WITHDRAWN:
A Comparison of SAA Levels in Children With Viral CNS Infection and Kawasaki's Disease

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background: The purpose of this study was to analyze the epidemiological differences of SAA in children with viral central nervous system (CNS) infection and Kawasaki's Disease(KD). The former is viral invasion of central nervous system, whereas the latter is a viral systemic vasculitis. Differences in the SAA concentration in the blood and the proportion of high level SAA cases reflected the influence of the blood-cerebrospinal fluid barrier (BCB) on the concentration of peripheral blood infection markers.

Methods: The SAA data comprised 226 consecutive cases of children, including 112 cases of viral CNS infection and 114 cases of KD. Differences in the proportion and concentration of SAA in the cases of the two groups were verified with a Kruskal-Wallis H-test and the chi-square test.

Results: The concentration of SAA differed between children with KD and viral CNS infection, and the high level SAA proportion was lower in children with viral CNS infection compared to that in the KD group.

Conclusions: The observed differences may be due to the *sequestration effect*, as the blood-cerebrospinal fluid barrier (BCB) can compartmentalize the pathogens at the site of disease. Therefore, other organs are unable to be stimulated to release additional SAA.

1. Background

Serum amyloid A (SAA) protein is a sensitive acute-phase reactant as a C-reactive protein (CRP) and procalcitonin (PCT), which plays an important role in infection and inflammation. Moreover, SAA is used in clinical laboratories as a diagnostic, prognostic, or therapeutic follow-up biomarker for many infectious diseases (1-3).The level of serum SAA of patients with infectious diseases and noninfectious inflammation was previously reported to be over 1,000 times higher than that of the noninflammatory groups (4). Moreover, SAA has been widely used to diagnose pediatric infectious diseases because it is a simple and rapid detection method, requiring only 40 L of fingertip blood. It has been previously reported that occasionally, SAA was not significantly increased in some types of acute inflammation (e.g., viral CNS infection) in which children may experience life-threatening events or severe sequela (5-11).Therefore, a retrospective case review was performed in this study to analyze the differences in SAA between two high risk pediatric diseases: viral CNS infection and KD (12-24).

2. Methods

2.1 Samples and Data

The SAA data contained 266 consecutive cases from January 2018 to October 2019 of the children aged from 1 months to 12 years, including 112 cases of viral CNS infection and 114 cases of KD. Table 1 lists the clinical characteristics of the patients in the two groups. The SAA were detected in the fingertip or venous blood of the children before they received regular treatment at the hospital. The composite cases

of the two diseases were excluded. The data included in the analysis were that recorded in the laboratory information system of Yu Huangding hospital.

SAA was tested using an Ottoman automatic protein analyzer, and the results were ensured by both daily internal and biannual external quality control. The normal range of serum SAA is 0 10 mg/mL, and the detection limit is between 4.8 mg/mL and 320 mg/mL due to an instrumental deficiency. Therefore, the SAA results below 4.8 mg/mL or above 320 mg/mL were recorded as 4.8 mg/mL or 320 mg/mL, respectively. The distribution of the data and proportion of the SAA blood concentration level are summarized in Table 2 and Fig.1.

2.2 Statistics

With the aid of IBMSPSS 24.0 (IBM corp, USA), the statistical analysis was conducted using a Kruskal-Wallis H-test (Table 3) and chi-square test (Tables 1 and 4), because the nonparametric tests were applicable for an analysis of non-normal distribution data of this thesis. A threshold of $p < 0.05$ was considered significant.

3. Results

3.1 Clinical characteristics of the patients

The sex, age, length of hospital stay, and the degree of infection were analyzed using a chi-square test (Table 1). There were no differences between the two groups regarding the sex, length of hospital stay, and degree of infection, with the exception of age. The 5 year 12 year group and the 1 year age groups had the highest disease incidence in the viral CNS and KD groups, respectively. The lowest disease incidence of both groups was in the 1 month age group ($P = 0.00 < 0.05$). This finding indicated that school-age children were more likely to infect each other.

3.2 Differences in the SAA concentration in the blood

The results of the pairwise comparisons via a Kruskal-Wallis H-test revealed that the concentration of SAA in the blood differed between the viral CNS infection and KD groups ($P < 0.05$) (Table 3).

3.3 Differences in the proportion of high level SAA cases

The results of the chi-square test presented in Table 4 demonstrated that the proportion of SAA increased with significant differences between the two groups. There was a lower proportion of patients in the viral CNS group with elevated SAA (SAA >10 mg/mL) compared to that in the KD group ($^2 = 43.4$; $P = 0.000 < 0.05$).

4. Discussion

SAA has been extensively studied over the past 50 years, since it was detected and characterized as an acute-phase reactant, and has become a common indicator for diagnosing infection and inflammation, especially in pediatric patients (1, 20). The SAA protein is widely expressed in many histologically normal human tissues and is predominantly localized to the epithelial components of tissues (e.g., brain neurons, breast lobule, and duct epithelium, thyroid follicular epithelium, pancreas glandular epithelium, kidney tubule epithelium, skin epidermis, as well as lymphocytes, plasma cells, and endothelial cells) (21).

CNS infection and KD are diseases that commonly feature febrile syndrome in children, and can lead to serious life-threatening complications. In addition, the two diseases often occur concurrently (e.g., meningitis can occur in conjunction with Kawasaki disease) (22-24).In this study, composite cases of the two diseases were excluded. A phenomenon was noted regarding the finding that the concentration of SAA significantly differed between children with KD and viral CNS infection. Moreover, there was a lower proportion of high-level SAA among the casesof viral CNS infection compared to that of the KD group. These results were verified with both a Kruskal-Wallis H-test and chi-square test.

These differences can be attributed to the fact that KD is an acute systemic pediatric vasculitis frequently caused by a virus, which may damage the coronary arteries, leading to a spectrum of injuries, including coronary artery aneurysms in the most severe cases(14).As a form of systemic inflammation, KD may lead to multiple organ damage and tends to involve the release of SAA from additional organs (15). In contrast, in the viral CNS infection cases, the pathogens are occasionally compartmentalized at the site of disease by BCB, which fails to stimulate the release SAA from other organs. This phenomenon was observed by Dieli et al., and termed the *sequestration effect*(25).The authors showed that polyclonal CD4 T cell lines from the peripheral blood failed to proliferate in a purified protein derivative (PPD)test and to the 16-or 38-kDa proteins of *Mycobacterium tuberculosis* for the *sequestration effect* by the BCB. In contrast, CD4 cell lines from the disease site responded to PPD, the 16-kDa and 38-kDa proteins, and derived epitopes in vitro. In our previous study, we reported that two cases of tuberculous meningitis with false negative results were due to the *sequestration effect*(26). Collectively, these may represent the reasons for both the lower SAA concentration and the lower proportion of high-level SAA cases within the viral CNS infection group compared to the KD group.

5. Conclusion

SAA is a valuable adjunct to the diagnosis of an infection with a poor performance of clinical signs or the presence of inflammation in conjunction with an unidentified pathogen. The concentration of SAA differed between children with KD and viral CNS infection and the high proportion of SAA in children with viral CNS infection was lower than that in the KD group. This difference may be due to the compartmentalization of BCB. Moreover, the epidemiological survey of SAA represents an important diagnostic aid. Together, while the above analysis provides theoretical support to explain this phenomenon, further experimentation is required to explain the source of these differences and elucidate the potential role of SAA as a biomarker of these infectious diseases in children.

Abbreviations

SAA Serum amyloid A; CNS central nervous system; KD Kawasaki's disease; BCB blood-cerebrospinal fluid barrier; CRP C-reactive protein; PCT procalcitonin; PPD purified protein derivative.

Declarations

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. All data generated or analysed during this study are included in this published article.

Authors' contributions

HY perception and design; drafting of the article; data analysis; final approval of the version to be published; RH language modification during writing and publishing of the whole article.

Ethics approval and consent to participate

Manuscripts was a retrospective case review, reporting studies without individual person's details, human tissue or animals. The data were summarized of the laboratory information system, sorted by EXCEL, and were checked repeatedly by the author. No patient privacy is involved, so that ethics approval and consent to participate not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Clinical characteristics of patients in the viral CNS and KD groups

Clinical parameter		CNS group(n=112)	KD group(n=116)	2	P
Gender	Male	65(58.04)	74(66.07)	1.13	0.34
	Female	47(41.96)	40(35.71)		
Age	1mo-	6(5.36)	28(25.00)	24.47	0.00
	1y-	42(37.50)	52(46.43)		
	5-12y	64(57.14)	34(30.36)		
Length of hospital stay,d	1–3	12(10.71)	5(4.46)	4.50	0.10
	4–7	49(43.75)	45(40.18)		
	7	51(45.54)	64(57.14)		
moderate cases		108(98.21)	112(101.79)	0.72	0.44
critical cases		3(0.89)	2(0.89)		
death cases		1(0.89)	0(0.00)		

Table 2. Data distribution of SAA for the three groups

Levels of SAA (mg/mL)	Cases	
	Viral CNS (% of category	KD (% of category
≤ 4.8*	39 (34.82)	6 (5.3)
4.9 ~ 10*	18 (16.07)	6 (5.3)
11 ~ 100	41(36.61)	28 (24.6)
101 ~ 200	8(7.14)	18 (15.8)
201 ~ 320	2(1.79)	27 (23.7)
> 320	4(3.57)	29 (25.4)
Total	112(100)	114 (100.0)

*The serum amyloid A (SAA) normal cases. CNS = viral CNS infection Group; KD = Kawasaki's Disease group.

Table 3: Kruskal-Wallis H-test pairwise comparisons and frequencies

Groups	Median	Percentiles		H	P
		25	75		
CNS	8.1	4.8	62.9	70.77	0.000
KD	189.8	62.0	320.0		

P values of groups: CNS-KD P = 0.000<0.05,

Significance values have been adjusted with a Bonferroni correction for multiple tests.

Table 4: Level group ^{2} testcross tabulation

			Groups		Total	2	P
			CNS	KD			
Level	H†	Count	55	102	157	43.40	0.00
		%within Group	49.10%	89.47%	69.47%		
	N†	Count	57	12	69		
		%within Group	50.90%	10.53%	30.53%		
Total		Count	112	114	226		
		%within Group	100.00%	100.00%	100.00%		

†The high-level [serum amyloid A (SAA) > 10 mg/mL] and the normal level (SAA ≤ 10 mg/mL).

Figures

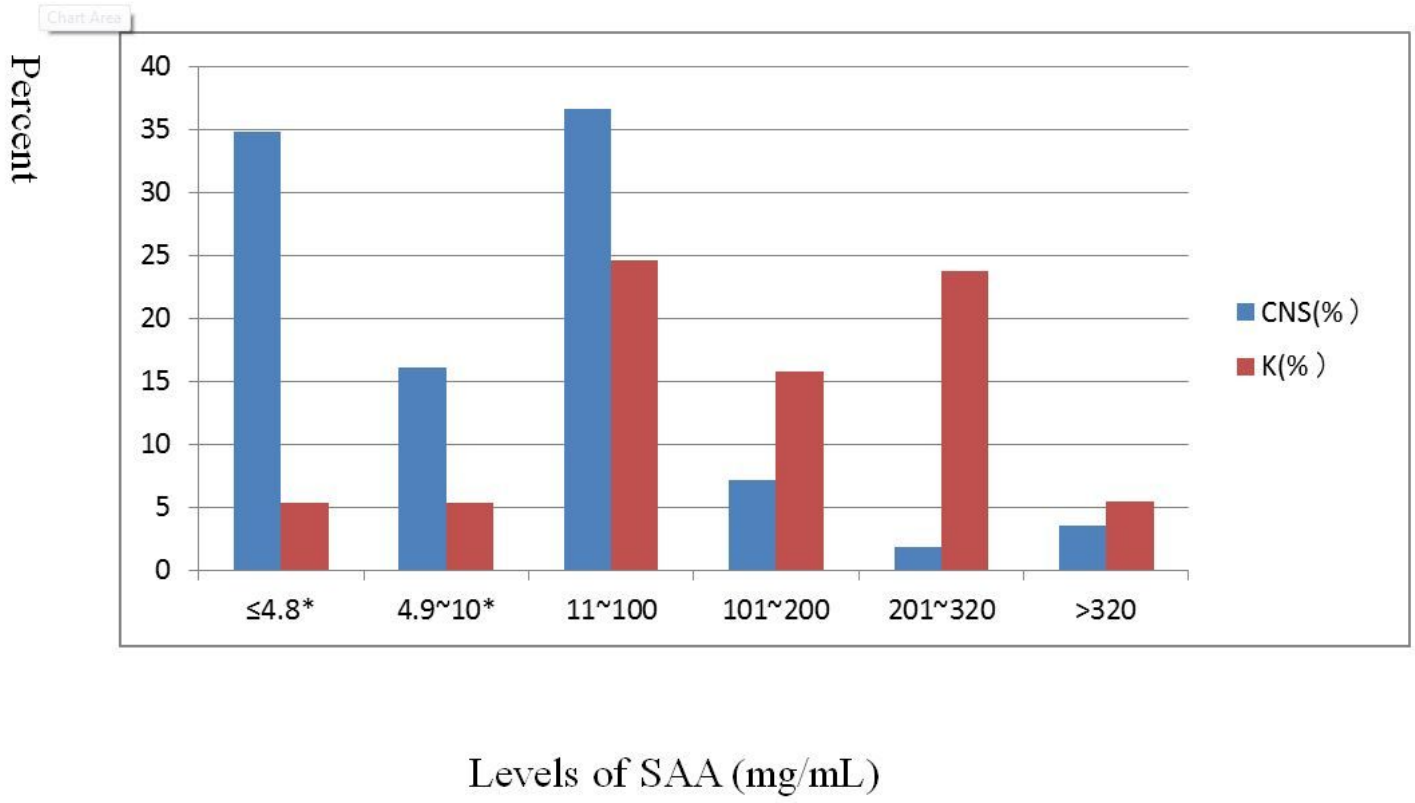

Figure 1

Serum amyloid A (SAA) concentration in the peripheral blood.