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Plasma TIMP-1 in patients with colorectal adenomas: a prospective study

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Abstract

Colorectal cancer patients have increased plasma levels of tissue inhibitor of metalloproteinases-1 (TIMP-1). However, it remains unresolved whether colorectal adenomas are associated with increased plasma TIMP-1. Plasma TIMP-1 levels were determined using an immunoassay in 121 patients prospectively enrolled from surveillance colonoscopy programmes. TIMP-1 levels were correlated to various clinicopathological parameters. No significant associations were found between plasma TIMP-1 and size, macro- or microscopic morphology or grade of dysplasia of the adenomas. No significant differences in TIMP-1 levels were found between patients with adenomas ($n = 36$), hyperplastic polyps ($n = 12$) or no pathology ($n = 68$) of the large intestine. However, patients with colonic ($n = 3$) or rectal ($n = 2$) adenocarcinomas had significantly increased TIMP-1 levels ($P = 0.02$). The present study demonstrates that measurement of plasma TIMP-1 cannot be used for the identification of adenomatous or hyperplastic polyps of the large intestine.

Keywords: Plasma TIMP-1; Colorectal adenomas; Colorectal cancer; Diagnosis

1. Introduction

According to the hypothesis presented by Vogelstein and colleagues [1], adenomatous polyps in the colon or rectum are neoplastic processes with a genetically based potential to develop into invasive adenocarcinomas. Thus, preventive polypectomy should lead to decreased numbers of subsequent malignancies of the large bowel in the population under surveillance. In keeping with this, data from case-control studies and surveillance studies with flexible sigmoidoscopy and colonoscopy with accompanying polypectomies suggest that the incidence of colorectal carcinomas is significantly decreased in the screening population compared with a representative background population [2–5].

At present, colonoscopy is the most sensitive endoscopic diagnostic procedure for the detection of adenomatous polyps in the colon or rectum; however, due to the cost and time required, the need of experts, and the risk associated with the procedure itself, colonoscopy will most likely not be introduced as a routine screening technology for intestinal adenomas. Many bowel tumours will bleed into the lumen of the intestine and based on this, alternative strategies for screening for adenomas and carcinomas of the colon or rectum have gained much interest. One of these is the faecal occult blood test (FOBT) and it has been shown that the FOBT as a screening regimen can result in decreased mortality from colorectal cancer (CRC) [6]. However, since only a

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few adenomas will bleed and since the bleeding that does occur may be only intermittent, the development of other methods for adenoma diagnosis is needed.

We have recently shown that measurements of total plasma levels of tissue inhibitor of metalloproteinases-1 (TIMP-1) give high specificity and sensitivity in the diagnosis of early stage, as well as of late stage colorectal cancer [7]. In order to further evaluate the clinical value of plasma TIMP-1 measurements, we extended our studies to include patients with colorectal adenomas. A prospective study was undertaken including a total number of 121 individuals referred to colonoscopy.

2. Materials and methods

2.1. Individuals

In this prospective study, a total of 121 individuals were included. All were referred to colonoscopy at the Department of Gastrointestinal Surgery, Odense University Hospital, Denmark and were enrolled consecutively in the study. Most of the participants were recruited from surveillance colonoscopy programmes after previous colorectal adenomas (84) or carcinomas (27). A plasma sample was drawn from the participants before the endoscopic procedure, and all signed an informed consent form. The study was approved by the local ethical committee. The median age (range) of the study population was 68 (33–82) years and there were 47 females and 74 males.

2.2. Blood collection

A blood sample (10 ml) was taken on the day of colonoscopy, immediately before the endoscopic procedure. The blood was subsequently processed into ethylene diamine tetra-acetic acid (EDTA) plasma and frozen at −70 °C until analysis for TIMP-1 content.

2.3. TIMP-1 ELISA

A sandwich immunoassay was used to measure the total levels of TIMP-1 in all of the samples. The assay has previously been thoroughly validated with demonstration of low intra- and interassay variations, high analytical specificity and good recovery [8]. In brief, the immunoassay employs a polyclonal sheep-anti-TIMP-1 antibody for capture and a monoclonal anti-TIMP-1 antibody for detection of bound antigen. An alkaline-phosphatase conjugated rabbit–anti-mouse antibody (DakoCytomation, Denmark) enables colour generation by enzymatic degradation of the substrate *p*-nitrophenyl-phosphate. A total of four enzyme-linked immunosorbent assay (ELISA)-plates were used for the determination of TIMP-1 levels in the plasma samples and on each plate three internal control samples with low, intermediate and high TIMP-1 levels, respectively, were included for assurance of assay reproducibility. The median TIMP-1 levels and interassay coefficients of variation (CV% v/v) for the low, intermediate and high controls samples were: 48.4 μg/l and 8.4% v/v (low), 100.6 μg/l and 5.0% v/v (intermediate), 145.7 μg/l and 2.4% v/v (high).

2.4. Statistical methods

The software package Statview (SAS) was used for data handling and statistical calculations. Plasma levels of TIMP-1 were log-transformed, thus approximating a normal distribution. Plasma TIMP-1 levels were correlated to age and gender. As a weak, but significant, association between plasma TIMP-1 and age was found, correction for this was accounted for in the one way analysis of variance (ANOVA) procedures used to test for differences in TIMP-1 levels between patient categories by including age as a covariate in the model. The level of significance was set at 5%.

3. Results

Table 1 shows the characteristics of the individuals, enrolled as well as the results of the present colonoscopy. Sixty-eight individuals had no pathology of the large bowel as determined by colonoscopy, whereas 36 individuals had at least one adenomatous polyp and 12 individuals had at least one hyperplastic polyp in the colon or rectum. Three individuals were identified with an adenocarcinoma of the colon and 2 individuals with an adenocarcinoma of the rectum. As appears from the table, there were no major differences among patients with or without pathology with respect to age and gender. Nineteen of the adenomas identified by the endoscopic procedure were located in the colon, 17 in the rectum. Six hyperplastic polyps were located in the colon, 6 in the rectum. The size (largest diameter) of the adenomatous polyps of the colon ranged from 2 to 20 mm and in the rectum from 3 to 19 mm. As some patients were found to have more than one adenomatous polyp, the cumulated diameter of the removed polyps was calculated. These figures, as well as the macroscopic and microscopic features of the adenomatous polyps, are given in Table 2.

All individuals had measurable plasma TIMP-1 levels (Table 1 and 2). Associations between plasma TIMP-1 levels and various included clinicopathological parameters were tested for, as illustrated in Table 3. Since a weak, but significant, correlation between TIMP-1 and age has been found in our previous studies [7,8] an association between these parameters was tested for in the present study. As shown in Fig. 1, a weak but signifi-

Table 1
Patient characteristics and plasma TIMP-1 levels

Parameter	Healthy individuals	Adenomatous polyps	Hyperplastic polyps	Colonic cancer	Rectal cancer
Number (n)	68	36	12	3	2
Age (years)					
Median (range)	69 (33–82)	67 (47–81)	71 (41–77)	62 (60–66)	70 (66–74)
Gender (m/f)	40/28	24/12	5/7	3/0	2/0
Location (n)					
Colon		19	6	3	
Rectum		17	6		2
TIMP-1 (μg/l)					
Median (range)	71.7 (47.8–126)	70.5 (55.7–102)	69.2 (51.3–97.1)	77.9 (71.7–183)	105 (97.3–113)
Mean (±SD)	73.7 (14.7)	73.1 (13.3)	74.7 (14.4)	111 (62.8)	105 (11.1)

m, male; f, female; SD, standard deviation.

Table 2
Pathological features of colorectal adenomas

Parameter		Colonic adenomas	Rectal adenomas
Number		19	17
Size (mm)	Median (range)	5 (2–20)	5 (3–19)
Cumulated size (mm)	Median (range)	10 (2–54)	6 (3–28)
Macroscopic feature	Sessile	1	2
	Pedunculated	18	15
Adenoma architecture	Tubular	17	15
	Tubulo-villous	1	1
	Villous	1	1
Degree of dysplasia	Mild	13	16
	Moderate	6	1
	Severe	0	0
TIMP-1 (μg/l)	Median (range)	68.8 (55.7–93.2)	77.4 (56.3–101)
	Mean (±SD)	64.6 (11.8)	78.3 (13.3)

Table 3
Association between plasma TIMP-1 and clinico-pathological parameters

Parameter	*P*-value	R^2-value
Age	<0.0001[a]	0.12
Colonoscopy: normal vs adenoma vs hyperplasia	0.9[b]	
Adenoma location: rectum vs colon	0.9[b]	
Adenoma size	0.2[a]	0.03
Cumulated adenoma size	0.4[a]	0.02
Adenoma type: pedunculated vs sessile	0.3[b]	
Adenoma architecture: tubular vs villous vs tubulo-villous	0.5[b]	
Grade of dysplasia: mild vs moderate[c]	0.8[b]	

TIMP-1, tissue inhibitor of metalloproteinases-1.
[a] Spearman's correlation.
[b] One way analysis of variance (ANOVA) procedure.
[c] No adenomas with severe dysplasia were identified.

cant, correlation was found; TIMP-1 increasing slightly with age ($R^2 = 0.12$, $P < 0.0001$). The regression line in Fig. 1 corresponds to an increase in plasma TIMP-1 of 5.2 μg/l over a 10-year period. Fig. 2 shows that there were no significant differences in plasma TIMP-1 levels between individuals with no pathology found and individuals presenting with adenomatous polyps or hyperplastic polyps ($P = 0.9$). Furthermore, median (range) TIMP-1 levels in individuals with colonic (68.8 (55.7–93.2) μg/l) or rectal (77.4 (56.3–101) μg/l) adenomas did not differ significantly ($P = 0.9$). Fig. 3 illustrates plasma TIMP-1 level as a function of adenoma size and as can be seen no correlation between these parameters were found ($R^2 = 0.03$, $P = 0.22$).In addition, when testing for an association between plasma TIMP-1 and cumulated adenoma size, no significant correlation was found

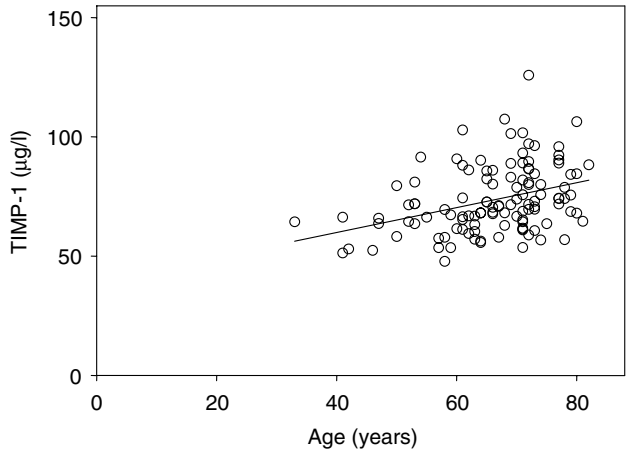

Fig. 1. Association between age (years) and plasma tissue inhibitor of metalloproteinases-1 (TIMP-1) values (µg/l) of all included individuals ($n = 121$).

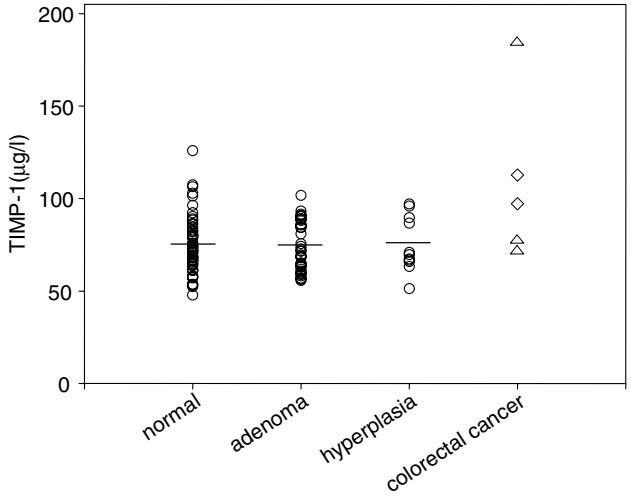

Fig. 2. Plasma TIMP-1 levels (µg/l) in individuals with no pathology, adenomatous polyps, hyperplastic polyps or malignant tumours of the colon or rectum.

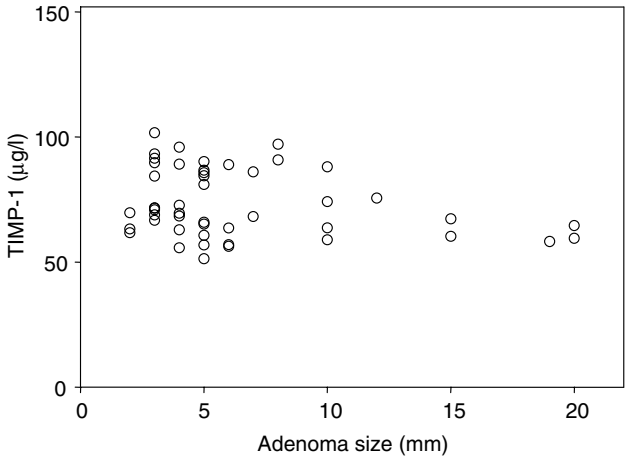

Fig. 3. Association between adenoma size (mm) and plasma TIMP-1 (µg/l).

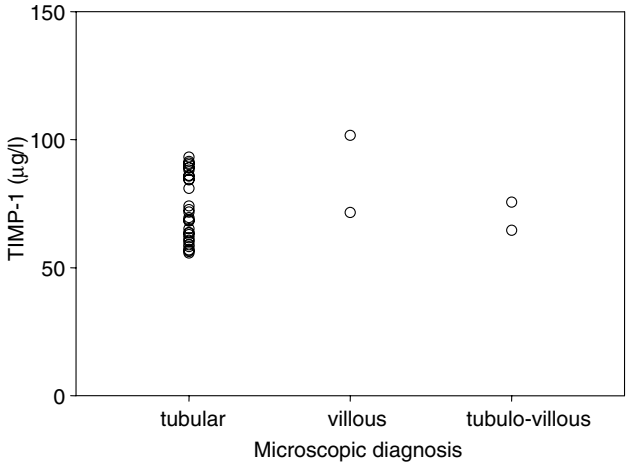

Fig. 4. Association between adenoma architecture and plasma TIMP-1 (µg/l).

($R^2 = 0.02$, $P = 0.4$). When looking at macroscopic characteristics of the adenomas (pedunculated vs sessile), no difference in plasma TIMP-1 levels was found ($P = 0.3$). However, the number of sessile adenomas was very low ($n = 3$). Likewise, no significant difference in plasma TIMP-1 levels was found when correcting for adenoma architecture (tubular, villous, tubulo-villous), as is demonstrated in Fig. 4 (ANOVA, $P = 0.5$), although the numbers of patients presenting with villous or tubulo-villous adenomas were low (both groups $n = 2$). Finally, the grade of dysplasia of the adenomas had no influence on plasma TIMP-1 levels (ANOVA, $P = 0.8$).

When including TIMP-1 levels for patients with colonic or rectal cancer in the ANOVA procedure, a significant difference in plasma TIMP-1 levels was found (ANOVA, $P = 0.02$) with increased TIMP-1 levels in patients with cancer of the bowel (Fig. 2). However, considering the low number of cancer patients in the study, the relevance of such statistical analysis is very limited. The mean (±SD) plasma TIMP-1 levels in patients with colorectal cancer were 111 µg/l (62.8) and 105 µg/l (11.1). As seen in Fig. 2, although the numbers of patients with colorectal cancer were low, their plasma TIMP-1 levels were higher than in healthy individuals or in patients with adenomatous or hyperplastic polyps.

4. Discussion

This prospective study shows that patients with colorectal adenomas have plasma TIMP-1 levels similar to those in individuals with no pathology found during colonoscopy. Thus, measurement of plasma TIMP-1

concentration does not provide diagnostic information about colorectal adenomas.We have previously described that measurement of plasma TIMP-1 level yields high specificity and sensitivity in the diagnosis of colorectal cancer [7]. Of particular interest was that high plasma TIMP-1 levels were also found in early stage colorectal cancer regardless of the primary tumour location; i.e. right- and left-sided cancers [7]. By including plasma TIMP-1 measurements from a large number of healthy blood donors, from patients with inflammatory bowel diseases and from patients with primary breast cancer, the data suggested a high degree of specificity of plasma TIMP-1 in the diagnosis of colon cancer [7]. However, in our previous study, we did not include patients with adenomas or hyperplastic polyps of the large bowel.

The present study clearly supports the specificity of plasma TIMP-1 in the diagnosis of colorectal cancer since none of the patients with colon adenomas presented with increased plasma TIMP-1 levels. We have previously reported on plasma TIMP-1 values in blood donors being in the range of 58.0–91.8 μg/l, with a median of 71.2 μg/l [8]. The present data thus support our previous finding that healthy individuals have low plasma TIMP-1 levels within a narrow range [7–9]. Of importance is that in the present study the non-cancer patients included have a median age comparable to the age where colorectal cancer has its highest incidence.

Traditionally, it has been regarded that TIMP-1 plays its role only during the late stages of cancer disease, namely invasion and metastasis, by inhibition of the matrix metalloproteinases. However, TIMP-1 has recently been shown to have several functions distinct from its anti-proteolytic activity. First, TIMP-1 has been demonstrated to promote growth of a variety of cell lines *in vitro* [10,11]. Secondly, TIMP-1 has been shown to inhibit apoptosis [12–14]. Finally, TIMP-1 has been shown to be involved in angiogenesis and to be upregulated upon malignant transformation [15–18]. Thus, it could be speculated that the high levels of plasma TIMP-1 in colorectal cancer patients are causally involved in the progression of malignant disease in the large bowel. In support of this notion are studies demonstrating a highly significant association between plasma TIMP-1 levels and colorectal cancer patient survival, with high plasma TIMP-1 levels predicting shorter survival [19,20], independent of the stage of disease.

It has been demonstrated that TIMP-1 expression is increased at both mRNA and protein levels in colorectal cancer tissue [21–24]. Both malignant epithelial cells and surrounding stromal cells responding to the presence of malignancy have been argued to be the origin of the TIMP-1 expression [20–23]. Based on the findings described above, it could be hypothesised that at some point during malignant transformation of benign intestinal adenomas, high expression of TIMP-1 is initiated. This results in increased levels of TIMP-1 protein in the tumour tissue, which will eventually find its way into the circulation, giving rise to increased plasma TIMP-1 levels. This could explain both our previous findings of increased plasma TIMP-1 levels in patients with colorectal cancer, as well as the present finding of no increase in plasma TIMP-1 levels in individuals with colorectal adenomas, irrespective of the size and grade of dysplasia, compared with individuals with no pathology found [7,8]. However, in order to further validate these results, we are currently performing studies of TIMP-1 in colon adenomas and early stage adenocarcinomas by *in situ* hybridisation and immunohistochemistry.

In conclusion, we have shown that patients with benign tumours of the colon or rectum have plasma TIMP-1 levels which are similar to those found in individuals with no colonic or rectal pathology. Thus, although based on a very limited number of subjects, this study supports our previous observation that patients with colorectal cancer have elevated plasma TIMP-1 levels [7,8].

Conflict of interest

None.

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