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Nucleosome assembly protein 1-like 1 (NAP1L1) in colon cancer patients: a potential biomarker with diagnostic and prognostic utility

M.A. AYDIN[1], G. GUL[2], R. KIZILTAN[3], S. ALGUL[4], O. KEMIK[3]

[1]Department of General Surgery, Altinbas University Faculty of Medicine Medical Park Bahcelievler Hospital, Istanbul, Turkey
[2]Clinic of General Surgery, Private Goztepe Hospital, Istanbul, Turkey
[3]Department of General Surgery Yuzuncu Yil University Faculty of Medicine, Van, Turkey
[4]Department of Physiology, Yuzuncu Yil University Faculty of Medicine, Van, Turkey

Abstract. – OBJECTIVE: To evaluate the diagnostic and prognostic utility of nucleosome assembly protein 1-like 1 (NAP1L1) in colon cancer patients.

PATIENTS AND METHODS: A total of 95 patients with colon cancer [mean (SD) age: 61.0 (1.7) years, 58.9% were males] and 50 healthy individuals [mean (SD) age: 61.0 (2.3) years, 52.0% were males] were included in this prospective multicenter study. Data on patient demographics (age, gender) and serum NAP1L1 levels were recorded in both control and colon cancer groups. In colon cancer patients, serum NAP1L1 levels were further analyzed with respect to TNM stages and tumor size.

RESULTS: Serum NAP1L1 levels were significantly higher in colon cancer patients as compared with control subjects [median (min-max) 14(12-16) *vs.* 2(1-2) ng/mL, $p<0.001$]. In colon cancer patients, serum NAP1L1 levels were significantly higher for tumor size of >4 cm *vs.* <4 cm [15(12-16) *vs.* 12(12-14) ng/mL), $p<0.001$] and for M1 *vs.* M0 stage [15(12-16) *vs.* 12(12-14) ng/mL), $p<0.001$]. Serum NAP1L1 levels were significantly higher in T4 stage tumors *vs.* T1, T2 and T3 stage tumors ($p<0.001$ for each), in T3 stage tumors *vs.* T1 and T2 stage tumors ($p<0.001$ for each) and in N2 stage tumors *vs.* N0 and N1 stage tumors ($p<0.001$ for each).

CONCLUSIONS: Our findings revealed for the first time the substantial rise in serum NAP1L1 levels among colon cancer patients as compared to controls and as correlated with the disease progression. Accordingly, NAP1L1 seems to be a potential biomarker for colon cancer, offering clinically important information on early diagnosis and risk stratification.

Key Words:
Colon cancer, NAP1L1, Marker, Early diagnosis, Risk stratification, Stage, Controls.

Introduction

Colon cancer is a common and highly fatal malignancy worldwide[1,2]. A mass screening of average-risk adults over age 50 for colon cancer is recommended due to asymptomatic course of early stage disease and the premalignant adenomatous polyps[1-3]. Accordingly, risk stratification is considered crucial in the management of colon cancer, to be able to implement therapeutic strategies tailored to select patients[4]. Exploring drivers of early-stage disease through high-predictive markers for the early recognition of colon cancer has therefore become increasingly addressed by substantial clinical investigation[4,5].

The colon cancer diagnosis is based on blood-test profiles for diagnostic biomarkers (i.e., serum cancer antigens, circulating tumor cells), colonoscopy, and barium meal imaging[6-10]. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are the most widely used cancer antigen biomarkers for monitoring colon cancer[11,12]. Circulating tumor cells originate from primary or metastatic tumor sites that can directly constitute the state of the disease, whereas the number of circulating tumor cells is very low[13,14]. DNA-based and RNA-based molecular methods are more sensitive than circulating tumor cells for the investigation of molecular heterogeneity and clonal disagreement, whereas they have limited use in clinical practice[15]. Although colonoscopy is the mainstay in diagnosis and monitoring of colon cancer, the patient discomfort during procedure limits its early diagnostic value[16]. Hence, there is a prompt need for a reliable and stable biomarker of prognostic value in the early diagnosis of colon cancer patients.

Corresponding Author: Mehmet Akif Aydin, MD; e-mail: drakifaydin@hotmail.com

NAP1L1 (nucleosome assembly protein 1-like 1), a member of NAP1L family, is a highly conserved protein involved in nucleosome assembly, cell cycle progression and cell proliferation.[17,18]Although it is expressed ubiquitously in most human tissues and cell lines, the increased levels are commonly detected in rapidly proliferating cells[17-20]. Oncogenic role of NAP1L1 has been suggested in several tumors based on its overexpression[21-24], while its diagnostic and prognostic role in colon cancer remains unclarified[25,26].

This study was therefore designed to evaluate the diagnostic and prognostic potential of NAP1L1 in colon cancer patients by analysis of serum levels in comparison to control subjects and in relation to clinicopathological variables.

Patients and Methods

Study Population

A total of 95 patients with newly-diagnosed colon cancer (56 men and 39 women; mean age, 61±1.7 years) and 50 healthy individuals (26 men and 24 women; mean age, 61±2.3 years) were included in this cross-sectional multicenter study. Colon cancer was diagnosed and staged using the American Joint Commission on Cancer (AJCC) TNM system[27]. Patients with other malignancies, active infection and connective tissue diseases and patients who received adjuvant/neoadjuvant radiotherapy or chemotherapy before surgery were excluded from the study. Control group consisted of age-matched and gender-matched individuals without clinical and laboratory signs of any malignant or inflammatory disease.

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the Ethical Principles stated in the "Declaration of Helsinki" and approved by the Institutional Ethics Committee.

Assessments

Data on patient demographics (age, gender) and serum NAP1L1 levels were recorded in both control and colon cancer groups. In colon cancer patients, serum NAP1L1 levels were further analyzed with respect to TNM stages and tumor size. Colon cancer staging was based on TNM staging system, using standard radiological examinations: chest x-ray, ultrasound (US) and computed tomography (CT) of the abdomen and, if necessary, other radiological imaging. In patients who underwent surgery, the pathological diagnosis was performed.

Serum NAP1L1 Analysis

Blood samples (3 mL) were collected using blood collection tube (Blood Collection Tubes with Polymer Gel for serum Separation), and then, immediately transported to the laboratory for centrifugation at 4°C for 20 min at 1000 g. The 1.5 mL supernatant was transferred into micro-tube and kept at -80°C until analysis.

Serum NAP1L1 levels were measured using an enzyme linked immunosorbent assay kit (ELISA) for *in vitro* quantitative measurement of NAP1L1 (Lifespan Biosciences, Inc. Catalog No: LS-F6872). Each well of the supplied microtiter plate has been pre-coated with a target specific capture antibody. Standards and samples were added to the wells and the target antigen bind to the capture antibody. After, an avidin-horseradish peroxidase conjugate, TMB substrate, and a sulfuric acid stop solution were added to terminate color development reaction, and then, the optical density of the well was measured by a wavelength of 450 nm±2 nm. Detection range is 0.313-20 ng/mL (intra-assay CV<10%; inter-assay CV<12%).

Statistical Analysis

Statistical analysis was made using NCSS 10 (2015, Kaysville, UT, USA) software program. Yates corrected Chi-square (χ^2) test was used for the comparison of categorical data, while numerical data were analyzed using Mann-Whitney U and Kruskal Wallis tests with post-hoc Dunn's test. Data were expressed as "mean (standard deviation; SD)", minimum-maximum and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

Results

Patient Demographics and Serum NAP1L1 Levels in Study Groups

In the colon cancer group, mean (SD) age was 61.3(1.7) years and 58.9% of patients were males, while in the control group, mean(SD) age was 61.0(2.4) years and 52.0% of subjects were males. No significant difference was noted between control and colon cancer groups in terms of age and gender (Table I).

Table I. Patient demographics and serum NAP1L1 levels in study groups.

		Control (n = 50)	Colon cancer (n = 95)	*p*-value
Age (year)	Mean (SD)	61.0 (2.4)	61.3 (1.7)	0.2991
	Median (min-max)	60 (57-67)	61 (58-67)	
Gender, n (%)	Female	24 (48.0)	39 (41.1)	0.5312
	Male	26 (52.0)	56 (58.9)	
NAP1L1 (ng/mL)	Mean(SD)	1.8 (0.4)	14.1 (1.5)	< 0.001[1]

[1]Mann-Whitney U test, [2]Chi square test.

Serum NAP1L1 levels were significantly higher in colon cancer patients as compared with control subjects (median (min-max) 14(12-16) *vs.* 2(1-2) ng/mL, $p<0.001$) (Table I, Figure 1). On ROC analysis a serum NAP1L1 cut-off value of 10.5 ng/ml provided the best discrimination between colon cancer patients and controls.

Preoperative Serum NAP1L1 Levels in Colon Cancer Patients According to Clinicopathologic Variables

Most of the patients had a tumor with >4 cm tumor size (70.5%) and M1 stage (78.9%), T4 stage (42.1%) and N2 stage (63.1%) tumors (Table II).

In colon cancer patients, serum NAP1L1 levels were significantly higher for tumor size of >4 cm *vs.* <4 cm [15(12-16) *vs.* 12(12-14) ng/mL, $p<0.001$] and for M1 *vs.* M0 stage [15(12-16) *vs.* 12(12-14) ng/mL, $p<0.001$] (Table II).

Serum NAP1L1 levels were significantly higher in T4 stage tumors than in T1, T2 and T3 stage tumors ($p<0.001$ for each) and in T3 stage tumors than in T1 and T2 stage tumors ($p<0.001$ for each) (Table II, Figure 2).

Serum NAP1L1 levels were significantly higher in N2 stage tumors than in N0 and N1 stage tumors ($p<0.001$ for each) (Table II).

Gender is distributed homogenously between T stages, tumor size, distant metastasis and lymph node metastasis ($p=0.793$, $p=0.834$, $p=0.746$, $p=0.823$, respectively).

Discussion

Our findings revealed significantly higher serum levels for NAP1L1 in colon cancer patients than in control subjects and association of high serum NAP1L1 levels with increasing tumor burden in terms of advanced tumor stage and presence of local and distant metastases. Accordingly, our findings provide data for the first time in the literature on potential diagnostic, as well as prognostic role of NAP1L1 as a non-invasive biomarker in colon cancer patients.

Similarly, in a past study on analysis of genes expressed in colon adenocarcinoma, authors noted that NAP1L1 genes are overexpressed in tumors than in the adjacent non-cancerous tissues along with presence of serological responses to NAP1L1 antigens mainly in serum of cancer patients[25]. However, in contrast to our findings, in past study[26] on assessment of Affymetrix transcriptional profiling by QRT-PCR in small intestinal carcinoid (SIC), liver and lymph node (LN) metastases, colorectal carcinomas and healthy tissues, authors[26] reported that NAP1L1 was significantly overexpressed in SIC compared with colorectal carcinomas and healthy tissue as well

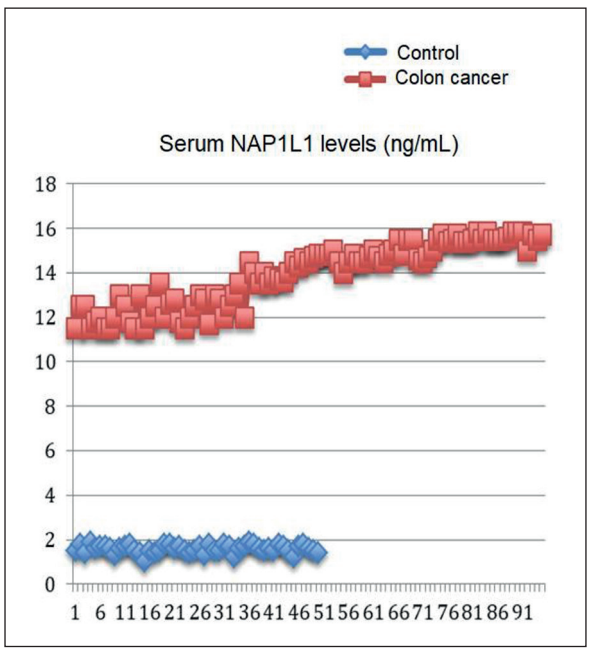

Figure 1. The histogram of NAP1L1 levels in study groups.

Table II. Preoperative serum NAP1L1 levels in colon cancer patients according to clinicopathologic variables (n=95).

	N	NAP1L1 levels (ng/mL), median (min-max)	*p*-value
Tumor size			
< 4 cm	28 (29.5)	12 (12-14)	< 0.001[1]
>4 cm	67 (70.5)	15 (12-16)	
M Stage			
M0	20 (21.1)	12 (12-14)	< 0.001[1]
M1	75 (78.9)	15 (12-16)	
T stage			
T1	19 (20.0)	12 (12-14)*,q	< 0.001[2]
T2	16 (16.8)	12 (12-14)*,q	
T3	20 (21.1)	14 (14-15)*	
T4	40 (42.1)	16 (14-16)	
N Stage			
N0	20 (21.1)	12 (12-14)w	< 0.001[2]
N1	15 (15.8)	12 (12-14)w	
N2	60 (63.1)	15 (14-16)	

*p<0.001 compared to T4 stage, qp<0.001 compared to T3 stage and wp<0.001 compared to N2 stage. [1]Mann-Whitney U test, [2]Kruskal Wallis test with post-hoc Dunn's test.

as in both liver and LN metastases compared with healthy tissue, whereas levels in colorectal carcinomas were similar to healthy mucosa.

Nonetheless, the association of NAP1L1 expression pattern with aggressive tumor pathology in colon cancer patients in the current study seems in line with data from a past study[23] among hepatocellular cancer (HCC) patients indicated that NAP1L1 expression was significantly upregulated in HCC when compared to the adjacent non-tumor hepatocytes along with association of high NAP1L1 expression with aggressive clinicopathologic features, such as serum AFP levels, larger tumor size and late clinical stage. The authors also reported upregulated NAP1L1 expression to be an independent determinant of poor prognosis in HCC after curative resection and to predict poorer overall survival for TNM stage I-II patients[23]. Likewise, higher NAP1L1 levels were noted in colon cancer patients, regardless of the disease stage, as compared to controls in the current study. Moreover, advanced-stage metastatic disease was apparent in most of our patients at the time of initial diagnosis along with significantly higher NAP1L1 levels in advanced-stage *vs.* early-stage disease. These findings seem to emphasize the clinical relevance of serum NAP1L1 in early detection for colon cancer.

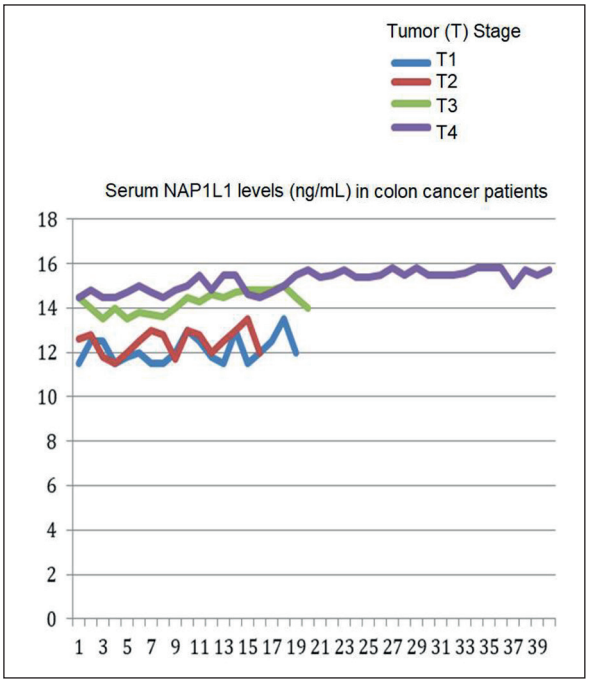

Figure 2. The histogram of the NAP1L1 levels in colon cancer patients according to tumor stage.

Indeed, the potential role of NAP1L1 in oncogenesis has been reported in the literature with several studies indicating high NAP1L1expression in some tumor tissues, such as renal cell carcinoma[21], pancreatic neuroendocrine neoplasm metastasis[24], appendiceal tumor[22] and hepatocellular carcinoma[23]. Moreover, NAP1L1 has been suggested to be involved in stress response and apoptosis, while NAP1L1 downregulation was reported to be associated with increased cell vulnerability to apoptotic cell death through attenuation of NF-κB transcriptional activity on the anti-apoptotic Mcl-1 gene[28].

NAP1L1 has also been suggested to be involved in AKT and ERK signaling in induced pluripotent stem cells (iPSC)[29], while NAP1L1 overexpression was noted in pancreatic neuro-

endocrine neoplasm metastases and considered to epigenetically promote the cell proliferation through regulation of p57 (Kip2) promoter methylation[24].

Given that NAP1L1 is considered a neoplasia-related gene associated with mitotic regulation, increase in serum levels with T3-T4 stage, N2 stage and M1 stage tumors as compared to lower TNM stages in the current study seems to emphasize the potential utility of NAP1L1 as an oncofetal protein in identifying the tumors' propensity to metastasize and poor oncological outcome among early stage colon cancer patients.

Certain limitations to this study should be considered. First, due to the cross-sectional design it is impossible to establish any cause and effect relationships. Secondly, given the relatively low sample size and the ethnic and racial diversity in colon cancer stage at diagnosis, our findings may not be generalizable to the entire colon cancer population. Third, serum NAP1L1 levels were measured only once at the time of initial diagnosis and the lack of data on longitudinal sequential measurements in relation to treatment is therefore another limitation which otherwise would extend the knowledge achieved in the current study. Nevertheless, despite these certain limitations, given the restricted amount of data available on the prognostic role of NAP1L1 in colon cancer, providing evidence regarding the utility of NAP1L1 as an oncofetal protein in diagnosis and identification of metastatic potential and poor oncological outcome among early stage colon cancer patients, our findings represent a valuable contribution to the literature.

Conclusions

In brief, our findings revealed substantial rise in serum NAP1L1 levels among colon cancer patients as compared to controls, as also correlated with the disease progression. Accordingly, NAP1L1 seems to be a potential biomarker for colon cancer, offering clinically important information on early diagnosis and risk stratification. Further larger scale prospective studies are required to achieve high level evidence on the potential utility of NAP1L1 in risk assessment of patients with early-stage colon cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

MAA, GG, RK contributed to conception/design of the research; MAA, RK and SA contributed to acquisition, analysis, or interpretation of the data; OK and MAA drafted the manuscript; OK and GG critically revised the manuscript. All authors read and approved the final manuscript.

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