreover, mild-to-moderate intimal thickening in coronary arteries has been observed yet in the absence of aneurysms: several studies have also revealed that alterations in the lipid profile and generalised endothelial dysfunction persist for a long-time after the clinical resolution of KD (6, 7). There is an increasing body of literature dealing with the risk of future atherosclerosis in post-KD adults without any substantiation of CAA in the course of the disease. Indeed, vascular endothelial damage is the crucial event in KD, leading to widespread arterial dysfunction and risk of atherogenesis (8, 9).

Fibroblast growth factor-23 (FGF23) is a hormone involved in the regulation of both phosphate homeostasis and bone mineralisation: it is the master phosphatonin acting through FGF-receptor 1 (FGFr1)/cofactor Klotho, which is present in several tissues, including vasculature and heart (10). An association between higher levels of serum FGF23 and impaired endothelium function has been found in patients with chronic kidney disease and even in subjects without known abnormalities in mineral metabolism (11-15).

In this present study we have measured intact serum FGF23 levels in a cohort of children with an established diagnosis of KD and we have evaluated their potential association with the development of CAA.

Patients and methods

Our study population included 109 consecutive patients with KD, 68 males and 41 females, with a median age of 30.5 months. A formal Ethical Committee approval from our hospital and informed consents were obtained by relatives or guardians of each patient. All patients were Caucasians, of Italian ancestry, and lived in Italy. Eighty-two out of 109 (75.2%) fulfilled the American Heart Association (AHA) criteria for the diagnosis of typical KD, while 27 of them had an incomplete KD. In the acute phase, 80 out of 109 patients, received intravenous immunoglobulins (IVIG, 2 g/kg over 10–12 hours) and aspirin (60–80 mg/kg/day in 4 divided doses) within day 10 from fever onset, while in 21 of them the same therapy was administered later due to the delay in the proper diagnosis.

All patients underwent 2D-echocardiogram at admission, at 15 days, at 2, 6 and 12 months, if coronary artery involvement was absent. Twenty-eight (25.7%) out of 109 children developed CAA, 9 (8.3%) aneurysms (with a coronary artery diameter ≤5 mm) and 19 (17.4%) dilatations within the first month of illness (see Fig. 1). Among the 88 patients who had received timely IVIG, 7 developed coronary artery dilatations and 2 coronary artery aneurysms, while in the group with delayed treatment 7 presented coronary aneurysms, including 2 giant aneurysms (with a coronary diameter ≥8 mm), and 12 coronary dilatations. Patients with CAA were controlled by the same cardiologist according to the specific entity of coronary artery damage. All patients with dilatations in the acute phase had normal coronary arteries at the 2nd month-cardiac assessment, while 5 out of the 9 children with coronary aneurysms showed a reduced cor-

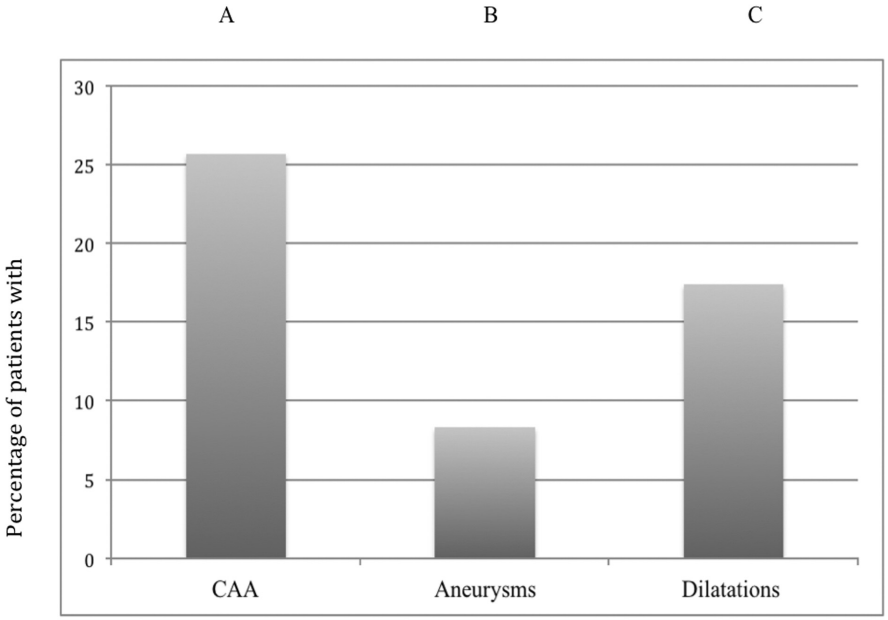

Fig. 1. Percentage of patients with Kawasaki disease (KD) displaying coronary artery abnormalities (CAA). Column A shows the total percentage of CAA, which were found in 25.7% of patients; column B shows the aneurysms (found in 8.3% of patients) and column C the dilatations (found in 17.4% of patients).

Table I. Demographic, clinical and laboratory details of patients in relationship with the presence or absence of coronary artery damage.

	Presence of coronary artery damage	Absence of coronary artery damage
Sex	19 males, 9 females	60 males, 41 females
Age (in months)	19.3 ± 7.0	53.6 ± 11.1
Fever duration (in days)	10.3 ± 1.4	7.9 ± 0.4
Erythrosedimentation rate	75 ± 6.0	70 ± 4.1
C-reactive protein	50.06 ± 7.43	45.21 ± 6.56
Platelet count	512 ± 113.81	425 ± 21.33
Fibrinogen	743 ± 33.07	690 ± 23.74
Total cholesterol	128 ± 14.06	167.5 ± 6.09
LDL-cholesterol	74 ± 11.48	100 ± 6.02
HDL-cholesterol	37.6 ± 6.74	42 ± 7.02
Tryglicerides	108 ± 4.32	110 ± 5.08

Values are expressed as means ± Standard Deviations.

onary artery diameter and 4 had stable coronary dimensions. Patients with aneurysms were taking low-dose aspirin, while 2 patients with giant aneurysms were receiving anticoagulant treatment (warfarin).

Sixty sex- and age-matched healthy children were studied as controls, after the clinical and laboratory exclusion of rheumatic, endocrinological and chronic renal diseases: in all these subjects general data from family history and evaluation of the lipid profile were collected to exclude any familiar predisposition to atherosclerosis.

Fibroblast growth factor-23 assay

Intact FGF23 was measured in the serum collected during routine blood tests in the acute phase of the disease (mainly when KD diagnosis was established) before IVIG infusion. Each sample had been stored at -20° until final evaluation. Testing was performed with an ELISA tool (Immunotopics Inc. San Clemente, CA, USA). The assay was standardised to measure serum FGF23 in picograms per millilitre (pg/ml), had an inter-assay coefficient of variation of 6.1–6.5% and a lower limit of detection of 1.0 pg/ml. Assays were done in triplicate. The same type of assessment was performed in healthy controls, after receiving an informed consent to the participation in the study.

Statistical analysis

Mann-Whitney U-test was applied to evaluate serum FGF23 levels between patients with KD and healthy controls, mostly in the evaluation of those who developed CAA and not, since all groups were not normally distributed. Level of significance was set at $p \leq 0.05$. Non.parametric tests were used, where necessary, due to the small size of our groups. A forward stepwise multiple logistic regression analysis was used to elucidate any association of serum FGF23 with several variables, including age at disease onset, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total fasting cholesterol, LDL-cholesterol, HDL-cholesterol and tryglicerides in the acute phase of the disease and CAA development. ESR and CRP were related to the disease onset. Odds ratios were determined to define the risk of developing coronary artery involvement in patients with high levels of serum FGF23. Table I lists the demographic, clinical and laboratory variables of KD patients in relationship with CAA presence or absence. Table II lists factors associated with the prediction of coronary artery damage in the multiple logistic regression analysis. Statistical 5.1 package was the software used (Stat soft Inc., Tulsa, OK, USA).

Results

Serum levels of intact FGF23 were significantly higher in all KD patients than in controls (72 pg/ml ± 40 SD *vs*. 12.3 pg/ml ± 3.2 SD; p=0.01) (normal range for intact serum FGF23: 13.3-19 pg/ml). The 28 KD patients with CAA displayed higher levels of serum FGF23 than those without any CAA (120 pg/ml ± 40 *vs*. 38.2 ± 5; p<0.0001) (see Fig. 2). The multiple logistic regression analysis showed that only serum FGF23 levels were significantly related to the occurrence of CAA (see Table II). The estimated Odds ratio of having CAA among patients with increased serum levels of FGF23 was 4.86.

Discussion

Endothelium dysfunction is an established risk factor for cardiovascular mortality in the general population and results highly prevalent in patients with KD (16). Endothelial derangement in KD has been demonstrated by increased release of microparticles, which contribute to the occurrence of systemic vascular complications through a still unravelled modality (17). It is not completely clear how FGF23 can be involved in the arterial wall disruption: the physiological role of FGF23 in modulating plasma phosphate concentrations and renal 1α-hydroxylase activities was elegantly described in the Fgf23 null mice (18), whose growth rate is reduced and for whom extra-skeletal calcifications in several tissues, including heart and vasculature, are reported. FGF23 exerts its bioactivity on selected target-tissues interacting with its receptors (FGFr), largely diffuse in bone tissue and in the endothelium, in the presence of Klotho co-factor (13). Klotho null mice that carry non-functional FGF23 activity, due to the lack of co-receptor, exhibit endothelial dysfunction which can be rescued by the administration of Klotho protein (19, 20): this is due to the fact that Klotho suppresses TNF-alpha-induced expression of adhesion molecules ICAM-1 and VCAM-1 in the endothelial cells (20).

In the present study we have observed that intact serum FGF23 levels were significantly higher in children with KD (p=0.003), mostly in those displaying CAA, in comparison with healthy controls; conversely the other evaluated parameters seem not to have influenced the coronary artery system (see Table II).

We might hypothesise that the vascular damage and senescence signs observed in patients with a history of KD might be in part the consequence of a reduction of Klotho activity in the vasculature. This should cause a lower control on TNF-α activity without a down-regulation of several factors, such as vascular NADPH oxidase, involved in the pathogenesis of atherosclerosis (21, 22). Higher levels of serum FGF23 observed in KD patients could also be a compensatory response to an insufficient Klotho activity and could worsen the vascular dysfunction. However, recently, a direct effect of FGF23 on the vasculature has been shown by several authors (12, 23, 24). Mirze *et al*. found the association between higher FGF23 levels and impaired endothelium function in subjects with normal renal function: in particular, they showed arterial stiffness and vascular hyporeactivity in subjects with high serum FGF23 levels (11). In addition, Gutierrez *et al*. observed that FGF23 is independently associated with mortality in patients on hemodialysis and that increased serum FGF23 concentrations were the main predictor of death (12). Lastly, in KD patients a higher number of circulating endothelial progenitor cells with low activity has been found to be crucial in the pathogenesis of endothelium dysfunction (20): endothelial progenitor cells are normally down-regulated by TNF-α, which is under control of Klotho/FGF23 axis. It is likely that a disequilibrium in the Klotho/FGF23 axis could in part explain how FGF23 contributes to the formation of CAA in children with KD.

In conclusion, our present data highlight a statistically significant association between serum FGF23 levels and the development of CAA: higher serum FGF23 levels in KD patients, especially those with CAA, support the hypothesis of FGF23 potential contribution to the pathogenesis of vascular complications

Table II. Factors associated with the prediction of coronary artery damage in the multiple logistic regression analysis.

	Coefficient (β)	p-level
Intercept	0.903859	0.006
Serum FGF23	0.016187	0.003*
Age at onset	0.001975	0.491
Erythrosedimentation rate	0.002689	0.449
C-reactive protein	-0.003334	0.151
Total cholesterol	-0.001064	0.721
LDL-cholesterol	0.007879	0.618
HDL-cholesterol	-0.000154	0.983

Intercept: mathematical constant without clinical interpretation. Coefficient (β): the mathematical weighting of each variable in the model. *statistically significant.

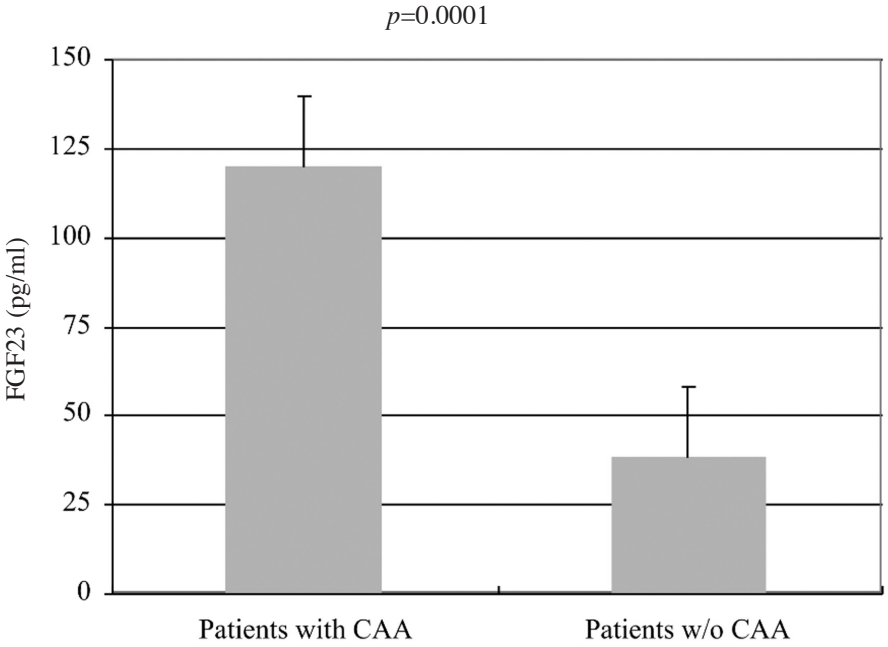

Fig. 2. Serum levels of intact FGF23 in KD patients with and without (w/o) coronary artery abnormalities (CAA).

in KD. To the best of our knowledge, this is the first study evaluating serum levels of FGF23 in children with KD: these preliminary results would reveal FGF23 as a suggestive marker to the recognition of coronary artery disease.

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