RESEARCH BRIEF

Plasma Endothelial Microparticles, TNF-α and IL-6 in Kawasaki Disease

ZHEN TAN, *YAN YUAN, SUN CHEN, YI CHEN AND #TONG-XIN CHEN

*From the Department of Pediatrics, Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, China, 200092; *Department of Public Health Sciences, University of Alberta, Canada T6G IC9; #Department of Clinical Immunology/Department of Nephrology and Rheumatology, Children's Hospital of Shanghai, Shanghai Jiao Tong University, Shanghai, China, 200040.*

Correspondence to:
Dr Tong-Xin Chen, Department of Clinical Immunology/Department of Nephrology and Rheumatology, Children's Hospital of Shanghai, Shanghai Jiao Tong University, No. 24, Lane 1400, Western Beijing Road, Shanghai 200040, China.
tongxinch@gmail.com;
Received: September 09, 2011;
Initial review: October 10, 2011;
Accepted: October 22, 2012.

We studied the levels of endothelial microparticles (EMPs), IL-6, and TNF-α in patients with Kawasaki disease (KD). EMPs were enumerated by flow cytometry, while IL-6 and TNF-α were measured using enzyme-linked immunosorbent assay. EMPs and IL-6 were elevated in KD, the level of TNF-α in KD was not different from disease controls, but higher than healthy controls. EMPs were positively correlated with TNF-α and negatively correlated with albumin. Elevated level of EMPs, a biomarker of endothelial cells damage, concomitant with increased levels of TNF-α and IL-6, is seen in patients with KD.

Key words: *Endothelial microparticles; Interleukin-6; Kawasaki disease; Tumor necrosis factor-α.*

PII: S097475591100751

Kawasaki disease (KD) is an acute febrile autoimmune vasculitis with unclear etiology. Its diagnosis is based on symptoms without specific diagnostic test. A delay in diagnosis could result in a delay in treatment, which is associated with a 25% probability of coronary artery lesions [1]. Therefore, an early diagnosis of KD is clinically important.

Endothelial microparticles (EMPs), budded from endothelial cells, have been suggested as a possible marker of endothelial disturbance [2]. Elevated EMPs were detected in patients from a number of vascular disorders, e.g. systemic vasculitis [3]. Few studies have assessed these particles in children with KD.

METHODS

Patients with KD under 36-month of age who met the diagnostic criteria [4] within 10 days of the onset of fever, were enrolled between March 2009 and April 2010. Age and sex matched patients with acute infectious febrile disease and healthy children were enrolled as disease controls and healthy controls, respectively. Following institutional ethics approval and informed parental consent, blood samples were collected from all subjects before therapy with intravenous immunoglobulin for KD group.

Clinical symptoms were obtained from observations and interviews of the primary caregivers. Laboratory results including white blood cell count, absolute neutrophil count, platelet count, erythrocyte sedimentation rate, C-reactive protein and albumin were obtained.

Citrated venous blood (1 mL) was centrifuged immediately twice for 5 minutes at 5000g at room temperature to obtain platelet poor plasma, which was stored at -80°C. A 50 μL aliquot was incubated with 10 μL of FITC labeled anti-CD31 BD Biosciences, Franklin Lakes, NJ, USA) and 10 μL of phycoerythrin labeled anti-CD146 (BD Biosciences) at room temperature for 20 minutes in dark. Samples were measured using Becton Dickinson FACSCalibur flow cytometry. The gate was standardized by Megamix beads, forward scatter parameters were plotted on logarithmic scales to <1.0 mm. All microparticles positive for CD31 and CD146 were counted as EMPs to a maximum value of 10,000.

Venous blood (2 mL) was collected in a gel coagulation-promoting vacuum tube and centrifuged immediately for 15 minutes at 4000rpm at room temperature for serum, which was stored at -80°C. IL-6 and TNF-α in serum were measured by enzyme-linked immunosorbent assay (R&D, Minneapolis, MN, USA) according to manufacturer's instructions.

Statistical analysis: Sample size assessment and power analysis was done by using OpenEpi (Version 2.3.1), with type I error 5% and power of 80%. All results were

expressed as mean±SD. One-way analysis of variance (ANOVA) followed by Test of least significant differences assuming equal variance or Tamhane's T2 test assuming unequal variances were used. Pearson correlation coefficients were reported for EMPs and other variables of interest. The diagnostic value of EMPs was assessed with receiver operating characteristic curves and area under curve.

RESULTS

We enrolled 20 KD patients, 18 disease controls and 20 healthy controls. 8 patients had atypical KD and one KD patient had coronary lesions. KD and disease controls had recurrent fever; mean duration of 6.1 and 8.1 days, respectively.

The percentage counts of EMPs were 28.07±14.16% in KD children, which were significantly higher (P<0.001) than that of disease controls (17.20±6.99%) and healthy controls (11.67±3.97%). The respective serum concentrations of IL-6 were 1247.11±1093.02 pg/mL, 495.66±281.49 pg/mL and 326.08±302.69 pg/mL. The concentrations of TNF-α were 54.32±25.59 pg/mL in KD children, 48.42±31.45 pg/mL in disease controls and 25.12±11.0 pg/mL in healthy controls (***Fig.* 1**). EMPs were positively correlated with TNF-α (P<0.001) and negatively correlated with albumin (P<0.001). There were no significant correlations between EMPs and IL-6, white blood cells and neutrophil count, C-reactive protein, erythrocyte sedimentation rate or platelet counts.

As shown in ***Web Fig.* 1**, the area under curve for predicting KD using EMPs among febrile patients (KD and disease controls) was 0.714. When a cutoff value of 20.99% was chosen based on highest Youden index of receiver operating characteristic curves for predicting KD, it generated a specificity of 0.72 and sensitivity of 0.60.

DISCUSSION

Microparticles are vesicles consisting of variable amounts of cytoplasmic components and surface phospholipids when compared to their parental cells [5]. In non-disease states, the release of microparticles is programmed, and increased microparticles levels are triggered by activation of apoptosis or cell lysis [6]. EMPs, microparticles from endothelial cells, have a varied group of surface biomarkers, such as CD31+, CD42b-, CD62E+, CD144+ or CD146+/CD105-. The combination of multiple biomarkers is a more specific assessment of EMPs due to the co-expression of these markers on the other cells or platelets. We choose CD31+/CD146+ to characterize EMPs [7,8]. The level of EMPs in KD patients was significantly higher than that of disease controls and healthy controls, and was negatively correlated with serum albumin. Since the decreased serum albumin represents the vascular leakage by the damage to the vascular endothelium, the results confirmed that the level of CD31+/CD146+ EMPs is a potential biomarker of endothelial cell damage and the damage starts in the early stage of KD. However, a relatively low sensitivity of 0.60 in ROC curve suggested that EMPs alone was not highly sensitive.

IL-6 and TNF-α are potent pro-inflammatory cytokines and have multiple immune-modulatory functions. High levels of IL-6 could inhibit the differentiation of Th1 cells while promoting Th2 cells activation and consequently increasing the production of Th2 cytokines. Subsequently, they activate the polyclonal B cells to produce autoantibody in patients [9]. Therefore, IL-6 is a critical cytokine in the KD pathogenesis of autoimmune vasculitis. The difference of serum IL-6 levels between KD and disease controls may represent the fundamental difference of the two diseases.

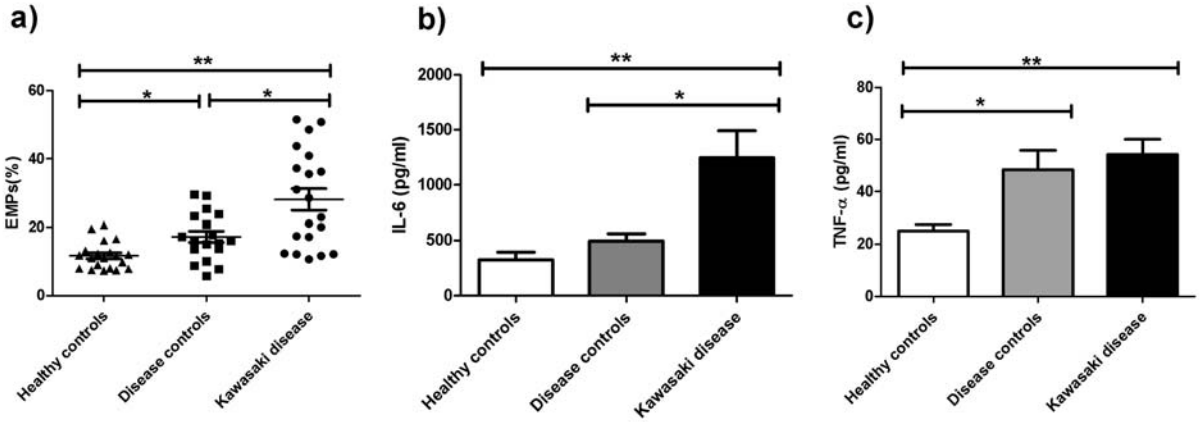

FIG. 1 *Levels of EMPs, IL-6 and TNF-α in Kawasaki disease, Disease controls and Healthy controls * P<0.05; ** P<0.01.*

WHAT THIS STUDY ADDS?

- Endothelial microparticles are a biomarker of endothelial cell damage in Kawasaki disease.

In an animal model of KD induced by *Lactobacillus casei* cell wall extract, the process of coronary arteritis and aneurysms could be ablated by blocking TNF receptor [10], suggesting that TNF-α is capable of inducing coronary artery lesions directly. It has been reported that TNF-α activation could trigger the release of EMPs from endothelial cell *in vitro* [11]. Consistently, we found that EMPs were correlated with TNF-α but not with IL-6. In our study, there were no differences in the levels of TNF-α between the KD and disease controls. So far, it is agreed by most of pediatricians that KD may be triggered by undefined infectious agents in genetically predisposed individuals [12]. Therefore, the levels of TNF-α were elevated in both KD and disease controls can be partly explained by overlapping disease progression. However, since the levels of IL-6 and EMPs were higher in KD than in disease controls one possible explanation is that combination of TNF-α with IL-6 will speed up the release of EMPs. An alternative is that some unknown agents help TNF-α to speed up the release of EMPs in KD.

To summarize, the level of EMPs, a potential biomarker of endothelial cell damage, was elevated in KD patients and was concomitant with high levels of IL-6 and TNF-α. An increased level of IL-6 denotes a potential autoimmune response, while elevated level of TNF-α induces endothelial cell activation. The combination of these three factors indicates that autoimmune vasculitis is fundamental in the pathogenesis of KD.

Contributors: All the authors have contributed, designed and approved the study.
Funding: None. *Competing interests*: None stated.

REFERENCES

1. Burns JC, Glode MP. Kawasaki syndrome. Lancet. 2004;364:533-44.
2. Jimenez JJ, Jy W, Mauro LM, Horstman LL, Bidot CJ, Ahn YS. Endothelial microparticles (EMP) as vascular disease markers. Adv Clin Chem. 2005;39:131-57.
3. Clarke LA, Hong Y, Eleftheriou D, Shah V, Arrigoni F, Klein NJ, *et al*. Endothelial injury and repair in systemic vasculitis of the young. Arthritis Rheum. 2010;62: 1770-80.
4. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Pediatrics. 2004;114:1708-33.
5. Enjeti AK, Lincz LF, Seldon M. Detection and measurement of microparticles: an evolving research tool for vascular biology. Semin Thromb Hemost. 2007;33:771-9.
6. Enjeti AK, Lincz LF, Seldon M. Microparticles in health and disease. Semin Thromb Hemost. 2008;34:683-91.
7. Widemann A, Sabatier F, Arnaud L, Bonello L, Al-Massarani G, Paganelli F, *et al*. CD146-based immunomagnetic enrichment followed by multiparameter flow cytometry: a new approach to counting circulating endothelial cells. J Thromb Haemost. 2008;6:869-76.
8. Harrison M, Murphy RP, O'Connor PL, O'Gorman DJ, McCaffrey N, Cummins PM, *et al*. The endothelial microparticle response to a high fat meal is not attenuated by prior exercise. Eur J Appl Physiol. 2009;106:555-62.
9. Kuo HC, Liang CD, Wang CL, Yu HR, Hwang KP, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. Acta Paediatr. 2010;99:1578-83.
10. Chang J, Lu JR, Liang D. The function of Th1/Th2 cells in children with acute Kawasaki disease. Zhonghua Er Ke Za Zhi. 2006;44:377-8.
11. Sofi MH, Li W, Kaplan MH, Chang CH. Elevated IL-6 expression in CD4 T cells via PKCtheta and NF-kappaB induces Th2 cytokine production. Mol Immunol. 2009;46:1443-50.
12. Galeotti C, Bayry J, Kone-Paut I, Kaveri SV. Kawasaki disease: aetiopathogenesis and therapeutic utility of intravenous immunoglobulin. Autoimm Rev. 2010;9:441-8.