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DR-70 as a novel diagnostic biomarker for gastric cancer

STOMACH

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Original Article

ABSTRACT

Background/Aims: To assess the utility of the DR-70 immunoassay in the diagnosis of gastric cancer.

Materials and Methods: A total of 29 patients with histologically proven malignant gastric tumor and 29 healthy blood donors were enrolled in this study. DR-70 immunoassay was performed using an enzyme-linked immunosorbent assay kit to quantify the serum levels of fibrin degradation products.

Results: The DR-70 values in patients with gastric cancer significantly differed from the values in controls ($p<0.0001$). Receiver operating characteristic curve analysis revealed ≥1.45 µg/mL as the best cut-off value to distinguish between patients with gastric cancer and healthy controls. The area under the receiver operating characteristic curve was 0.871. Using ≥1.45 µg/mL as the cut-off value, the DR-70 immunoassay showed a good clinical performance with a sensitivity of 82.8% and a specificity of 79.3%. The positive predictive value was 80.0%, and the negative predictive value was 82.1%.

Conclusion: The DR-70 immunoassay reliably differs between gastric cancer and healthy controls, promising to become a useful cancer detection tool in clinical practice.

Keywords: DR-70, fibrin degradation products, gastric cancer, tumor markers

INTRODUCTION

A cancer biomarker may be a molecular, cellular, tissue, or process-based alteration (1). In the clinical practice they can be used for screening, diagnosis, and prognostic assessment and determination of therapeutic strategy and monitoring. They can also be available during follow-up subsequent to treatment. Although cancer biomarkers have contributed to improved therapeutic outcomes, they are currently in routine use only in a limited number. Developing new biomarkers requires the identification of candidates and their validation. There must be convincing evidence that a biomarker has sufficient test characteristics and clinical performance to accurately diagnose a cancer in the screened population.

Gastric cancer is a leading cause of cancer death worldwide (2). Surgical resection remains the mainstay of treatment and cure in localized, non-metastatic gastric cancer cases, whereas there is no global consensus on the best treatment regimen to be used in advanced gastric cancer (3). In addition, novel treatment strategies are required, based on the discovery of predictive biomarkers, to improve the prognosis of patients with gastric cancer.

The aim of this study was to assess the utility of the DR-70 immunoassay, which is based on the immunochemical detection of fibrin degradation products (FDPs) in the diagnosis of gastric cancer. In addition, we investigated the potential of DR-70 as a parameter with respect to tumor dissemination in patients with gastric cancer.

MATERIALS AND METHODS

A total of 29 patients with a histopathological diagnosis of malignant gastric tumor (11 females, 18 males; median age: 57 years, range: 24–84 years) and 29 healthy blood donors (19 females, 10 males; median age: 42 years, range: 18–69 years) were prospectively and se-

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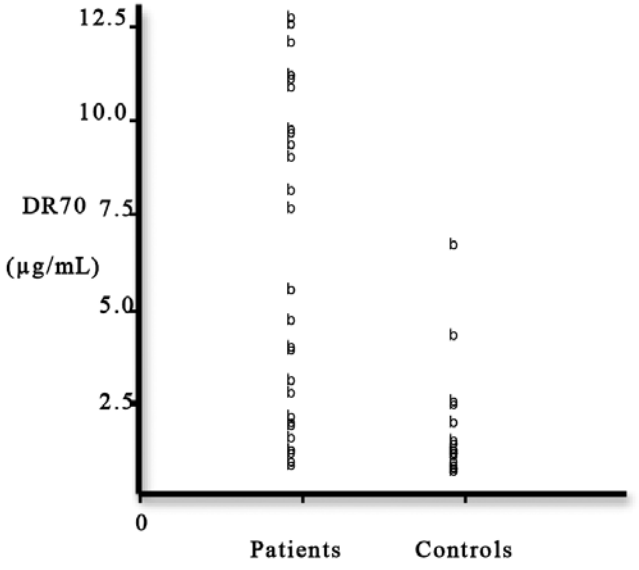

Figure 1. Amount of serum DR-70 in controls and gastric cancer patients.

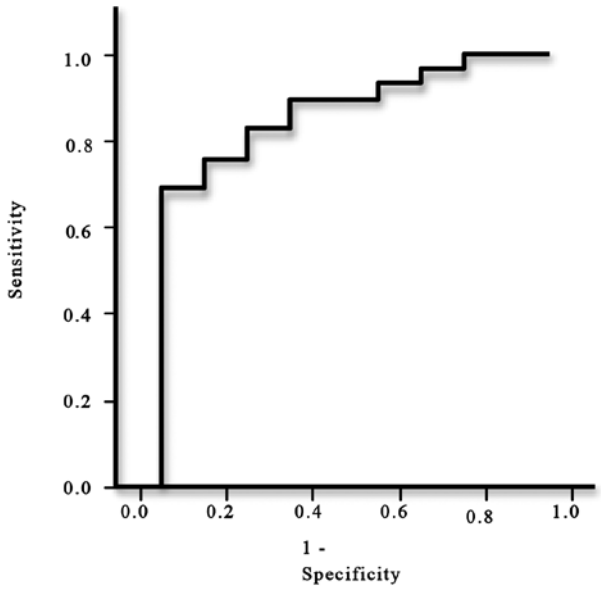

Figure 2. Receiver operating characteristic analysis evaluating different cut-off values for the DR-70 immunoassay.

quentially enrolled in this study between September 2013 and April 2014. Informed written consent was obtained from the participants, and the local ethics committee approved the trial.

On admission, blood samples were studied to assess blood cell count, aspartate aminotransferase, alanine aminotransferase, c-glutamyltranspeptidase, alkaline phosphatase, and creatinine levels using commercially available tests. Patients were additionally tested for carcinoembryogenic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). Ultrasonography, computed tomography of the abdomen, and chest X-ray were used for the staging evaluation. The organ-specific The TNM Classification of Malignant Tumours (TNM) classification was used to evaluate the tumor extension and spread.

DR-70 immunoassay

An enzyme-linked immunosorbent assay (ELISA) kit (AMDL Inc.; Tustin, CA, USA) was used to quantify the serum levels of FDP. The blood samples were stored in siliconized vacutainers with gel serum separators (SST vacutainer). Centrifugation of the serum samples was performed for 30 min–4 h after venipuncture (4). All samples were kept frozen at –80°C until assay. During the immunoassay, the immobilized rabbit anti-DR-70 (FDP) polyclonal antibodies and horseradish peroxidase-conjugated polyclonal anti-human fibrinogen antibodies were used. The serum levels of FDP were determined by interpolating the absorbance at 450 nm from the provided standard curve. When the obtained value exceeded the upper limit of the DR-70 kit (10 mg/mL), the test was performed again with two- and fourfold diluted sera.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc.; Chicago, IL, USA). Normality was assessed using the Shapiro–Wilks test. Accordingly, data were expressed as median (min-max). A comparison between the groups was performed using the Mann–Whitney U-test. Sensitivities and specificities of the immunoassay were calculated at various threshold concentrations. Thus, the best cut-off value for the DR-70 immunoassay was determined by receiver operating characteristic (ROC) curve analysis; 95% confidence intervals were given. P<0.05 was accepted as statistically significant. A two-sided p value of 0.05 or less was considered statistically significant.

RESULTS

The DR-70 values in patients with gastric cancer (5.41 µg/mL; range: 0.72–12.62) showed a significant difference from the values in controls (1.08 µg/mL; range: 0.62–6.64 µg/mL; p<0.0001). The results of the measured amounts of FDP in patients and controls are given in Figure 1.

ROC curve analysis revealed that ≥1.45 µg/mL was (Figure 2) the best cut-off value to differentiate between patients with gastric cancer and healthy subjects. The area under the ROC curve was 0.871. When ≥1.45 µg/mL was accepted as the cut-off value, the DR-70 immunoassay had a sensitivity of 82.8% (63.5%–93.5%) and a specificity of 79.3% (69.7%–91.3%). The negative predictive value was 80.0% (60.9%–91.6%), and the negative predictive value was 82.1% (62.4%–93.2%).

According to the organ-specific TNM classification, 6 cancer patients had stage 1 or 2 disease and the remaining 23 patients had more advanced tumor spread as stage 3 or 4. There was no significant difference between these two groups with respect to the serum DR-70 level [9.4 (1.1–12.5) vs. 4.6 (0.7–12.6) µg/mL p>0.05].

In total, 11 of 29 patients with gastric cancer showed elevated CEA values in serum. The sensitivity of the CEA assay to diagnose gastric cancer was 37.9% (22.1%–59.2%). In 13 of 29 patients, the level of CA 19-9 was higher than normal. Therefore, the sensitivity of CA 19-9 to detect gastric cancer was 44.8% (28%–65.8%). As a tumor marker, DR-70 was significantly superior to CEA and CA 19-9 in terms of sensitivity to detect the patients with gastric cancer ($p<0.01$). The combination of DR-70 with CEA and CA 19-9 increased the sensitivity that at least one of these tests was positive in 28 out of 29 patients (96.6%).

DISCUSSION

Highly accurate screening tools are required to facilitate a higher rate of curative resection in gastric cancer patients. In present clinical practice methods, screening for gastric cancer is based on upper gastrointestinal (GI) endoscopy and biopsy. Identification of a blood marker that is relatively non-invasive and cost efficient could be provided by the measurement of disease-specific markers in peripheral blood. Although such a marker would never eliminate the need for an upper GI endoscopy as the definitively diagnostic procedure, it may identify high-risk patients as candidates for an endoscopic examination. A tumor marker may also serve an important role in the determination of best endoscopic screening intervals in certain subgroups with preneoplastic lesions, such as atrophic gastritis, intestinal metaplasia, or low-grade gastric epithelial dysplasia.

The DR-70 immunoassay had been developed in the USA in 1995 as a commercial kit to detect serum levels of FDP. Although conventional FDP tests can detect only part of the degradation products, this polyclonal anti-FDP antibody-based immunoassay measures all the major products of the cancer-related FDP production pathways, including fragments D, E, and D-dimers. Therefore, it had a better sensitivity. The DR-70 ELISA test is simple to perform, rapid, and requires only 1.5 h to obtain the results. Compared with other ELISA tests that take 6 h to complete, the DR-70 test requires a significantly shorter time to complete and therefore can provide a clinical report in a shorter time. DR-70 has been shown to have the potential to indicate the presence of various malignant tumors involving the lung, tongue, gastrointestinal tract, breast, ovary, and liver (4-8). In our findings, DR-70 levels were significantly higher in patients with gastric cancer than in healthy controls. Although some of the previous studies included small numbers of gastric cancer patients, to the best of our knowledge, ours is the first study with the largest patient group that specifically focuses on the clinical value of DR-70 in gastric cancer. Based on the current literature, no marker is appropriate for the diagnosis of gastric cancer because of insufficient test characteristics (9). Studies on CEA or CA 19-9 in the follow-up of patients with gastric cancer concluded that these markers may show recurrence, but further clinical trials are required to confirm this data (9). Our results show that DR-70 may have the potential to be used as a diagnostic serum biomarker in gastric cancer. In addition, DR-70 can be used in combination with CEA and CA 19-9 to detect more than 90% of the cases.

An ideal tumor marker theoretically should be highly sensitive and specific. It should have low false-positive and -negative results and 100% accuracy in differentiating between healthy individuals and tumor patients. An ideal marker would predict early recurrence and have a prognostic value and positive correlation with tumor volume and extent. For a marker to be beneficial for cancer screening, its levels should begin to rise during the neoplastic process. Accordingly, when the test characteristics of DR-70 were considered (sensitivity: 82.8%, specificity: 79.3%; positive predictive value (PPV): 80.0%, negative predictive value (NPV): 82.1%), it cannot be claimed that DR-70 is an ideal tumor marker because none of the tumor markers have been reported to date. In addition, we could not observe a correlation between the DR-70 level and tumor extent. Despite these drawbacks, we think that it is a promising biomarker which may be used alone or as a complementary test in future.

There are several limitations in our study. Patients with preneoplastic conditions, such as atrophic gastritis, intestinal metaplasia, and dysplasia, may have been included. A prospective and long-term rather than a cross-sectional study design could have provided data on the relationship between the DR-70 level and response to therapy, early recurrence, and prognosis. We think that future studies need to address these issues.

In conclusion, the present study showed that the DR-70 immunoassay could reliably distinguish between patients with gastric cancer and healthy subjects. Therefore, it may be a good candidate to be used as a tumor marker and to be further tested in prospective studies with a large sample size for this purpose.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gazi University Ethics Comitttee.

Informed Consent: : Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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