Research Article [Araştırma Makalesi]

Urinary Telomerase Activity, C-erbB2, Malondialdehyde and Nitric Oxide as Possible Biological Markers of Bladder Cancer

[Mesane Kanserinde Biyolojik Belirteç Olasılıkları; İdrarda Telomeraz Aktivitesi, C-erbB2, Malondialdehit ve Nitrik Oksit]

[1]Saad M. El-Gendy,

[1]Abdel Salam.I.M.,

[2]Nahed Abd El-Wahab.,

[3]Hussein.M.K.,

[4]Gabry.M.S.,

[1]El-Aaser.A.A.

[1]Cancer Biology, [2]Clinical Pathology, [3]Medical Oncology Departments, National Cancer Institute, Cairo University. [4]Zoology Department, Faculty of Science Helwan University.

Yazışma Adresi
 [Correspondence Address]

Saad M. El- Gendy

Asis. Prof. Medical Biochem Cancer Biology dept., NCF, Cairo, Egypt.
Fax: +20-02-3644-720;
E-mail: saadelgendy2004@yahoo.com

ABSTRACT

The aim of the present study is to evaluate the possible diagnostic role of telomerase activity, c-erbB2, malondialdehyde and nitric oxide levels in the urine of bladder cancer patients. Eighty urine samples were taken from 3 groups of individuals; 1) Ten healthy age matched control subjects, 2) Twenty schistosoma haematobium infected patients and 3) Fifty preoperative bladder cancer patients. Fresh urine samples (50mL) were collected and subjected to the assay of telomerase activity in urine sediment. Urine telomerase activity was increased in 72 % of bladder cancer cases. Bilharzial nonmalignant group showed normal telomerase activity as compared to normal healthy controls. Its increase in bladder cancer patients with bilharzial infection was statistically insignificant compared to non bilharzial cancer group. Urine telomerase activity of bladder cancer patients were increased in an ascending manner with tumor grades. Malondialdehyde level increased in bladder cancer patients with bilharzial infestation rather than those without bilharziasis, but the difference was statistically insignificant. C-erbB2 expression increased in 27 % of bladder cancer patients; while no single case of the bilharzial group showed positive c-erbB2 expression. Regarding the stage of tumor, nitric oxide level in bladder cancer patients showed statistically significant difference between stage I, II and stage III (p=0.04). Statistically significant positive correlation between telomerase and c-erbB2 in bladder (r=0.456 & p=0.005) has been observed. The study of telomerase activity in the urine sediment of bladder cancer cases may be a useful marker for early detection of the disease.

Key Words: Bladder cancer, Bilharziasis, Urinary telomerase activity, C-erbB2, Malondialdehyde, Nitric oxide.

ÖZET

Bu çalışmanın amacı mesane kanser olgularının idrarında telomeraz aktivitesi, c-erbB2, malondialdehit ve nitrik oksit düzeylerinin diyagnostik katkısını değerlendirmektir. Üç grup hastadan seksen idrar örneği toplanmıştır; 1) on sağlıklı yaş uyumlu kontrol olgusu 2) yirmi schistosoma haematobium enfeksiyonlu hasta 3) elli ameliyat öncesi mesane kanserli hasta örneği. Her olgudan 50 ml taze idrar örneği toplanmış ve idrar çökeltisinde telomeraz aktivitesi ölçülmüştür. Mesane kanser olgularının % 72'sinde telomeraz aktivitesi yükselmiştir. Malinitesi bulunmayan bilharzial grubunun idrar telomeraz aktivitesi normal kontrollerden farksızdır. Bilharzial enfeksiyonu bulunan ve mesane kanseri geliştirmiş olgularda gözlenen aktivite, enfeksiyonu bulunmayan mesane kanserli olgulardan istatiksel olarak bir farklılık göstermemektedir. Mesane kanserli olgularda idrar telomeraz aktivite düzeyi tümör evreleri ile artış göstermektedir. Mesane kanseri ve bilharzial enfeksiyonu bulunan olgularda malondialdehit düzeyi bilharziasis bulunmayan olgulara göre yüksekse de bu farklılık istatistiksel olarak anlamlı değildir. Mesane kanser hastalarının % 27'sinde c-erbB2 ekspresyonu yükselmiş olmasına karşın bilharzial enfeksiyonlu hastaların hiç biri c-erbB2 ekspresyonu göstermemektedir. Mesane kanserli olgularda nitrik oksit düzeyi tümör evreleri arasında I, II ve III (p=0.04) istatistiksel olarak anlamlı farklılıklar göstermiştir. Telomeraz aktivitesi ve c-erbB2 arasında mesane kanserli hastalarda istatistiksel olarak anlamlı pozitif korelasyon (r=0.456 ve p=0.005) gözlenmiştir. Mesane kanser vakkalarının idrar sedimentinde telomeraz aktivitesinin ölçümü hastalığın erken teşhisinde faydalı olabilir.

Anahtar Sözcükler: Mesane kanseri, Bilharziasis (Shistozoma hematobium), İdrarda telomeraz aktivitesi, c-erbB2, Malondialdehit, Nitrik oksit

INTRODUCTION

Bladder neoplasm incidences are particularly high in the Nile river valley secondary to bilharzial infestation which is frequently associated with the development of squamous cell carcinoma (SCC). At the National Cancer Institute (NCI), Cairo, Egypt, bladder cancer constitutes 30 % of all malignancies (1).

Cystoscopic and cytologic examinations are the golden standard screening methods for detection and follow up of bladder carcinoma but they have some limitations especially in the low grade tumors (2).

A number of studies have focused on the evaluation of urinary markers that may hold promise as non-invasive methods of the detection with higher sensitivity and specificity for follow-up (3). Urinary sediment telomerase activity showed higher sensitivity in bilharzial bladder cancer than non bilharzial type, which indicates its clinical value in screening patients with urinary bilharziasis (4).

Telomerase is a ribonucleoprotein enzyme that can reconstitute the ends of chromosomes after cell division and thus circumvent the damage that occurs in normal adult somatic cells during successive mitotic cycles and compensates for the progressive erosion of telomeres (5). Its RNA protein complex can enzymatically extend telomere length (6). It is thought to be active in the transformation of normal somatic cells into immortal human tumor cells, and it is found predominantly in malignant cells and its detection provides new cancer diagnostic and therapeutic possibilities (7).

The c-erbB2 gene is activated by a point mutation which results in the change of amino acid residue 664 from valine to glutamic acid, and this change is associated with its ability to transform cells. Alteration and amplification of this gene have been reported in carcinoma of bladder and other human cancers (8).

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic (9,10).

Nitrite plays a major role in the process of bladder cancer development in schistosoma haematobium infected patients due to its possible role in the formation of potent carcinogen namely, nitrosamines (3,11). The aim of the present work is to determine the possible diagnostic role of urinary sediment telomerase activity and c-erbB2, MDA and nitric oxide (NO) levels in the urine of bladder cancer patients.

MATERIAL and METHODS

Eighty urine samples were taken from three groups; 1) Control group, which included 10 healthy age matched subjects, 2) Bilharzial group, which included 20 schistosoma heamatobium infected patients (cancer free), 3) Pre-operative bladder cancer group where urine samples were collected from 50 patients before bladder cystectomy, these cases were divided into 2 sub-groups according to the presence or absence of bilharzial infestation: 31 had history of bilharziasis and 19 were bilharzial free.

Urine samples (50 ml) were collected and each was divided into 4 aliquots, one of them was used for nitrite assay, the other three samples were centrifuged, then the pellet was washed with PBS and one aliquot of each sample was lysated for telomerase assay, and subjected to protein extraction and assay, then stored at -80°C till analysis. The other 2 aliquots were snapped frozen in liquid nitrogen and were stored at -80°C till use.

Telomerase activity in the urine sediment (TAU) was measured by PCR-ELISA technique using the telomerase repeat amplification protocol (TRAP) according to the Kit purchased from Boehringer Mannheim GmbH, D-68305 Mannheim.

Determination of lipid peroxides level (Malondialdehyde) is based on the spectrophotometric determination of thiobarbituric acid reactive substances (TBARS) which is based on the reaction of one molecule of malondialdehyde (MDA) with 2 molecules of thiobarbituric acid (TBA) at low pH (3.5) and temperature of 95°C for 60 min. The resulting pink colour product is extracted by a mixture of n-butanol and pyridine (15:1) and the absorbance was determined spectrophotometrically at 535 nm (12).

The measurement of nitrite is based on, in acid medium and the presence of nitrite the formed nitrous acid diazotize sulphanilamide and the product is coupled with N- (1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish- purple color which can be measured at 540nm (11).

The c-erbB2 activity was measured by the ELISA technique according to the kit purchased from Oncogene Research Product Co. (13).

Statistical Methods

SPSS package (version 10.0) was used for data analysis.

Qualitative data were expressed as frequency and percentage. T student test and chi- square test were used for comparative analysis. p-value is always two tailed and significant at 0.05 levels (14).

RESULTS

Age and sex distribution of patients and controls are shown in table 1. The clinicopathological characters of bladder cancer patients are shown in table 2.

Comparison of the mean levels of urinary telomerase, malondialdehyde, c-erbB2 and NO in the groups under investigation are shown in table (3). Telomerase activity in urine sediment (TAU) is increased in 72 % of bladder cancer cases, this increase was statistically significant compared to the control (p=0.002). The enzyme activity was higher in bladder cancer patient

Table 1. Age and sex distribution of patients and controls

Group	Number	S e x (M/F)	Age (years) Mean + SD
Control	10	7/3	45.18 ± 1.84
Bilharzial	20	20/0	51.7 ± 1.7
Bladder cancer patients	50	40/10	56.3 ± 2.44

Table 2. Clinicopathological features of bladder cancer patients

Patient characters	Bladder cancer patients Number (%)
Sex	
Male	40 (80)
Female	10 (20)
Age	
Median	56.3
Pathologic subtype	
Squamous Cell Carcinoma (SCC)	28 (56)
Transitional Cell Carcinoma (TCC)	22 (44)
Pathological grade	
I	4 (8)
II	35 (70)
III	11 (22)
Stage	
T1	2 (4)
T2	18 (36)
T3	30 (60)
L.N status	
Positive	34 (68)
Negative	16 (32)
Bilharzial Status	
Positive	31 (62)
Negative	19 (38)

associated with bilharzial infestation compared to those without bilharziasis, the difference was statistically insignificant, and meanwhile the activity was normal in bilharzial non malignant group compared to the normal healthy controls.

- Malondialdehyde was increased in 63 % of bladder cancer group, and 50 % of bilharzial non malignant group (p=0.002). c-erbB2 was increased in 27 % of bladder cancer patients while no significant difference was shown in the bilharzial non malignant group (p=0.07).

- NO level was increased in 100 % of bilharzial non malignant group and 90 % of bladder cancer group compared to control. The difference between the 3 groups was statistically significant.

As regards the relation between the studied parameters and the clinicopathological features of bladder cancer patients, there were no statistically significant differences regarding age, sex and lymph node status and pathological subtypes.

In bladder cancer patients, the level of telomerase increase is correlated with the tumor grade increase i.e. were highest in grade III (163 ± 51), followed by grade II (66.64 ± 9.37) and, lowest in grade I (62.5 ± 16.7) (p = 0.01). NO level was higher in grade III (9.07 ± 1.40), than grade II (4.33 ± 1.03) and that of grade I (2.65 ± 0.7) (p=0.08).

However, c-erbB2 and malondialdehyde showed no correlation with pathological grades (table 4).

As regards stage of the tumor, only NO level in bladder cancer patients showed statistical significant increase in different stages, being higher in stage 3 (6.5 ± 1.6) compared to stage 2 (3.25 ± 0.69) and stage 1 (3.1 ± 0.3), (p=0.04) (table 4). NO level was higher in bilharzial cancer patients compared to non-bilharzial cases (p=0.04).

A statistical significant positive correlation was noticed between telomerase and c-erbB2 in bladder cancer patients r = 0.456, p <0.005.

DISCUSSION

Bladder cancer is a common malignancy in Egypt and other developing countries in which infection with Schistosoma haematobium is prevalent. Bladder cancer caused by bilharziasis has different clinical and biological characters than that observed in the Western World (15). Carcinoma of bilharzial urinary bladder is the most common malignant tumor registered in the NCI Cairo (16).

Although cystoscopy remains the golden standard for diagnosis and follow up of bladder malignancies,

Table 3. Mean levels of urinary telomerase, malondiadehyde c-erbB2 and NO in different groups.

Group	Telomerase unit/mg protein	Malondi-aldehyde (μmol/mL)	c-erbB2 (ng/dL)	NO (μmol/mL)
Control	27.60±2.45 (a)	270±18.62 (a)	9±0.25 (a)	0.5±0.05 (a)
Bilharziral	21.5±1.65 (a)	404.5±18.36 (b)	8.2±0.33 (a)	21.6±2.29 (b)
Bladder cancer	74.27±10.08 (b)	552.27±68.19 (C)	24.59±7.3 (b)	4.3±0.7 (c)
P	0.002	0.002	0.07	0.001

*Groups with different letters are statistically significant.

Table 4. The mean levels of telomerase, c-erbB2, malondialdehyde and NO in different pathological grades and stages.

	Telomerase (unit/mg protein)	c-erbB2 ng/dl	Malondialdehyde (µmol/mL)	NO (µmol/L)
Grade				
I	62.5 ± 16.7 (a)	28.1 ± 5.1	340 ± 0.0	2.65 ± 0.7
II	66.64 ± 9.37 (a)	25 ± 16.23	561.4 ± 93.9	4.33 ± 1.03
III	163 ± 51 (b)	68.5 ± 61.5	601.6 ± 120.5	9.07 ± 1.49
P	0.01	0.15	0.6	0.08
Stage				
I	76.1 ± 7.3	21 ± 15.3	522 ± 79.1 (a)	3.1 ± 0.3 (a)
II	82.4 ± 24.8	25.5 ± 17.4	544.2 ± 109.7	3.25 ± 0.60 (a)
III	70.4 ± 9.8	24.1 ± 7.5	556 ± 88.4	6.5 ± 1.6 (b)
P	0.59	0.93	0.91	0.04

*Groups with different letters are statistically significant.

this invasive procedure is uncomfortable for patients, particularly in their follow-up (17). On the other hand, urine cytology can provide a useful tool only in high grade tumors and is particularly affected by subjective interpretation (2). For these reasons, there is a high necessity for a non-invasive test for early detection of bladder cancer before muscle invasion and monitoring tumor recurrence or new onset.

The role of telomerase in the diagnosis of bladder cancer has been widely reviewed, its potential relevance for diagnosis is derived from 2 main findings: telomerase has been detected in 90 % of bladder cancer samples in addition the sensitivity of telomeric repeat amplification protocol enables the detection of this enzyme in sediments collected from voided urine or in bladder (17,18).

In this study, the level of telomerase activity was found to be high in the urine sediment of 72 % of the preoperative bladder cancer patients (p <0.001). Previous studies, reported that the positive rate of telomerase activity of bladder cancer was 79.3 % (19). The non-malignant bilharzial infected group showed low telomerase activity compared to controls. Comparing telomerase level in bladder cancer patients with different clinicopathological features, there were no statistically significant difference with age, sex, LN status or tumor stage, this is in agreement with previous studies (5,2). However, we found that, the higher the tumor grade the higher the telomerase activity level. Therefore telomerase activity in urine sediment (TAU) is of important value in the diagnosis of bladder carcinoma and it may correlate with differentiation degree of carcinoma. This is in agreement with other researchers, who reported that, the detection of telomerase mRNA expression in urine was highly sensitive marker for the diagnosis of transitional cell carcinoma (TCC) of the bladder. However other researchers didn't find any correlation between the telomerase activity and tumor grades (17,20).

Telomerase activity was higher in bladder cancer patients with bilharziasis than those without infection but the differences didn't reach the statistical significant level, and this is also in agreement with previous reports (15).

In the present work no statistically significance in TAU level was found between squamous cell carcinoma (SCC) and TCC bladder cancer patients, which is not in accordance with the other findings (5).

In this study, c-erbB2 was found to increase in 27 % of bladder cancer patients which is in accordance with a recent study (21). However c-erbB2 was increased in grade III compared with its level in grade II and I; this increase was statistically insignificant, which is in agreement with previous studies (21,22). Some investigators have shown a relationship with high grade and stage of the tumors that over expressed c-erbB2 (8,23).

In this study, the level of NO was increased in bilharzial non malignant cases and in cancer bladder patients as compared to controls (p=0.001). However, others reporting that there was no significant difference in serum nitrite + nitrate levels among bladder cancer patients and control subjects [24]. We found an insignificant increase in grade II and III as compared with grade I (p=0.08). There was a significant increase in stage III as compared to stage II and I (p=0.04). These results are in agreement with previous reports (25).

In accordance with the previous reports (26), we found that the urinary levels of malondialdehyde were significantly increased in bladder cancer patients when compared to controls and bilharzial non malignant cases (p = 0.002). This increase was more obvious in bilharzial cancer patients than in the bilharzial free cancer cases. There was an increase in malondialdehyde in grade II and III as compared to grade I; also in stage II and III as compared to stage I, but, the differences were statistically insignificant. As malondialdehyde plays a

mutagenic and carcinogenic role, it is expected to be elevated in bladder cancer group (10).

Conclusion: The study of telomerase activity in the urine sediment of bladder cancer cases may be used as an indicator for early detection of the disease. For malondialdehyde, c-erbB2 and NO further study is needed to evaluate their role in early detection and/or follow up of bladder cancer patients.

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