BENHA MEDICAL JOURNAL bmj
Print ISSN 1110-208X.
Online ISSN 2357-0016

Assessment of Serum Amyloid A in Healthy Children from 4 Years to 7 Years

Ghada S. Abd Elmotaleb [a], Amira O. Abd El-ghafar [b],
Saneya M. Abdallah [a], Enas M. Nor Eldeen [a]

[a] Pediatrics Department, Faculty of Medicine Benha University, Egypt.

[b] Clinical and Chemical Pathology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to:
Dr. Saneya M. Abdallah.
Pediatrics Department, Faculty of Medicine Benha University, Egypt.
Email: soso01021992@gmail.com

Received:28 August 2024
Accepted: 9 November 2024

Abstract:

Background: Serum amyloid A (SAA) is a major conserved and sensitive acute phase protein that is highly expressed in response to inflammation and tissue injury. This study aimed to determine the value of SAA detected by a sensitive and automatized ELISA method in healthy children from 4 to 7 years and to investigate the correlation between SAA and CRP. Methods: This cross-sectional study included 85 healthy children. All studied cases were subjected to demographic data collection and history taking, complete clinical examination, laboratory investigations; Complete blood count (CBC), Serum amyloid A (SAA), and C-reactive protein (CRP). Results: There was a positive correlation between SAA and CRP (r=0.901 and P value<0.001). There was a positive correlation between SAA and Platelets count (r=0.670 and P value<0.001). There was no significant correlation between serum amyloid A and demographic data. There was no significant correlation between serum amyloid A and (hemoglobin%, RBCs, WBCs, lymphocytes, and neutrophil count.

Conclusion: The positive SAA-CRP correlation suggests that the selection of the best test should be based on practical utility and/or clinical performance.

Keywords: Serum Amyloid A; Healthy children; C-Reactive Protein.

Introduction

Serum amyloid A (SAA) is a major conserved and sensitive acute-phase protein that is highly expressed in response to inflammation and tissue injury [(1)].

Related to this wide function, SAA showed a highly conserved sequence of both gene and protein structure in mammals as well as in birds and other animals [(2)].

In humans, several isoforms have been identified. SAA is present in the blood of healthy subjects at generally quite low levels, but during the acute-phase response (APR), SAA hepatic production leads to remarkably increased serum values within the initial 24 hrs with very lower levels after the acute phase [(3)].

SAA exerts also immunological activity by activating immune cells and inducing cytokines and chemokines [(4)]. High levels of SAA are associated with several chronic inflammatory diseases such as atherosclerosis, rheumatoid arthritis, psoriasis, Alzheimer's disease, and Crohn's disease, and may also be a potential biomarker of several malignancies [(5)]. However, the exact role of SAA in physiological and pathological settings is only partially understood [(2)].

Since very long, acute-phase reactants such as erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) have been widely known and used as the most reliable markers of acute-phase inflammation [(6)].

Indeed, SAA has been shown to be of better correlation than other inflammatory markers with disease activity in ankylosing spondylitis and juvenile idiopathic arthritis and worked better than CRP as an activity indicator also in Crohn's disease and ulcerative colitis [(7)]. In cases of viral infections, SAA levels raised more than CRP and this latter may revert to the basal level faster than SAA [(8)]. These findings showed that the SAA test could be recommended in conditions in which CRP showed a scarce response.

ELISA was expected to measure low levels of SAA because of its high sensitivity; however, no widely accepted cutoff was defined for some commercially available immunoassays [(7)]. Hence, the growing interest in more sensitive, rapid enough, and standardized assays to quantify SAA proteins, prompts us to determine the reference value of SAA detected by a sensitive and automatized ELISA method in healthy subjects and to investigate the correlation between SAA and CRP.

The purpose of this study was to determine the value of SAA in healthy children aged 4 years to 7 years and to investigate the correlation between SAA and CRP.

Subjects and methods

This cross-sectional study included 85 children from 5 schools and nurseries in Behira governorate for six months in the period between July 2023 to December 2023 after approval of the Faculty of Medicine Benha University ethical committee, Benha, Egypt (Approval code: MS 26-4-2023).

Informed written consent was obtained from all participants. Research results were only used for scientific purposes. Any unexpected risks appearing during the research were clarified to the participants and to the ethical committee on time.

Inclusion criteria were Their age ranged from 4 to 7 years healthy children and both sexes.

Exclusion criteria were inflammatory disorders, ongoing infections, obesity, use of any medication, heart, kidney, and liver diseases (clinically), and individuals who refused to take part in the study.

All studied cases were subjected to the following: history taking, including, Personal history (name, age, sex, file number, weight, height, and body mass index (BMI). History of any systemic disease. **Complete clinical examination. Laboratory investigations;** Complete blood count (CBC), Serum amyloid A (SAA), and C-reactive protein (CRP).

Collection of diagnostic blood specimens

Blood was drawn from children by a single, expert phlebotomist.

All subjects were respected at a 12-hour fast and remained seated for at least 15 min before phlebotomy so that possible interferences of blood distribution due to the posture could be prevented.

Blood samples (2ml) were collected by a 20 G straight needle directly into BD vacutainer plastic serum collection tubes (Becton, Dickinson and Company, NJ USA).

About 2ml blood sample was taken from each subject.

All blood collection steps were appropriately standardized (e.g., use of needles and vacuum tubes of the same lot).

The samples were then centrifuged at 2500 g for 15 min at room temperature within one hour of collection and the sera were separated and stored at -20°C until analysis.

Serum SAA (Human Serum Human Serum amyloid A (SAA)ELISA Kit, catalogue No. 201-12-1226), SUNRED®) concentrations were detected and quantified with a commercial solid-phase sandwich enzyme-linked immunosorbent assay used on an automated analyzer according to the manufacturer's protocol [(9)].

Serum concentrations of the classic acute-phase CRP were measured with the Beckman Coulter AU System CRP Latex reagent (Beckman Coulter, Inc., USA).

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the data distribution. Quantitative parametric data were presented as mean and standard deviation (SD) and compared between the two groups using an unpaired Student's T-test. Qualitative variables were presented as frequency and percentage (%). Correlation between various variables was done using the Pearson moment correlation equation for linear relation of normally distributed variables. A two-tailed P value < 0.05 was considered statistically significant.

Results

Table 1 shows demographic data, Serum amyloid A, and C-reactive protein of the studied patients.

Table 1: Demographic data, Serum amyloid A, laboratory finding of the studied children

		N = 85
Age (years)		5.4 ± 1.06
Sex	**Male**	42 (49.41%)
	Female	43 (50.59%)
Weight/centile (kg)		19.4 ± 2.78
Height/centile (m)		1.1 ± 0.09
BMI/ centile (kg/m^2)		15.89 ± 0.96
Serum amyloid A (ng/ml)		3.2 ± 1.07
C-reactive protein (mg/L)		2.7 ± 1.85
Complete blood count		
Hemoglobin (gm/dl)		11.3 ± 0.57
RBCs (10^6/mcL)		4.8 ± 0.41
WBC (*1000/ml)		7.8 ± 1.15
Platelet (*1000/ml)		179.6 ± 18.96
Lymphocytes (%)		30.7 ± 7.66
Neutrophil (%)		48.9 ± 9.15

Data represented as Mean ± SD, BMI: Body mass index.

There was no significant correlation between serum amyloid A and demographic data, and no significant correlation between serum amyloid A and (hemoglobin, RBCs, WBCs, lymphocytes, and neutrophil count). There was a positive correlation between SAA and platelets count (r=0.670 and P<0.001). Table 2

There was a positive correlation between SAA and CRP (r=0.901 and P<0.001). Table 3

There was no significant difference between both genders concerning serum amyloid A level. Table 4

Table 2: Correlation between SAA and (weight/centile, height/ centile, and BMI/ centile) and laboratory parameters

		Serum amyloid A (ng/ml)
Age (years)	r	-0.0137
	P value	0.9
Sex	r	-0.046
	P value	0.672
Weight/centile (kg)	r	-0.038
	P value	0.727
Height/centile (m)	r	-0.04
	P value	0.713
BMI/centile (kg/m^2)	r	0.021
	P value	0.845
		Serum amyloid A (ng/ml)
Hemoglobin (gm/dl)	r	0.04
	P value	0.712
RBCs (10^6/mcL)	r	0.135
	P value	0.216
WBC (*1000/ml)	r	-0.046
	P value	0.673
Platelets (*1000/ml)	r	0.670
	P value	<0.001*
Lymphocytes (%)	r	0.084
	P value	0.439
Neutrophil (%)	r	0.118
	P value	0.281

Significant p-value <0.05, BMI: Body mass index.

Table 3: Correlation between serum amyloid A and C-reactive protein of the studied patients

		SAA (ng/ml)
C-reactive protein (mg/L)	**r**	0.901
	P value	< 0.001*

*: statistically significant as P value <0.05, SAA: Serum amyloid A.

Table 4: Gender comparison of relative SAA values of the studied patients

	Male gender (n=42)	**Female gender (n=43)**	**P value**
SAA (ng/ml)	3.22 ± 1.16	3.17 ± 0.99	0.851

Discussion

This study demonstrated that serum amyloid A levels ranged from 1.6 to 5.58 ng/ml, with a mean (± SD) of 3.19 (±1.07) ng/ml. C-reactive protein ranged from 0.4 to 6 mg/L with a mean (± SD) of 2.7 (±1.85) mg/L.

Additionally, Fellahi et al. conducted a study for 5 years (2014-2018), based on data from the population reference interval (SAA ≤ 6.4 mg/L) given by the manufacturer of the reagent when paired with CRP [(10)].

On the contrary, Ichihara et al. surveyed the SAA reference interval and showed that the upper limit of the SAA reference interval was higher than that of this study for either Asian or Chinese populations. The reasons for these differences may be as follows: (a) different detection methods; SAA was mainly detected by immunoturbidimetry, enzyme-linked immunosorbent assay, or microsphere capture enzyme immunoassay; (b) there were some differences in population characteristics and regional characteristics, such as race, environment, and living habits [(11)].

In this study, we found that there was no correlation between serum amyloid A and demographic data.

In agreement with our results, Carbone et al. showed no statistically significant differences when the subject ages were considered (P > .05) [(12)].

Our results are supported by Liu et al. who showed that there was no significant difference in the level of serum SAA among healthy children in terms of age (*P*> .05) [(13)].

This study demonstrated that there was a positive correlation between SAA and platelet count in CBC (r=-0.213 and P value=0.049).

Both SAA and platelets are key players in the acute-phase response to inflammation. Elevated SAA can interact with platelets, enhancing their activation and aggregation, which can contribute to the inflammatory process [(14)].

Siman-Tov et al. found that there was a negative correlation between SAA and platelets count (p <0.05) [(15)].

In this study, we illustrated that there was a positive correlation between SAA and CRP (r=0.901 and P<0.001).

In the same line, Carbone et al. found that the correlation between SAA concentration and CRP level was investigated, and a positive correlation was found, suggesting a good concordance between the two laboratory parameters [(12)].

This agrees with Higazi et al, who demonstrated a statistically significant positive correlation between SAA and CRP [(16)].

In this study, we found that there was no significant difference between both genders.

This came in line with Carbone et al. who found that there was no difference found when stratified by gender, indicating that gender-partitioned reference values are not indicated for this analyte [(12)].

Conclusion

In this study, we obtained a SAA reference value which ranges from 1.6 ng/ml to 5.58 ng/ml. The positive SAA-CRP correlation suggests that the selection of the best test should be based on practical utility and/or clinical performance.

References

1. Webb NR. High-Density Lipoproteins and Serum Amyloid A (SAA).Curr Atheroscler Rep.2021;23:7-9.
2. Sack GH, Jr. Serum amyloid A - a review.Mol Med.2018;24:46-9.
3. Li H, Xiang X, Ren H, Xu L, Zhao L, Chen X, et al. Serum Amyloid A is a biomarker of severe Coronavirus Disease and poor prognosis.J Infect.2020;80:646-55.
4. Lin Y-k, Zhu P, Wang W-s, Sun K. Serum amyloid A, a host-derived DAMP in pregnancy?Front immunol.2022;13:929-32.
5. Zhou J, Dai Y, Lin Y, Chen K. Association between serum amyloid A and rheumatoid arthritis: A systematic review and meta-analysis.Semin Arthritis Rheum.2022;52:943-5.
6. Keykavousi K, Nourbakhsh F, Abdollahpour N, Fazeli F, Sedaghat A, Soheili V, et al. A

Review of Routine Laboratory Biomarkers for the Detection of Severe COVID-19 Disease.Int J Anal Chem.2022;2022:1-14.
7. Carbone T, Pafundi V, Schievano C, Assunta D, Padula MC, Giordano M, et al. Serum amyloid A in healthy subjects: assessment of reference value using ELISA method.J Immunoassay Immunochem.2021;42:129-37.
8. Chen M, Wu Y, Jia W, Yin M, Hu Z, Wang R, et al. The predictive value of serum amyloid A and C-reactive protein levels for the severity of coronavirus disease 2019.Am J Transl Res.2020;12:4569-75.
9. Sipe JD, Gonnerman WA, Loose LD, Knapschaefer G, Xie W-J, Franzblau C. Direct binding enzyme-linked immunosorbent assay (ELISA) for serum amyloid A (SAA).Journal of immunological methods.1989;125:125-35.
10. Fellahi S, Béraud L, Capeau J, Grateau G, Hentgen V, Georgin-Lavialle S, et al. [Validation of the usual values provided by a supplier without access to a control population: example of serum amyloid A].Ann Biol Clin (Paris).2019;77:255-9.
11. Ichihara K, Ceriotti F, Kazuo M, Huang YY, Shimizu Y, Suzuki H, et al. The Asian project for collaborative derivation of reference intervals: (2) results of non-standardized analytes and transference of reference intervals to the participating laboratories on the basis of cross-comparison of test results.Clin Chem Lab Med.2013;51:1443-57.
12. Carbone T, Pafundi V, Schievano C, Assunta D, Padula MC, Giordano M, et al. Serum amyloid A in healthy subjects: assessment of reference value using ELISA method.J Immunoassay Immunochem.2021;42:129-37.
13. Liu Q, Li Y, Yang F, Xu T, Yao L, Sun J, et al. Distribution of serum amyloid A and establishment of reference intervals in healthy adults.J Clin Lab Anal.2020;34:e23120.
14. England BR, Tiong BK, Bergman MJ, Curtis JR, Kazi S, Mikuls TR, et al. 2019 Update of the American College of Rheumatology Recommended Rheumatoid Arthritis Disease Activity Measures.Arthritis Care Res (Hoboken).2019;71:1540-55.
15. Siman-Tov R, Shalabi R, Shlomai A, Goldberg E, Essa W, Shusterman E, et al. Elevated Serum Amyloid A Levels Contribute to Increased Platelet Adhesion in COVID-19 Patients.Int J Mol Sci.2022;23.
16. Higazi A, Higazi A, Mohamed D, Mahrous, Sayed S, Mohamed O, et al. Assessment of Urinary Interleukin-18 and Serum Amyloid A Efficacies against C-Reactive Protein in Diagnosis and Follow-up of Neonatal Sepsis.Journal of Clinical & Cellular Immunology.2016;7.

To cite this article: Ghada S. Abd Elmotaleb , Amira O. Abd El-ghafar , Saneya M. Abdallah , Enas M. Nor Eldeen . Assessment of Serum Amyloid A in Healthy Children from 4 Years to 7 Years. BMFJ 2025;42(1):302-308.