

Evaluation of *MUC1*, *CK20*, and *hTERT* expression in peripheral blood of gastrointestinal cancer patients in search of diagnostic criteria

Sibel KÜÇÜKYILDIRIM^{1*}, Beril ERDEM¹, Emel SAĞLAR¹, Zülfikar POLAT², Hatice MERGEN¹

¹Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey

²Department of Gastroenterology, Gülhane Military Medical Academy, Ankara, Turkey

Received: 19.03.2014

Accepted: 09.08.2014

Published Online: 24.11.2014

Printed: 22.12.2014

Abstract: The goal of this study was to identify the optimal marker or marker combinations for detection of gastrointestinal malignancies using reverse-transcriptase polymerase chain (RT-PCR) reaction. To detect the presence of tumors, we analyzed mucin 1 (*MUC1*), cytokeratin 19 (*CK19*), cytokeratin 20 (*CK20*), and human telomerase reverse transcriptase (*hTERT*) mRNA in the peripheral blood of 31 patients with gastrointestinal (esophagus, stomach, and colorectal) cancer and 30 healthy individuals. In RT-PCR analysis of the peripheral blood, 77.4% (24/31), 61.29% (19/31), and 45.16% (14/31) of cancer patients were positive for *MUC1*, *CK20*, and *hTERT* mRNA, respectively. According to our results, any one of these mRNA markers is a predictor of the presence of gastrointestinal tumors ($P < 0.001$) and colorectal tumors ($P < 0.05$). However, they did not have predictive potential for presence of metastasis in gastrointestinal tumors. As a result, combination of these 3 tumor-specific mRNA markers would increase the detection rate and may be clinically helpful in predicting tumor presence.

Key words: Gastrointestinal cancer, reverse-transcriptase PCR

1. Introduction

The gastrointestinal tract is the most common site of malignancies of any anatomic system in the body (Seregini et al., 2001); every year an estimated 2.5 million people are newly diagnosed (Boyle and Levin, 2008; Jemal et al., 2011). Gastrointestinal tumors are often diagnosed at late stages since patients with gastrointestinal tumors have a very broad range of dyspeptic symptoms. Therefore, the determining of sensitive and specific molecular markers can be a useful clinical tool for the early detection and management of diseases (Plaks et al., 2013). Because of the heterogeneity of gastrointestinal tumors, a panel of biomarkers for screening and diagnosis would be the most appropriate (Lurje et al., 2010). Recently published studies showed that detection of elevated circulating tumor cells (CTCs) was a prognostic factor for metastatic gastrointestinal cancers as well as an accurate indication of rapid progression and mortality (Rahbari et al., 2010; Rhim et al., 2012).

Detection of tumor cells in the blood could lead to a search for epithelial-specific mRNAs, which might be revealed in patients' blood samples via amplification by reverse transcription-polymerase chain reaction (RT-PCR). The use of RT-PCR allows sensitive and

reliable detection of a very small number of circulating tumor cells in blood or bone marrow. RT-PCR assays using epithelial markers are based on the principle that carcinoma cells detach from the site of the primary tumor and are distributed to hematopoietic or lymphatic tissue (Alix-Panabières and Pantel, 2013). This can lead to the appearance of gene transcripts that are not normally expressed in these host tissues (Wang et al., 2006; Tsouma et al., 2008).

Cytokeratins (CKs) such as *CK19* and *CK20* are generally used for the detection of most epithelial tumors and they belong to the epithelial subgroup of the intermediate filament protein family that is involved in cell structure and differentiation. Expression studies showed that *CK19* expressed in all epithelial cells; however, *CK20* is restricted to gastrointestinal epithelium, urothelium, and Merkel cells and this profile is maintained in malignant tumors of these cells. The malignant nature of CK-positive cells in the bone marrow has been confirmed through genomic analysis using fluorescence in situ hybridization (Muller et al., 1996; Stathopoulou et al., 2002). The relatively specific expression of CKs by epithelial tissues and conservation of this cell specificity following malignant transformation has led to their widespread use as reliable markers for diagnostic pathology.

* Correspondence: sibelkucukyildirim@gmail.com

Understandably, CKs have served as the most widely used target for detection of metastasis from epithelial-derived tumors in a variety of sites (Wlyd, 1998).

Tumor-associated protein mucin 1 (*MUC1*) is also very often used to trace metastatic cancer cells in gastrointestinal cancer (Uen et al., 2006; Lagoudianakis et al., 2009). Mucins are involved in a variety of physiologic functions including protection against mechanical and infectious insults, lubrication, and acid resistance. An increased expression of *MUC1* in human and colorectal cancer as a marker of progression and metastasis was demonstrated previously (Nakamori et al., 1994; Baldus et al., 2004). Telomerase, an enzyme responsible for continuous cell growth, is repressed in most somatic cells but activated in approximately 85% of human cancer tissues (Hiyama and Hiyama, 2003). There were several reports that human telomerase reverse transcriptase (*hTERT*) mRNA is present in the peripheral blood of patients with some cancer types (Chen et al., 2000; Shin et al., 2002; Wang et al., 2006).

The current study aimed to evaluate the feasibility of detection of *MUC1*, *CK20*, and *hTERT* mRNAs in the peripheral blood of gastrointestinal cancer patients based on RT-PCR amplification.

2. Materials and methods

2.1. Subjects

A total of 61 peripheral blood samples from 31 pathologically confirmed gastrointestinal cancer patients and 30 healthy subjects were collected from the Department of Gastroenterology of the Gülhane Military Medical Academy Hospital. The study was conducted in compliance with the Helsinki Declaration and it was approved by the institutional ethics committee; all patients and healthy subjects involved gave their written informed consent.

Clinicopathologic characteristics of the study population are summarized in Table 1. All patients suffered from adenocarcinomas. Clinical stages and pathologic features of primary tumors were defined according to the criteria of the American Joint Commission of Cancer (Greene et al., 2001). All patients with gastrointestinal cancer were found to have no evidence of other disease. Ten milliliters of peripheral blood was drawn into an EDTA tube and stored at 4 °C. As a positive control, RNA samples were also prepared from the human colon cancer cell line HT-29 (a gift from Dr G Selmanoğlu, Hacettepe University, Ankara, Turkey).

2.2. Total RNA isolation and cDNA synthesis

Blood tubes were centrifuged at 1000 × g for 5 min; the buffy coat was collected and transferred into 1.5-mL Eppendorf tubes. Total RNA extraction was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the

Table 1. Patients' characteristics.

Characteristic	n			
Sex				
Male	28			
Female	3			
Age				
Median	63.3			
Range	20–80			
Tumor location	Stage I	Stage II	Stage III	Stage IV
Esophagus	1	2	1	
Gastric	1	6	4	
Colorectal	1	3	8	4

manufacturer's instructions and the product was stored at –80 °C until use. To avoid contamination of DNA within RNA preparation, 1 µg of RNA was treated with RNase-free DNase I, as recommended by the supplier (New England BioLabs, Hitchin, UK). The first-strand cDNA was synthesized from total RNA by using MuLV reverse transcriptase (New England BioLabs). Total RNA (1 µg) was reverse-transcribed in a final volume of 50 µL containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.2 mM oligo(dT)₁₅, 0.2 mM dNTPs, and 100 U of MuLV reverse transcriptase according to the manufacturer's guidelines.

The reaction mixtures with RNA were incubated at 42 °C for 1 h, heated to 95 °C for 5 min, and then stored at –20 °C until analysis.

2.3. PCR amplification

The quantity of isolated RNA was confirmed by amplification of cDNA for house-keeping gene *GAPDH*. Each PCR reaction mixture contained 1X PCR buffer [67 mM Tris-HCl (pH 8.3), 16 mM (NH₄)₂SO₄, 0.1% Tween-20, 2.5 mM MgCl₂], 0.1 mM dNTP, 0.1 µM sense and antisense primers for target genes, and 1 U Taq DNA polymerase (Bioron, Ludwigshafen am Rhein, Germany) in a total volume of 25 µL. PCR products were analyzed in 2.5% agarose gel. The signals on UV transilluminator for each target gene and *GAPDH* expression levels were analyzed with a computing densitometer (Syngene GeneTools software) to calculate the relative mRNA density ratio. The primer sequences, PCR conditions, and predicted product size are given in Table 2.

2.4. Sequencing of PCR products

The amplified PCR products were sequenced using a Big Dye sequencing system (Applied Biosystems, Carlsbad, CA, USA) and determined to be identical to those expected.

Table 2. Primers and PCR conditions.

Primer	5'-3' sequence	PCR conditions	Size of PCR product (bp)
<i>MUC1</i> (sense)	ACCTACCATCCTATGAGCGAG	(94 °C/30 s, 60 °C/30 s, 72 °C/30 s) × 35 cycles	228
<i>MUC1</i> (antisense)	TGAAGAACCTGAGTGGAGTGGAAT		
<i>CK19</i> (sense)	TACCTGAAGAAGAACCATGAGGAG	(94 °C/30 s, 60 °C/30 s, 72 °C/30 s) × 35 cycles	446
<i>CK19</i> (antisense)	TGATTCTGCCGCTCACTATCAG		
<i>CK20</i> (sense)	CTGAATAAGGTCTTTGATGACC	(94 °C/30 s, 60 °C/30 s, 72 °C/30 s) × 35 cycles	295
<i>CK20</i> (antisense)	CTGTGACCTGTTGCTGCAGAACT		
<i>hTERT</i> (sense)	AAGTTCCTGCACTGGCTGAT	(94 °C/30 s, 60 °C/30 s, 72 °C/30 s) × 35 cycles	265
<i>hTERT</i> (antisense)	CACGACGTAGTCCATGTTCA		
<i>GAPDH</i> (sense)	CCTCAAGATCATCAGCAATGC	(94 °C/30 s, 60 °C/30 s, 72 °C/30 s) × 35 cycles	165
<i>GAPDH</i> (antisense)	GGAAACTGTGGCGTGATGG		

2.5. Statistical analysis

All analyses were conducted with SPSS 19. Potential association between the expression of molecular markers used in combination and the clinicopathologic features of the study subjects were evaluated using 2×2 or $2 \times k$ contingency tables and the significance of these comparisons was assessed using 2-tailed Fisher's exact tests.

To evaluate the usefulness of gene expression levels and presence/absence data of the 3 mRNA markers for predicting overall tumor presence, metastasis status, and colorectal tumor presence, we conducted separate logistic regression analysis. Tumor presence, metastasis status, and colorectal tumor presence were taken as binary dependent variables and coded using 0 (absence of tumor or metastasis) and 1 (presence of tumor or metastasis) as dummy variables. Significant fit of the regression equation to the data was assessed using Wald statistics; however, the cut-off for statistical significance was taken as $P < 0.008$ after Bonferroni correction for multiple comparisons. We also calculated odds ratios (ORs) determining the strength of association between predictive markers and tumor presence.

Receiver operating characteristic (ROC) curve analyses were done to analyze the RT-PCR data of *MUC1*, *CK20*, and *hTERT* gene expression in the peripheral blood of the subjects. The area under the ROC curve and the corresponding 95% confidence intervals (95% CI) were calculated for each marker. The cut-off value at the highest accuracy level (minimal false-negative and false-positive results) was determined.

3. Results

3.1. Expression of *MUC1*, *CK20*, and *hTERT* mRNAs in peripheral blood samples from gastrointestinal cancer patients

In analysis of the peripheral blood, 77.4% (24/31), 61.29% (19/31), and 45.16% (14/31) of cancer patients were positive for *MUC1*, *CK20*, and *hTERT* mRNAs, respectively. Seven (22.58%) of 31 patients were positive for all 3 mRNAs. The sensitivity and specificity for any one of the mRNAs detected in peripheral blood is 90.32% and 53.3%, respectively, with an accuracy of 68.85% (Table 3).

We used 2-tailed Fisher's exact tests to analyze the potential association between the expression of molecular markers used in combination and the clinicopathologic features of the study subjects. No association was found between the presence of mRNA markers and clinicopathologic characteristics of patients (Table 4).

For the 3 mRNA markers that we detected, there were no differences between patients with different stages ($P > 0.05$). Our results showed that combined analysis of *MUC1* and *CK20* helps to decrease the false-positive rate of detection (Table 3).

The predictive values of the 3 mRNA markers for tumor presence were analyzed using univariate logistic regression analysis (Tables 5 and 6). According to our results, any one of these mRNA markers is a predictor of the presence of gastrointestinal tumors ($P < 0.001$) and colorectal tumors ($P < 0.05$). These markers do not have predictive value for presence of metastasis in gastrointestinal tumors (Table 7).

ROC curve analyses of the RT-PCR data of the 61 subjects (31 cancer patients and 30 healthy individuals)

Table 3. Sensitivity, specificity, and accuracy of *MUC1*, *hTERT*, and *CK20* mRNA markers and their combination.

	Sensitivity	Specificity	Accuracy
<i>MUC1</i>	24/31 (77.4%)	8/30 (73.3%)	32/61 (52.4%)
<i>CK20</i>	19/31 (61.29%)	6/30 (80%)	25/61 (40.98%)
<i>hTERT</i>	14/31 (45.16%)	4/30 (86.6%)	18/61 (29.5%)
Any one mRNA	29/31 (93.54%)	14/30 (53.3%)	43/61 (70.49%)
<i>CK20+MUC1</i>	17/31 (54.84%)	2/30 (93.3%)	19/61 (31.14%)
<i>MUC1+hTERT</i>	8/31 (25.80%)	2/30 (93.3%)	10/61 (16.39%)
<i>CK20+hTERT</i>	8/31 (25.80%)	1/30 (96.6%)	9/61 (14.75%)
<i>MUC1+CK20+hTERT</i>	7/31 (22.58%)	1/30 (96.6%)	8/61 (13.1%)

were done. The ROC curves for each mRNA marker are shown in the Figure. Accordingly, the optimal cut-off value and area under the ROC curve for each mRNA marker was as follows: 2.00 and 0.795 (95% CI: 0.679–0.911) for *MUC1*, 1.50 and 0.748 (95% CI: 0.625–0.871) for *CK20*, and 3.00 and 0.673 (95% CI: 0.537–0.809) for *hTERT*.

3.2. Expression of *MUC1*, *CK20*, and *hTERT* mRNAs in peripheral blood samples from healthy volunteers

MUC1, *CK20*, and *hTERT* markers were detected in 30 healthy volunteers. *MUC1* expression was observed in 8 (26.6%), *CK20* expression in 6 (20%), and *hTERT* expression in 4 (13.3%) samples.

The positive ratios of *MUC1*, *CK20*, and *hTERT* mRNAs in gastrointestinal cancer patients are significantly higher than in healthy subjects (Table 3).

4. Discussion

Developments in biotechnology have made it possible to detect small numbers of tumor cells in peripheral blood, and these cells would provide a useful monitoring, diagnostic, and prognostic tool (Bednarz-Knoll et al., 2011; Wicha and Hayes, 2011). In recent years, there have been many reports on the RT-PCR detection of CTCs in the peripheral blood, bone marrow, and/or peritoneal lavage of patients with gastrointestinal cancer (Pantel et al., 1999; Yamaguchi et al., 2000; Stathopoulou et al., 2002; Gradilone et al., 2003; Dandachi et al., 2005; Uen et al., 2006). Compared with bone marrow and lymph nodes, blood collection is a minimally invasive procedure and blood can be sampled throughout the course of the disease (Böckmann et al., 2001; Huang et al., 2003; Pantel and Alix-Panabières, 2010; Bednarz-Knoll et al., 2011).

Because of the heterogeneity of gastrointestinal tumors, a panel of biomarkers could enhance the sensitivity of CTC detection compared with the single markers in use (Conzelmann et al., 2005; Lurje et al., 2010). The aim of this work was to evaluate the feasibility of detection of CTCs in the peripheral blood of gastrointestinal cancer

patients based on the RT-PCR amplification of *MUC1*, *CK20*, and *hTERT* mRNAs, aiming to use a standardized PCR method according to the World Health Organization criteria for a screening test: acceptability, practicability, high specificity, and high sensitivity (Kramer et al., 1999).

To evaluate the presence and predictive value of *MUC1*, *CK20*, and *hTERT* in the peripheral blood of gastrointestinal cancer patients, we analyzed 3 markers in 31 cancer patients and 30 healthy individuals, and we explored the correlation between expression of these markers and tumor presence.

Because of its high sensitivity, RT-PCR based on the amplification of the cell type-specific mRNA is increasingly used to detect CTCs. In previous studies, many target genes have been used to detect CTCs in gastrointestinal carcinoma, such as *CEA*, *MUC1*, *hTERT*, *cMet*, *CK18*, *CK19*, and *CK20* (Soeth et al., 1997; Yamaguchi et al., 2000; Katsumata et al., 2006; Uen et al., 2006; Wu et al., 2006; Xi et al., 2007; Tsouma et al., 2008; Lagoudianakis et al., 2009). Cytokeratins are frequently used to detect the CTCs in peripheral blood (Weitz et al., 1999; Gradilone et al., 2003; Wang et al., 2006; Wang et al., 2007). The mRNAs of *CK19* and *CK20* have been successfully used to detect CTCs in the peripheral blood of patients with breast cancer, lung cancer, and gastrointestinal carcinoma (Mori et al., 1996; Denis et al., 1997; Peck et al., 1998). Wlyd et al. (1998) suggested that *CK20* may be a useful target for RT-PCR detection of epithelial-derived cancers than previously described in cytokeratins.

In our study, in RT-PCR analysis of the peripheral blood, 77.4% (24/31), 61.29% (19/31), and 45.16% (14/31) of cancer patients were positive for *MUC1*, *CK20*, and *hTERT* mRNAs, respectively. When combining *MUC1*, *CK20*, and *hTERT* mRNA markers for the detection of CTCs in gastrointestinal cancer patients, the sensitivity of the detection rate increased up to 90.32% (28/31) and the rate of false positives was 3.33% (1/30). No correlation was found between the presence of *MUC1*, *CK20*, and *hTERT*

Table 4. Clinicopathological features of patients with or without mRNA expression.

	MUC1		CK20		hTERT		Any 1 mRNA		Any 2 mRNAs		All 3 mRNAs		P
	+	-	+	-	+	-	+	-	+	-	+	-	
Patients (n = 31)	24	7	19	12	14	17	29	2	21	10	7	24	
Healthy individuals (n = 30)	8	22	6	24	4	26	14	16	3	27	1	29	0.053
Age													
≥60	17	3	11	9	9	11	18	2	14	6	5	15	
<60	7	4	8	3	5	6	11	0	7	4	2	9	0.664
Tumor location													
Esophagus	3	1	1	3	1	3	4	0	1	3	0	4	
Gastric	9	2	6	5	6	5	11	0	8	3	2	9	
Colorectal	12	4	12	4	7	9	14	2	12	4	5	11	0.593
Stage													
1	1	0	1	0	1	0	1	0	1	0	1	0	
2	5	0	3	2	1	4	5	0	3	2	1	4	
3	12	3	10	5	9	6	15	0	12	3	4	11	
4	6	4	5	5	3	7	8	2	5	5	1	9	0.314
Metastasis													
M0	18	4	14	8	11	11	21	1	16	6	6	16	
M1	6	3	5	4	3	6	8	1	5	4	1	8	0.640

Table 5. Results of logistic regression analysis for predicting tumor presence based on gene expression and presence/absence data of mRNA markers.

Markers	B	SE	Wald	df	Sig.	Odds ratio exp(B)	Odds ratio (95% CI)	
							Lower	Upper
<i>MUC1/GAPDH</i>	2.454	0.827	8.809	1	0.003	11.631	2.301	58.792
<i>CK20/GAPDH</i>	3.004	1.002	8.987	1	0.003	20.165	2.829	143.721
<i>hTERT/GAPDH</i>	0.002	0.003	0.381	1	0.537	1.002	0.996	1.008
Any 1	-2.808	0.818	11.794	1	0.001	0.060	0.012	0.300
Any 2	-2.939	0.720	16.677	1	0.000	0.053	0.013	0.217
All 3	-2.135	1.104	3.740	1	0.053	0.118	0.014	1.029

Table 6. Results of logistic regression analysis for predicting colorectal tumor presence based on gene expression and presence/absence data of mRNA markers.

Markers	B	SE	Wald	df	Sig.	Odds ratio exp(B)	Odds ratio (95% CI)	
							Lower	Upper
<i>MUC1/GAPDH</i>	0.552	0.563	0.961	1	0.327	1.737	0.576	5.243
<i>CK20/GAPDH</i>	1.930	0.933	4.283	1	0.038	6.892	1.108	42.878
<i>hTERT/GAPDH</i>	-0.002	0.003	0.387	1	0.534	0.998	0.992	1.004
Any 1	21.411	2.85E+04	0.000	1	0.999	1.9E+09	0.000	Inf.
Any 2	-0.875	0.827	1.122	1	0.290	0.417	0.082	2.106
All 3	-1.253	0.934	1.801	1	0.180	0.286	0.046	1.780

Table 7. Results of logistic regression analysis for predicting the presence of metastasis based on gene expression and presence/absence data of mRNA markers.

Markers	B	SE	Wald	df	Sig.	Odds ratio exp(B)	Odds ratio (95% CI)	
							Lower	Upper
<i>MUC1/GAPDH</i>	-1.102	0.779	2.002	1	0.157	0.332	0.072	1.529
<i>CK20/GAPDH</i>	-0.485	0.867	0.313	1	0.576	0.616	0.112	3.370
<i>hTERT/GAPDH</i>	-0.002	0.005	0.173	1	0.677	0.998	0.989	1.007
Any 1	0.965	1.474	0.429	1	0.513	2.625	0.146	47.183
Any 2	1.001	0.842	1.413	1	0.235	2.720	0.522	14.165
All 3	1.099	1.164	0.891	1	0.345	3.000	0.307	29.353

markers and clinicopathologic characteristics of patients (Table 4).

In this study, in RT-PCR analysis of the peripheral blood of cancer patients and healthy individuals, 100% (61/61) were positive for *CK19*. Due to the high false-positive rate, *CK19* is not useful for CTC detection. Similarly to our results, other investigators have also detected *CK19* transcripts in the peripheral blood of healthy individuals (Krismann et al.,

1995; Stathopoulou et al., 2002; Wu et al., 2006). The false positive in healthy individuals might be attributed to the design of primers, pseudogenes, contamination of epithelial cells, or the handling of samples (Ruud et al., 1999; Wang et al., 2006; Tsouma et al., 2008). These may involve the ectopic expression of the *CK19* gene in hematopoietic cells or the expression of any gene in any cell type (Chelly et al., 1989; Novaes et al., 1997; Stathopoulou et al., 2002).

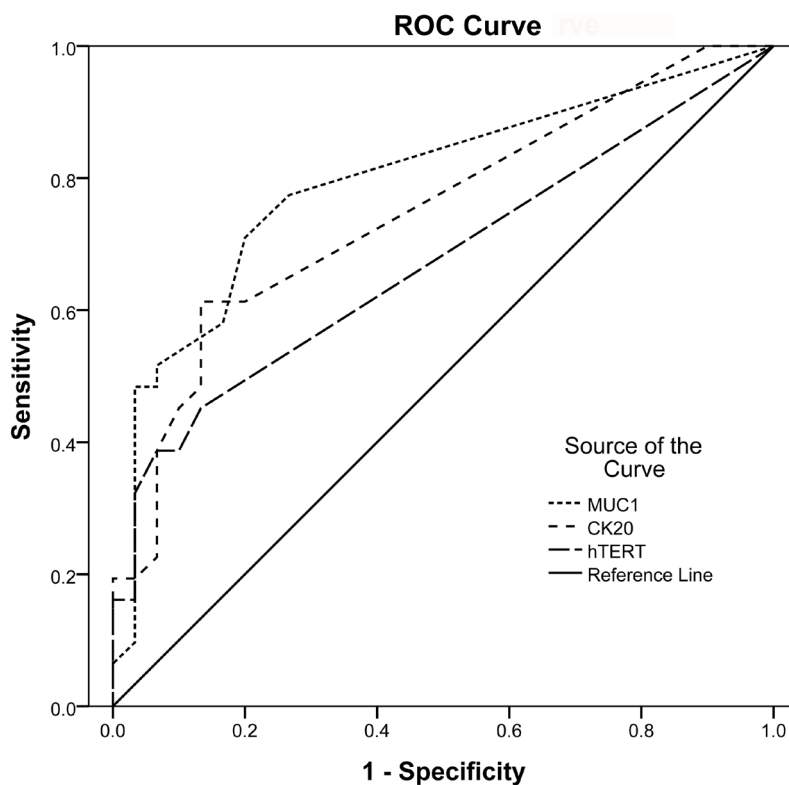


Figure. ROC curves for *MUC1*, *CK20*, and *hTERT* mRNAs from analysis of RT-PCR data of 61 subjects. Sensitivity (Y axis) was plotted against the false-positive fraction (1-specificity; X axis) for various cut-off values.

In the literature, different frequencies for *MUC1*, *CK20*, and *hTERT* in patients have been described (Noguchi et al., 1996; de Cremoux et al., 2000; Katsumata et al., 2006; Uen et al., 2006). The differences could be explained by the heterogeneous designs of these studies (number of samples, sampling methods, tumor sites, tumor stages, RNA extraction, and PCR protocols). Improved sensitivity in patients may be achieved by analysis of larger blood volumes and analysis of multiple samples from one individual. This method may reduce false-negative results (Wlyd et al., 1998). Jonas et al. (1997) reported an increased rate of CEA detection by RT-PCR in peripheral blood samples from patients with colorectal cancer when 3 separate samples were analyzed. CTC detection methods also need to be taken into account, as sensitivity and specificity are of major importance and may differ significantly. Thus, it will be important to define the critical variables in the methods and to introduce at least some level of standardization to allow for more reliable and reproducible results (Pantel et al., 1999; Vlems et al., 2002; Uen et al., 2006; Khair et al., 2007).

In our study, we found that *MUC1*, *CK20*, and *hTERT* mRNAs are significantly more frequently detected in gastrointestinal cancer patients than in healthy controls

and could serve as markers. Funaki et al. (1998) detected *CK20* mRNA in the peripheral blood of advanced colorectal carcinoma patients, which seems to present an indicator of possible recurrence in individual patients. It has been suggested that *CK20* mRNA detection by RT-PCR in the peripheral blood and bone marrow is a promising marker for circulating tumor cells of epithelial origin (Burchill et al., 1995; Johnson et al., 1995; Soeth et al., 1997; Katsumata et al., 2006).

In the univariate analysis, there was statistically significant association between the detection of any one mRNA and tumor presence ($P < 0.001$). These results clearly demonstrate the significant and prognostic/predictive value of *MUC1*, *CK20*, and *hTERT* mRNA detection by RT-PCR in the peripheral blood of patients with gastrointestinal cancers. Similar to our results, Msaouel and Koutsilieris (2011) reported that CTC evaluation can confirm tumor diagnosis.

Other studies showed *MUC1*, *CK20*, and *hTERT* expressions in peripheral blood of healthy individuals (de Cremoux et al., 2000; Gradilone et al., 2003; Uen et al., 2006; Wu et al., 2006; Wang et al., 2007). Illegitimate transcription of tissue-specific genes in blood from healthy individuals has been previously described by the

mononuclear fraction. Sources of illegitimate expression of *MUC1*, *CK20*, and *hTERT* mRNAs are macrophages, lymphocytes, and hematopoietic cells. Illegitimate transcription in blood cells is a well-known limitation and lowers the specificity of the RT-PCR analysis. While using RT-PCR in CTC detection could overcome the problems of lack of sensitivity associated with other methods, the selection of epithelial-specific mRNA is difficult (Solmi et al., 2006).

Our study demonstrated that *MUC1*, *CK20*, and *hTERT* gene expressions differ between individuals even if they have the same tumor type. Therefore, marker combinations maximize differences in expression in tumors compared with cells isolated from normal blood. Due to the heterogeneity of the expression of tumor-related genes, a multimarker assay is regarded as more reliable and sensitive than a single marker assay (Conzelmann et al., 2005; Lurje et al., 2010).

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- In this study, we found that the detection of *MUC1*, *CK20*, and *hTERT* mRNAs in the peripheral blood of patients with gastrointestinal cancers is a significant predictive/prognostic factor for determining tumor presence. This method could offer a simple, noninvasive, and promising tool for the detection of CTCs. However, a further study in larger patient and healthy populations is required to confirm the clinical usefulness of these molecular markers. Moreover, in order to arrive at a highly significant conclusion, a further study is to be performed with a particular tumor type, such as focusing on the stomach.
- Acknowledgments**
This work was funded by the Hacettepe University Scientific Research Unit (Project number: 0901-601010). This study is part of an MSc thesis submitted to Hacettepe University. We deeply thank Dr İsmail Kudret Sağlam for helping us during the statistical analysis.
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