Quantitative Evaluation of Telomerase Activity in the diagnosis of Urinary Bladder Cancer utilizing the Telomeric Repeat Amplification Protocol (TRAP) assay

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Abstract

Early diagnosis is one of the most determining factors for patient survival. The detection of telomerase activity is a potentially promising tool in the diagnosis of bladder and other types of cancer due to the high expression of this enzyme in tumor cells. We carried out an in vitro quantitative evaluation of telomerase activity in blood serum and biopsied tissue samples as a marker capable of identifying urinary bladder tumor in patients. Telomerase activity was quantified by photometric immunoassay, utilizing the Telomeric Repeat Amplification Protocol (TRAP) assay in blood samples from 21 healthy volunteers and in blood and biopsied tumor samples from 34 previously untreated urinary bladder cancer patients and expressed activity in µgm Protein/assay. Telomerase activity values in blood and tissue samples were found to be significantly higher in patients with urinary bladder cancer in comparison to healthy donors. Similar variation was also observed with the grade and stage of the disease. The sensitivity of the test is 56% with 100% specificity.

Keywords: telomerase activity, Urinary bladder tumor

Introduction

Early diagnosis of bladder cancer is one of the most determining factors for patient survival; the frequency of recurrence and tumor progression depends to a great extent on tumor grade and stage at time of diagnosis [1, 2]. Cystoscopy is the standard procedure routinely used for the detection of bladder cancer. However, it represents an invasive, uncomfortable, and expensive procedure and cannot be used for screening programs. Cytology is a cheaper, noninvasive procedure routinely used for diagnosis, but it has only a 50% sensitivity and many tumors, mainly of low grade, may be missed [3].Therefore, noninvasive methods for bladder cancer diagnosis are warranted.

In recent years, a great deal of information has been accumulated on the molecular alterations that take place during the development of bladder tumors, such as gene mutations or genomic rearrangements. Studies on p53 and ras gene mutations or CD44 variant have highlighted the possibility to detect tumor alterations in exfoliated urine cells [4–6]. Moreover, Mao et al. [7], Linn et al. [8], and Steiner et al. [9] have detected microsatellite alterations in urine samples, and new methodological approaches have recently been developed [10].However, individual tumors harbor specific mutations and the overall analysis of more than one gene mutation or microsatellite loci is needed to reach a high sensitivity.

A potentially promising tool for the diagnosis of bladder cancer is the detection of telomerase activity. The telomerase enzyme is a ribonucleoprotein reverse transcriptase that synthesizes the telomeric repeats located at the

ends of chromosomes [11, 12]. The majority of somatic cells do not have telomerase activity, and thus these repetitive sequences decrease with subsequent cell divisions due to the incomplete replication of linear DNA molecules. The progressive shortening of telomeres finally reaches a critical stage, probably correlated with cell senescence and death. The enzyme activity is assumed to favor telomere length maintenance and, as a consequence, to play an important part in cell immortalization and also tumor progression [13, 14]. Telomerase reactivation has, in fact, been observed in immortalized cell lines and in many tumor histotypes [15].The widespread association of telomerase activity with tumor cells has induced researchers to investigate and define the role of the enzymatic activity present in tumor tissue or biologic fluids as a diagnostic or prognostic marker [16,17]. In the present study, we have determined telomerase activity in blood samples as well as histologically verified biopsied tissue specimens from patients with cancer and healthy donors by using a quantitative evaluation, utilizing the Telomeric Repeat Amplification Protocol (TRAP) assay.

Materials and Methods

The present study has been carried out on 55 patients undergoing treatment in the Department of Urology, University Hospital, Institute of Medical Sciences, Banaras Hindu University. In a case control study, 34 cases of urinary bladder carcinoma and 21 cases of not having bladder carcinoma 'as control' were inducted in the study. Tissue and blood samples were obtained from both the patients and controls with informed consent. Diagnosis of tumor was histologically confirmed (WHO score).

Telomerase assay

Tissue samples and serum were suspended in 200 µl of ice-cold TRAP lysis buffer (Tris-HCl pH 7.5 10 mM, MgCl2 1 mM, EGTA 1 mM, phenyl methylsulfonyl fluoride 0.1 mM, β-mercaptoethanol 5 mM, 3-[(3-cholamidopropyl) dimethylamino]-1-propanesulfonate (CHAPS) 0.5% and glycerol 10%) and processed in the Department of Biophysics, Institute of Medical Sciences, Banaras Hindu University as per the supplier's protocol obtained from Roche Applied Science, Germany (Catalog no. 12 013 789 001) employing Telo TAGGG Telomerase PCR Elisa Plus kit. Protein concentrations of sample were measured with protein assay kit. Telomerase activity was expressed in µgm Protein/assay by observing the absorbance at 450 nm using a reference wavelength at approx.690 nm.

Statistical analysis

Data were expressed as the mean values ± SD. The t test for paired data was used to test the significance between the means of two groups. $P<0.05$ was considered as statistically significant.

Results

Telomerase activity has been determined in tumor tissue of 34 patients as well as in blood samples from 21 healthy donors employing Photometric Enzyme immunoassay for quantitative determination of telomerase activity, utilizing the Telomeric Repeat Amplification Protocol (TRAP) assay. The characteristics of the study group are summarized in table 1. Telomerase levels in the blood serum were found to have a mean value of 12.41 $\pm$ 4.99 µgm Protein/assay in healthy donors and 41.54$\pm$ 2.97 µgm Protein/assay in patients with cancer. And, in the tissue samples the mean value was found to be 14.85$\pm$ 5.86 µgm Protein/assay in healthy donors and 46.07$\pm$ 4.62 µgm Protein/assay in patients with cancer (Table 2). There was a highly significant positive correlation between serum telomerase activity and tissue telomerase activity. Telomerase activity values in blood and tissue samples were found to be significantly higher in patients with urinary bladder cancer in comparison to healthy donors. Similar variation was also observed with the grade and stage of the disease (table 3).

Sensitivity and specificity of telomerase activity data both in serum as well as in tissue specimens depicted telomerase activity was present in 34 cases with positive in 19 and negative in 15 cases. Telomerase activity on the other hand was absent in 21 cases with negativity in 15 cases. The sensitivity of the test is 56% with 100%

specificity (Table 4, 5). By using score discriminate function test cut –off score for serum telomerase was found to be 31.8 µgm Protein/assay and above for the patients.

Table 1: Characteristics of the Study Group

	Patients	**Control**
Number	34	21
Age	57.5 ± 8.7	56.0 ± 0.9
Male	25	15
Female	09	06
Grade		
I	10	
II	16	
III	08	
Stage Distribution		
Superficial	21	
Invasive	13	

Table 2: Telomerase Activity levels (pg/assay) in study subjects

Group	Serum (Mean ± SD)	't'	p	Tissue (Mean ± SD)	't'	p	Correlation co-efficient (r) (between serum and tissue telomerase)	**p**
Control	12.41 ± 4.99	5.71	<0.001	14.85 ± 5.86	5.69	<0.001	r=0.9926	<0.001
Patients	41.54± 2.97			46.07± 4.62			r= 0.9915	<0.001

Table3: Grade and stage of Tumor in relation to serum and tissue telomerase activity

Grade of patients	Serum (Mean ± SD)	Tissue (Mean ± SD)
I (n=10)	15.34 ± 4.80	17.79 ± 5.85
II (n=16)	46.99 ± 16.25	51.87 ± 17.01

III (n=08)	63.36 ± 17.32	69.80 ± 18.17
Stage		
Superficial (n= 21)	29.65±18.14	33.54± 19.53
Invasive(n= 13)	60.74± 16.01	66.3±17.69

Table 4: Sensitivity and specificity of Serum Telomerase

Disease	Positive	Negative	Total
Present	19	15	34
Absent	-	21	21
Total	19	36	

Sensitivity= 56% Specificity= 100%

Table 5: Sensitivity and specificity of Tissue Telomerase

Disease	Positive	Negative	Total
Present	19	15	34
Absent	-	21	21
Total	19	36	

Sensitivity= 56% Specificity= 100%

Discussion

In the present study the presence of telomerase activity was observed in blood samples as well as in histologically verified biopsied tissue specimens from patients with cancer and healthy donors by using a quantitative evaluation, utilizing the Telomeric Repeat Amplification Protocol (TRAP) assay. The quantitative determination and statistical analysis we used enabled us to identify and characterize the analysis by different sensitivity and specificity values.

Telomeres are protective DNA—protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere shortness in human beings is emerging as a prognostic marker of disease risk, progression, and premature mortality in many types of cancer, including breast, prostate, colorectal, bladder, head and neck, lung, and renal cell. The role of the telomeric repeat amplification protocol (TRAP) in evaluating the function of telomerase in telomere maintenance, cell proliferation, tumour development, and cell immortalization has been quintessential. Since TRAP introduction in 1994 [18], this sensitive PCR-based assay has been widely used for telomerase activity screening in human and other organisms.

Telomerase compensates the loss of telomeric repeats, through synthesizing new chromosomal telomeres along with every cycle of DNA replication (19). Absence of telomerase activity and the resultant progressive shortening of the telomeres characterize the process of cellular senescence in vivo and in vitro. On the other hand, the acquiring of immortal phenotype for tumor cells is associated with the reactivation of telomerase expression, leading to telomere elongation, enabling unlimited cell division. It is estimated that approximately 66-97% of assayed human tumor samples have shown telomerase activity, including cancers of the lung (20), colon (21), stomach (22), liver (23),

breast (24), prostate (25), brain (26), and endometrium (27). In the present study, we found the telomerase activity in all bladder cancer samples. This may indicate that telomerase assay may be more sensitive than cytology for fluid specimens containing only small numbers of malignant cells.

In conclusion, the quantitative evaluation method is a noninvasive approach, highly reproducible, does not miss low-grade tumors, and, more importantly, if performed after cytologic evaluation, succeeds in unmasking the presence of tumors in cytologically negative cases.

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