Original Article

Chirurgia (2016) 111: 414-421
No. 5, September - October

http://dx.doi.org/10.21614/chirurgia.111.5.414

The Diagnostic and Prognostic Significance of Serum Neutrophil Gelatinase-Associated Lipocalin Levels in Patients with Colorectal Cancer

Ibrahim Ali Ozemir[1], Sinan Aslan[1], Tunc Eren[1], Baris Bayraktar[1], Cagri Bilgic[1], Banu Isbilen[2], Haydar Yalman[1], Rafet Yigitbasi[1], Orhan Alimoglu[1]

[1]Istanbul Medeniyet University, School of Medicine, Goztepe Training & Research Hospital, Department of General Surgery, Istanbul, Turkey
[2]Istanbul Medeniyet University, School of Medicine, Goztepe Training & Research Hospital, Department of Biochemistry, Istanbul, Turkey

Rezumat

Semnificația diagnostică și prognostică a nivelului seric de lipocalină asociată gelatinazei neutrofilelor la pacienții cu cancer colorectal

Scop: Lipocalina asociată gelatinazei neutrofilelor (NGAL) este un biomarker inflamator depozitat în granulele neutrofilelor. Studii recente au revelat că expresia NGAL crește la nivelul țesuturilor pacienților cu boli inflamatorii ale sistemului gastrointestinal și la cei cu cancer. Scopul prezentului studiu este de a evalua semnificația diagnostică și predictivă a nivelului plasmatic de NGAL în stadii diferite ale secvenței adenom-carcinom ale cancerului colorectal.
Materiale și Metode: 80 de cazuri au fost incluse în studiu și separate în 3 grupuri. "Grupul de Cancer" a constat în 27 de pacienți cu cancer colorectal supuși rezecției curative, în timp ce 24 de pacienți cu polipi colorectali adenomatoși decelați prin colonoscopie au fost clasificați drept "Grupul de Polipi", iar 29 de pacienți cu rezultate normale la colonoscopie au fost catalogați drept "Grupul Control". Au fost determinate nivelurile serice de NGAL, CEA și CA19-9, precum și rezultatele examenelor histopatologice.
Rezultate: Nivelul seric mediu de NGAL pentru grupurile de control, polipi și cancer au fost: 91,5 ng/ml, 139,6 ng/ml, respectiv 184,3 ng/ml. Nivelurile plasmatice de NGAL identificate au fost semnificativ mai crescute în grupul de cancer comparativ cu grupul control (p:0,006). Nivelurile plasmatice de NGAL au prezentat semnificație statistică și corelație pozitivă cu diametrul tumoral și numărul de noduli limfatici cu invadare metastatică (p:0,047, r:%38,6 și p:0,026, respectiv r:%42,8) din grupul de cancer.
Concluzii: Suntem de părere că nivelul preoperator plasmatic de NGAL este un potențial biomarker de diagnostic pentru pacienții cu cancer colorectal. Deși mai multe studii aprofundate sunt necesare pentru o concluzie fermă, nivelul seric de NGAL poate fi utilizat ca biomarker diagnostic și/sau predictiv pentru prezența metastazelor în ganglionii limfatici la pacienții cu cancer colorectal.

Cuvinte cheie: secvență adenom-carcinom, biomarkeri pentru cancer, cancer colorectal, lipocalina asociată gelatinazei neutrofilelor, diagnostic

Abstract

Aim of the study: Neutrophil gelatinase-associated lipocalin (NGAL) is an inflammatory biomarker that is stored in neutrophil granules. Recent studies revealed that NGAL expression increases in tissue samples of patients with inflammatory gastrointestinal system diseases and cancers. The aim of this study was to evaluate the diagnostic and predictive significance of plasma NGAL levels in various stages of adenoma-carcinoma sequence of colorectal cancer.
Materials and Methods: Eighty cases were included in the study

Corresponding author: Ibrahim Ali Ozemir, MD
Istanbul Medeniyet University, School of Medicine
Department of General Surgery, Dr. Erkin Street,
Kadikoy, Istanbul, Turkey
E-mail: draliozemir@hotmail.com

and separated into 3 groups. “Cancer Group” consisted of 27 colorectal cancer patients who underwent curative resection, whereas 24 patients with colorectal adenomatous polyps detected by colonoscopy were classified as the "Polyp Group", and 29 patients with normal colonoscopy findings were classified as the "Control Group". The serum NGAL, CEA and CA19-9 levels and histopathology findings were determined.

Results: The mean plasma NGAL levels for control group, polyp group and cancer group were found to be 91.5 ng/ml, 139.6ng/ml and 184.3ng/ml, respectively. Plasma NGAL levels were found to be significantly higher in cancer group compared to the control group (p:0.006). Plasma NGAL levels were detected statistically significant and positive correlated with tumor diameter and number of metastatic lymph nodes (p:0.047, r:%38.6 and p:0.026, r:%42.8, respectively) in cancer group.

Conclusions: We are of the opinion that pre-operative plasma NGAL level is a potential diagnostic biomarker for colorectal cancer patients. Although more comprehensive studies are needed for definitive judgments, serum NGAL levels may be used as a diagnostic and/or predictive biomarker for lymph node metastasis in patients with colorectal cancer.

Key words: Adenoma-carcinoma sequence, cancer biomarkers, colorectal cancer, Neutrophil gelatinase-associated lipocalin (NGAL), diagnosis

Introduction

Colorectal cancer (CRC) is responsible for 8% of total cancer-related deaths worldwide (1). Most CRCs are known to develop as a consequence of the adenoma-carcinoma sequence (2-4). Diagnosis of early stage CRC has a critical importance in terms of 5-year survival rates alter from 5% to 96% according to stage of disease (5). The efforts for seeking a biochemical marker that has both diagnostic and predictive value for the extent of disease are still ongoing.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein that is stored in human neutrophil granules, which acts as a modulator of inflammation (6). It has been ascertained that NGAL is expressed in many types of cells and plays a role in various processes such as growth, development, and differentiation of these cells (7-11). The diagnostic and prognostic potential of NGAL appears variable and contradictory according to the type of cancer. Studies that performed on thyroid, breast and endometrial tissue samples showed that NGAL expression increases in the presence of neoplasia or dysplasia (12-17). Immunohistochemical staining experiments revealed that NGAL expression in normal tissue was null or weak in 98% of cases, whereas it was moderate or intense in 74% of cancer tissue (18). NGAL expressions are increased in CRC and benign inflammatory diseases such as inflammatory bowel disease (19-22). Several studies observed the prognostic importance of NGAL expression on tissue samples (19,23-25).

Recent studies regarding to plasma NGAL level showed that, increased plasma levels are related with cancer patients than in healthy controls (26,27). But examined serum NGAL levels revealed discordant results. Sun et al, did not show ay significance increase in plasma NGAL levels in CRC patients. Conversely, Fung et al (28), reported moderately higher plasma NGAL level without correlation with "T" stage. There are only few studies in current literature that evaluates the relationship between serum NGAL level and CRC (18,28,29). The aim of this study was to evaluate the significance of serum NGAL levels as a diagnostic and predictive biomarker in patients with CRC.

Materials and Methods

This study was performed in General surgery department of Istanbul Medeniyet University, Goztepe Education and Research hospital, between January 2011 and December 2012. Ninety patients were planned to classified into 3 groups as the "Cancer group (CAG)", the "Polyp group (PG)", and the "Control group (COG)". Subjects with pre-operatively known distant metastasis or if they have active infection, history of renal or pancreatic diseases, concomitant other organ cancers, hemolysis blood samples, and patients had received neo-adjuvant chemotherapy or radiotherapy were excluded from the study. Plasma creatinine levels were detected simultaneously, to avoid biases from NGAL increases during renal failure. Patients with less than 12 harvested lymph node and patients with polyps smaller than 1 cm diameter on pathological examination were also excluded from the assessment.

Totally 80 patients were included the study. Twenty-seven patients consecutively admitted for elective surgery and underwent curative resections for CRC were classified as CAG. Twenty-four patients who were detected colorectal polyps greater than 1cm in diameter during colonoscopy were classified as PG, and colonoscopic polypectomy was performed to all patients. We performed colonoscopy to the patients in control group to exclude the presence of inflammatory bowl disease, and 29 patients with no pathological findings during colonoscopy were classified as the COG.

Blood samples were obtained before the surgery for the CRC patients, and before the polypectomy for patients at polyp group. Blood samples were immediately stored at +4°C, and analyses were blinded. Serum NGAL, carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9) levels were detected to evaluate the significance of serum NGAL levels regarding to the adenoma-carcinoma sequence of CRC. Serum NGAL levels were determined using Human NGAL/Lipocalin-2 ELISA kit (Santa Clara, CA Cat. No.SK00233-01) in accordance with the manufacturer's protocol.

Tumor size and grade, cancer stage, presence of lymphovascular invasion, number of metastatic lymph nodes and presence of distant metastasis in CRC patients were investigated in the study. The degree of dysplasia of colorectal polyps was determined in PG.

Descriptive characteristics were presented as percentages for categorical variables or medians with interquartile range for

continuous variables. The software NCSS (Number Cruncher Statistical System) 2007 and PASS (Power Analysis and Sample Size) 2008 statistical software (Utah, USA) were used for statistical analyses in this study. The Kruskal Wallis test was used for the comparisons of descriptive statistical methods (Mean, Standard deviation) as well as quantitative data, and parameters showing abnormal distribution between the groups. The Mann Whitney U test was used in the determination of the groups, which caused the difference and the compassions of the parameters to show abnormal distribution between the groups. Spearman's rho correlation coefficient was used for the correlation of the analysis between the parameters. ROC curve was performed to represent a sensitivity/specificity pair corresponding to a particular decision threshold. Results were reviewed in a confidence interval of 95%, and a value of $p < 0.05$ was accepted as statistically significant.

This study was approved by the Scientific Ethics Committee of the Istanbul Medeniyet University, School of Medicine. All the procedures were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, which was revised in 1983.

Results

Eighty patients, 42 (52.5%) male and 38 (47.5%) female, with a mean age of 55.9±14.7 years were included in the study. There were no statistically difference between control group and other groups according to age and sex.

Serum NGAL levels of the patients in CAG, PG and COG were determined and presented on a scatter plot graph (*Fig. 1*). Mean NGAL levels of these three groups were calculated as 184.29 ng/mL, 139.57 ng/mL and 91.46 ng/mL; respectively and compared statistically (*Table 1*). NGAL levels were statistically significant difference between groups (p:0.013). The Mann-Whitney U test was used to perform binary group analysis to determine the reason for statistical significance. Hereby, serum NGAL levels of the patients in CAG were detected significantly higher than the patients in COG (p: 0.006). Five of the 80 patients' NGAL levels were higher than the "mean serum NGAL value+2SD". They excluded from the groups to standardize the results, and re-analysis demonstrated that NGAL levels of patients in CAG was still statistically higher than COG (p: < 0.001). Patients separated into two groups such as patients with CRC or polyp with high-grade dysplasia considered as a group and patients with low-grade dysplasia or normal colonoscopy findings the other group. ROC analysis was performed to define the cut-off value of serum NGAL (*Fig. 2*). ROC analysis revealed that the discriminative power of serum NGAL level between CRC and control group assessed by an area under the curve (AUC) of

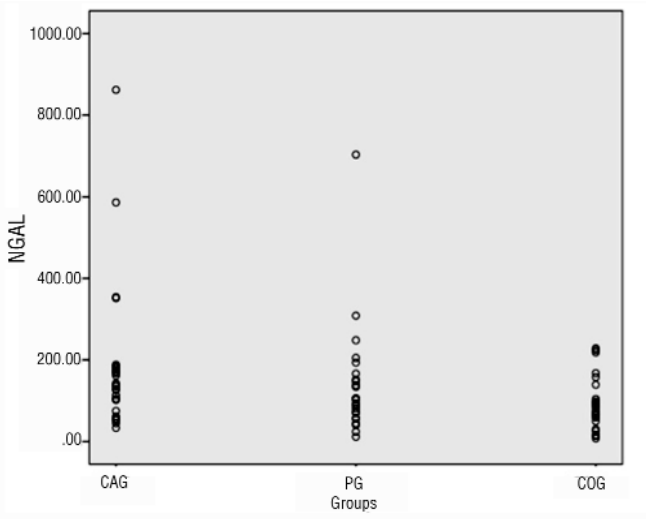

Figure 1. *NGAL values of all patients as a scatter plot graph*

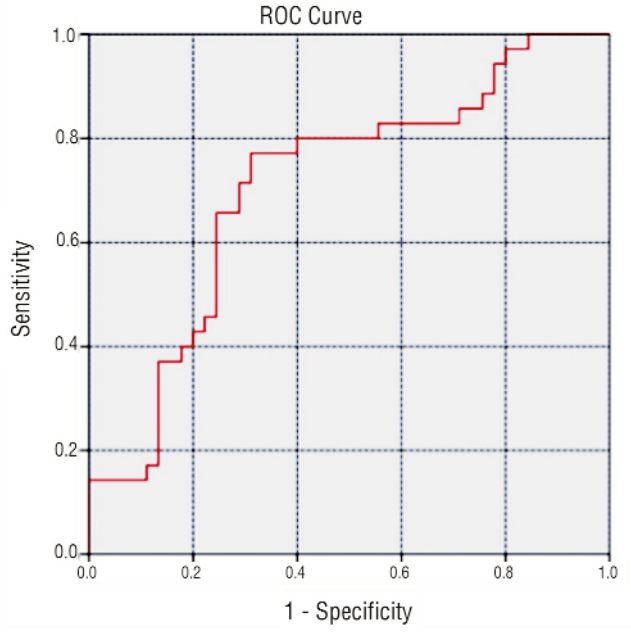

Figure 2. *ROC curve graph of serum NGAL levels of patients*

Table 1. *Comparison of serum NGAL, CEA and CA 19-9 levels of the patients in CAG with the patients in COG and PG*

	CAG (n=27)	COG (n=29)		PG (n=24)	
	Mean±SD	Mean±SD	p	Mean±SD	p
NGAL (ng/ml)	184.29±177.5	91.46±59.43	0.006**	139.57±139.84	0.248
CEA (ng/ml)	9.3±15.17	1.46±1.007	0.001**	2.53±1.48	0.404
CA19-9 (U/ml)	32.47±62.91	7.93±6.99	0.003**	13.34±16.48	0.156
Mann Whitney U test	**p<0.01				

Table 2. *Evaluation of the serum NGAL levels of the patients in PG, according to their dysplasia degrees*

Polyp Group (n=24)		**NGAL (ng/ml)**	**p**
		Mean±SD	
Dysplasia	Low grade (n=16)	110.33±83.97	0.154
	High grade (n=8)	198.04±220.85	
Mann Whitney U test		Kruskal Wallis test	

0.71 (95% CI:0.59-0.83). The comparison of these groups revealed a 103.55ng/mL cut-off value of serum NGAL, with the 71.4% sensitivity and 68.9% specificity.

Serum CEA and CA19-9 levels of patients in CAG were determined significantly higher than patients in COG (p: 0.001 and p: 0.003; respectively). But there was no significant difference between patients in CAG and patients in PG according to serum CEA and CA19-9 levels (*Table 1*). Although not statistically significant difference, the patients in PG had lower NGAL levels compared to CAG, and had higher NGAL levels compared to COG (p: 0.248 and p: 0.166, respectively). On the other hand serum CEA levels of the patients in PG were detected significantly higher when compared with COG (p:0.008).

Patients in PG were separated into two subgroups according to their degrees of dysplasia, such as "Low-grade" and "High-grade" (*Table 2*). Eight of the 24 patients in PG had "High-grade" dysplasia and the mean NGAL level was detected 198.04ng/ml. The mean NGAL level of patients with "Low-grade" dysplasia was detected 110.33ng/ml. Statistically significant difference was not detected between these subgroups in terms of serum NGAL levels (p:0.154).

Table 3. *Correlation between the serum NGAL levels and age, tumor diameter, and the number of metastatic lymph nodes of patients in the cancer group*

Cancer Group (n=27)	**NGAL (ng/ml)**	
	r	**p**
Age	-0.165	0.420
Tumor Diameter	0.386	0.047*
Number of Metastatic Lymph Nodes	0.428	0.026*
r: Spearman's rho correlation coefficient	*p<0.05	

Eight of the patients with CRC underwent laparoscopic surgery, while the others open surgery. There was no statistically significant correlation detected between serum NGAL levels and age in CAG (*Table 3*). When we compared serum NGAL levels with tumor-related features, statistically significant and positive correlational relationship was detected with tumor size (r:0.38, p:0.047) and the number of metastatic lymph nodes (r:0.42, p:0.026) (*Fig. 3*). But we did not detect significant correlation between NGAL other tumor features such as tumor

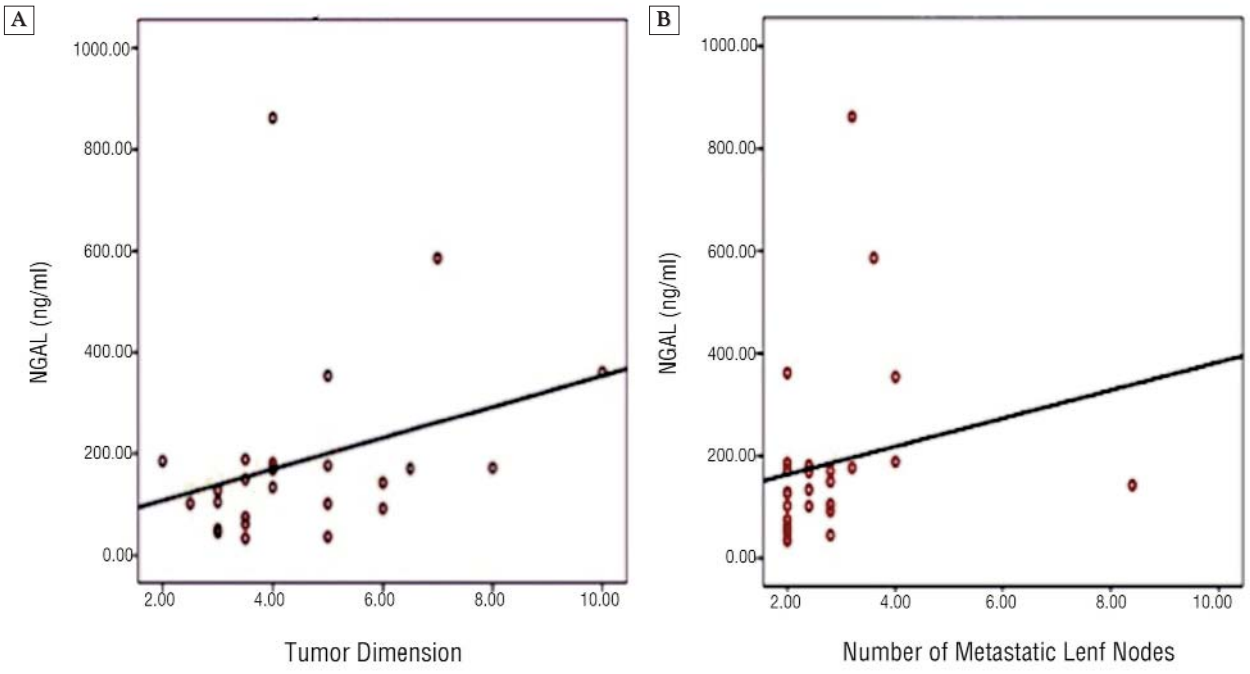

Figure 3. ***(A)*** *Correlation between serum NGAL levels and tumor diameter in colorectal cancer patients.*
(B) *Correlation between serum NGAL levels and the number of metastatic lymph nodes.*

Table 4. *Comparison of the serum NGAL levels and tumor localization, presence of lymphovascular and perineural invasion and tumor differentiation degree in CAG*

Cancer Group (n=27)		**NGAL (ng/ml)** Mean±SD	**p**
+Tumor Localization	Right colon (n=7)	270.09±294.55	0,188
	Left colon (n=4)	107.89±25.23	
	Sigmoid colon (n=6)	112.52±55.47	
	Rectum (n=10)	196.25±143.80	
Lenfovascular Invasion	No (n=15)	167.87±123.20	0.614
	Yes (n=12)	203.47±235.53	
Perineural Invasion	No (n=21)	206.64±195.87	0.195
	Yes (n=6)	103.38±53.7	
+Differentiation	Poor (n=5)	305.91±333.85	0.152
	Moderate (n=17)	158.66±134.38	
	Well (n=5)	146.58±32.36	

Mann Whitney U test was used +Kruskal Wallis test

Table 5. *Evaluation of serum NGAL levels of the patients in CAG, according to T, N and M categories of the TNM staging system*

Cancer Group (n=27)		**NGAL (ng/ml)** Mean±SD	**p**
"T" categories	T2 (n=2)	138,57±66,02	-
	T3 (n=22)	189,77±197,62	
	T4 (n=3)	169,22±23,74	
"N" categories	N0 (n=13)	123,74±88,07	0,048*
	N1 (n=8)	216,89±264,48	
	N2 (n=6)	269,32±172,57	
"M" categories	M0 (n=24)	184,29±190,07	-
	M1 and M2 (n=3)	178,91±8,55	

Mann Whitney U test was used **p<0.05

localization and presence of lymphovascular or perineural invasion (p:0.188, p:0.614, and p:0.195, respectively). Despite revealing no statistical significance, an association detected between increased serum NGAL level and poor cancer differentiation (p:0.152) (*Table 4*).

There was statistically significant difference and positive correlation detected between serum NGAL level and "N" stage according to the TNM classification system. In consequence of the comparisons that were carried out to determine the group which caused the statistically difference, serum NGAL levels of patients with "N2" stage were significantly higher than patients with "N0" stage (p:0.017) (*Table 5*). Statistical analysis of serum NGAL levels could not be carried out with the "T" stages because of the limited number of patients at "T2" and "T4" stages.

Discussion

NGAL is a protein that stored in neutrophil granules with a weight of 25-kDa5. NGAL is characterized by the ability to bind and transport lipophilic substances. It is included in the lipocalin family, which has more than 50 members (8). NGAL delivers iron to the cytoplasm, and also provides its uptake through the specific membrane receptors (30,31). NGAL also plays a strategic role for immune defense against bacterial pathogens by reducing the amount of iron when released from activated neutrophils (32). Additionally, NGAL regulates critically important genes that effect cellular differentiation. Therefore, it participates in the growth, development and differentiation of tissues, beginning from the embryonic period (7-10). Hu et al. determined that NGAL decreases E-cadherin mediated cell-cell adhesion, so increases cellular motility and invasion property of carcinoma cells (21). It is revealed that synthesis of NGAL is induced by various factors that may cause neoplasia (8,10,33).

Sun et al. stated a statistically significant increase of NGAL expression on tissue samples of 526 patients during adenoma-carcinoma sequence. The increase was particularly evident at the stage of transition zone from low-grade to high-grade dysplasia (25). In our study, no statistically significant difference was observed between the high-grade and low-grade dysplasia subgroups regarding to serum NGAL levels (p:0.154). Also we did not detect any statistically significant differences between PG and CAG or COG (p:0,248, and p:0.166, respectively). We need more comprehensive studies to determine whether increased serum NGAL levels occur during the adenoma-carcinoma sequence or not.

Lee et al. reported that elevated CEA levels accompanied by anemia has a predictive significance for CRC, but it did not constitute significance for patients with colon polyps (34). In

our study, CEA levels in PG were significantly higher than COG (p:0,008). Serum NGAL levels in CAG were also higher than the other groups, as consistent with CEA and CA19-9. Positive correlation between the serum NGAL levels and other cancer biomarkers such as serum CEA and CA19-9 levels may support the predictive and diagnostic significance of serum NGAL level.

Tumor size and number of metastatic lymph nodes are known to be the most important prognostic indicators for patients with CRC. However, necessity of biomarker that has prognostic significance for preoperatively staging of CRC is ongoing. Recent articles have revealed that NGAL plays an important role in development of gastrointestinal system cancers such as esophagus and gastric cancer. However, its role in CRC is still unclear (19,23-25). Recent studies have revealed an increased level of NGAL expression in the resection specimens of cancer patients (22,35-37). Some other studies have shown that NGAL mRNA levels have a positive correlation with advanced stage CRC (19,21). Additionally, İt's demonstrated that overexpression of NGAL decreases intercellular connections, and increases tumor invasion, cell-matrix combination and cellular motility in vitro (21). Nevertheless, there are inconsistent and even contradictory reports on the role of NGAL in the development and progression of cancer. Various studies were presented in the literature about genetic and immunohistochemical analyses on tissue samples to evaluate the diagnostic value of NGAL in CRC (19,21,25,38-40). However, there is only one study available in current literature to evaluate the relationship between serum NGAL level and CRC. Researches are still being conducted to find easily applicable blood tests in order to diagnose either early stage colorectal cancer or ongoing adenoma-carcinoma sequence. There are only few studies reported that evaluates differences of serum NGAL levels during adenoma-carcinoma sequence. Duvillard et al (18) demonstrated that serum NGAL levels are detected statistically significant higher in patients with CRC, especially elevated in patients with large tumors. Marti et al (29) reported that metastatic and non-metastatic colorectal cancer patients had higher serum NGAL levels than healthy controls. Likewise, our study revealed that existence of CRC, larger tumor size and number of metastatic lymph nodes are statistically significant correlated with serum NGAL levels. Duvillard et al. detected optimal NGAL cut-off value was 106 ng/mL, with 60% specificity and 70% sensitivity. We revealed similar results that an optimal cut-off value was 103.55 ng/mL, with 68.9% specificity and 71.4% sensitivity. Increased tumor size and the number of metastatic lymph nodes are known to be associated with advanced stage disease and poor prognosis in CRC (41,42). Sun et al. reported that there was no statistically significant correlation between tumor diameter and NGAL expression levels in tissue samples of patients with CRC (25). Our study revealed that serum NGAL level has a statistically significant and positive correlational relationship with tumor size (r:0.38, p:0.047) and the number of metastatic lymph nodes (r:0.42, p:0.026) (*Fig. 3*).

The presence of lymphovascular and perineural invasion increases the risk of lymph node metastasis, as an independent prognostic factor adversely affecting survival (43,44). Similar studies have been carried out to investigate the correlation between prognosis and lymphovascular or perineural invasion (44,45). Our study has limited number of patients with lymphovascular or perineural invasion. As a result, we did not detect statistically significant correlation between serum NGAL levels and lymphovascular or perineural invasion (p:0.614, p:0.195, respectively).

Studies have been carried out to compare the NGAL expression levels in CRC with other tumor features such as cancer differentiation and localization of the tumor. As a result of these studies there were no significant correlations between NGAL expression level and tumor localization or cancer differentiation (25,39). Likewise, we did not find a correlation between serum NGAL levels and these features.

TNM staging system is the most commonly used staging system to predict the prognosis of patients with CRC (41,42). The correlation between TNM staging system and NGAL expression was also examined in several studies and it was concluded that advanced stage tumors were associated with higher NGAL expression levels and poorer prognosis (25,40). Serum NGAL level and "N" stage of patients with CRC was compared in our study. Increased serum NGAL levels were significantly associated with increased number of metastatic lymph nodes (p:0.026). Also statistically significant difference was found between advanced "N" stages and increased serum NGAL levels (p:0.048). We ascertained that the serum NGAL levels of the patients with "N2" stage were significantly higher than those with "N0" stage (p:0.017).

This study revealed no statistically significant difference between the patients with low-grade dysplasia and high-grade dysplasia in PG according to serum NGAL levels. Additionally, no significant correlation between NGAL levels and the adenoma-carcinoma sequence was revealed. Although this study has some limitations such as the number of patients, serum NGAL level was significantly higher in patients with CRC, suggests that the diagnostic value of serum NGAL for CRC. Moreover, the significant positive correlation between serum NGAL levels and well-known prognostic indicators such as the number of metastatic lymph nodes and tumor size may also supports the prognostic value of serum NGAL levels for CRC patients.

Conclusions

In conclusion, preoperative detection of serum NGAL level is an easy applicable test, and 103.55ng/mL cut-off value of serum NGAL for CRC or high-grade dysplasia is detected with 68.9% specificity and 71.4% sensitivity. Although more comprehensive studies are needed for definitive judgments, serum NGAL levels may be used as a diagnostic biomarker and/or predictive test for lymph node metastasis in patients with CRC.

Acknowledgements

We are grateful to Tureng Medical Translation and Empair Medical Statistic for their support and for providing necessary guidance concerning this study.

Declaration of interest

Authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Clinical trial registration number:
ACTRN12614000849695

Conflicts of interest

Ibrahim Ali OZEMIR and other co-authors declare no conflict of interest.

Authors' contribution

Conception: Ibrahim Ali OZEMIR, Sinan ASLAN, Rafet YIGITBASI, Orhan ALIMOGLU.
Design: Ibrahim Ali OZEMIR, Sinan ASLAN, Tunc EREN, Baris BAYRAKTAR.
Supervision: Ibrahim Ali OZEMIR, Orhan ALIMOGLU.
Data collection: Sinan ASLAN, Cagri BILGIC, Banu ISBILEN.
Analysis: Ibrahim Ali OZEMIR, Sinan ASLAN, Banu ISBILEN, Haydar YALMAN.
Writer: Ibrahim Ali OZEMIR, Sinan ASLAN, Tunc EREN.
Critical review: Ibrahim Ali OZEMIR, Haydar YALMAN, Rafet YIGITBASI, Orhan ALIMOGLU.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010 Dec 15;127(12):2893-917. doi: 10.1002/ijc.25516.
2. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319(9):525-32.
3. Ilyas M, Straub J, Tomlinson IP, Bodmer WF. Genetic pathways in colorectal and other cancers. Eur J Cancer. 1999;35(14):1986-2002.
4. Salazar R, Roepman P, Capella G, Moreno V, Simon I, Dreezen C, et al Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. J Clin Oncol. 2011;29(1):17-24. doi: 10.1200/JCO.2010.30.1077. Epub 2010 Nov 22.
5. Sanjoaquin MA, Choodari-Oskooei B, Dolbear C, Putcha V, Sehgal A, Key TJ, et al. Colorectal cancer incidence, mortality and survival in South-east England between 1972 and 2001. Eur J Cancer Prev. 2007 Feb;16(1):10-6.
6. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics. 1997;45(1):17-23.
7. Gwira JA, Wei F, Ishibe S, Ueland JM, Barasch J, Cantley LG. Expression of neutrophil associated-gelatinaselipocalin regulates epithelial morphogenesis in vitro. J Biol Chem. 2005;280(9):7875-82. Epub 2005 Jan 6.
8. Bratt T. Lipocalins and cancer. Biochim Biophys Acta. 2000;1482(1-2):318-26.
9. Zerega B, Cermelli S, Michelis B, Cancedda R, Cancedda FD. Expression of NRL/NGAL (neu-related lipocalin/neutrophil gelatinase-associated lipocalin) during mammalian embryonic development and in inflammation. Eur J Cell Biol. 2000;79(3):165-72.
10. Bolignano D, Donato V, Lacquaniti A, Fazio MR, Bono C, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. Cancer Lett. 2010;288(1):10-6. doi: 10.1016/j.canlet.2009.05.027. Epub 2009 Jun 18.
11. Tong Z, Wu X, Ovcharenko D, Zhu J, Chen CS, Kehrer JP. Neutrophil gelatinase-associated lipocalin as a survival factor. Biochem J. 2005;391(Pt 2):441-8.
12. Wang HJ, He XJ, Ma YY, Jiang XT, Xia YJ, Ye ZY, et al. Expressions of neutrophil gelatinase-associated lipocalin in gastric cancer: a potential biomarker for prognosis and an ancillary diagnostic test. Anat Rec (Hoboken). 2010;293(11):1855-63. doi: 10.1002/ar.21230.
13. Iannetti A, Pacifico F, Acquaviva R, Lavorgna A, Crescenzi E, Vascotto C, et al. The neutrophil gelatinase-associated lipocalin (NGAL), a NF-kappaB-regulated gene, is a survival factor for thyroid neoplastic cells. Proc Natl Acad Sci U S A. 2008;105(37):14058-63. doi: 10.1073/pnas.0710846105. Epub 2008 Sep 3.
14. Miyamoto T, Kashima H, Suzuki A, Kikuchi N, Konishi I, Seki N, et al. Laser-captured microdissection-microarray analysis of the genes involved in endometrial carcinogenesis: stepwise up-regulation of lipocalin2 expression in normal and neoplastic endometria and its functional relevance. Hum Pathol. 2011;42(9):1265-74. doi: 10.1016/j.humpath.2010.07.027. Epub 2011 Feb 21.
15. Sung H, Choi JY, Lee SA, Lee KM, Han S, Jeon S, et al. The association between the preoperative serum levels of lipocalin-2 and matrix metalloproteinase-9 (MMP-9) and prognosis of breast cancer. BMC Cancer. 2012;12:193. doi: 10.1186/1471-2407-12-193.
16. Wenners AS, Mehta K, Loibl S, Park H, Mueller B, Arnold N, et al. Neutrophil gelatinase-associated lipocalin (NGAL) predicts response to neoadjuvant chemotherapy and clinical outcome in primary human breast cancer. PLoS One 2012;7:e45826.
17. Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL, et al. Lipocalin 2 promotes breast cancer progression. Proc Natl Acad Sci U S A. 2009;106(10):3913-8. doi: 10.1073/pnas.0810617106. Epub 2009 Feb 23.
18. Duvillard L, Ortega-Deballon P, Bourredjem A, Scherrer ML, Mantion G, Delhorme JB, et al. A case-control study of preoperative levels of serum neutrophil gelatinase-associated lipocalin and other potential inflammatory markers in colorectal cancer. BMC Cancer. 2014;14:912. doi: 10.1186/1471-2407-14-912.
19. Zhang XF, Zhang Y, Zhang XH, Zhou SM, Yang GG, Wang OC, et al. Clinical significance of neutrophil gelatinase-associated lipocalin (NGAL) expression in primary rectal cancer. BMC Cancer. 2009;9:134. doi: 10.1186/1471-2407-9-134.
20. Bousserouel S, Kauntz H, Gosse F, Bouhadjar M, Soler L, Marescaux J, et al. Identification of gene expression profiles correlated to tumor progression in a preclinical model of colon carcinogenesis. Int J Oncol. 2010;36(6):1485-90.
21. Hu L, Hittelman W, Lu T, Ji P, Arlinghaus R, Shmulevich I, et al. NGAL decreases E-cadherin-mediated cell-cell adhesion and

increases cell motility and invasion through Rac1 in colon carcinoma cells. Lab Invest. 2009;89(5):531-48. doi: 10.1038/labinvest.2009.17. Epub 2009 Mar 23.
22. Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M, Kjedsen L. Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut. 1996 Mar;38(3):414-20.
23. Kubben FJ, Sier CF, Hawinkels LJ, Tschesche H, van Dujin W, Zuidwijk K, et al. Clinical evidence for a protective role of lipocalin-2 against MMP-9 auto degradation and the impact for gastric cancer. Eur J Cancer. 2007;43(12):1869-76. Epub 2007 Jul 2.
24. Zhang H, Xu L, Xiao D, Xie J, Zeng H, Wang Z, et al. Upregulation of neutrophil gelatinase-associated lipocalin in oesophageal squamous cell carcinoma: significant correlation with cell differentiation and tumour invasion. J Clin Pathol. 2007;60(5):555-61. Epub 2007 Apr 5.
25. Sun Y, Yokoi K, Li H, Gao J, Hu L, Liu B, et al. NGAL Expression Is Elevated in Both Colorectal Adenoma-Carcinoma Sequence and Cancer Progression and Enhances Tumorigenesis in Xenograft Mouse Models. Clin Cancer Res. 2011;17(13):4331-40. doi: 10.1158/1078-0432.CCR-11-0226. Epub 2011 May 27.
26. Cho H, Kim JH. Lipocalin2 expressions correlate significantly with tumor differentiation in epithelial ovarian cancer. J Histochem Cytochem. 2009;57(5):513-21. doi: 10.1369/jhc.2009.953257. Epub 2009 Feb 2.
27. Porta C, Paglino C, De Amici M, Quaglini S, Sacchi L, Imarisio I, et al. Predictive value of baseline serum vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in advanced kidney cancer patients receiving sunitinib. Kidney Int. 2010;77(9):809-15. doi: 10.1038/ki.2009.552. Epub 2010 Feb 10.
28. Fung KY, Priebe I, Purins L, Tabor B, Brierley GV, Lockett T, et al. Performance of serum lipocalin 2 as a diagnostic marker for colorectal cancer. Cancer Biomark. 2013;13(2):75-9. doi: 10.3233/CBM-130335.
29. Martí J, Fuster J, Solà AM, Hotter G, Molina R, Pelegrina A, et al. Prognostic value of serum neutrophil gelatinase-associated lipocalin in metastatic and nonmetastatic colorectal cancer. World J Surg. 2013;37(5):1103-9. doi: 10.1007/s00268-013-1930-z.
30. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, et al. An iron delivery pathway mediated by a lipocalin. Mol Cell. 2002;10(5):1045-56.
31. Goetz DH, Willie ST, Armen RS, Bratt T, Borregaard N, Strong RK. Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. Biochemistry. 2000;39(8):1935-41.
32. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell. 2002;10(5):1033-43.
33. Hannai J, Mammoto T, Seth P, Mori K, Karumanchi SA, Barasch J, et al. Lipocalin 2 diminishes invasiveness and metastasis of Ras-transformed cells. J Biol Chem. 2005;280(14):13641-7. Epub 2005 Feb 3.
34. Lee JH, Hong SP, Jeon TJ, Kang GH, Choi WC, Jeon SM, et al. Should a colonoscopy be recommended for healthy individuals with increased carcinoembryonic antigen levels? A case-control study. Dig Dis Sci. 2011;56(8):2396-403. doi: 10.1007/s10620-011-1606-1. Epub 2011 Feb 17.
35. Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N, Friedl A. Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. Breast Cancer Res Treat. 2008;108(3):389-97. Epub 2007 Jun 7.
36. Lannetti A, Pacifico F, Acquaviva R, Lavorgna A, Crescenzi E, Vascotto C, et al. The neutrophil gelatinase-associated lipocalin (NGAL), a NF-kappaB-regulated gene, is a survival factor for thyroid neoplastic cells. Proc Natl Acad Sci U S A. 2008;105(37):14058-63. doi: 10.1073/pnas.0710846105. Epub 2008 Sep 3.
37. Barresi V, Ieni A, Bolignano D, Magno C, Buemi M, Barresi G. Neutrophil gelatinase-associated lipocalin immunoexpression in renal tumors: correlation with histotype and histological grade. Oncol Rep. 2010;24(2):305-10.
38. Barresi V, Luciano R, Vitarelli E, Labate A, Tuccari G, Barresi G. Neutrophil gelatinase-associated lipocalinimmuno expression in colorectal carcinoma: A stage-specific prognostic factor? Oncol Lett. 2010;1(6):1089-1096. Epub 2010 Sep 23.
39. Lv Z, Xu L, Shen ZY, Zhang FR, Xu XE, Li EM. Overexpression of neutrophil gelatinase-associated lipocalin and its receptor in colorectal carcinoma: Significant correlation with cell differentiation and tumour invasion. Oncol Lett. 2010;1(1):103-108. Epub 2010 Jan 1.
40. Barresi V, Reggiani-Bonetti L, Di Gregorio C, Vitarelli E, Ponz De Leon M, Barresi G. Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP9) prognostic value in stage I colorectal carcinoma. Pathol Res Pract. 2011;5:479-86.
41. Rosai J. Gastrointestinal tract. In: Rosai and Ackerman's surgical pathology, volume 1, 9th ed, Mosby. 2004. p. 776-855.
42. Fenoglio-Preiser CM, Noffsinger AE, Stemmermann GN. Carsinomas and other epithelial and neuroendocrine tumors of the large intestine. In: Gastrointestinal pathology an atlas and text, 2nd ed, Lippincott-Raven, Philadelphia; 1999. p. 909-1068.
43. Lim SB, Yu CS, Jang SJ, Kim TV, Kim JH, Kim JC. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. Dis Colon Rectum. 2010;53(4):377-84. doi: 10.1007/DCR.0b013e3181cf8ae5.
44. Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. Ann Surg Oncol. 2010;17(8):2066-72. doi: 10.1245/s10434-010-0982-7. Epub 2010 Feb 25.
45. Yamamoto S, Watanabe M, Hasegawa H, Baba H, Yoshinare K, Shiraishi J, et al. The risk of lymph node metastasis in T1 colorectal carcinoma. Hepatogastroenterology. 2004;51(58):998-1000.