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**Elevated Fibrinogen-Fibrin Degradation
Products (FDP) in Serum of Colorectal
Cancer Patients**

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ABSTRACT

Serum fibrinogen-fibrin degradation product (FDP) levels of healthy control subjects and of patients with colorectal cancer have been measured by DR-70[®] ELISA in Frankfurt, Germany (Germany study) and in Tustin, California (U.S. study). Serum FDP levels of patients with colorectal cancer were significantly higher than those of the healthy controls. The median serum FDP levels in healthy control groups of the German and U.S. studies were, respectively, 0.37 $\mu\text{g}/\text{mL}$ and 0.61 $\mu\text{g}/\text{mL}$. The median serum FDP levels in the colorectal cancer groups of the German and U.S. studies were, respectively,

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1.25 $\mu\text{g}/\text{mL}$ and 1.60 $\mu\text{g}/\text{mL}$. The results are consistent with enhanced fibrinolysis in serum of patients with colorectal cancer.

Key Words: Colorectal cancer; Fibrin-fibrinogen degradation products (FDP); DR-70[®] ELISA; CEA.

INTRODUCTION

The invasiveness and metastasis of tumors require the degradation of an extracellular matrix and fibrin surrounding the tumor. Locally generated and released proteases must prepare the migration of tumor cells out of the tumor.^[1] Malignant cells generally possess high levels of plasminogen activator, which induces local fibrinolysis.^[2] Local thrombin generation and fibrin deposition and dissolution appear to be important in tumor growth and dissemination.^[3-5] It has previously been shown that plasma FDP levels, primarily measured as D-dimer, were elevated in colorectal patients and other cancers.^[6-8] Wu et al.,^[9,10] using AMDL DR-70[®] Immunoassay kit, found that serum FDP levels of lung cancer patients were significantly elevated. AMDL DR-70[®] ELISA is an immunoassay developed to measure serum FDP. The aim of this study was to use DR-70[®] Immunoassay kit to measure serum FDP levels in patients with colorectal cancer and compare to the levels in healthy individuals.

EXPERIMENTAL

Current studies were conducted at two independent sites: Site 1: Frankfurt/Main, Germany; Site 2: Tustin, CA. They are, respectively, referred to as German study and U.S. study.

Germany Study

Healthy Control Subjects: Sera from 100 healthy blood donors (68 male, 32 female; median age: 37 years; range: 18-66 years) were used as the healthy controls in this study.

Cancer Patients: Sera from 30 patients diagnosed with colorectal cancer were collected. There were seven female patients with age ranges from 59-82 years old and 23 male patients with age ranges from 38-82 years old. On admission to the hospital, all patients underwent routine biochemical evaluation of the blood that includes blood cell count, aspartate aminotransferase,

alanine aminotransferase, γ -glutamyltranspeptidase, alkaline phosphatase, total bilirubin, albumin, and prothrombin activity. Staging examinations were performed with sonography and computer tomography of the abdomen and chest x-ray. The serum carcinoembryonic antigen (CEA) levels of 24 of the 30 colorectal cancer patients were determined by enzyme-linked immunosorbent assay (ELISA). All cancer patients were histologically proven, with fine needle puncture guided by endosonography or with operative resection, to have malignant tumor.

U.S. Study

Healthy Control Subjects: Sera from 100 healthy volunteers were used as the healthy controls in this study. There were 40 male volunteers with age ranges from 20–69 years old and 60 female volunteers with age ranges from 21–81 years old.

Cancer Patients: Sera from 44 patients diagnosed with colorectal cancer were purchased commercially. There were 20 male patients with age ranges from 22–80 years old and 24 female patients with age ranges from 49–76 years old. The malignancy of these patients was confirmed histologically, and stage of the colorectal cancer was assigned to each patient. There were three patients with Duke's stage 1 cancer, 17 with Duke's stage 2 cancer, 19 with Duke's stage 3 cancer, and five with Duke's stage 4 cancers.

Sera Collection

All sera were obtained by the following procedures: Approximately 5 mL venous blood was drawn into a serum separation vacutainer tube. (This tube contained SST gel and clot activator, the Tiger Top tube.) The blood in the tube was left at room temperature for 30 min in an upright position, then centrifuged at 2,000 rpm for 15 min. The clear serum was transferred to a separated tube for analysis. When not in use, the sera was aliquoted and stored at -20°C .

Measurement of FDP Using DR-70[®] ELISA Kit

Serum FDP levels were measured with an ELISA kit (DR-70[®] ELISA, AMDL Inc., Tustin, Ca 92780) manufactured by AMDL, Inc. The kit contains a microwell plate of 12×8 well strips coated with affinity-purified rabbit anti-FDP antibodies, a vial of peroxidase-antibody conjugate, one vial of

each of diluent, substrate solution, stop solution, wash buffer, low control, high control, and five calibrators.

The Assay Principle

DR-70[®] ELISA used affinity-purified anti-FDP antibodies that were immobilized on the bottom of the microwell to capture FDP in serum. These serum FDPs were products of fibrinolysis and proteolysis, presumably by enzymes secreted by tumor cells. The captured FDPs were then complexed by peroxidase labeled antibodies to form an immuno-sandwich. The bound enzyme-antibody conjugates are quantitatively measured with TMB substrates. The amount of bound enzyme-antibody conjugate is directly proportional to the amount of captured FDP. Upon stopping the enzymatic reactions, the absorbance is read at 450 nm.

Assay Procedures

Serum was diluted 200-fold with diluent solution supplied in the kit. For example, 10 μL of serum was added to 2000 μL diluent. Upon mixing, the diluted serum was added to two adjacent wells of a dilution plate. Each microwell of the dilution plate received 200 μL of the diluted serum in duplicate. 100 μL of the diluted serum from microwells in the dilution plate was transferred to the corresponding microwells of the antibody-coated plate using an eight-channel pipettor. The plate was incubated at room temperature for 30 min. Then, the microwells were washed six times each with 300 μL of wash buffer, inverting the plate and tapping it on a clean absorbent paper. This was immediately followed by adding 100 μL of peroxidase-antibody conjugate to each microwell using an eight-channel pipettor. The plate was incubated at room temperature for 30 min. Again, the microwells were washed six times each with 300 μL of wash buffer, inverting and tapping it on a clean absorbent paper. Then, 100 μL of TMB substrate solution was added to each microwell using an eight-channel pipettor. The plate was then covered with a piece of aluminum foil and incubated at room temperature for 15 min. The reactions in the microwells were stopped by adding 100 μL of stop solution to each microwell using an eight-channel pipettor. The absorbance in each microwell was immediately read at 450 nm. From the absorbance of the five calibrators, a standard curve was constructed. The FDP level of the serum was read from this standard curve.

AMDL DR-70[®] ELISA kit can also be run in automated ELISA equipment.

Statistical Methods

Data are given as median and ranges. Receiver operating characteristic (ROC) curves were performed, calculating sensitivities and specificities for variable cutoff values.

The term "sensitivity" is used in the context of diagnostic immunoassay to characterize the incidence of true positive results obtained when the assay is applied to patients known to have a cancer. The term "specificity" is, on the other hand, used to characterize the incidence of true negative results obtained when an assay is applied to subjects known to be free of cancer. [16] Mathematically, the sensitivity and specificity can be presented as follows:

$$\text{Sensitivity} = \frac{\text{TP} \times 100}{\text{TP} + \text{FN}}$$

$$\text{Specificity} = \frac{\text{TN} \times 100}{\text{FP} + \text{TN}}$$

In the above, TP = true positive = number of cancer patients correctly classified by the test. TN = true negative = number of noncancer patients correctly classified by the test. FP = false positive = number of noncancer patients misclassified by the test. FN = false negative = number of cancer patients misclassified by the test.

Data between groups were compared using the unpaired student *t*-test. Results show that $P \leq 0.05$ was considered statistically significant.

RESULTS

Results of Germany Study

Healthy Controls: The serum FDP levels of healthy controls were obtained by measuring the serum FDP level for 100 healthy blood donors using DR-70[®] ELISA. The assay results showed that the serum FDP level of the healthy blood donor group ranges from 0.1–1.11 $\mu\text{g}/\text{mL}$ with a median value of 0.37 $\mu\text{g}/\text{mL}$.

Cancer Patients: The serum FDP levels of patients with colorectal cancer were obtained by measuring the serum FDP levels for 30 patients with histologically proven colorectal cancer using DR-70[®] ELISA. The results showed that the serum FDP levels of the colorectal cancer range from 0.4–7.8 $\mu\text{g}/\text{mL}$ with a median value of 1.25 $\mu\text{g}/\text{mL}$.

The results from the study of site 1 in Germany on the FDP levels of both healthy controls and colorectal cancer patients are presented as a scattergram in Fig. 1.

Results of U.S. Study

Healthy Controls: The serum FDP levels for healthy controls were obtained by measuring the serum FDP levels of 100 healthy volunteers using DR-70[®] ELISA. The assay results showed the serum FDP levels of the healthy group range from 0.25–2.2 $\mu\text{g}/\text{mL}$ with a median value of 0.61 $\mu\text{g}/\text{mL}$.

Cancer Patients: The serum FDP levels for patients with colorectal cancer were obtained by measuring the serum FDP levels of 30 patients with histologically proven colorectal cancer using DR-70[®] ELISA. The results showed that the median serum FDP level of the colorectal cancer ranges from 0.31–10 $\mu\text{g}/\text{mL}$ with a median value of 1.60 $\mu\text{g}/\text{mL}$.

The results of the study of site 2 in Tustin, California, on the FDP levels of both healthy controls and colorectal cancer patients are presented as a scattergram in Fig. 2.

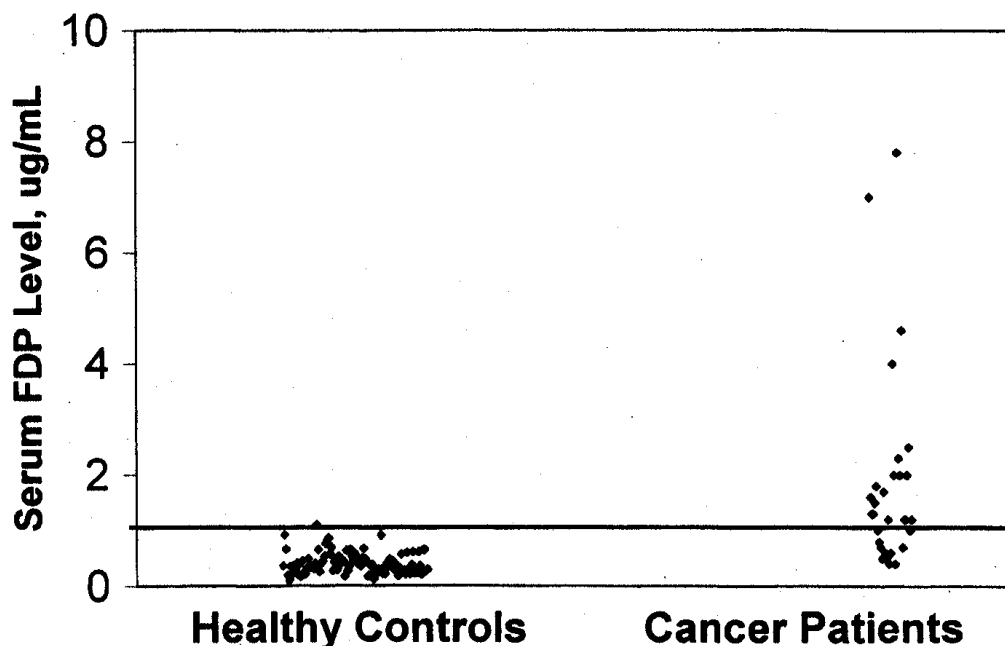


Figure 1. Scattergram for serum FDP level of healthy controls and of patients with colorectal cancer (Germany study). The heavy horizontal line represents a serum FDP cutoff level at 1 $\mu\text{g}/\text{mL}$.

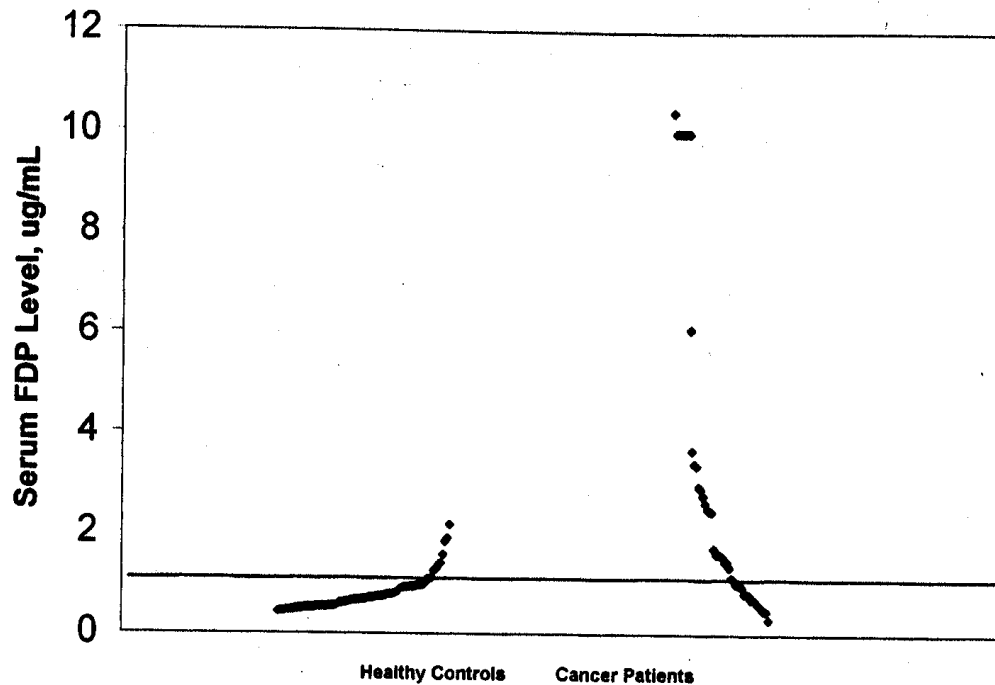


Figure 2. Scattergram for serum FDP levels of healthy controls and of patients with colorectal cancer (U.S. study). The heavy horizontal line represents a serum FDP cutoff level at $1\mu\text{g/mL}$.

The above-mentioned results are summarized in Table 1, which also includes some statistical parameters such as the median values, standard deviations of the median, and the *P* values for statistical significance.

Table 2 shows the relationship between serum FDP cutoff level and the sensitivity and specificity of the assay. Depending on the level serum FDP used as the cutoff value, a range of specificity values and their corresponding sensitivity values can be obtained for colorectal cancer. The relationship between a series of specificity and sensitivity can be presented as ROC curve, as shown in Fig. 3.

The relationship between the assay sensitivity and the stages of colorectal cancer for results obtained in Tustin, California, are shown in Table 3.

DISCUSSION

Independent studies conducted in Frankfurt, Germany, and Tustin, California, showed that the serum FDP levels in patients with colorectal cancer were significantly higher than the levels in their respective healthy

Table 1. Summary of the serum FDP levels of healthy controls and patients with colorectal cancer.

Category	Study in Frankfurt, Germany			Study in Tustin, California			
	Number of subjects	Median FDP level $\mu\text{g}/\text{mL}$	Std. dev.	Number of subjects	Median FDP level $\mu\text{g}/\text{mL}$	Std. dev.	<i>P</i>
Healthy controls	100	0.37	0.19	100	0.61	0.35	≤ 0.0001
Colorectal cancer patients	30	1.25	1.8	44	1.60	3.42	—

Table 2. The specificity and sensitivity of DR-70[®] ELISA for colorectal cancer at different serum FDP cutoff concentrations.

FDP cutoff µg/mL	Study in Frankfurt, Germany		Study in Tustin, California	
	Specificity	Sensitivity	Specificity	Sensitivity
0.4	57%	100%	—	—
0.5	74%	93%	33%	93%
0.6	83%	87%	49%	89%
0.7	93%	80%	62%	84%
0.8	95%	73%	74%	80%
0.9	97%	70%	78%	73%
1.0	99%	70%	88%	71%
1.1	99%	63%	91%	64%
1.2	100%	63%	92%	60%
1.3	100%	50%	94%	60%
1.4	100%	43%	95%	58%
1.5	100%	43%	96%	53%

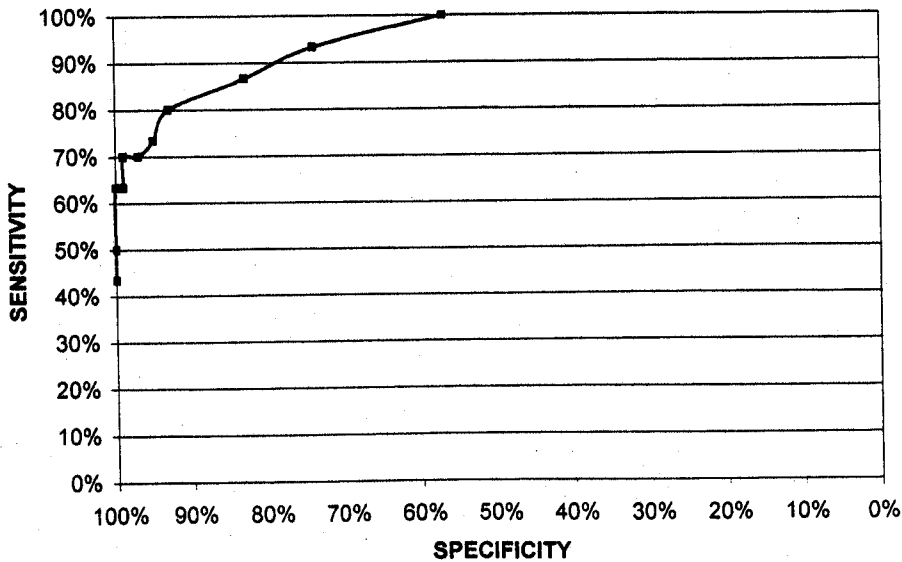


Figure 3. Receiver operating characteristic (ROC) curve for measurements of serum FDP levels using DR-70[®] ELISA kit.

Table 3. The relationship between the assay sensitivity and the stages of colorectal cancer.

Stage of colorectal cancer	Study in Tustin, California
	Sensitivity of DR-70 [®] ELISA
1	71%
2	51%
3	70%
4	52%

control groups (Figs. 1,2). The FDP levels for the colorectal cancer patients for both the German and California studies range from 1.25–1.60 $\mu\text{g}/\text{mL}$. In contrast, the corresponding values for healthy controls range from 0.37–0.61 $\mu\text{g}/\text{mL}$. The differences in the median serum FDP levels between the cancer and healthy control groups were highly significant with *P* values less than 0.0001 in both the German and U.S. studies (Table 1). Based on our current studies, Table 2 was constructed to show the relationship between the specificity and sensitivity of DR-70[®] ELISA for colorectal cancer testing. At a serum FDP cutoff level of 1 $\mu\text{g}/\text{mL}$, the German study shows a specificity of 99%, whereas the U.S. study shows 88%. The sensitivity obtained from both studies was similar, being 70% and 71%, respectively, for the German and U.S. studies. Fig. 3 shows the ROC curve based on data obtained from the German study. Although the number of colorectal cancer patients was not sufficiently large enough to allow for a definitive conclusion to be made on the sensitivity of DR-70 ELISA for different stages of colorectal cancer, nevertheless, it is of interest to analyze current preliminary results obtained from the U.S. study. Such preliminary analysis showed that the DR-70–ELISA was able to detect all stages of cancer with sensitivity values ranging from 51–71% (Table 3).

Data presented in this report are consistent with the notion that fibrinolysis is often associated with oncogenic transformation^[2] and with the findings that plasma D-dimer level, a component of FDP, was elevated in breast cancer and in colorectal cancers.^[3,4,6–8] Wu et al.^[9,10] and others^[11–13] have demonstrated that the serum FDP levels in several different cancers, including colorectal cancer, were also elevated. Furthermore, it has been shown for more than two decades that the measurement of urinary FDP was as accurate or more accurate than other available tests, including urine cytology.^[14] Determination of urinary FDP by immunoassay is an efficient,

reliable, noninvasive, as well as quantitative or qualitative method that can be a useful adjunct on the surveillance of superficial bladder cancer and for monitoring the course of the disease.^[11,15]

In conclusion, we have shown that serum FDP levels, measured by using DR-70[®] ELISA, were significantly higher in colorectal cancer than those of the healthy control. It is therefore worthy to investigate the potential use of this assay in the management of colorectal cancer.

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