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Serum CEA, TIMP-1, and E-selectin Levels for Screening of Liver Metastasis in The Patients with Colorectal Cancer

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Serum CEA, TIMP-1, and E-selectin Levels for Screening of Liver Metastasis in The Patients with Colorectal Cancer

Directed by Professor Nam Kyu Kim

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

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<ABSTRACT>

Serum CEA, TIMP-1, and E-selectin Levels for Screening of Liver Metastasis in The Patients with Colorectal Cancer

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(Directed by Professor Nam Kyu Kim)

Introduction: Liver metastases (LM) of colorectal cancer (CRC) occur about half of the patients somewhere in the course of the disease. Although with curative resection of LM, 5-year survival rate of 35-40% can be achieved, only 15-25% of the patients with CRC LM present resectable disease. Thus, early detection of LM by simple blood test would be beneficial. The purpose of this study was to determine the usefulness of 7 secretomes (CEA, VEGF, TIMP-1, MMP-7, selectin, HGF, and osteopontin) screening markers for detecting CRC LM.

Materials and Methods: A total of 70 patients with CRC were prospectively enrolled and divided into LM(+) and LM(-) group. Blood from peripheral vein before the operation and drain vein (SMV or IMV) before the resection of primary tumor were collected. ELISA was performed to detect serum levels of 7 biologic markers. We applied 3 different models for screening: model 1 where only the serum level of the molecules from peripheral vein (PV) was used, model 2 where the ratio of PV to that from drain vein (DV) was used (PV/DV), and model 3 where DV was replaced with a value (cDV) calculated

from a correlation equation between PV and DV (PV/cDV). For each model, sensitivity and specificity were obtained and compared with those from each other model.

Results: Univariate analyses revealed no difference between LM(+) and (-) groups in clinical variables such as T and N categories, age, gender-ratio, the size of primary tumor, histologic grade, and the location of primary tumor. For model 1, only PV of TIMP-1 was found to be an independently predictive of liver metastasis ($p<0.001$; ODD 1.038; 95% CI 1.010-1.057), and a sensitivity of 84.8%, a specificity of 89.2%, and an accuracy of 87.1% were achieved. For model 2, the PV/DV of TIMP-1 ($p<0.001$; ODD 1.040; 95%CI 1.017-1.063) and E-selectin ($p=0.007$; ODD 0.875; 95%CI 0.794-0.964) were found to be independently predictive and a sensitivity of 87.9%, a specificity of 94.6%, and an accuracy of 91.4% were achieved. For model 3, we first performed correlation analyses between PV and DV, and found significant correlations in CEA ($R^2=0.812$; $p<0.001$), TIMP-1 ($R^2=0.491$; $p<0.001$), MMP-7 ($R^2=0.944$; $p<0.001$), osteopontin ($R^2=0.460$; $p<0.001$), and HGF ($R^2=0.477$; $p<0.001$). Then we calculated the serum molecular level from DV with that from PV by the equations derived from the correlation analyses and calculated the ratio of PV to calculated DV (PV/cDV). The PV/cDV of TIMP-1 ($p<0.001$; ODD 5036.77; 95% CI 42.56-596156) and CEA ($p=0.004$; ODD 130.98; 95% CI 4.612-3719.8) were independently predictive and a sensitivity of 87.9%, a specificity of 94.6%, and an accuracy of 91.4% were achieved.

Conclusion: The serum level of CEA, E-selectin and TIMP-1 might be used as a tool for screening LM in the patients with CRC. More accurate screening may be possible using the PV/DV.

Key words: carcinoembryonic antigen; E-selectin; tissue inhibitor metallomatrix proteinase-1; liver metastasis; colorectal neoplasm

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I. INTRODUCTION

Colorectal cancer is the third most frequent cancer worldwide[1]. Nearly 0.6 million new cases of colorectal cancer are diagnosed each year. During the past few decades, the Asian population has experienced a two- to four-fold increase in the incidence of colorectal cancer, and South Korea has not been an exception[2]. Colorectal cancer is the fourth leading cause of cancer-related mortality in South Korea.

Approximately 50% of patients with colorectal cancer ultimately develop liver metastasis and, unfortunately, only 10–25% of these patients are candidates for liver resection. The five-year survival rates of 30-35% can only be expected for patients who have underwent complete resection of all liver metastases[3, 4]. For rest of the patients who are not the candidates for liver resection, their prognosis remains poor so that the median survival is only about 20 months[4]. Therefore, early screening of the liver metastasis before it

progresses and becomes unresectable is important to achieve improved treatment outcomes.

Conventional radiologic methods such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) for detecting liver metastasis have limitations. Small sized lesions (less than 1 cm in diameter) may not be easily detected and the cost is relatively high. Serologic test with peripheral blood may provide more easier and cost-effective screening. Liver metastases as early as conventional radiologic studies cannot found are detected by measuring serum carcinoembryonic antigen (CEA) level for relatively lower cost. However, rise of serum CEA level is not metastasis-specific and in about 25% of the patients serum CEA level remains within normal limit despite disease progression. Thus to use a serologic test as a valuable screening method, improvement of sensitivity and specificity for liver metastasis is essential, which might be possible in two ways: the one is adding other molecular markers that are sensitive and specific for liver metastasis and the other is drawing blood from where the influence of metastatic tumor can be differentiated.

To satisfy the latter condition, blood was drawn from the peripheral vein (PV) as well as the principal drainage vein (DV) that drains primary tumor. In absence of liver metastasis, a serum level of molecular markers detected from PV reflects the tumor burden only from primary lesion. Meanwhile in the presence of liver metastasis, a serum level of molecular markers from PV reflects tumor burdens not only from primary lesion, but also from metastatic lesion, thus not specific for liver metastasis. However, a serum level of molecular markers detected from DV constantly reflects the tumor burden from primary lesion only. We postulated that a ratio of the serum level of molecular markers detected from PV to that detected from DV is more specific

for liver metastasis than a serum level of molecular markers measured from PV.

Molecular markers that are sensitive and specific for liver metastasis are probably the molecules that are considered to be involved in the stepwise mechanism of liver metastasis. Liver metastasis from colorectal cancer is known to be the result of an integration of complex mechanisms of tumor progression and this can be interpreted as stepwise phenomena roughly categorized as tumor growth/angiogenesis, migration, proteolysis and adhesion. We choose 7 molecules which are deeply studies and are known to represent each step: VEGF and HGF for tumor growth and angiogenesis[5], MMP-7 and TIMP-1 for proteolysis[6], and CEA, e-selectin, and osteopontin for migration and adhesion [7]. Among those 7 molecules we tried to determine markers that are sensitive and specific for liver metastasis.

The aim of this study is to determine the usefulness of serum molecular markers for screening liver metastasis in the patients with colorectal cancer using the ratio of the level of serum markers detected from PV to those from DV.

II. MATERIALS AND METHODS

1. Patient

We prospectively enrolled patients with colorectal carcinoma who underwent surgery between Aug. 2004 and Jun. 2006. The patients were divided into 2 groups: the patients without distant metastasis (M0) regardless T and N stages were classified into the control group (LM-) and those who were with initially resectable synchronous liver metastasis were classified into the liver metastasis group (LM+). The patients were considered eligible as long as primary tumor and their liver metastasis were resectable regardless T and N stages. Resectability was determined by the Colorectal Tumor Board at Severance Hospital, Yonsei University Health System composed of surcigal and medical oncologists as well as radiologists and pathologists. Extrahepatic metastasis was ruled out by abdominal CT scan and/or PET. The patients with either unresectable liver metastasis or extrahepatic liver metastasis were excluded.

2. Study design

Our hypothesis was that the ratio of the serum level of molecular markers from PV to that from DV would reflect more precisely metastases than PV alone and thus screening method using the ratio of PV to DV would provide more accurate information. DV was defined as SMV in the patients with cecal, right colon and proximal transverse colon cancer and it was defined as IMV in the patients with distal transverse, descending, and sigmoid colon cancer and rectal cancer. The ratio of the serum levels of molecular marker from PV to those from DV was calculated as follows:

Ratio (PV/DV) = the serum level of a molecular marker from PV) / the serum

level of a molecular marker from DV

However, drawing blood from DV via percutaneous route is so difficult and invasive that it is not adequate for screening method. Thus we set another hypothesis that there might be a correlation between the serum levels of molecular markers from PV and DV and that the serum level of markers from DV could be calculated on the basis of that correlation. The calculated ratio was defined as:

Calculated ratio (PV/DVc) = the serum level of a molecular marker from PV / the serum level of a molecular marker from DV calculated by the formula derived from correlation analysis between the serum levels of a molecular marker from PV and DV

To prove our hypothesis we tested 3 different models for screening liver metastasis in the patients with colorectal cancer. Model 1 was using serum levels of molecular markers from PV only. Model 2 was using the ratio of serum levels of molecular markers from PV to those from DV, and Model 3 was using the ratio of serum levels of molecular markers from PV to those calculated from correlation formula between PV and DV. We compared sensitivities, specificities, and positive predictive values of 3 models and determined which one is the most accurate screening model for liver metastasis from colorectal cancer.

3. Blood Sampling and Enzyme-Linked Immune Specific Assay (ELISA)

Five milliliter of blood was drawn from PV and DV respectively and contained in plain tubes in all patients. PV blood was obtained within 1 hour

before surgical incision. DV blood was obtained before the ligation of any branch of SMV in proximal colon cancer and before the ligation of IMV in distal colon and rectal cancer. Sampled blood was immediately centrifuged and plasma and serum were separately stored in -70℃ until used. ELISA for all 7 molecules was performed using commercially available kits (VEGF, HGF, E-selectin, MMP-7, TIMP-1, osteopontin: R&D Systems, Inc., MN, USA; CEA: IBL-Hamburg GmbH, Hamburg, Germany) as instructed. Diluted serum was prepared in each well of a plate pre-coated with primary antibody. After recommended incubation period, the plate was washed with buffer solution. Then, substrate solution was prepared in each well and incubated as instructed. After washing, the wells were treated with color-reagent. After period of incubation, stop solution was added to each well and optical density was measured at 450 nm using automatized optical densitometry. A triplet test was performed for each sample and the mean value was used for the analysis. If R^2 of standard solutions was less than 0.98, entire data from the plate was abandoned.

4. Statistical Analysis

To establish each model, we performed univariate analyses by comparing the means of serum levels of each marker of LM- group vs. LM+ group using T-tests. Using logistic regression, we performed multivariate analysis to find out independently significant markers and to establish a formula for calculating the probability of the presence of liver metastasis. We applied the formula to the enrolled patients and obtained sensitivity, specificity, and positive predictive value. All tests were two-sided and a P value less than 0.05 was considered significant.

III. RESULTS

1. Patient characteristics and serum levels of 7 markers

There were no significant differences between LM- and LM+ group in clinical and pathologic parameters such as age, gender, the location of primary tumor, histologic grades and the distribution of T and N stages (Table 1).

The mean serum levels of 7 molecular markers from PV and DV are summarized in Figure 1 and Table 2. Only the mean serum levels of TIMP-1 fitted our hypothesis that in LM- group, there was no difference between the serum level of TIMP-1 from PV and DV, but that in LM+ group the serum level of TIMP-1 from PV was significantly higher than that from DV ($p<0.001$). Meanwhile, the mean serum levels of E-selectin was on the contrary to our hypothesis. The mean serum level of E-selectin from PV was significantly higher than from DV in LM- group ($p<0.001$), whereas there was no difference in LM+ group.

Table 1. Patient characteristics

Clinical variables		LM(-) (n=37)	LM(+) (n=33)	*P*
Mean age (years)		56.5±10.0	58.8±10.2	0.357
Male-to-female ratio		19 : 18	21 : 12	0.300
pT	pT2	1	0	>0.999
	pT3	33	300	
	pT4	3	3	
pN	pN0	12	10	0.544
	pN1	9	5	
	pN2	16	18	
Location	Right colon	6	5	0.903
	Left colon	31	28	
Histology	Well / Mod. diff.	30	26	0.811
	Poorly / mucinous	7	7	

LM, liver metastasis; right colon includes from appendix and cecum to proximal 2/3 of transverse colon; left colon includes from distal 1/3 of transverse colon to rectum

Figure 1. Distribution of the serum level of molecular markers (box-whistker plots)

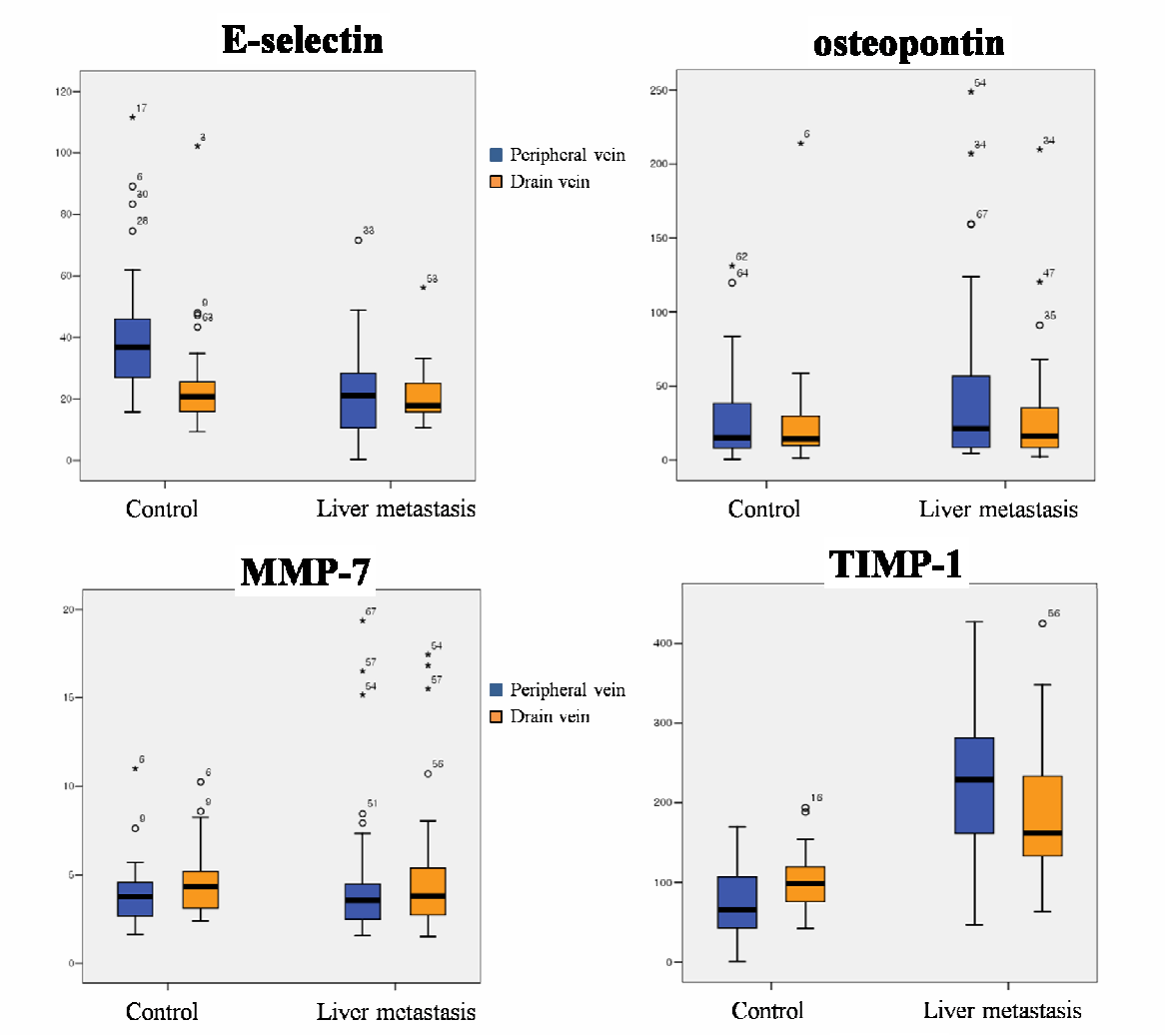

Figure 1. (Cont'd)

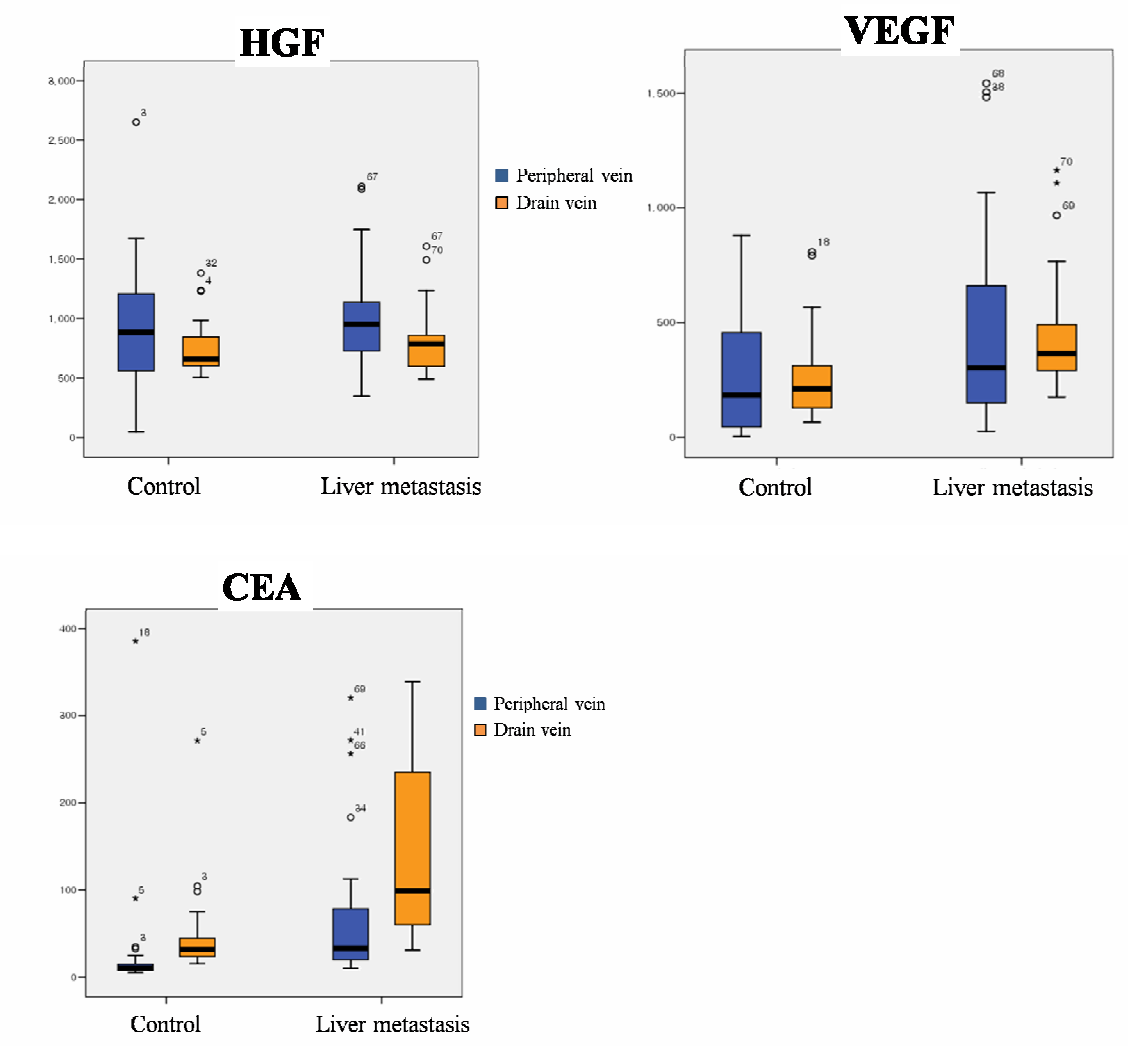

Table 2. The mean serum levels of 7 markers

	CEA		E-selectin		osteopontin		VEGF		HGF		MMP-7		TIMP-1	
LM	-	+	-	+	-	+	-	+	-	+	-	+	-	+
PV	45.15 ± 32.19	116.70 ± 100.31	41.54 ± 19.38	20.38 ± 12.01	27.21 ± 27.12	50.17 ± 40.70	258.80 ± 179.33	459.15 ± 319.82	891.87 ± 712.39	1015.1 ± 921.63	3.90 ± 1.17	4.88 ± 2.61	74.05 ± 31.42	233.71 ± 97.71
P	0.116		0.049		0.068		0.024		0.266		0.229		<0.001	
DV	135.46 ± 91.31	350.11 ± 189.12	24.37 ± 10.01	21.16 ± 13.45	25.44 ± 18.48	31.24 ± 19.78	246.58 ± 134.29	454.17 ± 184.18	748.91 ± 532.47	805.69 ± 591.17	4.63 ± 1.21	5.15 ± 2.04	101.9 ± 87.27	194.01 ± 90.73
P	0.057		0.311		0.528		<0.001		0.338		0.517		<0.001	

CEA, Carcinoembryonic antigen; VEGF, Vascular endothelial growth factor; HGF, Hepatocyte growth factor; MMP-7, Metallomatrix proteinase-7; TIMP-1, Tissue inhibitor of metallomatrix proteinase-1; LM, Liver metastasis; PV, the serum level of a molecule from peripheral blood; DV, the serum level of a molecule from drain vein; Units are: ng/mL for CEA, E-selectin, osteopontin, MMP-7, and TIMP-1; pg/mL for HGF, and VEGF

2. Model 1

Univariate analyses revealed the mean serum levels of E-selectin (p=0.049), VEGF (p=0.024), and TIMP-1 (p<0.001) were significantly different between LM- and LM+ group (Table 3). Multivariate analysis, however, showed the only the serum level of TIMP-1 was independently different between the two groups (p<0.001; HR 1.038; 95% CI 1.019-1.057). The probability of liver metastasis could be calculated as followed:

Probability (liver metastasis) = ez / (1 + ez), where z = 1.038 X the serum level of TIMP-1 from PV + (-5.043)

When applied to the enrolled patients, given the cut-off value of 0.5, this formula achieved the sensitivity of 84.8% , the specificity of 89.2%, and the accuracy of 87.1% (Table 4).

Table 3. Univariate analyses of Model 1

	E-selectin		OSN		CEA		HGF		VEGF		MMP-7		TIMP-1	
	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+
Mean	41.54 ± 19.38	20.38 ± 12.01	27.21 ± 27.12	50.17 ± 40.70	45.15 ± 32.19	116.70 ± 100.31	891.87 ± 712.39	1015.1 ± 921.63	258.80 ± 179.33	459.15 ± 319.82	3.90 ± 1.17	4.88 ± 2.61	74.05 ± 31.42	233.71 ± 97.71
P	0.049		0.068		0.116		0.266		0.024		0.229		<0.001	

CEA, Carcinoembryonic antigen; VEGF, Vascular endothelial growth factor; HGF, Hepatocyte growth factor; MMP-7, Metallomatrix proteinase-7; TIMP-1, Tissue inhibitor of metallomatrix proteinase-1; LM, Liver metastasis; PV, the serum level of a molecule from peripheral blood; DV, the serum level of a molecule from drain vein; Units are: ng/mL for CEA, E-selectin, osteopontin, MMP-7, and TIMP-1; pg/mL for HGF, and VEGF

Table 4. Classification table for Model 1.

		Predicted	
		LM(-)	LM(+)
observed	LM(-)	33	4
	LM(+)	5	28

LM -/+, liver metastasis negative/positive; cut-off value was probability of liver metastasis greater than 0.5.

3. Model 2

Univariate analyses revealed the mean PV/DV of TIMP-1 (p=0.002), MMP-7 (p=0.025), and E-selectin (p=0.001) was significantly different between LM- and LM+ group (Table 5). Multivariate analysis showed them mean PV/DV of TIMP-1 (p<0.001; HR 1.040; 95% CI 1.017-1.063) and E-selectin (p=0.007; HR 0.875; 95% CI 0.794-0.964) were independently different between the two groups. The probability of liver metastasis could be calculated as followed:

Probability (liver metastasis) = ez / (1 + ez), where z = 0.039 X PV/DV of TIMP-1 + (-0.134) X PV/DV of E-selectin + (-1.456)

When applied to the enrolled patients, given the cut-off value of 0.5, this formula achieved the sensitivity of 87.9%, the specificity of 94.6%, and the accuracy of 91.4% (Table 6).

Table 5. Univariate analyses for Model 2.

	E-selectin		OSN		CEA		HGF		VEGF		MMP-7		TIMP-1	
	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+
Mean PV/DV	2.162 ± 1.481	1.109 ± 0.782	1.795 ± 1.081	2.853 ± 1.412	2.918 ± 2.690	1.111 ± 0.792	1.216 ± 0.876	1.299 ± 0.751	1.420 ± 1.027	1.374 ± 1.010	0.854 ± 0.307	0.967 ± 0.571	0.784 ± 0.100	1.445 ± 0.157
P	0.001		0.201		0.306		0.563		0.904		0.025		0.002	

PV/DV, the ratio of the mean serum level of molecule from peripheral vein to that from drainage vein; OSN, osteopontin; CEA, carcinoembryonic antigen; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; MMP-7, metalomatrix proteinase-7; TIMP-1, tissue inhibitor of metallomatrix proteinase-1; LM -/+, liver metastasis negative/positive.

Table 6. Classification table for Model 2

		Predicted	
		LM(-)	LM(+)
observed	LM(-)	35	2
	LM(+)	4	29

LM -/+, liver metastasis negative/positive; cut-off value was probability of liver metastasis greater than 0.5.

4. Model 3

We established a hypothesis that if a molecular marker is expressed at high level specifically by tumor and relatively at ignorable level by normal tissue, then there might exist a certain linear correlation between the levels of a certain molecular marker from peripheral vein and from drain vein, and that using that correlation we can derive a equation and using that equation, we can calculate estimated drain vein level of a certain molecular marker from measured peripheral level of the molecular marker. To derive formulas for calculating PV/DVc of each marker, the analyses of linear correlation between serum levels of markers from PV and DV were performed. Significant correlations between serum levels of markers from PV and DV were found for CEA (R^2=0.812; p<0.001), osteopontin (R^2=0.460; p<0.001), TIMP-1 (R^2=0.491; p<0.001), MMP-7 (R^2=0.944; p<0.001), and HGF (R^2=0.477; p<0.001) (Figure 2). For those 5 markers, the levels from drain vein could be calculated from the levels from peripheral vein using correlation equations. The other 2 markers (E-selectin and VEGF) showed no significant correlation between the levels from PV and DV. On the basis of these correlations, PV/DVc's for 5 markers were calculated. Using PV/DVc, Univariate (Table 7) and multivariate analyses were performed and revealed that the mean PV/DVc

of CEA ($p<0.001$; HR 130.98; 95% CI 4.612-3719.8) and TIMP-1 ($p<0.001$; HR 5036.77; 95% CI 42.56-596156) were significantly different between LM- and LM+ group. The probability of liver metastasis could be calculated as followed:

Probability (liver metastasis) = ez / (1 + ez), where z = 5036.77 X PV/DVc of TIMP-1 + 130.98 X PV/DVc of CEA + (-11.675)

When applied to the enrolled patients, given the cut-off value of 0.5, this formula achieved the sensitivity of 87.9%, the specificity of 94.6%, and the accuracy of 91.4%(Table 8).

Figure 2. The correlation of the serum levels of molecular markers from drainage vein with those from peripheral vein.

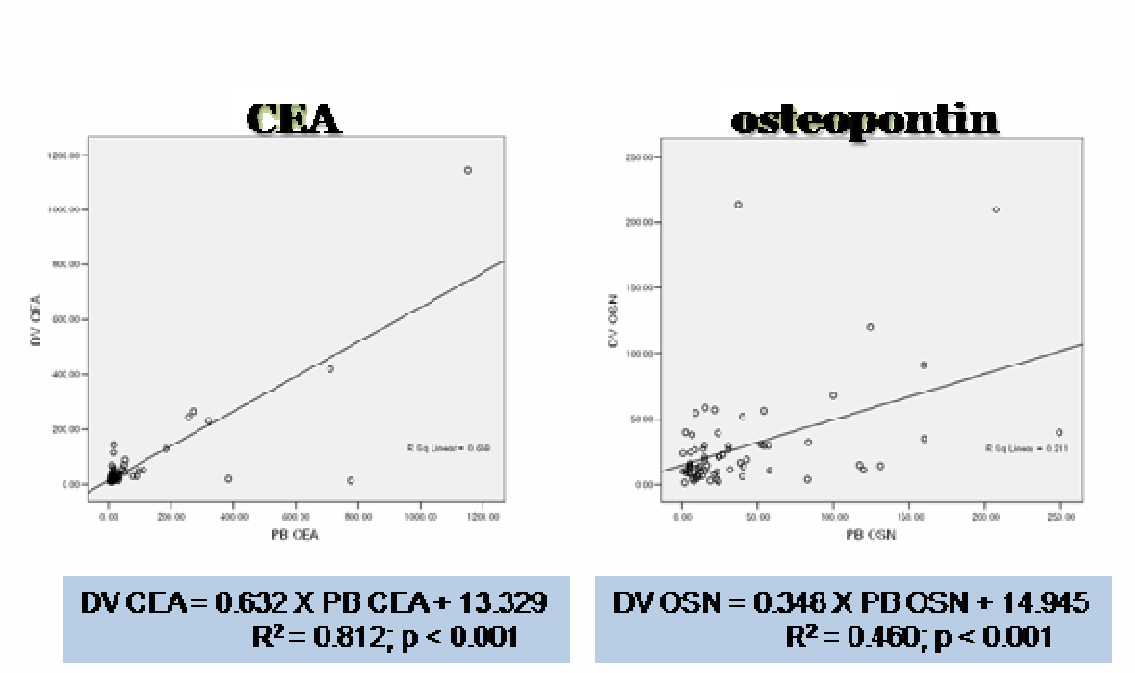

Figure 2 (cont'd)

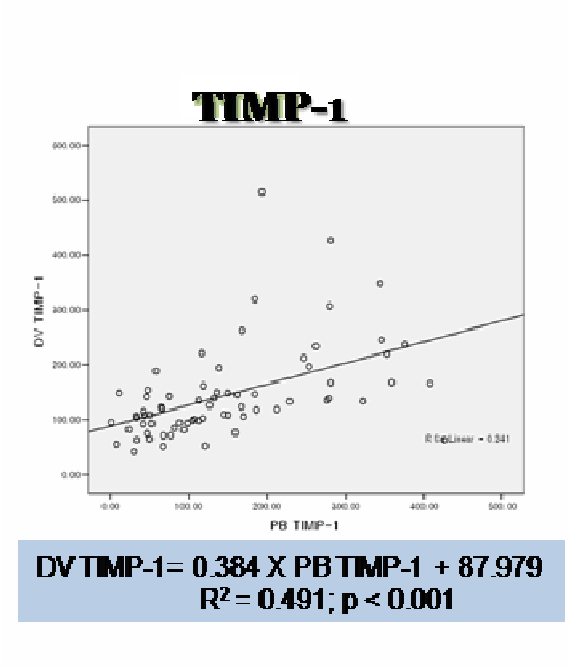

Table 7. Univariate analyses for Model 3

	OSN		CEA		HGF		MMP-7		TIMP-1	
	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+	LM-	LM+
Mean PV/DVc	0.89 ± 0.20	1.11 ± 0.45	0.62 ± 0.12	1.00 ± 0.11	1.10 ± 0.24	1.24 ± 0.27	0.86 ± 0.23	0.86 ± 0.19	0.60 ± 0.10	1.26 ± 0.17
P	0.163		<0.001		0.148		0.965		<0.001	

PV/DVc, the ratio of the mean serum level of molecule from peripheral vein to the calculated serum level of the molecule from drainage vein; OSN, osteopontin; CEA, carcinoembryonic antigen; HGF, hepatocyte growth factor; MMP-7, metalomatrix proteinase-7; TIMP-1, tissue inhibitor of metallomatrix proteinase-1; LM -/+, liver metastasis negative/positive.

Table 8. Classification table for Model 3.

		Predicted	
		LM(-)	LM(+)
observed	LM(-)	35	2
	LM(+)	4	29

LM -/+, liver metastasis negative/positive; cut-off value was probability of liver metastasis greater than 0.5.

5. Application of model 2 and 3 in patients showing high or normal serum CEA level from peripheral vein

Among 70 enrolled patients, 16 patients showed peripheral serum CEA level within normal limit (less than 5ng/mL). To determine whether our hypothetical model 2 and 3 fitted to those patients, we applied the probability equations derived from model 2 and 3 respectively. With the cut-off value of 0.5, model 2 achieved sensitivity, specificity, and accuracy of 100%. Model 3 demonstrated sensitivity of 92.3%, specificity of 100%, and accuracy of 93.75%.

Similarly, we applied the probability equations derived from model 2 and 3 to the 43 patients who showed peripheral serum CEA level more than 50.0 ng/mL. With model 2, the sensitivity of 87.9%, the specificity of 94.6%, and the accuracy of 90.7% could be achieved and with model 3, the sensitivity, the specificity, and the accuracy were 84.8%, 89.2%, and 87.3% respectively.

IV. DISCUSSION

The results of current study suggest potential validity of TIMP-1, CEA, and E-selectin as molecular markers for screening liver metastasis in patients with colorectal cancer. TIMP-1 is the primary inhibitor of MMP-9 and an imbalance in the MMP-9/TIMP-1 ratio had been proposed to be a potential reason for progression of adenomas to carcinomas [6, 8]. Several researchers reported that TIMP-1 tumor levels were increased in patients with colon cancer and correlated with poor prognosis [9]. TIMP-1 and -2 expression have been localized overwhelmingly to peri cancer stromal cells, while malignant and normal mucosal cells were weak or negative in in-situ hybridization studies in colorectal cancer [10]. Although clear evidences exist about the correlation between TIMP-1 expression level and cancer progression, the evidences are still lacking about the role of TIMP-1 in liver metastasis of colorectal cancer. In the present study, TIMP-1 was found to be a molecule that was the most strongly related to liver metastasis. The patterns of serum levels of TIMP-1 also satisfied our hypothesis: in the absence of liver metastasis, the serum level of TIMP-1 was the same between peripheral and drainage vein, but in the patients with liver metastasis the serum level of TIMP-1 from peripheral vein was higher than that from drainage vein, which reflected the secretion of TIMP-1 by metastatic tumor. Thus, TIMP-1 was considered to be an ideal molecular marker for liver metastasis from colorectal cancer.

Carcinoembryonic antigen (CEA) is a membrane glycoprotein normally present on fetal gastrointestinal and liver cells. However, it can become inappropriately expressed in a number of malignancies especially colorectal cancer[7, 11]. The involvement of CEA in the development of liver metastasis is

mediated by the induction of altered cell adhesion properties leading to increased retention of metastasized cells in the liver [7, 11-13]. Gangopadhyay et al.[12] demonstrated that CEA was responsible for modifying the hepatic environment, making it more conducive for colon cancer cell survival. The recent finding is that culture of Kupffer cells in the presence of CEA results in the release of TNF-alpha in the culture medium, and that treatment of endothelial cells with the latter results in increased expression of E-selectin and enhanced adhesion of colorectal cancer cells to the endothelium [13, 14]. According to our data, serum CEA level was significantly higher in the patients with liver metastasis, however it was not site-specific. Not only the serum CEA level from peripheral vein, but also that from drain vein was higher in liver metastasis group than non-metastasis group. This finding suggested that although CEA was an effective molecular marker that sensitively reflect tumor burden, it was not specific for liver metastasis.

However, as to E-selectin, we encountered an embarrassing result. Theoretically, E-selectin expression and secretion should be increased in metastasizing tumors, thus its serum level should be higher from peripheral vein of the patients with liver metastasis than from drain vein of the same patients or than from peripheral vein of the patients without liver metastasis. On the contrary, however, was our result. We found the peripheral serum E-selectin level of the patients without liver metastasis was the highest. Although no supporting evidences so far, one possible speculation might be that colorectal adenocarcinoma with high metastatic potential suppresses the expression of E-selectin by hepatocyte or Kuffer cell, which are, by adenocarcinoma with low metastatic potential, stimulated to express high level of E-selectin. This might also imply that a negative role of E-selectin as to formation of liver metastasis by an unknown mechanism of liver metastasis.

However, no supporting evidences can be provided in the present study and the explanation remained undetermined.

In stage IV colorectal cancer, an elevated peripheral serum CEA level can be detected in only 65-86%[15]. For the rest of 14-35% patients, peripheral serum CEA level remains within normal range, and thus it cannot be used as a screening test. We applied our screening models using the ratio of PV to DV and yielded acceptable sensitivity. We also demonstrated that our screening models were still valid for the patients who showed peripheral serum CEA levels more than 50ng/mL, which suggests that our model specifically reflect metastatic tumor burden.

There has been a ‘paradigm-shift’ in the search for screening markers for metastasis. Most previous works relied upon an ‘old paradigm’ experimental approach of a ‘single marker predictive model’. With recent advances in biotechnologies, however, identification of gene expression differences between the patients with and without metastasis, by screening tens of thousands of genes probed using commercially available chips, has become possible. Non-invasive proteomic techniques, using serum rather than tissue samples, have also emerged recently. These state-of-the-art techniques will allow better tumor fingerprinting, and will impact on individualized treatment planning. The use of such new techniques in routine clinical practice remains difficult at present. After thorough validation in large-scale prospective studies, it will be desirable to introduce the best predictive techniques to front-line clinical situations.

In conclusion, Serum levels of E-selectin, CEA and TIMP-1 are considered as important markers for screening liver metastasis in patients with colorectal cancer. More accurate screening model could be drawn from using the ratio of the serum concentration from peripheral blood (PV) to that from primary

tumor drainage vein (DV).

V. CONCLUSION

Serum levels of E-selectin, CEA and TIMP-1 are considered as important markers for screening liver metastasis in patients with colorectal cancer. More accurate screening model could be drawn from using the ratio of the serum concentration from peripheral blood (PV) to that from primary tumor drainage vein (DV).

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< ABSTRACT(IN KOREAN)>

대장직장암의 간전이 선별검사를 위한 표지자로서 혈중 CEA, E-selectin 및 TIMP-1 농도의 유용성

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도입: 대장직장암의 간전이는 전체 환자의 약 절반 정도에서 발생한다. 근치적 절제술을 통하여 35-40%에 이르는 5년 생존율을 기대할 수 있으나 전체 간전이 환자의 15-25%만이 근치적 절제술을 시행 받을 수 있다. 따라서 간단한 혈액 검사만으로 간전이를 조기에 진단할 수 있다면 간전이의 치료 성적을 향상 시키는데 도움이 될 것이다. 따라서, 본 연구의 목적은 대장직장암 환자의 혈중에서 7개의 분비성 단백질 (CEA, VEGF, TIMP-1, MMP-7, selectin, HGF, osteopontin)을 검출하여 간전이 선별 검사에 유용한 표지자를 발굴해내는 것이다.

방법: 전향적으로 70명의 대장직장암 환자들을 등록하여 간전이가 있는 환자군 (간전이군)과 없는 환자군 (대조군)으로 나누었다. 등록된 모든 환자들에서 수술 절개직전 말초혈액과 개복 후 원발암 제거전에 원발암의 배액정맥 (상장간막정맥 또는 하장간막정맥)에서 혈액을 채취하였다. 채취된 혈액은 원심분리 후 혈청만을 분리하여 -70°C에 보관하였다. 표지자는 ELISA를 통하여 검출하였으며 표지자를 발굴하기 위해서 말초혈액만을 이용하는 방법 (방법1)과 말초혈액과 배액정맥의

비를 이용하는 방법 (방법2) 및 말초혈액과 배액정맥과의 상관관계식에 따라 치환된 말초혈액의 비를 적용하는 방법 (방법3) 각각에 대하여 민감도와 특이도를 계산하였다.

결과: 전체 70명의 환자 중 간전이군 33명과 대조군 37명이 등록되었고 두 군간에 T 및 N 병기, 연령, 성별비, 원발종양의 크기, 원발종양의 조직학적 분화도, 원발종양의 위치 등의 임상적 지표에 있어 유의한 차이는 없음을 확인하였다. 말초혈액만을 이용하는 방법1의 결과 혈중 TIMP-1의 농도 (표1)만이 통계학적으로 유의한 독립적 예측인자로 나타났으며 ($p<0.001$) 로지스틱 회귀분석 결과 민감도 84.8% 특이도 89.2%, 정확도 87.1%로 간전이를 선별할 수 있는 것으로 나타났다 (ODD 1.038 (95% CI 1.019-1.057); $p<0.001$). 방법2의 결과 TIMP-1의 농도비 ($p<0.002$; ODD 1.040; 95% CI 1.017-1.063)와 E-selectin의 농도비 ($p=0.007$; ODD 0.875; 95% CI 0.794-0.964)가 유의한 독립 예측인자로 나타났으며 로지스틱 회귀분석을 통해 추출한 확률식에 의한 결과 민감도 87.9%, 특이도 94.6%, 정확도 91.4%로 간전이를 선별할 수 있었다. 방법3을 위해서 먼저 말초혈액과 배액정맥간의 선형회귀도를 분석한 결과 혈중 CEA ($R^2=0.812;p<0.001$), TIMP-1 ($R^2=0.491;p<0.001$), MMP-7 ($R^2=0.944;p<0.001$), osteopontin ($R^2=0.460;p<0.001$), HGF ($R^2=0.477;p<0.001$)에서 말초혈액과 배액정맥 간에 유의한 상관관계를 보였으며 이를 통해 말초혈액에서 검출된 혈중농도로 배액정맥의 혈중농도를 계산하여 이를 통해 배액정맥에 대한 말초혈액의 비를 구하였다. 다변량분석 결과 CEA (ratio: 대조군 0.62, 간전이군 1.00; ODD 130.98 (95% CI 4.612-3719.8); $p=0.004$)와 TIMP-1 (ratio: 대조군 0.60, 간전이군 1.26; ODD 5036.77 (95% CI 42.56-596156);

p<0.001)의 말초혈액과 배액정맥의 비만이 유의한 독립 인자로 나타났으며 민감도 87.90%, 특이도 94.6%, 정확도 91.4%로 간전이를 선별할 수 있었다.

결론: 혈중 CEA, E-selectin과 TIMP-1 농도는 대장직장암의 간전이를 선별하는데 유용한 표지자로 생각되며 말초혈액의 농도와 계산된 원발암 배액정맥 농도의 비를 이용하여 더 정확한 선별검사 모델을 제시할 수 있었다.

	CEA		E-selectin		osteopontin		VEGF		HGF		MMP-7		TIMP-1	
	대조	전이	대조	전이	대조	전이	대조	전이	대조	전이	대조	전이	대조	전이
말초	45.15	116.70	41.54	20.38	27.21	50.17	258.80	459.15	891.87	1015.1	3.90	4.88	74.05	233.71
p	0.116		0.052		0.068		0.024		0.266		0.229		<0.001	
배액	135.46	350.11	24.37	21.16	25.44	31.24	246.58	454.17	748.91	805.69	4.63	5.15	101.9	194.01
p	0.057		0.311		0.528		<0.001		0.338		0.517		<0.001	

표 1 각각의 표지자 검출치 (단위 CEA, E-selectin, osteopontin, MMP-7, TIMP-1은 ng/mL; HGF, VEGF는 pg/mL)

핵심되는 말: carcinoembryonic antigen; E-selectin; tissue inhibitor metallomatrix proteinase-1; liver metastasis; colorectal neoplasm