

REVIEW ARTICLE

Serum hTERT Level as Sensitive Biomarker With Prognostic Implications in Breast, Lung, Gastric and Liver Cancers

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ABSTRACT

Human telomerase reverse transcriptase (hTERT) plays an important role in telomere restitution and gene regulation. Evidences suggest that hTERT is linked with the risk and progression of several types of malignancies. Detection of hTERT mRNA levels, as one of tumor markers, may reflect the tumor burden and the clinical status of the patient. Present paper emphasizes the potency of hTERT mRNA detection in serum as a sensitive tumor biomarker in different types of cancer. Detection of serum hTERT mRNA levels has been found highly sensitive and specific for varied cancers. A number of reports reflect its superiority to other conventional tumor markers including alfa-fetoprotein, EGFR, lens culinaris agglutinin-reactive AFP and Des-gamma carboxy prothrombin. Serum hTERT has been found linked with the risk and progression of different cancer types. hTERT levels in combination with other tumor markers may be used to improve cancer detection, tumor size and level of cancer progression.

Keywords: hTERT, Tumor marker, Cancer diagnosis, Serum levels, Telomerase, hTERT mRNA

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INTRODUCTION

Human telomerase embraces two RNA subunits including a telomerase RNA template and telomerase reverse transcriptase protein (hTERT) as its catalytic component (1). Later being a catalytic unit, plays a significant role in telomere restitution and gene regulation (2). hTERT also plays an important role in cancer (3) as evidences suggest that it is associated with risk and progression of several types of malignancies (2). Moreover, hTERT mRNA levels have been found to present the tumor burden and clinical status of the patient (4). The estimation of serum hTERT mRNA levels has been found useful for early detection of pancreatic cancer (5).

A number of currently available tumor markers for different types of cancers include Lens culinaris agglutinin-reactive AFP (AFP-L3), Alpha fetoprotein (AFP), and Des-gamma carboxy prothrombin (DCP) (6). Sometimes, these conventional markers fail to diagnose early stage cancers. Thus, there is a need to discover and develop sensitive markers which can be detected at early stages in different cancer types. These additional biomarkers should have high sensitivity and specificity. These should also have highly relevant clinical significance in comparison to presently used tumor

markers for a particular type of cancer. Telomerase reverse transcriptase protein hTERT mRNA has been reported to be detectable with the help of reverse transcription polymerase chain reaction (RT-PCR) (7). This assay has also been reported as superior to other tumor markers for different cancer types. Serum hTERT has been found useful for determining and evaluating the clinical stage of breast cancer (8). Due to high sensitivity and specificity, the present review highlights various aspects of hTERT mRNA detection that makes it a suitable, novel and reliable tumor marker in different cancer types.

Breast Cancer

Ribo-nucleic acids (RNA) are noticeable in the serum of breast cancer patients and tumor-derived mRNA can also be isolated and amplified using RT-PCR (1). Studies have demonstrated significantly higher mean values of serum hTERT levels in breast cancer patients than healthy control individuals (1,8). A number of studies have been retrieved reporting hTERT expressions in breast cancer patients (9-11). The expression of hTERT has also been found to significantly correlate with telomerase activity in breast cancer tissues (8,11). Its expressions have been reported to be much greater in carcinoma than those in normal breast tissue nearby cancer (12). hTERT mRNA was investigated for its presence in the sera of 18 breast cancer patients, 2 benign breast disease patients, and 21 control subjects using RT-PCR. hTERT was detected in 94% tumors (17 out of 18 tumors) and in 25% of serum samples (4 of 16 samples). No hTERT

was detected in tissues and sera of two benign breast disease patients and in the sera of all control subjects (1). Investigative implications of hTERT in the serum of breast cancer patients were investigated in 159 patients with breast cancer and 41 control volunteers. Here, the evaluation of hTERT, cancer antigen 15.3 and carcinoembryonic antigen was performed by enzyme-linked immunosorbent assay (ELISA) rather than by PCR. Elevated serum hTERT levels were found in 51.9% patients (27 of 52 cases) with breast cancer of stage I, 77.5% patients (31 of 40 cases) with stage II cancer and 88.2% patients (30 of 34 cases) with stage III breast cancer. Significantly higher sensitivity (68.9%) and specificity (83.3%) of hTERT in cancer diagnosis was found in comparison to conventional markers (8).

Lung cancer

hTERT mRNA expression was analyzed in lung cancer in a wide number of studies using different kinds of samples (13-16). hTERT mRNA in serum has been quantified by RT-PCR to reveal its clinical significance as a biomarker for lung cancer (17). Serum hTERT mRNA was measured along with epidermal growth factor receptor (EGFR) mRNA levels in 112 lung tumor patients and 80 control individuals. Serum hTERT mRNA copy numbers were found to be in a significant correlation with tumor number, tumor size, and metastasis. The sensitivity of hTERT mRNA was found to be higher than EGFR mRNA (89.0% vs. 71.3% respectively) (17). Further surgical treatment was found associated with decreased copy number of hTERT mRNA. On the other hand, in advanced non-small cell lung cancer (NSCLC), high serum hTERT levels may fail to be a good prognostic indicator for overall survival prediction. In a study on NSCLC, serum hTERT quantification by RT-PCR was done as a reference of the total quantity of free DNA in blood in 99 patients with advanced NSCLC. The median hTERT level for patients in NSCLC stage IIIB was found to be 70.7 ng/ml in comparison to 53.1 ng/ml for patients in stage IV ($p = 0.35$). Furthermore, no association was found between therapy response and hTERT levels (18). But as for pulmonary malignancies, hTERT mRNA, especially in combination with EGFR mRNA, can prove to be an excellent and novel biomarker (17).

Gastric cancer

hTERT is a potential biomarker for the early detection of gastric cancer also (19). hTERT mRNA quantification in sera of patients with gastric cancers has previously been reported by some of the researchers (19-23). Nowadays, positron emission tomography-computed tomography (PET/CT) assisted by 18F-fluorodeoxyglucose (FDG) has become an outstanding method for detecting cancer. A comparative study of FDG-PET/CT and hTERT mRNA quantification (with RT-PCR) in patients with varied types of cancer including gastric cancer was designed. 470 subjects were enrolled in this study. hTERT mRNA

and FDG-PET/CT were demonstrated to be in a highly significant correlation with the clinical parameters of recurrence and metastasis. Though FDG-PET/CT was found superior to hTERT mRNA quantification in the early detection of cancer but for the detection of viable tumor cells, the combined use of both techniques exhibited a positivity of 94.4%. Hence, hTERT quantification can be important in improving the results derived by FDG-PET/CT alone (22). A substantial correlation between level of hTERT mRNA expression in gastric carcinomas and the degree of differentiation has also been reported presenting the significance of this assay (19).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the frequently occurring malignant tumors all over the world. Common used tumor markers for HCC are AFP-L3, AFP and DCP (6). Some other recent markers include glypican 3, heat shock protein 70, glutamine synthase, cytokeratin 19, HEP Par 1 and Golgi protein 73. It has been showed that AFP-L3 and DCP excel AFP in differentiating hepatocellular carcinoma from non-malignant hepatopathy and detecting small HCC (24). Miura et al. in 2007, introduced a newly developed quantitative serum hTERT mRNA detection method having a clinical significance in HCC diagnosis (6). Serum hTERT mRNA level was found to be elevated in patients with HCC when compared to chronic liver disease patients. Moreover, its level positively correlated with that in HCC tissue. Its expression was also found to be independently correlated with degree of differentiation ($p < 0.001$). A sensitivity of 88.2% and a specificity of 70% was found for hTERT mRNA diagnosis in HCC patients. hTERT mRNA detection emerged to be superior to conventionally used markers including AFP, AFP mRNA and DCP (6). Similarly, another study confirmed serum hTERT mRNA detection to be more sensitive and specific than AFP in the early detection of HCC (25). In this study, 35 HCC patients and 15 patients with liver cirrhosis were undertaken. hTERT mRNA was assayed using RT-PCR technique. Results of the study revealed that hTERT was exceeding the cutoff point (>144 copies/ml) in 27 patients with HCC with a specificity of 100% and a sensitivity of 77.14% whereas, AFP was above the cutoff point (>50 ng/ml) only in 23 HCC patients with a specificity of 96% and a sensitivity of 65.71%. Thus, hTERT showed a high sensitivity as well as a high specificity. Moreover, hTERT correlated with the size of the tumor (25).

hTERT mRNA expression was also shown to be independently correlated with clinical variables such as degree of differentiation, tumor size, and number in HCC ($p < 0.001$, each) (26). Another study by Miura et al. (7) presented serum hTERT mRNA as an excellent biomarker in HCC. The study assayed this biomarker in 78 HCC patients, 10 patients with liver cirrhosis (LC), 12 patients with chronic hepatitis (CH) and 34 healthy

control individuals. 89.7% of the HCC patients (70 of 78), 70% of the LC patients (7 of 10) and 41.7% of CH patients were found to be positive for hTERT expression. 100% of the healthy control individuals exhibited a nil hTERT expression. Thus, higher sensitivity makes hTERT an excellent biomarker in HCC.

Association with clinical course and gene variants

Serum hTERT mRNA level has been presented to be in correlation with the clinical course. It was estimated in serum and exosomes derived from serum from 133 patients suffering from varied malignancies and 45

Table I: Studies involving detection of serum hTERT mRNA in different cancer types

Authors	Year	Cancer type studied	Patients taken	Control subjects	Parameters/markers taken along with hTERT mRNA levels	hTERT mRNA detection method	Sensitivity	Specificity	Inference	hTERT superiority #	Reference
de Martino <i>et al.</i>	2016	RCC	243 RCC patients	420	7 polymorphic hTERT gene variants	-	-	-	hTERT serum levels increased with every G allele in rs2736098 (p = 0.008)	↑	[2]
Ping <i>et al.</i>	2015	Varied types	345 patients	125	FDG-PET/CT	RT-PCR	Positivity of 94.4% when used along FDG-PET/CT	-	FDG-PET/CT is a superior method to hTERT mRNA quantification in early cancer detection	↓	[22]
El-Manzy <i>et al.</i>	2014	HCC	35 HCC patients 15 LC patients	10	AFP	RT-PCR	hTERT mRNA: 77.14% AFP: 65.71%	hTERT mRNA: 100% AFP: 96%	Detection of hTERT mRNA is more sensitive parameter than AFP in early HCC	↑	[25]
Porika <i>et al.</i>	2011	Breast cancer	159 patients	41	CA-15.3 Carcinoembryonic antigen	ELISA	68.9%	83.3%	Pretreatment serum hTERT levels positively correlated with clinical stage of cancer	↑	[8]
Porika <i>et al.</i>	2011	Cervical cancer	192 SSC or adenocarcinoma of uterine cervix patients	38	SCC antigen CA-125	ELISA	-	-	Pretreatment serum hTERT levels positively correlated with clinical stage, tumour size and lymph node metastasis	↑	[27]
Miura <i>et al.</i>	2007	HCC	HCC patients and Chronic liver disease patients	-	AFP AFP-L3 DCP	New method developed	82.2%	70%	hTERT mRNA quantification is superior to AFP and DCP in HCC diagnosis	↑	[6]
Miura <i>et al.</i>	2007	Gynecological cancer	47 Ovarian lesions, 63 uterine lesions, 2 malignant and 62 benign lesion patients	20	EGFR mRNA CA-125	RT-PCR	74.4%	74.1%	EGFR mRNA failed to display any differences between disease types. hTERT is a superior marker than conventional tumour markers.	↑	[3]
Camps <i>et al.</i>	2006	NSCLC	99 NSCLC patients	-	-	RT-PCR	-	-	High serum hTERT levels may not be a very useful indicator of TTP and overall survival in advanced NSCLC	↓	[18]
Miura <i>et al.</i>	2006	Lung cancer	112 lung tumour patients	80	EGFR mRNA	RT-PCR	hTERT mRNA: 89% EGFR mRNA: 71.3%	hTERT mRNA: 72.7% EGFR mRNA: 80%	hTERT mRNA was found in positive correlation with tumour size number and metastasis. hTERT mRNA quantification along with EGFR mRNA can be an excellent biomarker for pulmonary cancer	↑	[17]
Leelawat <i>et al.</i>	2006	ChC	33 ChC and 41 BBTD patients	10	CA-19-9	RT-PCR	hTERT mRNA: 84.85% (ChC); 21.9% (BBTD) CA-19-9: 60.6% (ChC); 19.5% (BBTD)	-	hTERT mRNA can be used as a marker for ChC. No correlation found with tumour size.	↑	[28]
Miura <i>et al.</i>	2005	HCC	64 HCC, 20 LC and 20 CH patients	50	AFP mRNA	RT-PCR	hTERT mRNA: 88.2% AFP mRNA: 71.6%	hTERT mRNA: 70% AFP mRNA: 67.5%	hTERT mRNA levels higher in HCC patients than chronic liver disease patients; positively correlated with tumour size, tumour number and degree of differentiation; superior to AFP mRNA	↑	[26]
Miura <i>et al.</i>	2003	HCC	78 HCC, 10 LC and 20 CH patients	34	AFP	RT-PCR	89.7% (HCC) 70% (LC) 41.7% (CH)	-	hTERT mRNA positively correlated with tumour size and degree of differentiation	↑	[7]
Hu <i>et al.</i>	2004	Gastric cancer	35 gastric cancer patients	-	-	RT-PCR	-	-	hTERT mRNA significantly correlated with degree of differentiation; potential marker for early GC detection	↑	[19]

Continue.....

Table I: Studies involving detection of serum hTERT mRNA in different cancer types (continued)

Authors	Year	Cancer type studied	Patients taken	Control subjects	Parameters/markers taken along with hTERT mRNA levels	hTERT mRNA detection method	Sensitivity	Specificity	Inference	hTERT superiority *	Reference
Kim <i>et al.</i>	2012	HCC	23 HCC patients	06 LC patients 04 Liver donors	-	RT-PCR			No significant difference in the levels of hTERT mRNA between the HCC and control groups; PB-hTERT mRNA is not a likely marker for the diagnosis of HCC, especially in patients undergoing resection.	↓	[29]
March-Villalba <i>et al.</i>	2012	Prostate cancer	105 patients with higher PSA levels	68	PSA	RT-PCR	hTERT mRNA: 85% PSA: 83%	hTERT mRNA: 90% PSA: 47%	Plasma (not serum) hTERT mRNA is a significant independent predictor of prostate cancer	↑Plasma hTERT ↓Serum hTERT	[30]
Chen <i>et al.</i>	2000	Breast cancer	18 breast cancer patients and 2 benign breast disease patients	21	hTR	RT-PCR	hTERT: 28% hTR: 25%	-	Tumour-derived mRNA can be use as a reliable marker in diagnosis of breast cancer patients	↑	[1]

*Up arrow depicts serum hTERT as a beneficial marker, and vice versa; LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; RCC, Renal cell carcinoma; FDG, 18F-fluorodeoxyglucose; PET, Positron emission tomography; CT, computed tomography; RT-PCR, Real time reverse transcription polymerase chain reaction; AFP, Alfa fetoprotein; CA, Cancer antigen; SCC, Squamous cell carcinoma; ELISA, Enzyme-linked immunoassay; AFP-L3, Lens culinaris-reactive AFP; DCP, Des-gamma-carboxy prothrombin; NSCLC, Non-small cell lung cancer, TTP, Time to progression; ChC, Cholangiocarcinoma; BBTD, Benign biliary tract disease; CH, Chronic hepatitis; GC, Gastric cancer; PSA, Prostate specific antigen; hTR, Telomerase RNA template; PB, Peripheral blood

healthy control individuals (4). hTERT transcript was detected in 67.5% of patients with different cancer types. All the 45 control samples were found negative for hTERT mRNA. Numerous cases presented a positive correlation between levels of hTERT transcript and the clinical course. Another study investigated several hTERT gene variants relative to different cancer types. De Martino *et al.* (2) assessed seven polymorphic hTERT gene variants (MNS16A, rs2853677, rs13172201, rs2736100, rs2736098, rs7726159, and rs10069690) and hTERT serum levels in 243 patients with renal cell carcinoma (RCC) and 420 healthy controls which were age- and gender-matched. Serum levels of hTERT were found to significantly rise with every G allele in rs2736098 ($P=0.008$). Out of the seven observed variants, telomere length restitution was found to be possibly associated with only rs2736098 gene variant. Table I presents various studies reporting quantification of serum hTERT mRNA in relation to different cancer types.

hTERT and tumor size

hTERT mRNA levels in serum appear to be in correlation with the size of the tumor in different types of cancer. In some of the studies, hTERT has been demonstrated to have a greater correlation value with tumor size than many conventional tumor markers. El-Manzi *et al.* (25) compared the association of hTERT and AFP with tumor size and reported hTERT to be more correlated with the size of the tumor than AFP in case of HCC. On the same line, hTERT mRNA expression was shown to be independently correlated with clinical variables including tumor size ($p < 0.001$, each) (26). Porika *et al.* (27) also reported a significant correlation of pre-treatment serum hTERT levels with tumor size in case of cervical cancer. But in case of breast cancer, this

association has not been reported to be significant. Porika *et al.* (8) described only a marginal correlation. Correspondingly, the expression of hTERT mRNA did not correlate with tumor size ($p > 0.05$) in breast cancer patients (12) (Table II). Further studies are required to find out more about the association of hTERT and tumor size in this case.

Superiority to conventional markers

There are a number of conventional tumor markers used for the above discussed cancer types and researchers constantly seek newer and better markers. AFP is a fetal specific glycoprotein chiefly produced by the fetal liver and is a commonly used tumor marker in the detection of patients with HCC (24). However, other tumor markers like alpha-L-fucosidase, gamma-glutamyl transferase II, glypican-3, tumor-specific growth factor, and transforming growth factor-beta1 have been shown to be available supplementaries to AFP determination (24). Circulating DNA derived from tumor tissue is found in the plasma of cancer patients which can also be used as a tumor marker (1). Likewise, EGFR mRNA has also been reported as a tumor marker but it failed to display any variances between the disease types in case of gynecological cancers (3). Some other markers, such as vascular endothelial growth factor, gamma-glutamyl transferase mRNA, and interleukin-8, have also been used as prognostic indicators (24). hTERT mRNA detection has been found superior to other markers in cases of gynecological cancers and HCC (3,20,24). Serum hTERT mRNA has been considered novel and available biomarker for gynecologic malignancies (3). The activity levels of hTERT mRNA have been found to be more diagnostically sensitive than the cytological results in several cancerous tissues. Moreover, these correlate well with different confounders including

Table II: Correlation table showing significant and non-significant correlation of serum hTERT levels with different confounders

Author	Year	Age	Tumour Size	Tumour number	Tumour stage	Degree of differentiation	Recurrence	Reference
Hu <i>et al.</i>	2004					++		[19]
Ping <i>et al.</i>	2015			++			++	[22]
El-Manzy <i>et al.</i>	2014		++	--				[25]
Porika <i>et al.</i>	2011	--	+		++			[8]
Porika <i>et al.</i>	2011	--	++		++			[27]
Miura <i>et al.</i>	2007					++		[6]
Miura <i>et al.</i>	2007				++			[3]
Miura <i>et al.</i>	2006		++	++			++	[17]

(++), Significant Correlation; (+), Marginal Correlation; (--), No correlation

clinical disease stage (20). Its sensitivities could achieve levels of 76.9% in detecting HCC patients (24). Its levels may help differentiating patients with HCC from liver disease patients. Serum hTERT mRNA showed higher values in patients with HCC as compared to patients of chronic liver diseases (26). In HCC, hTERT mRNA achieved higher sensitivity (88.2%) and specificity (70%) than AFP mRNA (71.6% and 67.5% respectively). Thus, hTERT mRNA detection proved to be superior to other tumor markers. Hence, quantification of serum hTERT may be used as an aid along with conventionally used tumor markers to improve cancer detection and progression.

CONCLUSION

Human telomerase reverse transcriptase (hTERT) has been found associated with risk and progression of several malignancy types. hTERT mRNA is detectable in the serum of cancer patients and can be extracted and amplified using RT-PCR method. Serum levels of hTERT mRNA have been reported to be more diagnostically sensitive than cytological results in many cancer types. After going through an extensive literature search, it has been found that its serum levels are highly sensitive in early prognosis of different cancer types. High serum levels have been found corresponding to tumor size and differentiation in liver and cervical cancer. hTERT levels show high sensitivity and selectivity in diverse cancer types. Many reports reflect its superiority to other tumor markers including alfa-fetoprotein, EGFR, lens culinaris agglutinin-reactive AFP and Des-gamma carboxy prothrombin. Conclusively, serum hTERT level quantification along with other tumor markers may be used to improve cancer detection and progression. Further studies are recommended to augment the prognostic implications of serum hTERT mRNA in different cancer types.

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