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The clinical significance and diagnostic value of serum Dickkopf1 and CKAP4 levels in patients with gastric cancer: a prospective study

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Abstract. – OBJECTIVE: Gastric cancer is diagnosed at an advanced stage in most patients, and the prognosis is poor. Novel biochemical markers of high diagnostic value for the detection of the disease are therefore important. Dickkopf1 (DKK1) and cytoskeleton-associated protein 4 (CKAP4) have been extensively studied as biomarkers in cancer patients.

PATIENTS AND METHODS: Serum DKK1 and CKAP4 levels in 58 patients with gastric cancer and 41 healthy controls were examined using an ELISA kit in this prospective study. The patients were subdivided into groups based on pathological TNM staging and histological grades. Serum levels of both proteins in the patients with gastric cancer were measured preoperatively, 10 and 30 days after surgery.

RESULTS: Serum DKK1 and CKAP4 levels were significantly higher in the gastric cancer group compared to the healthy controls ($p<0.05$). Serum levels of both proteins increased in line with the pathological stage and histological grade of the gastric cancer. Serum CKAP4 and DKK1 levels decreased after surgical resection. Both serum levels also decreased significantly on day 30 after surgery compared to day 10 ($p<0.05$). Serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) positivity rates were below 20% in the gastric cancer group, while the diagnostic value (sensitivity and specificity) of serum CKAP-4 and DKK1 exceeded 80%.

CONCLUSIONS: DKK1 and CKAP4 are biomarkers of high diagnostic value that can be used to diagnose and predict the severity of gastric cancer. These proteins can also be employed for disease monitoring after surgical resection. The diagnostic value of these proteins is higher than that of biomarkers such as CEA and CA19-9, which are routinely used in clinical practice.

Key Words:
Gastric cancer, DKK1, CKAP4, Biomarker.

Introduction

Gastric cancer is the second leading cause of cancer-related deaths and the fourth most prevalent type of cancer worldwide[1]. The disease is diagnosed at an advanced stage in most cases, and the prognosis is poor[2]. However, recent advances in surgical techniques (endoscopic resection) and treatment (chemotherapy and radiotherapy) have made significant contributions to survival. However, while the five-year survival rate is 90% in early gastric cancer, this declines to 40% in advanced disease. It is, therefore, vitally important to detect and treat the disease in its early stages[3]. The lack of diagnostic tools or biomarkers capable of monitoring the pathological progression of the disease and predicting early diagnosis is the greatest barrier to the development of effective therapeutic modalities[4]. The discovery of a suitable biomarker will play an important role in the diagnosis and early treatment of the disease[5]. Tumor markers can be used for the diagnosis of malignancy, determination of prognosis, and prediction of recurrence and response to treatment. Although cancer tissue samples are the optimal material for tumor marker evaluation, blood samples are more easily accessible through non-invasive procedures[6]. Although many biomarkers have been identified to date, no biomarker of high diagnostic value that is easily detectable in the blood and that can be used to diagnose gastric cancer early, predict the stage of the disease, and monitor the disease course has to date been discovered[7,8]. Markers of high diagnostic value for new clinical uses are therefore needed.

The proteins Dickkopf1 (DKK1) and cytoskeleton-associated protein 4 (CKAP4) may serve as

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biomarkers for the diagnosis of gastric cancer and predict its clinical course. The DKK1-CKAP4 signal axis is the subject of research in many cancers, and it is likely that these markers and signaling pathways will be discussed for many years to come. Studies[9] have emphasized this and investigated how antibodies to both proteins can be used in the treatment of cancer.

This study investigated the serum levels of DKK1/CKAP4 proteins, which have been the subject of numerous studies in recent years, particularly in oncological research, in patients with gastric cancer. The findings will shed light on the relationship between these proteins and the disease stage and histological grade of the tumor and how the serum levels of these biomarkers change after gastric resection.

Patients and Methods

This prospective study was approved by the Atatürk University Faculty of Medicine Institutional Research Ethics Board, Türkiye (No. 24.06.2021, B.30.2.ATA.0.01.00/), and informed consent was provided by all the patients. All procedures in this study involving human participants were performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

This study involved patients with gastric cancer admitted to the Atatürk University Medical Faculty Research Hospital general surgery clinic and healthy adults who presented to the general surgery outpatient clinic. Fifty-eight patients with preoperative histopathological diagnoses of gastric adenocarcinoma and 41 healthy controls were enrolled. In order to minimize the effects on serum DKK1/CKAP4 levels, patients with a primary tumor other than stomach cancer, with significant systemic disease, with a history of chemotherapy and radiotherapy other than for gastric cancer, with more than one comorbid disease, with a history of drug use capable of affecting laboratory values, and pediatric patients under the age of 18 were excluded from the study. The control group consisted of healthy individuals (Table I).

Blood was drawn from gastric cancer patients immediately before surgery and 10 days and one month after surgery for DKK1/CKAP4 level measurement. Blood was collected once only from the healthy control group. The carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels of patients diagnosed with gastric cancer were subjected to routine study in the preoperative period. Serum levels of proteins were analyzed in three different ways, based on 1) pre- and post-operative values, 2) pathological TNM staging, and 3) histological grade (Table II, Figure 1 and 2). Tumor staging was based on postoperative pathology reports. The staging was performed in accordance with the TNM staging system for gastric cancer, and TNM staging was carried out in line with the 8th American Joint Committee on Cancer (AJCC)[10]. Tumors were classified histologically as a pathological complete response to neoadjuvant therapy (Grade 0), or (Grade 1), moderately (Grade 2), or poorly (Grade 3) differentiated based on the predominant cell type[10]. Receiver operating characteristic (ROC) analyses were applied to compare the cancer patients with the healthy controls. The data were retrieved from the patient files and the hospital's electronic software system.

Biochemical Analysis

Serum obtained from whole blood samples collected at admission were analyzed by enzyme-linked immunosorbent assay (ELISA) using the Human CKAP4 ELISA Kit (BT LAB, Cat. No. E4664Hu, China) and Human DKK1 ELISA Kit (BT LAB, Cat. No. E0630Hu, China) according to the manufacturer's instructions. The inter-assay and intra-assay coefficients of variance given by the manufacturer are <10% and <8%, respectively. Briefly, the samples and standards were added to wells pre-coated with human CKAP4 and DKK1 antibodies. The CKAP4 and DKK1 present in the samples were bound by the antibodies coating the wells. Biotinylated human CKAP4 and DKK1 antibody was then added to bind to the CKAP4 and DKK1 bound, followed by streptavidin-horseradish peroxidase (HRP) to bind to the biotinylated CKAP4 and DKK1 antibody. After incubation, the unbound streptavidin-HRP was washed away. Substrate solution was added, and color developed in proportion to the amount of human CKAP4 and DKK1 in the well. The reaction was terminated by adding an acidic stop solution and absorbance was measured at 450 nm. CKAP4 and DKK1 concentrations were determined by comparing the optical density in the sample wells with the standard curve. The results were expressed as ng/L and ng/mL, respectively.

Statistical Analysis

In the statistical analysis, numerical data for descriptive statistics were expressed as mean and standard deviation and categorical data as numbers and percentages. The distribution of numer-

Table I. Demographic and clinicopathologic features of gastric cancer and healthy control patients.

Parameters	n	
	GC patients	Healthy controls
Age (years)	61.48±10.18 (35-79)	43.1±18.6 (24-78)
Sex		
Female	16 (27.6%)	25 (61%)
Male	42 (72.4%)	16 (39%)
Body Mass Index (BMI)	24.91 (13.06-37.9)	30.03 (22.8-32.4)
Symptoms		
Weight loss	38 (65.5%)	
Abdominal pain	34 (58.6%)	
Nausea/vomiting	17 (29.3%)	
Dysphagia	14 (24.1%)	
Melena	8 (13.8%)	
Comorbidity		
Yes	24 (41.4%)	
No	34 (58.6%)	
Covid-19 history	15 (25.9%)	
CEA (ng/mL) (0-5)	3.42±2.95	
CA19-9 U/ml (0-39)	16.66±16.1	
Preoperative Chemotherapy		
Yes	47 (81%)	
No	11 (19 %)	
Type of surgery		
Distal gastrectomy	17 (29.3%)	
Total gastrectomy	41 (70.7%)	
TNM classification (pStage)		
Stage 0 (Complete response to chemotherapy)	9 (15.5%)	
Stage 1	11 (19%)	
Stage 2	21 (36.2%)	
Stage 3	17 (29.3%)	
Grade/differentiation		
Grade 0	9 (15.5%)	
Grade 1	3 (15.2%)	
Grade 2	29 (50%)	
Grade 3	17 (29.3%)	
Postoperative complications	15 (25.9%)	

HR: hazard ratio. CI: confidence interval. SCC: squamous cell carcinoma. FIGO: International Federation of Gynecology and Obstetrics. DFS: disease-free survival. OS: overall survival. $p<0.05$ and $p<0.01$ values were accepted for the significance level of the tests.

ical data was analyzed using a normality test and histogram graphics. Numerical data in more than two groups were compared using the One-Way ANOVA Post Hoc Tukey test. Student's *t*-test was used to compare paired groups in terms of independent samples. Additionally, the status of two numerical data points was analyzed using Pearson's correlation analysis. The Chi-square test was applied when comparing categorical data. ROC curves were produced to measure the ability of laboratory values to distinguish between healthy control and gastric cancer status. The area under the curve (AUC) and cut-off value were determined for each measurement. Specificity, sensitivity, and predictive values were calculated and evaluated together. SPSS version 23.0 for Windows software (IBM Corp., Armonk, NY, USA) was used for data recording and statistical evaluations. *p*-values <0.05 were considered statistically significant.

Table II. DKK1 and CKAP4 levels of gastric cancer and control group patients.

		n	Mean	Standard deviation	Minimum	Maximum	p-value
CKAP4	Preoperative period	58	1,243	574	342	3,056	<0.05*
	Postoperative day 10	58	971	531	259	2,731	
	Postoperative day 30	58	661	430	123	2,196	
	Healthy controls	41	196	74	102	444	
DKK1	Preoperative period	58	1,400	510	618	2,776	<0.05*
	Postoperative day 10	58	1,070	429	341	2,440	
	Postoperative day 30	58	770	352	196	2,178	
	Healthy controls	41	229	115	112	687	

*There is a significant difference between all groups (One-Way ANOVA test was applied).

Results

One hundred adult patients underwent surgery for gastric adenocarcinoma in the Atatürk University Medical Faculty Gastrointestinal Surgery Unit in 2021-2022. Forty-two patients with multiple comorbid diseases, who were deemed non-resectable at the time of surgery, who could not be followed up postoperatively for DKK1/CKAP4 blood level measurement, and for whom biochemical analysis could not be performed, were excluded from the study. The laboratory values of 58 patients with gastric adenocarcinoma were thus evaluated before surgery and 10 days and one month postoperatively. Fifty patients who presented to the general surgery outpatient clinic with benign diseases such as abdominal hernias and gallbladders but with no chronic diseases were enrolled as the control group. The blood results of 41 members of the control group were subjected to analysis.

Men represented 42 (72.4%) of the gastric cancer patients and 16 women (27.6%), while the control group consisted of 16 (39%) men and 25 (61%) women. Mean ages were 61.48±10.18 (35-

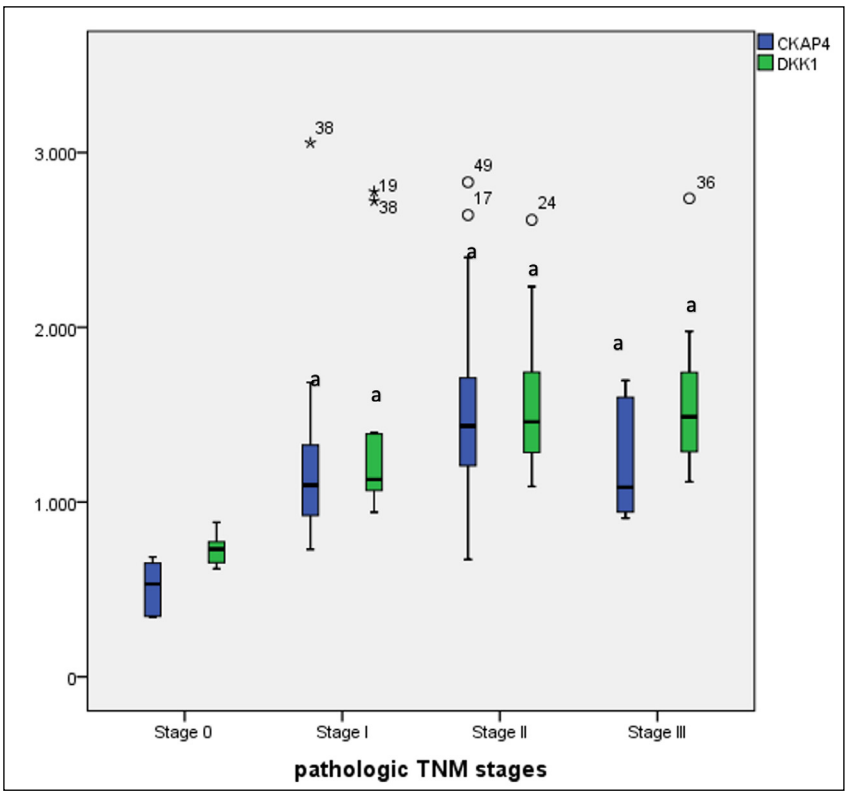

Figure 1. DKK1- CKAP4 serum levels according to pathologic TNM stages in gastric cancer patients. a: $p<0.05$ compared to stage 0. *While there is a significant difference between stage 0 and other stages, there is no significant difference between other stages (One-Way ANOVA test was applied).

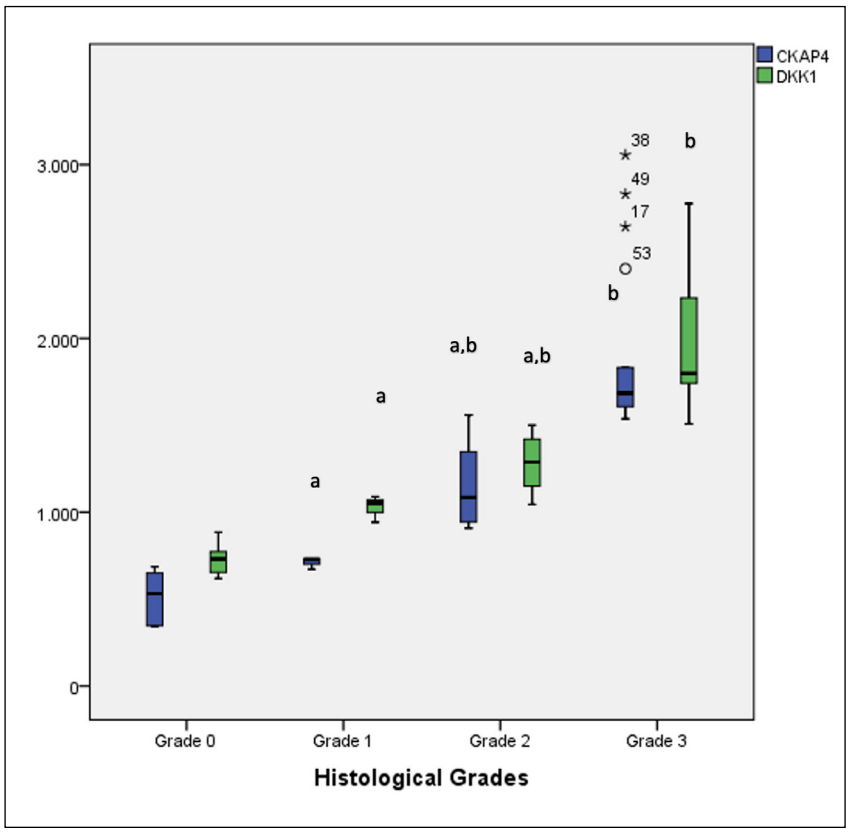

Figure 2. Serum DKK1- CKAP4 levels according to histological grades in gastric cancer patients.a: $p<0.05$ compared to grade 3, b: $p<0.05$ compared to grade 0. *While there is no significant difference between Grade 0 and 1 and between Grade 1 and 2, there is a significant difference between other groups.

79) in the gastric cancer group and 43.1±18.6 (24-78) in the control group. Comorbid diseases were present in 41.4% of the gastric cancer patients (Table I). The most common complaints among the patients were weight loss (65.5%), abdominal pain (58.6%), nausea/vomiting (29.3%), dysphagia (24.1%), and melena (13.8%). Serum CEA concentrations >5 ng/ml or serum CA19-9 concentrations >39 U/ml were regarded as positive for gastric cancer. The findings of 11 (19.29%) of the 57 patients were positive for gastric cancer based on serum CEA concentrations, and those of seven (12.28%) were positive based on CA19-9 concentrations. The CKAP4 reaction rate was 92%, while the DKK1 reaction rate was 95%. Neoadjuvant chemotherapy was administered to 81% of the locally advanced gastric cancer patients. Total gastrectomy was performed in 70.1% of cases and distal gastrectomy in 29.9% (Table I).

Based on the postoperative histopathological evaluation of the gastrectomy materials (TNM staging system), nine cases were evaluated as stage 0 complete response to chemotherapy, 11 as stage 1, 21 as stage 2, and 17 as stage 3. In terms of the degree of differentiation of the tumor, nine cases were evaluated as grade 0, three as grade 1, 29 as grade 2, and 17 as grade 3[10].

Serum levels of CKAP4 and DKK1 were significantly higher in patients with gastric cancer than in the control group ($p<0.05$). The gastric cancer patients' serum levels were higher in the preoperative period, decreased on the 10th postoperative day, and reached their lowest level on the post-operative 30th day ($p<0.05$) (Table II).

Blood levels of CKAP4 and DKKI may be expected to increase in line with the stage of the disease. Although this was not observed in our stage 3 gastric cancer patients, serum DKK1 and CKAP4 levels were significantly lower in stages 0 and 1 than in stages 2 and 3 ($p<0.05$). While a significant difference was observed between stage 0 and the other stages, no difference was determined between stages 1 and stages 2 and 3, and none between stages 2 and 3 (Figure 1).

The examination of the histological grades for DKK1 and CKAP4 levels revealed no significant difference between grades 0 and 1 nor between grades 1 and 2 ($p>0.05$), but significant differences were determined between grade 0 and grades 2 and 3, and between grade 3 and all the other groups ($p<0.05$) (Figure 2).

The diagnostic efficiency of CKAP4 and DKK1 in differentiating between gastric cancer patients and healthy controls was evaluated using ROC analysis. The Youden index was used to calculate optimum cut-off values, determined as 257 ng/l for CKAP4 and 386 ng/ml for DKK1. Accordingly, the area under the curve (AUC) value for CKAP4 was calculated as 0.899 (p<0.001) in distinguishing between the control and gastric cancer groups. The sensitivity and specificity of CKAP4 were 85% and 81%, respectively. The AUC value of DKK1 was calculated as 0.989 (p<0.001) in distinguishing between the healthy control and gastric cancer groups. The sensitivity of DKK1 was 97%, and the specificity was 93%, as shown in Figure 3 and Table III.

A significant positive correlation was observed between DKK1 and CKAP4 (r=.903, p<0.05). Since the patients received neoadjuvant therapy, the relationship between tumor diameter and marker levels could not be evaluated. The relationship between tumor diameter and serum protein levels can only be optimally evaluated in patients who have not received chemotherapy but have undergone surgery. Postoperative complications were scored and classified using the Clavien-Dindo Classification (CDC). Morbidity included all postoperative complications until discharge or up to 30 days[11]. Complications developed in 25.9% of the patients. Five patients were classified as grade 1, five as grade 2, three as grade 3, and two as grade 4, according to the CDC.

Discussion

Despite advances in modern surgical techniques and chemoradiotherapy, prognosis in gastric cancer is still poor. The disease is frequently detected late[12]. Novel biochemical markers with high diagnostic efficiency capable of use in the detection of the disease are, therefore, urgently needed. DKK1 and CKAP4 have recently been proposed as biomarkers and extensively studied in cancer patients. These proteins may also be promising in the diagnosis of gastric cancer[13].

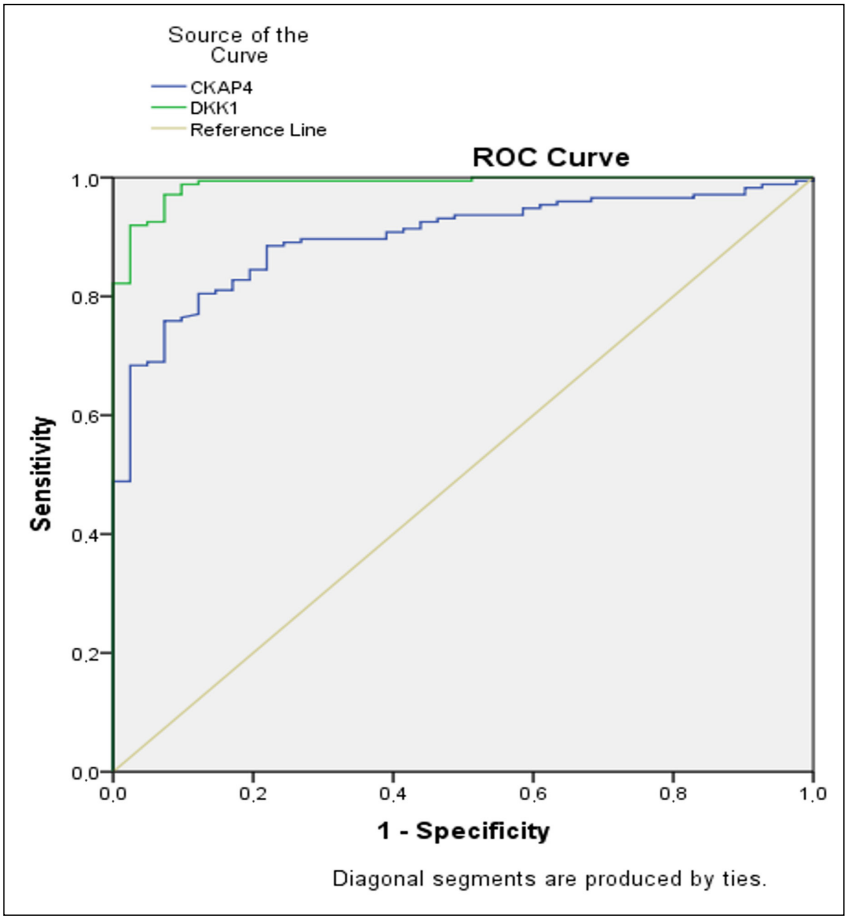

Figure 3. ROC curve analyses of DKK1/CKAP4 for the diagnosis of gastric cancer.

Table III. Comparison of healthy controls and gastric cancer groups (ROC curve test).

Parameters	Cut-off value (p)	AUC	Sensitivity (%)	Specificity (%)	PPV	NPV
CKAP4	257	0,899 (<0.001)	85	81	81.7	84.3
DKK1	386	0,989 (<0.001)	97	93	93.2	96.8

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

CKAP acts as the DKK1 receptor, and these proteins form the DKK1/CKAP4 signaling pathway, which is involved in both normal and cancer cell proliferation[9,14]. DKK1 is a secretory protein and a member of the DKK family. It inhibits the Wnt signaling pathway by binding to the Wnt coreceptor low-density lipoprotein receptor-related protein 6 (LRP6)[13,15]. DKK1 binds with similar affinities to CKAP4 and LRP6[9]. While DKK1 is overexpressed in cancers such as breast, lung, esophageal, and ovarian cancers, multiple myeloma, Wilms tumor, hepatoblastoma, and hepatocellular cancer, decreased DKK1 expression is observed in colon cancer, renal cell cancer, and leukemia. DKK1 expression thus differs depending on the tumor site[16-18].

The degree of expression of proteins affects cancer survival. High DDK1 serum levels and overexpression have been associated with poor prognosis in many types of malignancy[18-20]. Studies of DKK1 have yielded contradictory results. Some studies[21] have reported that it exhibits tumor-suppressor characteristics, while others have suggested oncogene features. These inconsistent results show the presence of unknown areas in DKK1 expression, and the scarcity of studies on gastric cancer patients highlights the need to investigate this. The expression status of DKK1/CKAP4 proteins has been investigated in the literature, although few studies have examined serum levels. The measurement of serum DKK1 levels in esophageal and lung cancer patients using an ELISA system revealed significantly higher values in cancer patients compared to healthy control groups. However, serum levels decreased dramatically following surgical resection[19]. The Wnt/ß-catenin pathway is known to be activated in gastric cancer[3]. The DKK1 protein may thus represent a guiding marker in that form of cancer. In the present study, serum DKK1 levels were significantly higher in the gastric cancer group than in the control group. When gastric cancer patients were classified based on pathological stage and histopathological grades, serum DKK1 levels increased in line with the disease stage. Although no statistical significance was observed between some stage groups, when the stages were combined, a significant difference was observed between groups I (stage 0-1) and II (stage 2-3). DKK1 serum levels decrease after surgical resection. The protein levels in serum decrease in line with the diminution of the tumor burden.

Examination of the second protein of the DKK1-CKAP4 pathway in the light of current literature shows that higher CKAP4 serum levels have been reported[22,23] in patients with esophageal squamous cell cancer and lung cancer compared to in healthy controls. CKAP4 is also highly immunohistochemically expressed in lung cancer. Expression of CKAP4 has also been implicated as a predictor of favorable clinical outcomes and prognosis in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. CKAP4 can thus be regarded as a tumor suppressor[9]. CKAP4 immunohistochemical positivity in tumor tissues has been reported in 94% of serum-positive patients. Since the serum level provides information concerning the expression level, this makes it possible to evaluate the effectiveness of anti-CKAP4 antibody therapy in these patients. In the current study, the findings suggested that Osimertinib and anti-CKAP4 antibody therapy combinations may represent a new therapeutic strategy in the treatment of lung cancer. Similarly, decreased serum levels of CKAP4 have been observed following resection of the primary lesion. Researchers have thus described CKAP4 as a marker capable of use in the follow-up of lung cancer patients[14]. CKAP4 may also represent a novel therapeutic target for cancers that express both CKAP4 and DKK1[24]. In the present study, serum CKAP4 levels in gastric cancer patients were significantly higher than those in the control group. Similarly to DKK1 levels, when gastric cancer patients were classified on the basis of pathological stage and histological grades, serum CKAP4 levels increased in line with the disease stage. Although no statistical significance was observed between some stage groups, when the

stages were combined, a significant difference was observed between group I (stage 0-1) and group II (stage 2-3). Serum CKAP4 levels also decreased after surgical resection.

Studies[9,24] have examined the serum levels or expression levels of both proteins together. Analysis of esophageal squamous cell carcinoma (ESCC) specimens in one study[24] showed that the mRNA levels of CKAP4 and DKK1 were more than twice as high in the tumor lesions than in non-tumor regions. The DKK1-CKAP4 signaling axis promotes the proliferation of ESCC cells. Those cells expressing both proteins exhibited poor prognosis and relapse-free survival. DKK1 and CKAP4 expressions have been shown[9] in pancreatic and lung cancers, and the simultaneous expression of both proteins adversely affects prognosis and relapse-free survival. CKAP4 was detected in 66-74% of lung and pancreatic cancer patients in one study[9], whereas a minimal positive signal (expression) was detected in non-tumor areas. Positive DKK1 expression was found[9] at similar rates to that of CKAP4 in the same tumors. In the present study, although CKAP4 reacted slightly less than DKK1, the positive measurement of both proteins exceeded 90%. A positive correlation was observed between the serum levels of both proteins in gastric cancer patients.

CEA-CA19-9 is the most commonly used traditional cancer biomarker for diagnosing gastric cancer in clinical practice[25,26]. Reported[27-29] CA19-9 positivity rates in gastric cancer patients are 8.7-50.0%, and CEA positivity rates are 10.6-57.6%. Although the sensitivity rates of CEA and CA19-9 are reported[6] at up to 50%, these figures are more usually in the region of 20%. Although these markers are used in routine gastric cancer screening and diagnosis, they are not yet of sufficient diagnostic value. While the serum CEA levels of the gastric cancer patients in this study were consistent with the previous literature, the sensitivity rate was 19.29% and the rate of CA19-9 was 12.28%. Although DKK1 and CKAP4 proteins are routinely used markers, their diagnostic efficiency in gastric cancer is much higher than that of conventional markers.

The objective of chemotherapy is to inhibit cell proliferation and tumor multiplication, thus preventing invasion and metastasis. The aim of neoadjuvant therapy (NAT) is to shrink primary tumors and eliminate microscopic metastatic lesions in order to degrade the staging and improve the R0 resection rate. The use of NAT in gastric cancers has increased considerably in recent years[30]. In our own daily clinical practice, we apply NAT to locally advanced gastric cancers. The levels of tumor markers due to downstaging are expected to decrease after NAT. In the present study, the stage 0 and grade 0 patient group represented the locally advanced gastric cancer patients who fully responded to chemotherapy. Both protein levels decreased to the lowest level in this patient group. The negative effect of NAT on the protein production of tumor tissue was clearly visible. However, our study did not yield detailed information concerning the serum levels of both markers with NAT. Prospective studies with larger patient numbers are now needed on this subject.

Study Limitations and Strengths

This study has a number of strengths and limitations. The principal limitation is that protein expression in tissues was not evaluated. With the available project budget, only the proteins' serum levels could be examined. The second limitation is that there was insufficient time for patient follow-up, and no relationship could be established between protein values and surveillance. Another limitation is the small number of patients studied due to the high cost of the markers investigated using the ELISA method. In our next study, we intend to perform immunohistochemical and molecular analyses on pathological specimens and examine the expression level of proteins in tissues. Examining the correlation between serum protein levels and tissue expression levels is another objective. Despite these limitations, we also think that our study also has several particular strengths. Previous studies have generally compared cancer patients with a healthy control group. In the present study, however, gastric cancer patients were subdivided into stages and compared on the basis of these. One of the particular strengths of this study is that serum protein levels were examined according to the severity of the disease. The fact that the two proteins were investigated together and the correlation between them was measured may represent another important aspect of this study.

Conclusions

Consistent with the abovementioned studies, our results also showed that higher DKK1 and CKAP4 values were identified more frequently in sera from gastric cancer patients than in those from the healthy controls. Serum DKK1 and CKAP4 levels also increased in line with the

stage of the disease and decreased significantly after surgical resection. We think that DKK1 and CKAP4 are biomarkers that can be used in the diagnosis of gastric cancer and in predicting the severity of the disease. Further studies examining the serum and tissue expression levels of these proteins and the correlation between tissue and serum levels may shed more light on this subject.

Authors' Contributions

R.P: Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; visualization; writing–original draft; writing – review and editing. ED: Conceptualization; data curation; formal analysis; investigation; methodology; validation; writing – review and editing EL: Data curation; formal analysis; investigation; methodology; software; supervision; writing – review and editing. MY: Data curation; investigation; methodology; software; resources; validation; EA: Data curation; investigation; validation; writing – review and editing. ZH: Conceptualization; investigation; methodology; validation; visualization; writing – original draft. SSA: Conceptualization; investigation; resources; supervision; writing – review and editing. All authors have read and approved the manuscript and ensure the accuracy of the information presented.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Availability of Data and Materials

The data supporting this article are available from the corresponding and senior author on reasonable request.

Ethics Approval

This study was approved by the Institutional Research Ethics Board of Atatürk University Faculty of Medicine (No.: 24.06.2021, B.30.2.ATA.0.01.00/). All procedures in this study involving human participants were performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

Informed Consent

Informed consent was provided by all the patients.

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