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Changes in OxPLs and eNOS during the Acute Phase of Kawasaki Disease: Their Association with Coronary Artery Lesions

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Abstract

Objective: This study aims to investigate the role of serotype oxidized phospholipids (OxPLs) and Endothelial Nitric Oxide Synthase (eNOS) in Coronary Artery Lesions (CAL) of Kawasaki Disease (KD) in children.

Methods: The study included ninety-five children diagnosed with acute KD in the KD group, twenty-seven children with fever from respiratory tract infections in the fever group, and thirty children with no health issues in the control group. Serum concentrations of OxPLs and eNOS were measured in all three groups, and cardiac ultrasound was conducted to assess the severity of CAL during the acute phase of KD. The groups were categorized based on different grades of coronary lesion severity, and differences in OxPLs and eNOS concentrations were compared among the groups. Receiver Operating Characteristic (ROC) curves was utilized to evaluate the diagnostic efficacy of OxPLs and eNOS for CAL in KD.

Results: The levels of OxPLs in the acute phase of the KD group were significantly higher than those in the fever group and the control group, while the concentrations of eNOS were significantly lower in the KD group compared to the fever group and the control group, with statistical significance ($P < 0.05$). The CAL group exhibited markedly higher OxPLs concentration and significantly lower eNOS concentration compared to the non-CAL (NCAL) group. Moreover, as the severity of CAL increased, the concentration of OxPLs also increased significantly, with a statistical difference ($P<0.05$). Pearson's correlation analysis demonstrated a negative correlation between serum OxPLs and eNOS levels in children with KD during the acute phase ($r = -0.363$, $P < 0.05$). The area under the ROC curve (AUC) for diagnosing KD using OxPLs and eNOS was 0.984 (95% CI: 0.965-1.000, $P < 0.001$) and 0.9816 (95% CI: 0.9623-1.000, $P < 0.001$).

Conclusions: Elevated concentrations of OxPLs and decreased concentrations of eNOS characterize the acute phase of KD. Deviations in OxPLs and eNOS levels are linked to KD CAL and its severity.

Keywords: Kawasaki disease; Coronary artery lesions; Oxidized phospholipids; Endothelial Nitric Oxide Synthase

Introduction

The primary adverse effect of Kawasaki Disease (KD) is the dilation of the coronary artery and the development of coronary artery aneurysms due to inflammatory damage. An essential aspect of KD pathogenesis is the damage to endothelial cells. Existing literature indicates that oxidized phospholipids (OxPLs) play a crucial role in inducing endothelial cell dysfunction [1]. OxPLs, formed under inflammatory and oxidative stress conditions, can impair endothelial function through mechanisms that include pro-inflammatory, pro-oxidative, pro-apoptotic activities, and the inhibition of reverse cholesterol transport. Research in adult atherosclerosis has demonstrated that OxPLs can trigger endothelial dysfunction and cell apoptosis by inhibiting endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production, thereby causing vascular dilation abnormalities and an increase in superoxide anion ($O2^{-}$) production, which exacerbates coronary artery disease [2]. However, the parallels in KD remain under-investigated. Thus, this study aimed to measure the expression levels of OxPLs and eNOS in children with acute KD and to preliminarily investigate the association between OxPLs, eNOS, and coronary artery lesions (CAL) in KD, as well as the influence of OxPLs on these lesions.

Object and Method

a. Research objectives

The study aimed to include KD patients admitted to the Children's Medical Center of the First Affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital) between September 2022 and September 2023. Inclusion criteria required patients to meet the 2022 Chinese expert consensus for KD diagnosis and treatment [3], have complete medical information, laboratory data, and relevant materials, not have other underlying conditions influencing the study results, and be in the acute phase of KD (<2 weeks). Exclusion criteria encompassed patients beyond the acute phase upon admission, incomplete medical records, comorbidities affecting the research outcomes like congenital vascular abnormalities, dyslipidemia, or genetic metabolic disorders, and refusal of participation. The study received approval from the Ethics Committee of Hunan Provincial People's Hospital (Approval No. [2023] -69).

b. Participant Selection

The study included 95 children with acute KD in the KD group, 27 children with fever due to respiratory tract infection as the fever group, and 30 healthy children undergoing physical examinations as the control group. Excluded were diseases affecting the cardiovascular, immune, hematological, and endocrine systems, along with recent treatments involving immunoglobulins, hormones, or antiplatelet drugs.

c. Specimen collection

In the KD group, blood samples were obtained within 24 hours of admission before initiating intravenous immunoglobulin, while healthy and febrile children had blood drawn upon admission. Residual blood samples of 2ml were collected into serum separation tubes. These samples were then left to rest overnight at 4 °C, centrifuged at 1000 × g for 20 minutes, and 0.5ml of the supernatant transferred to EP tubes for storage at -80 °C. It was emphasized to avoid repeated freezing and thawing until sample collection completion.

d. Sample processing

Serum phospholipid oxide and endothelial NO synthase levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) kit provided by Shanghai Hengyuan Biotechnology. The procedure involved sample addition, incubation, preparation, washing, enzyme addition, color development, termination, and final determination.

e. Clinical data collection

The data collection process included recording the visit time and basic information (gender, age) of all participants (comprising KD patients and the control group). Additionally, pertinent clinical and laboratory data were collected from all children diagnosed with KD, along with obtaining cardiac ultrasound images upon admission.

f. Evaluation of coronary artery lesions [4]

Transthoracic echocardiography was utilized for evaluating Coronary Artery Aneurysm (CAA) classification in KD cases. The criteria for CAA categorization were as follows: small CAA or coronary artery dilation when the coronary artery diameter exceeds 3 mm but is ≤ 4 mm, or in older children (≥ 5 years old), the inner diameter of coronary artery dilation is less than 1.5 times the normal value. Medium-sized CAA is defined as coronary arteries > 4 mm and ≤ 8 mm, or in older children (≥ 5 years old), when the diameter of coronary artery dilation exceeds 1.5-4.0 times the normal value. Giant CAA is characterized by a coronary artery diameter > 8 mm or a coronary artery dilation diameter > 4 times the normal value in older children (≥ 5 years old). Based on the risk categorization of coronary artery lesions (CAL), children with CAL are graded as follows: Grade I indicates no coronary artery dilation at any stage (Z-value < 2); Grade II signifies mild coronary artery dilation during the acute phase; Grade III represents a single small or medium-sized CAA, with IIIa denoting a small CAA (2.5 < Z-value < 5 or absolute inner diameter ≤ 4 mm), and IIIb indicating a medium-sized CAA (5 < Z-value < 10, and the absolute inner diameter value < 8 mm); Grade IV entails ≥ 1 large CAA (including giant CAA) or multiple CAAs within a single coronary artery but not meeting the criteria for Grade V; Grade V is diagnosed when coronary angiography reveals occlusion or stenosis, with Va being without myocardial ischemia and Vb being with myocardial ischemia.

g. Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. Measurement data following a normal distribution were expressed as mean ± standard deviation, and comparison between groups was conducted using two independent sample tests. For non-normally distributed data, represented by M (P_{25}~P_{75}), intergroup comparisons were made using rank sum tests, Wilcoxon rank sum tests, and Kruskal-Wallis rank sum tests, with a significance level set at $P < 0.05$. ROC curves of OxPLs and eNOS were employed to evaluate the predictive efficacy of CAL in KD, with statistical significance considered for $P < 0.05$.

Results

a. General Participant Overview

The study included 95 children with KD, comprising 58 males and 31 females, with a median age of 2 years and 11 months (range: 1 year and 1 month to 4 years and 9 months). Within the CAL group, 37 cases were identified, with 19 males and 18 females, having a median age of 2 years and 11 months (range: 1 year and 5 months to 4 years and 5 months). Additionally, the non-CAL (NCAL) group consisted of 58 cases, including 39 males and 19 females, with a median age of 2 years and 9 months (range: 9 months to 4 years and 11 months). The CAL group further delineated as 28cases at grade II, 5 cases in the III group, and 4 cases in the IV group.

In the fever group, 27 cases were identified, with 15 males and 12 females, and a median age of 3 years and 5 months (range: 1 year and 5 months to 5 years and 5 months). The control group consisted of 30 participants, with 17 males and 13 females, and a median age of 4 years and 6 months (range: August to 8 years and 4 months).

b. Comparison of OxPLs and eNOS Levels

The investigation involved a comparative analysis of OxPLs and eNOS levels across the KD group, fever group, and control group. Notably, the OxPLs concentration in the KD group exhibited a substantial elevation compared to both the fever group and the control group, alongside a significantly reduced concentration of eNOS relative to the fever group and control group, with statistical significance observed ($P<0.05$). Conversely, no statistically significant differences were discerned in the comparison of OxPLs and eNOS levels between the fever group and the control group. Refer to table 1 for detailed data.

Comparison between acute-phase CAL and NCAL groups in KD: Statistically significant differences ($P < 0.05$) were observed in the comparison of serum levels of OxPLs and eNOS between the two groups. Detailed data is presented in table 2.

Table 1: Comparison of OxPLs and eNOS Levels Across KD Group, Fever Group, and Control Group [M(P_{25}~P_{75})].

	OxPLs	eNOS
KD group(N = 95)	140.5[ab](125.3~159.8)	48.9[ab](46.2~56.5)
Fever group (N = 27)	87.0(78.5~91.0)	80.7(76.3~85.8)
Control group(N = 30)	74.05(65.75~82.05)	77.95(71.325~82.975)
H	104.257	104.376
P	P<0.05	P<0.05

Table 2: Comparison of OxPLs and eNOS Levels between the CAL and NCAL Groups in the Acute Phase of KD [M(P_{25}~P_{75})].

	OxPLs	eNOS
CAL group N = 37	148.1(133.1~166.45)	47(45.85~51.45)
NCAL group N = 58	133.5(119.2~152.125)	49.95(46.775~57.55)
Satistic	-2.876	-2.225
P	<0.01	<0.001

Inter-group Comparison of Different Severity Levels of CAL in the Acute Phase of KD: The grouping was done based on varying severity grades of coronary artery lesions, followed by an inter-group comparison. The results suggest a statistically significant difference in the levels of OxPLs and eNOS among the different CAL groups ($P < 0.05$). Additionally, as the severity of KD increases, there is a corresponding increase in the concentration of OxPLs, highlighting a potential relationship between the severity of coronary artery disease and OxPLs. Refer to table 3 for detailed data.

c. Correlation Analysis and Diagnostic Efficacy

The correlation analysis between OxPLs and eNOS in children with KD during the acute phase revealed a negative correlation ($r = -0.363$, $P < 0.05$), as illustrated in figure 1.

The diagnostic efficacy of OxPLs and eNOS in the acute phase of KD was not statistically significant ($P > 0.05$) due to variations in serum OxPLs and eNOS levels between the healthy and febrile groups. Therefore, they were combined into a control group for ROC curve analysis to evaluate the effectiveness of these two indicators in diagnosing KD in the acute phase. To facilitate comparison, we will denote eNOS as -eNOS in the figure. The AUC for OxPLs was 0.984 (95% CI: 0.965-1.000, $P < 0.001$), and the negative value of eNOS yielded an AUC of 0.9816 (95% CI: 0.9623-1.000, $P < 0.001$), demonstrating both indicators' strong diagnostic capabilities (Figure 2).

Discussion

OxPLs, a complex mixture of phospholipid oxidation products generated during normal or pathological processes, exhibits the capacity to stimulate endothelial cells to release chemokines and activate phagocytes, as well as to generate pro-inflammatory cytokines, thereby disrupting the endothelial barrier [5]. Increasingly abundant evidence supports its involvement in regulating vital processes such as inflammation, thrombus formation, angiogenesis, endothelial barrier function, and immune tolerance [6]. eNOS, an isoenzyme predominantly expressed in endothelial cells, plays a pivotal role in endothelial function and cardiovascular health. This study examined the serum levels of OxPLs and eNOS in children with KD. The results revealed significantly higher levels of OxPLs in the KD group compared to both the fever and control groups, while eNOS levels were notably lower in the former. Furthermore, a negative correlation was observed between OxPLs and eNOS levels, indicating a potential involvement of OxPLs and eNOS in the pathogenesis of KD during the acute phase. The underlying mechanism may be attributed to OxPLs inhibiting eNOS phosphorylation at the Ser1177 site, promoting phosphorylation at the Thr495 site, and disrupting the association between heat shock proteins and eNOS, consequently inhibiting eNOS activation and production [2]. In studies focusing on adult atherosclerosis, it has been observed that OxPLs can diminish Nitric Oxide (NO) production by inducing uncoupling and inhibiting eNOS, leading to impairment of the contractile function of endothelial

Table 3: Comparison of OxPLs and eNOS in Acute KD with Varying CAL Sever [M(P_{25}~P_{75})].

Severity	OxPLs	eNOS
Level I (N = 58)	133.5(119.2~152.13)	49.95(46.76~57.55)
Level II (N = 28)	146.9(134.78~167.5)	46.7(45.65~48.55)
Level III (N = 5)	148.1(131.15~170.05)	49.8(47.7~57.85)
Level IV (N = 4)	159.5(121.13~170.43)	54.15(45.33~60.35)
H	9.216	10.02
P	<0.05	<0.05

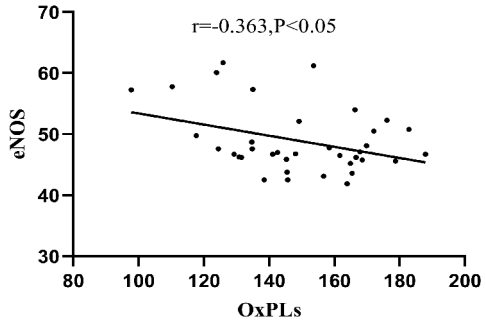

Figure 1: Correlation between OxPLs and eNOS.

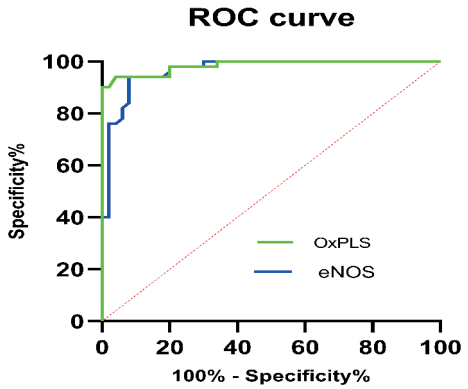

Figure 2: ROC curves for diagnosing KD using OxPLs and eNOS.

vessels and resultant vascular dysfunction [7-9]. KD, distinct in onset age and symptoms from adult atherosclerosis, may share a common pathogenesis of vascular inflammation. During the acute phase of KD, a substantial amount of oxidative stress-related molecules such as phospholipids or low-density lipoproteins are formed, interacting with immune cells, notably macrophages, and endothelial cells, thereby precipitating endothelial dysfunction [10]. Emerging evidence underscores that endothelial dysfunction serves as a crucial precursor in the cascade of events that culminate in cardiovascular disease development in KD [11].

OxPLs can induce cell response, promote inflammation in the blood vessel wall, activate inflammatory cells to enter the blood vessel wall, and destroy the endothelial cells in the blood vessel wal l [12]. Plasma OxPLs levels predict the presence and extent of anatomical atherosclerotic cardiovascular disease, and elevated levels are associated with disease in multiple arterial beds; measurement of OxPLs improves prognostication of peripheral artery disease, as well as incident and recurrent myocardial infarction and stroke, and improves risk reclassification, particularly in

patients in intermediate risk categories, for whom improving decision-making is most impactful [13]. In addition, relevant preclinical animal experimental data have shown that targeting OxPLs and reducing OxPLs level alone can effectively inhibit atherosclerosis [14].

In the present study, it was observed that in the acute phase of KD, the OxPLs level in the CAL group surpassed that in the NCAL group, while the eNOS level in the CAL group lagged behind that in the NCAL group. When assessing the severity of CAL in KD, a positive correlation was noted between higher OxPLs levels and increased severity of coronary artery lesions, suggesting a close association between OxPLs alterations and CAL severity during the acute phase of KD. An implication arises that these changes in OxPLs levels hold diagnostic potential for identifying coronary lesions in KD. Exploring the role of OxPLs in KD, a significant correlation was established by Japanese scholar Yasuhiro Nakamoto's team in 2021 between OxPLs levels and the occurrence of acute CAL in patients with KD, as evidenced by lipid analysis of their serum. Specifically, OxPLs levels in the CAL group of KD patients notably exceeded those in the control group, implicating OxPLs involvement in the pathogenesis of coronary arteritis in KD [15].

Through pathological immunohistochemical analysis of coronary artery aneurysm blood vessels in deceased children with KD, YU conducted an investigation revealing the presence of eNOS immunoreactivity in endothelial cells, a finding correlated with the severity of coronary artery wall injury and the progression of acute KD in patients [16]. Moreover, a notable reduction in the expression of eNOS in endothelial cells compared to the normal control group was observed, indicating suppressed eNOS expression in KD. This study further confirmed that during the acute phase of KD, there was a significant elevation in OxPLs levels and a considerable reduction in eNOS levels in coronary artery lesions, with a concomitant increase in the severity of CAL corresponding to the magnitude of these alterations.

In conclusion, this study identified a notable increase in OxPLs levels and a significant decrease in eNOS levels during the acute phase of KD. These findings suggest that specific pathways may be implicated in initiating and inhibiting eNOS, consequently diminishing its protective role on blood vessels in affected children, rendering their vascular walls more vulnerable and predisposed to coronary artery disease. Among these potential initiating pathways, the robust inflammatory response characteristic of KD might trigger lipid peroxidation, leading to amplified OxPLs production and subsequent suppression of eNOS synthesis and activation.

It is found that phospholipid oxide can be used as a reliable biomarker to identify oxidative stress in blood vessel wall, which is helpful for early diagnosis of coronary artery disease and evaluation of disease prognosis [17]. The prospective Bruneck study performed with a 5 year interval demonstrated high predictive value of OxPLs measured by E06 antibody also for the presence, extent and development of carotid and femoral atherosclerosis. [18] In the MIRACL Trial atorvastatin treatment decreased total OxPLs on all apoB particles in patients with acute coronary syndromes [19].

The aberrant alterations in OxPLs and eNOS could exacerbate endothelial dysfunction, thereby fostering the onset and progression of coronary artery disease in KD. Both markers could serve as valuable early diagnostic tools for coronary artery disease in KD and enable ongoing monitoring of changes in the condition of KD vasculitis. Targeting the reduction of oxidative phospholipid production and alleviating vascular oxidative stress during the acute stage of KD through pharmacological interventions might potentially mitigate CAL in affected individuals. Further clinical investigations are warranted to elucidate the efficacy of such therapeutic approaches.

Declaration of interest

The author declares no conflict of interest.

Ethical Statement

This study was approved by the Ethics Committee of Hunan Provincial People's Hospital (Approval No. [2023] -69).

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Acknowledgment

None.

Data Availability

All data can be provided as needed.

Author's Contributions

Zhu Liurong and He Xuehua designed the study and wrote the manuscript, Yuan Yonghua and Luo Lanzhu analyzed the data and revised the manuscript, Liang Daomiao collected the data. All authors reviewed and approved the final version of the manuscript.

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