# Evaluation of preoperative and postoperative total serum sialic acid levels in patients with colon cancer

*Ann. Ital. Chir., 2020 91, 6: 649-657*
pii: S0003469X20033485

Ümit Haluk İliklerden*, Çağhan Pekşen**, Tolga Kalayci***, Özgür Kemik*

**Department of Surgery, School of Medicine, Van Yuzuncu Yil University, Van, Turkey*
***Department of Surgery, School of Medicine, Istinye University, İstanbul Turkey*
**Department of Surgery, Igdir State Hospital, Department of General Surgery, Igdir, Turkey*

READ-ONLY COPY
PRINTING PROHIBITED

**Evaluation of preoperative and postoperative total serum sialic acid levels in patients with colon cancer**

AIM: *The aim of this study was to compare the preoperative and postoperative (48th hour) total serum sialic acid levels of the patients with colon cancer and to investigate if the total serum sialic acid levels can be used as a tumor marker in colon cancer.*
METHODS: *Preoperative and postoperative (48th hour) total serum sialic acid levels of 100 patients that were diagnosed with colon cancer and 70 healthy individuals were examined. All total serum sialic acid levels were determined by the methods of Warren.*
RESULTS: *Total sialic acid levels of both patient groups were significantly higher when compared to the control group ($p<0.0001$). Also, highly significant difference was found between preoperative and postoperative total serum sialic acid levels ($p<0.001$).*
CONCLUSION: *Evaluation of total serum sialic acid levels may play a critical role in colon cancers. Total serum sialic acid levels may serve as a non-invasive tool for early diagnosis of colon cancer.*

KEY WORDS: Colon cancer, Preoperative, Postoperative, Total sialic acid

## Introduction

As one of the most common malignancies all around the world, colon cancer constitutes a global health problem that affects approximately more than 1 million people per year [1]. When colon cancer is evaluated together with rectal cancer, colorectal cancers (CRC) are the third most common in men after prostate and lung cancers and also the third most common in women after breast and lung cancers.
The pathogenesis of colon cancer usually occurs after a period of 7 to 10 years, when normal colonic mucosa cells progressively turn into adenoma and finally carcinoma [2]. This period provides an opportunity for early diagnosis and treatment.[3] Classical screening methods include fecal occult blood test, fecal immunochemical tests, double-contrast barium enema, flexible sigmoidoscopy and colonoscopy as invasive and non-invasive methods [4]. Screening with an effective method, early diagnosis and follow-up modalities are needed because of the disadvantages of these methods such as being invasive, their specificity and sensitivity being insufficient, the cost, patient discomfort during the application and possible complications. Therefore, a simple and noninvasive tumor marker is needed in early diagnosis and the follow-up of the progress in the treatment process.

*Pervenuto in Redazione Maggio 2020. Accettato per la pubblicazione Giugno 2020*
*Correspondence to: Ümit Haluk İliklerden, MD, Department of Surgery, School of Medicine, Van MD, Department of Surgery, School of Medicine, Van Yuzuncu University, 65090 Van, Turkey (e-mail: dr.umitiliklerden@gmail.com)*

Carcinoembryonic antigen (CEA) is a tumor marker widely used in the screening of colon cancers worldwide [5]. Measurement of serial serum CEA levels has been developed in order to predict the recurrence and prognosis of CRC. However, CEA is known to have low sensitivity and specificity. Approximately in aproximately 30% of all CRC recurrences serum CEA levels are not increased. Since any marker alone is not sufficient, the combination of different tumor markers with different tumor biology will provide a better prognostic evaluation. For this purpose, new cancer markers should be developed in order to detect and evaluate the diagnosis of the disease [6].

Sialic acids (SA) has been attempted in various malignancies [7-13]. SA are acylated neuraminic acids found in glycoproteins and glycolipids of the cell membrane and in other parts of the cell [14]. SA has many biological functions: it provides additional electronegativity to the cell, is a main compound of many receptors on cell surface and affects the macromolecular structure of glycoconjugates and prevents their degradation [15]. Aberrant glycosylation in malignant or transformed cells results in increased synthesis of carbohydrates following that there is increased levels of SA on their surfaces. These glycoconjugates are released into the circulation through increased turnover, secretion, or shedding from malignant cells [16-18].

In this study we aimed to assess the importance of SA as a helpful and alternative tumor marker in the differentiation of precancerous and cancerous diseases of the colon and by evaluating its preoperative and postoperative serum levels in colon cancer patients.

## Material and Methods

This research was designed as a multi-center and prospective study. Patients with familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, inflammatory bowel disease, and patients with a history of cancer or previous surgery due to colon cancer were not included in the study. All colon cancer patients and healthy control groups were screened by colonoscopy for diagnostic purposes. Blood samples from all the patients with colon cancer whose pathological diagnosis was confirmed by colonoscopy were collected the day before the surgical intervention. None of the participants were treated with neoadjuvant chemoradiotherapy. Clinical and pathological factors for each patient were evaluated by age, gender, tumor size and tumor-node-metastasis (TNM) stage (Table I).

The control group was carefully constituted from individuals who did not have precancerous colorectal diseases such as ulcerative colitis or colon-derived polyps, as confirmed by colonoscopy and laboratory tests. None of the members of the control group developed cancer in the following period. The age and gender distribution between the healthy control group and patients with colon cancer has matched on a large scale and demographic data was shown in Table II. After all these selections, serums were analyzed without the knowledge of the disease levels.

One hundred patients with colon cancer (mean age: 57±(5.3) years, 55% women) and 70 healthy control group members (mean age: 56±(5.4) years, 50% women) were included in this study (Table II). Written informed consents explaining the objectives of the study in detail were obtained from the participants. The protocol of the study was carried out in accordance with the ethical principles in the 'Helsinki Declaration' and was approved by the ethics committee.

The study was approved by the internal institutional review board (Yuzuncu Yil University Faculty of Medicine, approval number: 2014/9).

The blood samples were collected by puncturing the vein without inhibiting coagulation, stored at room temperature and centrifuged. The serum was stored at -20°C until analysis.

Serum total SA levels were determined using the periodate-thiobarbituric acid method.[19] Serum samples (100 µl) were hydrolyzed at 80°C for 1 hour in 2 ml 0.05 mol/l $H_2SO_4$. After hydrolysis, the proteins were precipitated with 1.0 ml 10% trichloroacetic acid and waited for 30 minutes at 37°C with supernatant 0.025 N periodic acid.

Table I - Clinical and pathological variables of the patients and the control groups.

| | | n |
|---|---|---|
| Tumor size | < 3cm | 23 |
| | > 3cm | 77 |
| TNM stage | TI | 20 |
| | TII | 17 |
| | TIII | 22 |
| | TIV | 41 |
| Invasion | T1 | 22 |
| | T2 | 18 |
| | T3 | 23 |
| | T4 | 37 |
| Lymph node metastasis | N0 | 20 |
| | N1 | 20 |
| | N2 | 60 |
| Metastasis | Absent | 85 |
| | Present | 15 |

Table II - *Demographic features of patients and controls (p<0.05).*

| | Control Group (n=70) | Patients (n=100) | p |
|---|---|---|---|
| Age (y) (Mean/ Std. Dev.) | 60.77±7.07 | 59.06±6.15 | 0.096 |
| Gender (M/F) | 30/40 | 48/52 | |
| Gender (M/F) % | 42.8%/57.2% | 48%/52% | |

READ-ONLY COPY PRINTING PROHIBITED

The reaction was finalized with the addition of 2% sodium arsenite. Then 6% thiobarbituric acid was added and the mixture was kept in a boiling water bath for 7.5 minutes. 1.5 ml of dimethyl sulfoxide was added to increase the stability of the chromophore. For each determination, spectrophotometric reading plates were performed at 550 and 532 nm, preventing them from being affected by 2-deoxy-D-ribose. The inter-study and intra-study coefficients of the variation in total SA estimates were 4.5% and 5.3%, respectively.

### Statistical Analysis

Normality control was performed by Shapiro Wilk test, one sample Kolmogorov-Smirnov tests and histogram charts. Data were presented as mean, standard deviation, median, minimum, maximum, frequency and percentage. The variables that showed normal distribution in the comparison of the two groups were compared with independent groups T test and the others with Mann Whitney U test. Group comparisons of 3 or more were made with Kruskal Wallis one-way variance analysis. Afterwards, binary comparisons were evaluated with Bonferroni corrected Mann Whitney U test (significance limits were taken as $p<0.0167$ and $p<0.0083$). The gender variable was compared with Chi-square test. The differences between Preoperative sialic acid (PreSA) and postoperative sialic acid (PostSA) values were evaluated with Wilcoxon test. The significance limit was taken as $p<0.05$ and bidirectionally. Analyzes were perfomed using the SPSS 21 package program.

## Results

There was no statistically significant difference between the mean age of 100 patients with colon cancer (mean age: 57 (5.3) years, 55% women) and 70 healthy control group members (mean age: 56 (5.4) years, 50% women). In other words Age was found to be similar in patients and control groups (p = 0.096) (Figs. 1, 2).

Table III - *Preoperative and postoperative serum sialic acid levels of all the patients and the control group (Mean ± SD) (Min-Max).*

| | Control Group | Patients | p |
|---|---|---|---|
| Preoperative Sialic acid (mg/dL) | 12.05±1.31 | 47.29±7.6 | < 0.001 |
| Min-Max | 9.0-15.8 | 32.5-65.8 | |
| Postoperative Sialic acid (mg/dL) | | 24.75±2.0 | <0.001 |
| Min-Max | | 19.0-28.5 | |

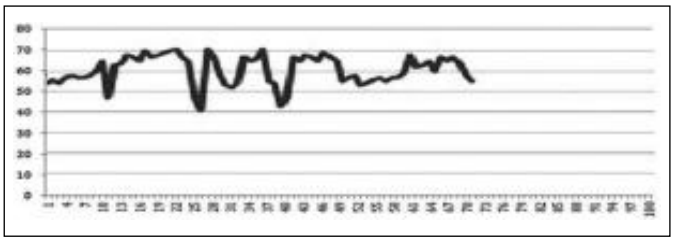

Fig. 1: Age graph of the control group.

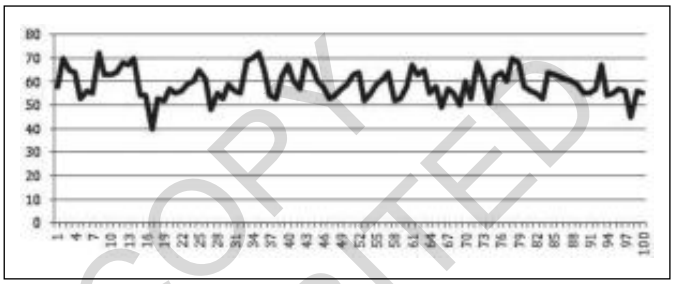

Fig. 1: Age Fig. 2: Age graph of the patients with colon cancer.

When the ages of the control group are examined, it is seen that they are in the 50-60 age range. Similarly, it is seen in the second graph that the age of the patient group is between the ages of 50-60. The ratio of women and men in the patient and control groups included in the study is approximately 50%. Gender was found to be similar in patients and control groups (p = 0.508). PreSA values were higher in patients with colon cancer than the control group ($p<0.001$) (Table III).

The value of the difference between PreSA and PostSA measurements was defined as "sialic acid difference" (SAD). When the SA value was compared to the preoperative tumor size, it was found to be similar (p = 0.538). Both the SAD value (p = 0.427) and the PreSA value (p = 0.181) were found to be similar between metastatic and non-metastatic patients.

PreSA (p = 0.019) and SAD (p = 0.011) were found to be different between N values. Binary comparisons were made with Bonferroni-corrected Mann Whitney U test. The significance limit was taken as $p<0.0167$. The SA difference and PreSA was found to be similar between N0 and N1. The SAD and PreSA was found to be similar between N0 and N2. SAD (p=0.003) and PreSA (p=0.011) was found to be different between N1 and N2.

PreSA and PostSA comparisons were found to be different in patients with tumors smaller than 3cm ($p<0.001$). PreSA and PostSA comparisons were also found to be different in patients with tumors larger than 3cm ($p<0.001$) (Figs. 3, 4).

Preoperative and postoperative sialic acid values were found quite different from each other. Although this is clearly seen in Table III, this is evident in graphs 5 and

READ-ONLY COPY PRINTING PROHIBITED

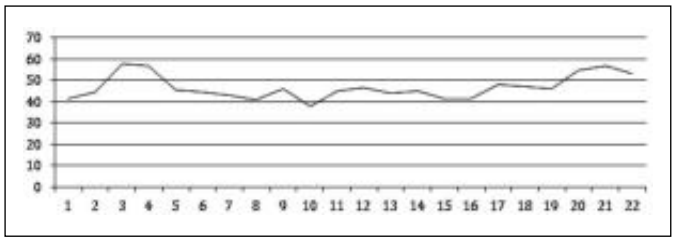

Fig. 3: Patients with a tumor size < 3 cm.

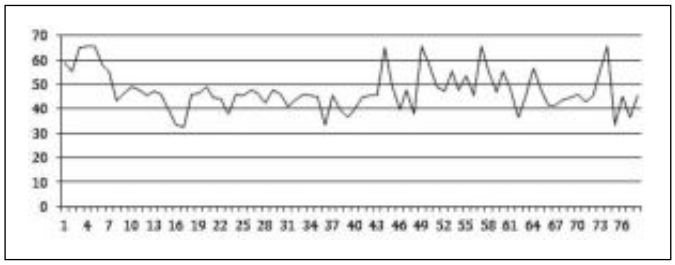

Fig. 4: Patients with a tumor > 3 cm.

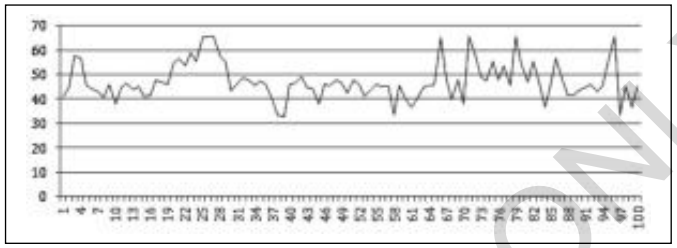

Fig. 5: Graph of preoperative sialic acid levels.

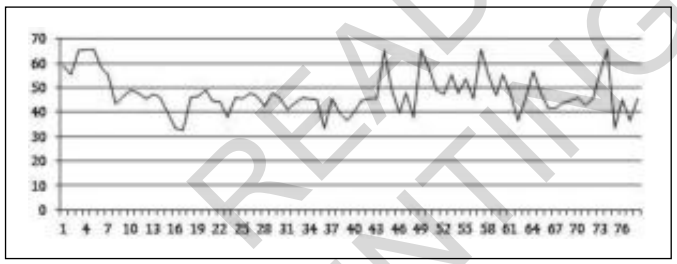

Fig. 6: Graph of postoperative sialic acid levels.

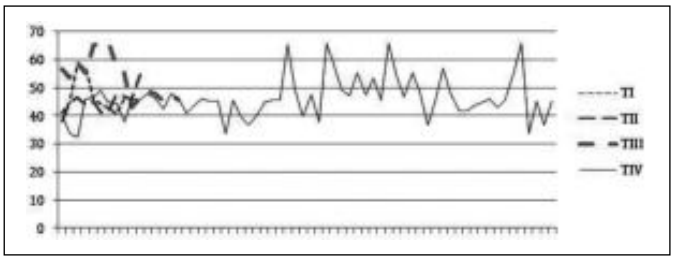


Fig. 7: Graph of TI-IV stages.

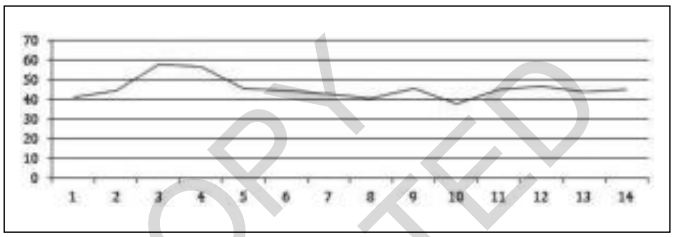

Fig. 8: Graph of the non-metastatic patient group.

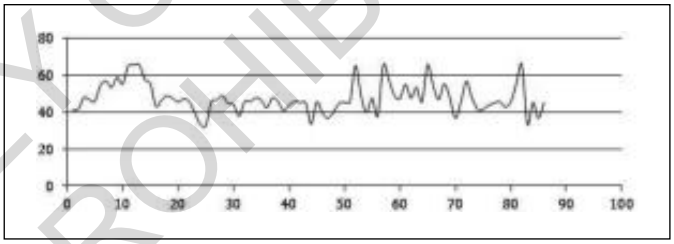

Fig. 9: Graph of the metastatic patient group.

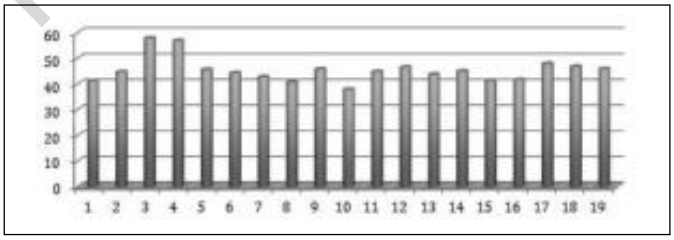

Fig. 10: Graph of N0 patients.

6. Sialic acid levels decreased significantly in patients after the operation. While the preoperative sialic acid level was 47.29, the postoperative level decreased to an average of 24.75 mg / dL (Figs. 5, 6).

The PreSA (p=0.004) and SAD (p=0.003) was found to be different between T values. Binary comparisons were made with Bonferroni-corrected Mann Whitney U test. The PreSA (p=0.004) and SAD (p=0.003) was found to be different between T values. Binary comparisons were made with Bonferroni-corrected Mann Whitney U test. The significance limit was taken as p<0.0083. The SAD and PreSA was found to be similar between T1 (p=0.569) / T2 (p=0.849), T1 (p=0.027) / T3 (p=0.015), T1 (p=0.669) /T4 (p=0.814) and T2 (p=0.816) / T4 (p=0.747). However, the SAD and PreSA values were different between T2 / T3 and T3 / T4. (P values are 0.005, 0.003, 0.001 and 0.001 respectively).

Fig. 7 shows the sialic acid levels of the patients according to TI-IV stages. The calculated SAD and PreSA values were compared in the TI-TIV stages of the tumor. As a result of comparing these stages with each other in terms of SAD and PreSA values, no difference was found between TI and TIV, TII and TIII, TII and TIV.

(p values are respectively 0.749, 0.842, 0.004, 0.005, 0.719, 0.835). However, TIII and TIV phases were found different from each other (p values are 0.001, 0.001 respectively).

READ-ONLY COPY
PRINTING PROHIBITED

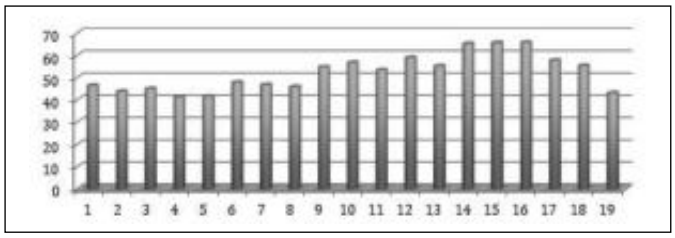


Fig. 11: Graph of N1 patients.

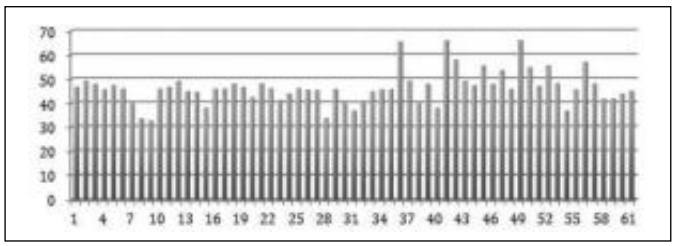


Fig. 12: Graph of N2 patients.

Fig. 8-9 shows the sialic acid levels of the non-metastatic and metastatic patient group. Sialic acid levels were compared before and after the operation (PreSA and PostSA). Sialic acid levels differ significantly between non-metastatic and metastatic patient groups according to p values calculated by Bonferroni-corrected Mann Whitney U test (p values are 0.001, 0.001 respectively). There is a statistically significant difference between the PreSA and PostSA values calculated before and after the operation of patients in stages N0, N1 and N2 (p values are 0.003 0.001, 0.001 respectively).

When preoperative sialic acid levels are evaluated according to N0, N1 and N2 stages in Lymph Node Metastasis classification, there is no obvious difference between these three stages as can be seen from the graphs (Figs. 10-12).

In the N0 stage, there is only 1 patient below the sialic acid level of 40 mg / dL, in the N1 stage, there are no patients below the sialic acid level of 40 mg / dL (Figs. 10,11).

## Discussion

Tumor markers are substances that are specific to a certain tumor or cancer cells. The plasma membrane is an important cell structure that plays role in controlling cell behavior and proliferation and expressing genetic changes [20]. In 1976, Nicolson described various types of plasma membranes as being related to cellular transformation [21]. The plasma membrane consists of phospholipids, glycoproteins and glycolipids. The carbohydrate portion of these glycoproteins and glycolipids is located on the outer surface of the membrane and covers the cell and shapes it. This cell jacket is made of mainly from glycoproteins containing SA [22]. SA plays an important role in cell-cell recognition, invasion, adhesion and immunogenetics [23]. The main structural component of the cell surface and glycoproteins undergo changes in the neoplastic transformation of the cells. One of these changes is the increase in SA levels on the cell surface. Due to increased turnover, secretion and circulation of metabolism, these glycoconjugates are released from malignant cells causing an increase in SA levels in the blood. The sialylation of sugar chains in particular has been identified as an important entity in the process of cancer evolution, progression, and development, and SA is often blamed for tumor-associated antigenicity [24-25]. Recent studies have shown that SA levels are higher in cancer patients compared to the control groups and SA levels are correlated with the disease stage, the degree of metastasis and the recurrence of the disase [17,18,26,27]. The data of the present study is in agreement with those previously reported which described an increase in the total SA content of serum in several types carcinomas [7,10,28-33].

Even though the mechanism of the increase in serum SA levels in inflammatory processes and malignancies is not fully known, some explanations have been recommended. These are reasons such as spontaneous release of abnormal SA containing cell surface glycoconjugates, increased serum glycoproteins due to increased glycolysis and/or concentration and increased inflammatory response of hepatic output of acute phase proteins by secondary inflammatory response [34,35]. In addition, detection of increased sialyltransferase (SIT) enzyme activity in some cultures of cancer cells that secrete glycoprotein into the growth medium can be counted as one of these reasons. Tumor cells also release their membrane components into intracellular fluid and this secretion process may explain increased serum SA concentrations in cancer [36,37].

It has been advocated since 1957 that an increase in serum glycoproteins can occur in cancers [38]. Studies have shown that tumor cells carry different types of glycoprotein-synthesizing enzymes and the synthesized substances contain large amounts of SA [38-41]. After Macbeth et al. determined that carbohydrates that are connected to plasma proteins increased in plasma in malignant diseases in 1962, Brozmanova et al. (1971), Khadopkar et al. (1975), Mrochek et al. (1976), Silver et al. (1976), Shearer et al. (1977), Coombes et al. (1977), Katapodis et al (1982) and some other researchers determined that SA started to increase in the serum of cancer patients in the early period of the disease. These studies concluded that SA increased in plasma also in benign diseases however this increase did not reach the same levels as it did in cancer [39,42-50]. Furthermore, it has also been suggested that SA is a mediator in malignant transformation. SA was also reported to protect the malignant cell against the host's immune system by creating a barrier in the cell membrane [42,45,48,51,52]. After the thought of SA leading the cells to escape from immune

READ-ONLY COPY PRINTING PROHIBITED

control by masking antibody receptors on the tumor cell surface which makes it directly responsible for the development of malignancy [53-55]. Horgan showed that the increase in LSA (lipid-bound sialic acid) in cancer tissue suppresses the host's immune system and increases the activity of the malignant cell [56].

After the understanding that SA protects the cell from the host's immune system by masking the tumor cell surface [48], studies investigating the effects of removing SA from the tumor cell were also conducted. In their experimental studies, Irimura et al. and Kijima-Suda et al. showed that increased SIT enzyme activity increased the rate of colon cancer metastasis to the lung. In the same studies, it was shown that the rate of metastasis decreased by inhibiting SIT activity [40,57]. It is known that SIT is an enzyme involved in SA metabolism and plays a role in its synthesis [58]. It is reported that SIT activity increases in parallel with the increase in plasma SA in patients with cancer [40,57]. Coombes et al. showed that SIT activity increased in parallel with plasma SA levels in stomach cancer [39]. Then, Kijima-Suda et al. showed that with the inhibition of SIT activity of the cells with KI-8110 (Disaccharide nucleotide), the adhesion of these cells to the other tissues decreased [40]. After being reported that cancer cells with a low amount of SA on the surface have low metastatic activity [57], SA was also reported to be important in cell-cell and cell-matrix relationship and in regulation of the relationship between the tumor cell and its surroundings [41]. Ogoshi et al. reported that tumor cells with a low amount of SA metastasized later and these cancers responded better to treatment [59]. SA synthesized in the malignant cells can also be passed onto the neighboring cells. SA increase in plasma is considered as a reflection of malignant cell destruction. The increase of SA in the cell membrane allows the reduction of cell adhesion and the tumor cell to invade easily. Thus, SA are effective in the development of near and distant metastases of the tumor cell by invading the vessels [51,60].

The changes in the cell surface proteins and glycoproteins may play a key role in determining the metastasis of the tumor cells. This is supported by the detection of cell surface sialoprotein changes in metastatic mouse colon cancer cell lines in an experimental animal model selected for colon cancer metastasis [61]. Moreover, after the detection of abnormal sugar components containing SA in the glycoprotein fraction in the extract obtained from the metastasis of the liver of sigmoid colon cancer in humans [62], and after showing the expression of a different sialoglycoprotein with a molecular weight of approximately 900.000 on the colon cancer cells that metastasize in humans in both tumor tissue and in the growth medium, the popularity of SA and glycoconjugates has gradually increased [57].

All these findings supported the view that sialylation of the cell membrane is important in determining the potential for cancer cells to metastasize. Therefore, the idea arises that determining the increase in blood SA levels may also be useful in evaluating the presence of a metastasis. In our study, the comparison of preoperative and postoperative SA in patients with metastatis was found to be different ($p<0.001$) (Table III, Figs. 8-9).

In our study, when serum total SA levels were evaluated between healthy control group and cancer patients, they were found to be significantly increased in favor of cancer patients [63] and this findings supports that SA levels can serve as a diagnostic and prognostic marker in malignant diseases (Table III).

By reviving this data in patients with colon cancer, we examined the role of SA which is one of the glycoconjugates, in colon cancer. Our study showed that serum total SA levels were higher in patients with colon cancer compared to the healthy control group. At the same time, there was a significant difference between PreSA values and PostSA values. Furthermore, as seen in the previous studies, SA levels were found to be proportional with malignant potential and the degree of metastasis[12, 17,18] (Tables I, III - Figs. 8, 9).

One of the causes of death from CRC is the delay in making the diagnosis [64]. Despite of the early diagnosis and clinical studies that are accelerated to prolong the five-year life span, the diagnosis of colon cancer is possible with the emergence of 85% of the findings [65]. Therefore, any marker alone will not be sufficient in the early diagnosis of colon cancer and its treatment and the combination of different tumor markers with different tumor biology will provide a better prognostic evaluation. For this purpose, new cancer markers should be developed in order to detect and evaluate the diagnosis of the disease [6]. In this sense, SA, which we think may be an alternative tumor marker, has been tried before in various malignancies [7,69]. It was found that our study's data coincided with the data of other previously reported studies which is "increased serum total SA levels in various cancer types" [7,11,70-75].

## Conclusion

In our study, we detected that total serum SA levels were a very good marker that was tested before. The data that increased SA levels were proportional to malignant potential and the degree of metastasis shows that in the follow-up of colon cancer treatment, the role of SA and other glycoconjugates in malignancies is worth investigating. Although this marker is highly sensitive, its low specificity can be attributed to pathological control in cancer and very small subgroup samples in cancer. However, these results suggest a significant increase in total SA in malignant diseases and suggest that SA may be useful in the follow-up, progression, and treatment process. Accordingly, we believe that SA will provide useful information about the presence of the cancer as well as the response to treatment, cancer behav-

READ-ONLY PROOF PRINTING PROHIBITED

ior and recurrence. In addition, we think that the idea of using SA and related enzymes in cancer treatment is promising with the studies to be carried out on this subject.


## Riassunto

SCOPO: Lo scopo di questo studio; Per determinare se i livelli sierici di acido sialico totale possono essere un marker nei tumori del colon, per verificare se i livelli sierici di acido sialico totale possono essere un marker nei tumori del colon confrontando i livelli sierici di acido sialico totale preoperatorio e postoperatorio.
PAZIENTI E METODI: Questa ricerca è stata progettata come uno studio multicentrico e prospettico. I pazienti con poliposi adenomatosa familiare, carcinoma colorettale ereditario non poliposo, malattia infiammatoria intestinale e pazienti con anamnesi di cancro o precedente intervento chirurgico a causa di tumore del colon non sono stati inclusi nello studio. Sono stati esaminati i livelli sierici preoperatori e postoperatori (48 ore) di 100 pazienti con diagnosi di carcinoma del colon e 70 individui sani. I livelli totali di acido sialico in tutti i sieri sono stati determinati con il metodo Warren.
RISULTATI: I valori di acido sialico preoperatorio (PreSA) e di acido sialico postoperatorio (PostSA) sono stati valutati con il test di Wilcoxon. I livelli totali di acido sialico di entrambi i gruppi di pazienti erano significativamente più alti rispetto al gruppo di controllo ($p < 0,0001$). Allo stesso modo, è stato riscontrato un alto grado di significatività tra i livelli totali di acido sialico nei sieri preoperatori e postoperatori ($p < 0,001$).
DISCUSSIONE: La valutazione dei livelli sierici di acido sialico totale può svolgere un ruolo critico nei tumori del colon. I livelli sierici di acido sialico totale possono servire come metodo non invasivo nella diagnosi precoce del carcinoma del colon. Di conseguenza, riteniamo che la SA fornirà informazioni utili sulla presenza del cancro, nonché sulla risposta al trattamento, sul comportamento del cancro e sulla recidiva. Inoltre, riteniamo che l'idea di utilizzare SA e gli enzimi correlati nel trattamento del cancro sia promettente con gli studi da svolgere su questo argomento.


## References


1. Siegel RL, Miller RD, Jemal A: *Cancer statistics, 2016*. CA Cancer J Clin, 66 (1) (2016), pp. 7-30, https://onlinelibrary.wiley.com/doi/full/10.3322/caac.21332.

2. Hofstad B, Vatn M: *Growth rate of colon polyps and cancer*. Gastrointest Endosc Clin N Am, 1997; 7: 345-63.

3. Diallo Owono FK, Nguema Mve R, Ibaba J, Mihindou C, Ondo N'dong F: *Epidemiological and diagnostic features of colorectal cancer in Libreville*, Gabon Med Trop (Mars), 2011; 71: 605-07.

4. São Julião GP, Habr-Gam A, Vailati BB, Araujo SEA, Fernandez LM, Perez RO: *New strategies in rectal Cancer.* Surgical Clinics, 97 (3) 2017; 587-604, 10.1016/j.suc.2017.01.008.

5. Duffy MJ: *CEA as a marker for colon cancer: Is it clinically useful?* Clin Chem, 2001; 47: 624-30.

6. Duffy MJ, Van dalen A, Haugland C, Hanson L, Holinski-Feder E, et al.: *Tumor markers in colorectal cancer: European Group on tumor markers guidelines for clinical use.* Eur J Clin, 2007; 43: 1348-360.

7. Gruszewska E, Chrostek L, Cylwik B, Tobolczyk J, Szmitkowski M, Kuklinski A, Kedra B: *Serum sialic acid as a marker of pancreatic cancers.* Clin Lab, 2013; 59 (7-8): 781-88.

8. Taqi SA*: Clinical evaluation of total and lipid bound sialic acid levels in oral precancer and oral cancer.* Indian J Med Paediatr Oncol, 2012; J; 33(1): 36-41.

9. Lopez-Morales D, Reyes-Leyva J, Santos-Lopez G, Zenteno E, Vallejo-Ruiz V: *Increased expression of sialic acid in cervical biopsies with squamous intraepithelial lesions.* Diagn Pathol, 2010; 5: 74.

10. Sandhu R, Lal H, Kundu ZS, Kharb S: *Serum fluoride and sialic acid levels in osteosarcoma.* Biol trace Elem Res, 2011; 144(1-3): 1-5.

11. Basoglu M, Atamanalp SS, Yildirgan MI, Aydinli B, Öztürk G Akcay F, Oren D: *Correlation between the serum values of soluble intercellular adhesion molecule-1 and total sialic acid levels in patients with breast cancer*. Eur Surg Res, 2007; 39(3): 136-40.

12. Basoglu M, Yildirgan MI, Taysi S, Yilmaz I, KiziltuncA, Balik AA, Celebi F, Atamanalp SS: *Levels of soluble intercellular adhesion molecule-1 and total sialic acid in serum of patients with colorectal cancer*. J Surg Oncol, 2000; 83(3): 180-84.

13. Uslu C, Taysi S, Akcay F, Sutbeyaz MY, Bakan N: *Serum free and bound sialic acid and alpha-1-acid glycoprotein in patients with laryngeal cancer.* Ann Clin Lab Sci, 2003 Spring; 33(2): 156-59.

14. Cook GM: *Techniques for the analysis of membrane carbohydrates.* In: Maddy AH, editor. *Biochemical analysis of membranes*. 1st ed. London: Wiley & Sons; 1976; 287-92.

15. Schauer R: *Chemistry, metabolism and biological function of sialic acids.* Adv Carbohydr Chem Biochem. 1982; 40: 131-234. [PubMed]

16. Sebzda T, Saleh Y, Gburek J, Warwas M, Andrzejak R, Siewinski M, Rudnicki J: *Total and lipid-bound sialic acid as a diagnostic markers in colorectal cancer patients: correlation with cathepsin B expression in progression go Dukes stage.* J Exp Ther Oncol, 2006; 5(3): 223-29.

17. Verazin G, Riley WM, Gregory J, Tautu C, Prorok JJ, Alhadeff JA: *Serum sialic acid and carcinoembryonic levels in the detection and monitoring of colorectal cancer.* Dis Colon Rectum, 1990; 33(2):139-42.

18. Plucinsky MC, Riley WM, Prorok JJ, Alhadeff JA: *Total and lipid-associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement.* Cancer, 1986; 58: 2680-85.

19. Smets LA, Van Beek WP: *Carbohydrates of the tumor cell surface.* Biochim Biophys Acta, 1984; 738: 237-49.

20. Nicolson GL: *Trans-membrane control of the receptors on normal and tumor cells: II: Surface changes associated with transformation and malignancy.* Biochim Biophys Acta, 1976; 458:1-72. STAD


READ-ONLY COPY PRINTING PROHIBITED

21. Ham AW, Cormack DH: *Cytoplasm and its organelles*. In: Ham AW, Cormack DH, (eds): *Textbook of histology*. 8th ed. Philadelphia: J B Lippincott; 1979; 107-65.

22. Baxi BR, Patel PS, Adhvaryu SG, Dayal P: *Usefulness of serum glycoconjugates in precancerous and cancerous diseases of oral cavity.* Cancer, 1991; 67:135-40.

23. Hakomori S, Handa K, Iwabuchi K, Yamamura S, Prinetti A: *New insights in glycosphingolipid function: "glycosignaling domain," a cell surface assembly of glycosphingolipids with signal transducer molecules, involved in cell adhesion coupled with signaling.* Glycobiology, 1998; 8:11-19.

24. Vallejo V, Reyes-Leyva J, Hernández J, Ramírez H, Delannoy P, Zenteno E: *Differential expression of sialic acid on porcine organs during the maturation process.* Comp Biochem Physiol B, 2000; 126: 415-24.

25. Krasnodebski IW: *Usefulness of biochemical tumor markers (CEA, Ca 19-9, ferritin and sialic acid) in diagnosis and prognosis of colonic neoplasms.* Wiad Lek, 1998; 51(3-4):132-41. Polish.

26. Cunietti E, Locatelli E, Vaiani G, Gandini R, Gandini MC, Reggiani A, Monti M: *Carcinoembryonic antigen, ferritin, anionic glycoproteins, and their sialic acid content in advanced colorectal cancer.* Cancer Detect Prev, 1985; 8(1-2): 227-32.

27. Tewarsan SL, Mintal VP, Singh M, et al.: *Serum sialic acid an important cancer marker.* Indian J Cancer, 1993; 30:125-31.

28. Krecicki T, Leluc M: *Acute phase reactant proteins and aid to monitoring surgical treatment of laryngeal carcinoma.* J Laryngeal Otol 1992; 106: 613-615.

29. Rajpura KB, Patel PS, Chawda JG, Shah RM: *Clinical significance of total and lipid bound sialic acid levels in oral pre-cancerous conditions and oral cancer.* J Oral Pathol Med, 2005; 34(5): 263-67.

30. Bektemür G, Ozer F, Kanat F, Imecik O: *Diagnostic efficiency of serum lipid-bound sialic acid level in malignant pleural effusions.* Tuberk Toraksk, 2003; 51(3): 265-70. Turkish.

31. Wongkham S, Bhudhisawasdi V, Chau-in S, Boonla C, Muisuk K, Kongkham S, Wongkham C, Boonsiri P, Thuwajit P: *Clinical significance of serum total sialic acid in cholangiocarcinoma.* Clin Chim Acta, 2003; 327(1-2): 139-47.

32. Diamantopoulou S, Stagiannis KD, Vasilopoulos K, Barlas P, Tsegenidis T, Karamanos NK: *Importance of high-performance liquid chromatographic analysis of serum N-acylneuraminic acids in evaluating surgical treatment in patients with early endometrial cancer.* J Chromatogr B Biomed Sci Appl, 1999; 732(2): 375-81.

33. Stefenelli N, Klotz H, Engel A, Bauer P: *Serum sialic acid in malignant tumors, bacterial infections, and chronic liver diseases.* J Cancer Res Clin Oncol, 1985; 109:55-59.

34. Turner GA, Skillen AW, Buamah P, et al.: *Relation between raised concentrations of fucose, sialic acid and acute phase proteins in serum from patients with cancer.* J Clin Pathol, 1985; 38:588-92.

35. Akamatsu S, Yazawa S, Tachikawa T, Fururata T, Okaichi Y, Nakamura J, Assao T, Nagamachi Y: *Alpha-2-3 Sialyltranferase associated with synthesis of CA19-9 in colorectal tumors.* Cancer, 1996; 77-9.

36. Dall'Olio F, Malagolini N, di Stefano G, Minni F, Marrano D, Stefani-Cessi F: *Incerased CMP-NeuAc-b1, 4-Glc Nac-R alpha2-6 sialytransferase activity in human colorectal cancer tissue.* Int J Cancer, 1989; 44:434-39.

37. Igen U. Alp I: *Kadin Genital Kansetlerinde Serum Lipid-Bağli Sialik Asit (LSA) Ölçümlerinin Yeri.* G A T A Bülteni, 1984; 26:63-6.

38. Coombes RC, Powles TJ, Gazet JC, et al.: *A Biochemical approach to the staging of human breast cancer*. 1987; 40:93/4.

39. Kijirna-Suda I, Miyazawa T, Itch M, et al.: *Possible mechanism of inhibition of experimental pulmonary metastasis of mouse colon adenocarcinom a 26 sublines by a sialic acid: nucleosid conjugate.* Cancer Res, 1988; 48:3728-732.

40. Wagner HE, Thomas P, Wolf BC, et al.: *Inhibition of sialic a cid incorporation prevents hepatic metastases.* Arch Surg 1990; 125:351-54.

41. Brozrrtanova E, Skrovina B: *Sialic Acid and Bone Tumors*. Neoplasmaa, 1972; 115-24.

42. Dnistrian AM, Schwartz MK: *Plasma lipid-bound sialic acid and carcinoembryonic antigen in cancer patients.* Clin Chern, 1981; 27:1737-739.

43. Katapodis N, Hirshaut Y, Geller NL, Stock CC: *Lipid associated sialic-acid test tor the dectection of human cancer.* Cancer Res, 1982; 42:5270-75.

44. Khadapkar SV, Sheth NA, Shide SV: *Independence of sialic acid levels in normal and malignant growth.* Cancer Res, 1975; 35:1520-23.

45. Khanderia U, Keller JH, Grossman HB: *Serum sialic acid is a biologic marker for malignant disease.* J Surg Oncol, 1983; 23.163-66.

46. Macartney JC: *Lecitin Histochemistry of Galactose and N-Acetylgalactosamine glycoconjugates in normal gastric mucosa and gastric cancer and the relationship with ABO and secretor status.* J Pathology, 1986; 150:135.

47. Shearer WT, Gottlieb C, Kornfeld S: *Humoral immunostimulation. Vil. sialic acid masks antigenic sites on an antibody-selected varían: cell line.* J Immunol, 1977; 119:614-17.

48. Silver HKB, Rangel DM, Morton D: *Serum sialic acid elevations in malignant melanoma patients.* Cancer, 1978; 41:1497-499.

49. Tseng PC, Sprance HE, Carcangiu ML, et al.: *CA-125,NB/70K, and lipid associated sialic acid in monitoring uterine papillary serous carcinoma*. Obstet Gynecol, 1989; 74:384-87.

50. Mrochek JE, Dinsmore SR, Tormey DC, et al.: *Protein-bound carbohydrates in breast cancer. Liquld-chromatographic analysis for mannose, galactose, fucose, and sialic acid in serum.* Clin Chemistry 1976; 22:1516-512.

51. Warren L: *Sialic acid in human semen and in the male genital tract.* J Clin Invest, 1959; 38:755-61.

52. Doğan P, Muhtaroğlu S: *Pre-Eklampsive eklampside serum total ve lipide bağli siyalik asid seviyeleri*. Erciyes Tip Dergisi, 1990; 12:10-6.

53. Muhtaroğlu S, Paşaoğlu H: *Tümör markerleri*. Erciyes: Tip Dergisi Ek-1. 1992; 326-32.

54. Vîlarem JM, Jouanneau J, Bourrillon R: *Differences in sialic acid contents of low cancer cells, high cancer cells and normal mouse lung counterparts*. Biochem and Biophys Res Com, 1981; 98-7-14.

READ-ONLY COPY PRINTING PROHIBITED

55. Horgan IE: *Total and lipid - bound sialic acid levels in sera from patients with cancer*. Climea Chemica Acta, 1982; 118:327-31.

56. Irirnura T, Carlson DA, Pricej, et al.: *Differantial experissiono! a siaiogiycoprotein with an approximate molecular weight of 900,000 on metastatic human colon carcinoma cells crowing in culture and in tumor tissues.* Cancer Res.

57. Dwivedi C, Dixit M, Hardy RE: *Plasma Sialyltransterase as a Tumor Marker*. Cancer Detection and Prevention, 1988; 11:191-96.

58. Ogoshi K, Iwata Y, Hara S, et al.: *Concentrations of alpha-1 - antichymotripsin and other Acute Phase Beactants in Patients with Gastric Cancer*. Toka, J Exp Clin Med,1988; 13:355-60.

59. Tibil KM, Jones JD, Klee GG: *Use and Limitation of serum total and lipid-bound sialic acid concentration as markerslor colorectal cancer.* Cancer, 1985; 55:404-09.

60. Bresalier RS, Rockwell RW, Dahiya R, Duh Qy, Kim YS: *Cell surface sialoprotein alterations in metastatic murine colon cancer cell lines selected in an animal model for colon cancer metastasis*. Cancer Res, 1990; 15:1299-307.

61. Otsuka K, Ohkuma S, Nakajima T: *Isolation and partial characterization of two glycoproteins from human liver metastases of sigmoid colon carcinoma.* Int J Biochem, 1990; 22: 653-58.

62. Kakari S, Stringou E, Toumbis M, Ferderigos AS, Poulaki E, Chondros K, et al.: *Comparison of serum TSA and LSA with CEA, ferritin and NSE as tumor markers for lung cancer.* Anticancer Res. 1998; 8: 1147.

63. Quinn M, BP, Brock A, Jones J: *Cancer Trends in England and Wales 1950- 1999. The* Statinery Office: London, 2001.

64. Speights VO, Jhonson MV, Stoltenberg PH, Rapaport ES, Helbert B, Riggs M: *Colorektal cancer: Current trends in initial clinicel manifestations*. South Med J, 1991; 84:575-80

65. O'Connell JB, Maggard MA, Ko CY: *Colon cancer survival rates with the new AJCC sixth edition staging.* JNCI 2004; 96(19):1420-24.

66. Compton CC: *Colorectal carcinoma: Diagnostic, prognostic, and molecular features.* Mod Pathol, 2003; 16(4):376-88.

67. Yardim N, Mollahaliloğlu S, Bora Başara B: *Türkiyede Kanser Durumu ve Uluslararasi Göstergeler İle Uyumun Değerlendirilmesi. İçinde: Türkiye'de Kanser Kontrolü.* Eds. Tuncer AM, Özgül N, Olcayto E, Gültekin M.T.C. Sağlik Bakanliği, Kanserle Savaş Dairesi Başkanliği, Koza Matbaacilik, Ankara, 2009; 51-63.

68. Cornelissen LAM, Blanas A, van der Horst JC, Kruijssen L, Zaal A, O'Toole T, Wiercx L, van Kooyk Y, van Vliet SJ: *Disruption of sialic acid metabolism drives tumor growth by augmenting CD8(+) T cell apoptosis.* Int J Cancer, 2019; 144(9):2290 302. https://doi.org/10.1002/ijc.32084

69. Corfield AP, Myerscough N, Warren BF, Durdey P, Paraskeva C, Schauer R: *Reduction of sialic acid O-acetylation in human colonic mucins in the adenoma-carcinoma sequence.* Glycoconj J; 1999; 16:307-17.

70. Pearce OM, Läubli H: *Sialic acids in cancer biology and immunity.* Glycobiology, 2016; 26:111-128. doi:10.1093/glycob/cwv097.

71. Shen Y, Kohla G, Lrhorfi AL, Sipos B, Kalthoff H, Gerwig GJ, Kamerling JP, Schauer R, Tiralongo J: *O-Acetylation and de-O-acetylation of sialic acids in human colorectal carcinoma.* Eur J Biochem, 2004; 271:281-90.

72. Wang F, Xie B, Wang B, Troy FA II: *LC-MS/MS glycomic analyses of free and conjugated forms of the sialic acids, Neu5Ac, Neu5Gc and KDN in human throat cancers.* Glycobiology, 2015; 25:1362-374.

73. Soliman C, Chua JX, Vankemmelbeke M, McIntosh RS, Guy A.., Spendlove I, Ramsland PA: *The terminal sialic acid of stage-specific embryonic antigen-4 has a crucial role in binding to a cancer-targeting antibody.* Journal of Biological Chemistry, 2020; 295(4), 1009-20.

74. Boligan KF, Mesa C, Fernandez LE, von Gunten S: *Cancer intelligence acquired (CIA): Tumor glycosylation and sialylation codes dismantling antitumor defense.* Cell Mol Life Sci, 2015; 72:1231-248.

READ ONLY COPY PRINTING PROHIBITED