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Elevated Serum Amyloid A is Associated with Colorectal Carcinoma Invasiveness

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Abstract

Searching for clinical sensible biomarkers that can be served as an alternative tool for colorectal cancer (CRC) early diagnosis and prognosis is still a great challenge. This study aimed to evaluate serum amyloid A (SAA) potential role in CRC diagnosis and prediction of poor disease progression. Results revealed that elevated SAA was significantly (P<0.0001) associated with CRC (33.15 (30.1-35.5) ng/mL) compared to patients with benign polyps (14.2 (10.8-19.7) ng/mL) and healthy (5.0 (3.2-7.0) ng/mL) controls. It had a great ability (AUC=0.995) to differentiate CRC patients from all non-cancer individuals. SAA levels was significantly (P<0.05) impacted on CRC invasiveness as its elevated levels were associated with including tumor late stages, lymph node invasion, distant metastasis, high grades and large tumor size. Moreover, SAA was significantly correlated with CEA (r=0.644; P=0.0001) in CRC patients. In conclusion, SAA protein appears to be a reliable marker for CRC, which could be recommended for initial differentiation of CRC from benign polyps. It also could act in clinical routine as a non-specific tumor marker for monitoring CRC progression to prevent poor outcomes.

Key words: Colorectal carcinoma; Serum Amyloid A; Biomarker; Severity; Inflammation

Introduction

Worldwide, colorectal cancer (CRC) is one of the most frequent digestive disorders [1]. In terms of mortality and incidence, it has steadily ascended to the top 3 tumors [2]. CRC prognosis varies according to the stage at diagnosis. Compared to late-stage tumors, early-stage tumors have greater overall survival rates [3]. For improving quality of life and survival rates, timely diagnosis, regular follow-up care and suitable therapy are important [3].

Despite progress made in management and detection and the novel treatment options that improved disease survival, metastatic CRC continues to have poor long-term prognosis and significant challenges still due to treatment failure and advanced stage diagnosis [4]. Thus, evaluating and searching for alternative optimal techniques

or more sensitive markers to assess tumor proliferation with potential prognostic significance seems very important [5].

A highly sensitive acute phase reactant is serum amyloid A (SAA) that is a family of proteins and is implicated and associated with a number of chronic inflammatory disorders including inflammatory bowel disease (IBD) [6, 7]. Besides IBD, these proteins also are related to other inflammation-related pathologies such as amyloidosis, rheumatoid arthritis, Crohn's disease and diabetes mellitus [7]. Moreover, many reports also have demonstrated elevated SAA tissue expression and serum levels in various tumors, including CRC [8], pancreatic [9], breast [10] and lung [11] cancers. Although such mechanistic studies remain very limited, reports suggesting the possibility of SAA direct role in malignancy have suggested that SAA can promote tumor metastasis [12, 13]. In pre-operative CRC stage, a very interesting study of Glojnarić et al. reported that SAA showed the most powerful reaction compared to other acute phase reactants [14]. Also, they found that mean SAA levels were declining during the post-operative clinical course, but never returned to the normal values [14].

This study aimed to evaluate the potential role of SAA levels as a reliable biomarker and parameter for CRC diagnosis and prediction of poor disease progression. We evaluated the association of SAA with tumor severity including advanced stages, lymph node invasion, large tumor size, high histological grades, distant metastasis and elevated levels of established tumor markers carcinoembryonic antigen (CEA).

Material and methods

Patients

Serum samples from 125 Egyptian participants (70 CRC patients, 25 cases with benign colon diseases and 30 healthy controls) were collected. CRC patients were gender- and

age-matched with benign and healthy controls. All cases were recruited from Mansoura Oncology center, Mansoura University hospital, Egypt. Diagnosis of CRC and benign disorders was based on colonoscopy. Cancer features were registered
based on the international Tumor-Node-Metastasis (TNM) classification system [15]. There were no one of healthy individuals or cases with benign disorder had a history of any tumors.

Samples collection and laboratory tests

After centrifugation (4000 rpm, 20 minutes), serum samples were obtained from blood samples collected from all participants. Fresh serum was used for testing alanine and aspartate aminotransferase (ALT and AST), total bilirubin, albumin, urea and creatinine using commercial kits and automated closed biochemistry analyzer (Hitachi, Tokyo, Japan). EDTA-K3 treated blood was used for complete blood count in an automated analyzer (Sysmex, Japan). By commercial ELISA assay kits, SAA (Bioneovan, Beijing, China) and CEA (MyBioSource, San Diego, USA) according to the industrial prescript.

Statistical analysis

Variables were expressed as mean±SD, median (interquartile range) or absolute numbers, appropriately. All analyses were carried out using SPSS version 20 and GraphPad version 6.0. *ANOVA* and *t-test* or the *Kruskal-Wallis* tests were used to evaluate differences in cases of parametric and nonparametric variables, respectively. $P<0.05$ is defined as significant. SAA diagnostic utility was evaluated using area under the receiver operating characteristic (ROC) curve. Correlation between CEA and SAA was assessed by Pearson correlation coefficient.

Results

Patients' characteristics

Healthy controls were age- and sex-matched to CRC cases and patients with benign diseases (P>0.05). The patient-related clinical and hematological parameters are summarized in Table 1. Due to the exclusion of any cases with other chronic diseases, there was no significant difference (P>0.05) between CRC patients and controls in hematological, liver functions and kidney functions related parameters. CRC patients were associated with significant (P<0.016) increased CEA levels (Table 1). All CRC patients were staged and classified according to the TNM staging system, tumor differentiation degree and tumor size, as illustrated in Table 2.

Table 1. Clinical characteristics of colorectal cancer patients and controls

Variables	Colorectal cancer	Benign	Healthy	*P* value
Number	70	25	30	—
Gender (males/females)	49/21	18/7	20/10	0.314
Mean age ±SD, years	51.4±12.2	49.1±10.4	47.8±5.0	0.323
Hemoglobin (g/dL)	11.52±1.8	11.51±2.13	11.65±1.81	0.240
RBCs ($\times 10^{12}$/L)	4.13±0.72	4.31±0.55	4.51±0.60	0.633
WBCs ($\times 10^{9}$/L)	8.1±3.2	7.9±1.8	6.99±1.6	0.201
Platelet count ($\times 10^{9}$/L)	240.2±51.1	255±65.1	270±61.6	0.193
ALT (U/L)	26.7±10.1	24.5±4.2	25.2±8.1	0.426
AST(U/L)	46.15±10.1	42.11±8.5	37.13±7.3	0.096
Bilirubin (mg/dL)	0.8±0.21	0.71±0.15	0.66±0.19	0.591
Albumin (g/dL)	3.73±0.41	3.92±0.44	4.1±0.35	0.123
Creatinine (mg/dL)	0.88±0.28	0.78±0.15	0.76±0.13	0.398
Urea (mg/dL)	27.12±5.30	24.8±5.9	23.6±4.7	0.621
CEA (U/L)	3.66 (2.0-23.0)	2 (1.1-4.6)	2 (1-3.6)	0.016

Normally and non-normally distributed data were expressed as mean± SD and median (interquartile range), respectively. RBC: red blood cell; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CEA: carcinoembryonic antigen. Significant differences were determined using *ANOVA* and *Kruskal-Wallis* test, appropriately. $P<0.05$ was significant.

Table 2. Classification of colorectal cancer patients

Clinicopathological features	Number (%)
Primary tumor stage	
Early stage (T1–T2)	30 (42.9%)
Late stage (T3–T4)	40 (57.1%)
Lymph node invasion	
Negative (N0)	21 (30%)
Positive (N1)	49 (70%)
Metastasis	
Negative (M0)	51 (72.9%)
Positive (M1)	19 (27.1%)
Histological grade	
Low grade (G1–G2)	29 (41.4%)
High grade (G3)	41 (58.6%)
Tumor size	
Small (≤ 5 cm)	30 (42.9%)
Large (>5 cm)	40 (57.1%)

Elevated SAA was associated with tumor development and severity

Elevated SAA was related to CRC development (Figure 1A), as CRC patients (33.15 (30.1-35.5) ng/mL) were associated ($P<0.0001$) with high SAA levels compared to patients with benign polyps (14.2 (10.8-19.7) ng/mL) and healthy (5.0 (3.2-7.0) ng/mL) controls. SAA had great ability (AUC=0.995) to differentiate CRC patients from all non-cancer individuals (Figure 1B).

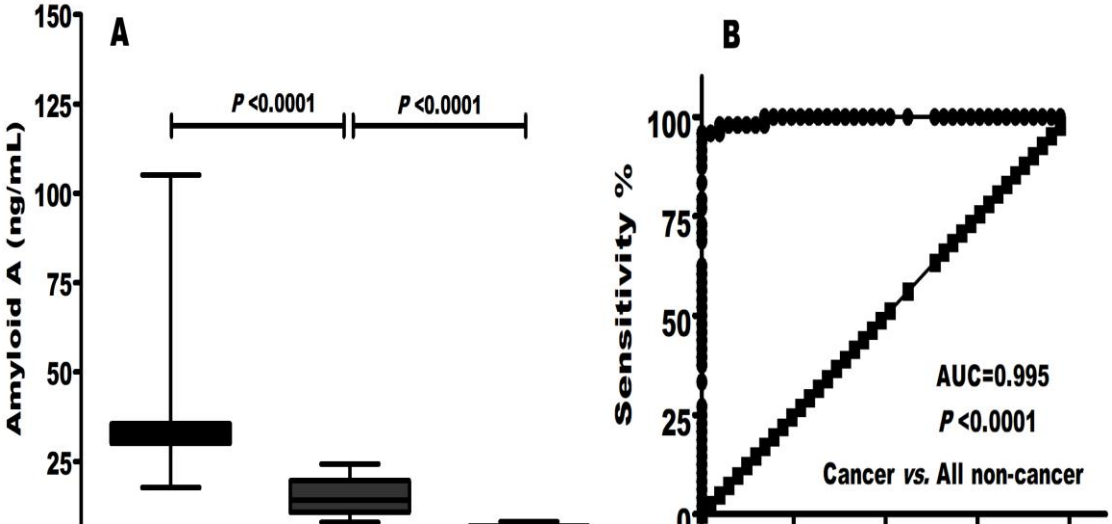

Figure 1. Serum Amyloid A in colorectal cancer. (A) Elevated levels of SAA were significantly associated with CRC patients compared to cases with benign polyps and healthy individuals. (B) ROC curve analysis revealed the great diagnostic power of SAA in differentiating CRC from all non-cancer individuals (benign and healthy combined).

In CRC patients, SAA levels (ng/mL) was significantly (P<0.05) affected the disease progression including tumor late stages (34.7 (32.0-47.8)), lymph node invasion (33.9 (32.1-42.5)), distant metastasis (34.2 (33.1-66.2)), high grades (40.2 (33.5-66.2)) and large size (34.1 (30.1-47.8)) (Table 3). Moreover, SAA was significantly correlated with CEA (r=0.644; P=0.0001; Figure 2) in CRC patients.

Table 3. Impact of SAA levels on CRC progression. Data were expressed as median (inter quartile range).

Categories	**Amyloid A (ng/mL)**	***P* value**
Primary tumor stage		
Early stage (T1-T2)	31.2 (29.6-33.4)	0.0010
Late stage (T3-T4)	34.7 (32.0-47.8)	
Lymph node invasion		
Negative (N0)	30.5 (29.2-34.1)	0.0045
Present (N1)	33.9 (32.1-42.5)	
Metastasis		
Negative (M0)	31.6 (29.8-34.0)	0.0105
Present (M1)	34.2 (33.1-66.2)	
Tumor histological grade		
Low grade (G1-G2)	31.6 (29.8-34.0)	0.0009

High grade (G3)	40.2 (33.5-66.2)	
Tumor size		
Small (≤ 5 cm)	31.8 (30.0-33.9)	0.0401
Large (>5 cm)	34.1 (30.1-47.8)	

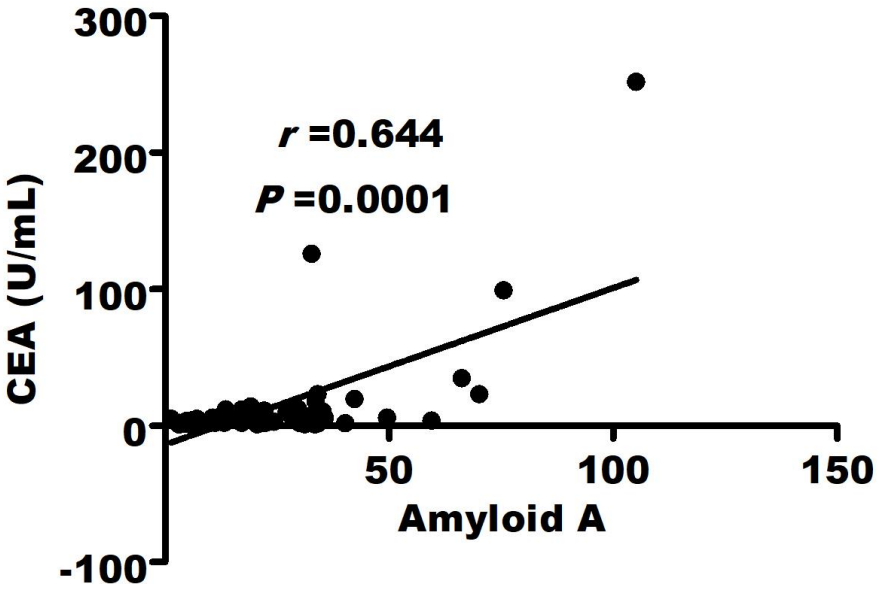

Figure 2. Correlation between serum amyloid A and CEA serum levels in colorectal cancer patients.

Discussion

It was recently reported that most of the CRCs start from non-cancerous or benign form, called polyps [16]. If these polyps are diagnosed at an early stage and removed from the body, it can prevent CRC development [16]. Moreover, CRC prognosis is stage dependent and disease early-stages have higher overall survival compared to advanced stages [3]. Thus, it is precisely important to evaluate alternative biomarkers, particularly blood markers, to detect CRC at an early stage and also to evaluate their association with tumor severity to prevent poor progression of the disease [17]. As a reliable biomarker, this study revealed the potential role of SAA levels in CRC early

differentiation from benign colonic diseases and its role in prediction of poor disease progression.

Our results showed that elevated SAA (ng/mL) was significantly (P<0.0001) related to CRC (33.15 (30.1-35.5)) development compared to patients with benign polyps (14.2 (10.8-19.7) and healthy (5.0 (3.2-7.0)) controls. It had great power (AUC=0.995) to differentiate CRC patients. Moreover, great SAA levels significantly (P<0.05) affected CRC severity including tumor late stages (34.7 (32.0-47.8)), lymph node invasion (33.9 (32.1-42.5)), distant metastasis (34.2 (33.1-66.2)), high grades (40.2 (33.5-66.2)) and large size (34.1 (30.1-47.8)). Moreover in CRC patients, SAA was significantly correlated with CEA (r=0.644; P=0.0001).

SAA persistent activation is widely related to inflammation-related pathologies, including IBD such as Crohn's disease and ulcerative colitis [18, 19]. Furthermore, many reports also have demonstrated elevated SAA tissue expression and serum levels in many solid tumors [20], including CRC [8], pancreatic [9], breast [10] and lung [11] cancers. In pre-operative CRC stage and compared to other major acute phase reactants: alpha1-acid glycoprotein, alpha1-antichymotrypsin and C-reactive protein, SAA protein exhibited the most powerful reaction [14]. During the post-operative clinical course, SAA protein concentration was declining until the 6th chemotherapy cycle [14]. Among all mentioned acute phase proteins, SAA showed the best specificity for CRC (83-100%) [14].

In a trial to unravel molecular mechanism underlying early CRCs invasiveness, Sudo et al., using both RNA-sequencing and immunohistochemical analysis, recently found a significant upregulation of SAA in poorly differentiated components (PORs) isolated from T1 CRC tissues and at the invasive front of T1b CRCs [21]. In CRC cells,

SAA upregulation promoted cell invasion and migration [21]. By coculture experiments using CRC cell lines, they demonstrated that SAA expression is induced by interleukin (IL)-1β generated from tumor-associated macrophages [21]. In another important study for the same authors, they found using immunohistochemical analysis neutrophils accumulation at the SAA-positive invasive front of T1 CRCs. This induced neutrophil migration and expression of matrix metalloproteinase-9 (MMP-9) and chemokine CXC motif ligand 8 (CXCL8) in neutrophils and consequently promote CRC cell invasion and migration. Immunohistochemistry confirmed accumulation of CXCL8- or MMP-9-positive neutrophils at the SAA1-positive invasive front of T1 CRCs [22].

In consistent of these findings, in a mouse model of colitis-related tumor, Davis et al. reported that SAA stimulates inflammation-related damage and tumorigenesis [7]. In mice deficient for SAA, they reported attenuated disease activity as confirmed by declined colitis-associated tissue damage, reduced rectal bleeding, elevated stool consistency and reduced weight loss [7]. In the distal colon of SAA knockout mice, levels of IL 4 and 10 and tumor necrosis factor-α were reduced and macrophage infiltration was attenuated [7]. These models also showed a reduced tumor burden, and cancers were reported to have reduced proliferation markers and elevated apoptotic activity [7]. Similar to our findings, former studies reported significant correlation between SAA and CEA in with sever COVID-19 [23] and gastric cancer [24].

Conclusions

Although SAA is not a tumor-specific biomarker, our obtained results revealed that SAA has a potential power in the early initial differentiating CRC from benign polyps. Thus in a case with negative other available markers (such as CEA), it could be helpful

and may be added to a panel of other biomarkers to increase the diagnostic ability. Its elevated levels were associated with CRC severity and thus could be helpful in preventing poor disease progression.

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Disclosure statement

None

Funding

None

Data availability

Data is available via request the corresponding author

Ethics approval

The study protocol was approved by the ethics and scientific committees of Faculty of Medicine, Port Said University and was in accordance with the ethical guidelines of the "Helsinki Declaration". Informed consent was get from each participant to involve in this work.

References

1. Roshandel G, Ghasemi-Kebria F, Malekzadeh R, Colorectal Cancer: Epidemiology, Risk Factors, and Prevention. Cancers (Basel) 2024; 16(8):1530.

2. Zhang Y, Wang Y, Zhang B, Li P, Zhao Y, Methods and biomarkers for early detection, prediction, and diagnosis of colorectal cancer. Biomed Pharmacother 2023; 163:114786.
3. Escrich V, Romero-Aranda C, López R, de Toro M, Metola Á, Ezcurra B, et al., Unprocessed snRNAs Are a Prognostic Biomarker and Correlate with a Poorer Prognosis in Colorectal Cancer. Cancers (Basel) 2024; 16(13):2340.
4. Ogunwobi OO, Mahmood F, Akingboye A, Biomarkers in Colorectal Cancer: Current Research and Future Prospects. Int J Mol Sci 2020; 21(15):5311.
5. Kasprzak A, Prognostic Biomarkers of Cell Proliferation in Colorectal Cancer (CRC): From Immunohistochemistry to Molecular Biology Techniques. Cancers (Basel) 2023; 15(18):4570.
6. Chen R, Chen Q, Zheng J, Zeng Z, Chen M, Li L, et al., Serum amyloid protein A in inflammatory bowel disease: from bench to bedside. Cell Death Discov 2023; 9(1):154.
7. Davis TA, Conradie D, Shridas P, de Beer FC, Engelbrecht AM, de Villiers WJS, Serum Amyloid A Promotes Inflammation-Associated Damage and Tumorigenesis in a Mouse Model of Colitis-Associated Cancer. Cell Mol Gastroenterol Hepatol 2021; 12(4):1329-1341.
8. Gutfeld O, Prus D, Ackerman Z, Dishon S, Linke RP, Levin M, et al., Expression of serum amyloid A, in normal, dysplastic, and neoplastic human colonic mucosa: implication for a role in colonic tumorigenesis. J Histochem Cytochem 2006; 54(1):63-73.
9. Djurec M, Graña O, Lee A, Troulé K, Espinet E, Cabras L, et al., Saa3 is a key mediator of the protumorigenic properties of cancer-associated fibroblasts in pancreatic tumors. Proc Natl Acad Sci U S A 2018; 115(6):1147-1156.

10. Yang M, Liu F, Higuchi K, Sawashita J, Fu X, Zhang L, et al., Serum amyloid A expression in the breast cancer tissue is associated with poor prognosis. Oncotarget 2016; 7(24):35843-35852.

11. Cho WC, Yip TT, Cheng WW, Au JS, Serum amyloid A is elevated in the serum of lung cancer patients with poor prognosis. Br J Cancer 2010; 102(12):1731-1735.

12. Lee JW, Stone ML, Porrett PM, Thomas SK, Komar CA, Li JH, et al., Hepatocytes direct the formation of a pro-metastatic niche in the liver. Nature 2019; 567(7747):249-252.

13. Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, et al., The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. Nat Cell Biol 2008; 10(11):1349-1355.

14. Glojnarić I, Casl MT, Simić D, Lukac J, Serum amyloid A protein (SAA) in colorectal carcinoma. Clin Chem Lab Med 2001; 39(2):129-133.

15. Greene FL, TNM staging for malignancies of the digestive tract: 2003 changes and beyond. Semin Surg Oncol 2003; 21(1):23-29.

16. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi J, John A, et al., Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. Cancers (Basel) 2022; 14(7):1732.

17. Das V, Kalita J, Pal M, Predictive and prognostic biomarkers in colorectal cancer: A systematic review of recent advances and challenges. Biomed Pharmacother 2017; 87:8-19.

18. Yarur AJ, Quintero MA, Jain A, Czul F, Barkin JS, Abreu MT, Serum Amyloid A as a Surrogate Marker for Mucosal and Histologic Inflammation in Patients with Crohn's Disease. Inflamm Bowel Dis 2017; 23(1):158-164.

19. Niederau C, Backmerhoff F, Schumacher B, Niederau C, Inflammatory mediators and acute phase proteins in patients with Crohn's disease and ulcerative colitis. Hepatogastroenterology 1997; 44(13):90-107.

20. Lin HY, Tan GQ, Liu Y, Lin SQ, The prognostic value of serum amyloid A in solid tumors: a meta-analysis. Cancer Cell Int 2019; 19:62.

21. Sudo G, Aoki H, Yamamoto E, Takasawa A, Niinuma T, Yoshido A, et al., Activated macrophages promote invasion by early colorectal cancer via an interleukin 1β-serum amyloid A1 axis. Cancer Sci 2021; 112(10):4151-4165.

22. Yoshido A, Sudo G, Takasawa A, Aoki H, Kitajima H, Yamamoto E, et al., Serum amyloid A1 recruits neutrophils to the invasive front of T1 colorectal cancers. J Gastroenterol Hepatol 2023; 38(2):301-310.

23. Abdelhakam DA, Badr FM, Abd El Monem Teama M, Bahig Elmihi NM, El-Mohamdy MA, Serum amyloid A, ferritin and carcinoembryonic antigen as biomarkers of severity in patients with COVID-19. Biomed Rep 2022; 16(2):13.

24. Hou Y, Zhao W, Yang Z, Zhang B, Serum amyloid A (SAA) and Interleukin-6 (IL-6) as the potential biomarkers for gastric cancer. Medicine (Baltimore) 2022; 101(43):e31514.