

AN EARLY MARKER OF MONOCLONAL LIGHT CHAIN PROTEIN IN BLADDER CANCER

By

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سلسلة بروتينية من النوع الخفيف وحيدة المنشأ واستخدامها كدليل للكشف المبكر عن سرطان المثانة

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تمثل البلهارسيا في مصر ومضاعفات الإصابة بها سببا للإصابة بمرض سرطان المثانة . وعادة ما يصيب الفلاحين الذين يعانون من التهابات المثانة المزمنة والمتكررة الناتجة عن الإصابة بمرض البلهارسيا . والتأخر في تشخيص مرض سرطان المثانة غالبا ما يكون نتيجة تشابه أعراض الإصابة بالبلهارسيا مع أعراض مرض سرطان المثانة . وفي السنوات الأخيرة أصبح هناك إهتمام لدراسة هذا المرض والتحكم فيه على المستوى الطبي والإقتصادي . وقد وجد أنه من الممكن تحسين نتائج العلاج لهذا السرطان الذي يصيب المثانة ، إذا أمكن تشخيصه ومعالجته في مراحله الأولى ولذلك مازال هناك جهداً كبيراً يبذل لإيجاد طرق تشخيص ومتابعة هذا المرض في مراحله . وتتناول الدراسة حالياً دراسات على مستوى تغير البروتينات الكلية وأجزائها الرئيسية وبعض مكوناتها الدقيقة وكذلك أنواع السلاسل البروتينية النوعية الخفيفة في كل من مصل وبول مرضى سرطان المثانة الناتج عن مضاعفات مرض البلهارسيا بالإضافة إلى خمسة عشر حالة من الأشخاص الأصحاء البالغين جميعهم في نفس مستوى معدل العمر والجنس والحالة الإجتماعية والإقتصادية وذلك لاستخدام نتائجها كمرجع قياسي لمقارنة نتائج هذه الحالات السابقة . وعند تقييم نتائج دراسة التقدير الكمي للبروتينات الكلية وأجزائها الرئيسية ومكوناتها النوعية من الجلوبيولينات المناعية ذات الوزن الجزيئي الخفيف (L) أو الثقيل (H) في بول مرضى سرطان المثانة وجد إرتفاع ملحوظ في بعض الجلوبيولينات المناعية الفردية وبالأخص نوع الـ IgG في عينات بول مرضى سرطان المثانة ذات المراحل المتقدمة للورم عنها في المراحل المبكرة مما يفسر عملية ظهور مثل هذه الاجسام المناعية في البول هي في الغالب ضد نواتج الأنتيجينات لخلايا الأورام تفرز مع البول والتي تعمل كالأجسام غريبة في الجسم . وكما أمكن اكتشاف وجود سلاسل بروتينية من النوع الخفيف (L) من نوع كابا (K) أو لداي (λ) في عينات بول مرضى سرطان المثانة يمكن إستخدامها كدليل أو مؤشر للتعرف على المرض في مرحلة المبكرة وذلك بإستخدام طريقة التقدير الكمي للروكت الكترولفوريس (Rocket immunoelectrophoresis) .

Key Words: Bladder carcinoma, Detection, An immunoprotein marker

ABSTRACT

Bladder cancer complicating schistosomiasis is a major cause of cancer mortality in Egypt. It affects farmers who suffer from repeated and severe chronic schistosomal cystitis. The late presentation of this disease is probably due to the overlapping and similar symptoms of simple bilharzial cystitis and bladder carcinoma. The present work deals with study of the variation of low molecular weight proteins, immunoprotein-patterns present in both serum and urine in different groups of bladder cancer patients in an attempt for predicting and screening the early and late stages of bladder carcinoma. The increased urinary excretion of high molecular weight proteins, the immunoglobulins (IgA, IgM, IgG) especially IgG and low molecular weight proteins such as light chain protein of kappa (κ) as well as B-2-microglobulin demonstrated in our patients of bladder carcinomas in the present study may be caused by the admixture of proteins to urine from tumour surface. The increased level of IgG type kappa in urine could also be related to the high degree of plasma cell infiltration usually associated with bladder tumours, or it may be due to alteration in the genetic material present in the chromosomes of the nucleic acid components of cells which is the basis for their transformation into neoplasms. On conclusion, our results demonstrated that detection of monoclonal light chain proteins can be used for diagnosis and predicting the early or late stages of the disease and its different pathological types.

INTRODUCTION

Carcinoma of the bladder is the most common malignant disease in Egypt. It also occurs in high frequency in some parts of Africa and the Middle East.

In Egypt, despite all measures and efforts directed towards the control of bilharziasis and the improvement of socio-economic standard in general, there is no evidence indicating a significant decrease in the incidence of bladder cancer[1].

The only certain means of bladder cancer detection is by urine cytology, cystoscopy and biopsy of the lesion which are invasive and expensive procedures. An easily applied screening test for detecting bladder cancer in early stages should significantly increase curability and patient survival.

The immunogenic nature of bladder cancer has been demonstrated by several investigations[2,3]. Production of antibodies as one of the immune response believed to be activated in patients with transitional cell carcinoma of the bladder[4]. Dearnely et al.[5] reported that the antigen is present in most primary and secondary carcinoma and can be used in differential diagnosis of tumours and the detection of micrometastases.

In recent studies, certain tumour associated antigen or neoantigen have been shown to be present on cell surface of a variety of tumours[6].

Our present work is an attempt to find out immunoprotein marker in patients with bladder cancer that can be used for diagnosis and differentiation between early and late stages of the disease.

MATERIAL AND METHODS

Material of study consisted of:

- a) Fifteen normal adult subjects with no evidence of malignancy or past history of bilharzial affection.
- b) Fifty six patients of both sex of bilharzial bladder cancer.

All samples were taken from in patient wards of the National Cancer Institute. The age of normal subjects and patients ranged between 26 to 58 years with a mean of 48 years old.

All the bilharzial bladder cancer patients were diagnosed clinically and followed by histopathological examination of

a biopsy taken postoperative[1]. All the patients included in this study were divided into three groups according to the histopathological classification as described by the World Health Organization[7].

Group I Included 26 cases of squamous cell carcinoma were classified into three grades as follows:

- a) Grade I: Verrucous squamous carcinoma, (10 cases).
- b) Grade II: Verrucous and invasive carcinoma, (10 cases).
- c) Grade III: Invasive squamous carcinoma, (6 cases).

Group II Included 25 cases of transitional cell carcinoma.

The cases were also classified into three grades as follows:

- (a) Grade I: Papillary non-invasive carcinoma, (10 cases).
- (b) Grade II: Papillary and invasive carcinoma, (10 cases).
- (c) Grade III: Invasive transitional carcinoma, (5 cases).

Group III Included 5 cases of adenocarcinoma. They were of grade II and of tubular type.

In this study, the following investigations were performed:

1. Full history and clinical examination.
2. Urine analysis.
3. Complete blood picture.
4. Renal profile.
5. Urinary total protein[8] and its electrophoretic fractions[9].
6. Urinary immunoglobulins i. e. (IgA, IgM and IgG) were detected and determined[10,11].
7. Urinary light chain proteins of both types, kappa(κ) and lambda(λ), also were determined[10,11].
8. Urinary B₂-microglobulin by Elisa procedure[12].

Twenty four urine samples were collected without the use of any preservatives to avoid interference with the nature and properties of the antigens present. Then, urine samples were centrifuged at 3000 r.p.m. for 15 minutes to get rid of the sediments and dialyzed overnight against physiological saline solution at 4°C, then concentrated 10 times, by using visking dialysis tubing and polyethylene glycol.

RESULTS

The mean value of urinary total protein in normal subjects was 286 ± 60 mg/gm creatinine in squamous cell carcinoma, 10735 ± 1396 mg/gm creatinine in transitional cell carcinoma and 17065 ± 2061 mg/gm creatinine in adenocarcinoma as shown in Table 1. Also it shows the

mean values for the individual protein fractions of alpha-1, alpha-2, beta- and gamma globulins in urine of normal subjects and the three different groups of bladder cancer patients.

(kappa and lambda) in urine of normal healthy subjects and the three groups of bladder cancer patients. Also, it shows the mean values of urinary B₂-microglobulin, light chain proteins (kappa lambda ratio) in the three different pathological types of bladder cancer and their related grades.

Table 2, shows the quantitative determination of IgA, IgM and IgG immunoglobulins and light chain proteins

Table 1

Urinary total proteins and their electrophoretic fractions in different groups of bladder cancer patients as compared to normal subjects.

	Total Protein	Albumin	α_1 -Glob.	α_2 -Glob.	β -Glob.	γ -Glob.	Total Glob.
Control							
M \pm SE	286 \pm 52	282 \pm 52	0.00	0.00	0.00	0.00	0.00
Group I							
M \pm SE	6298 \pm 600	3596 \pm 340	333 \pm 77	171 \pm 66	408 \pm 138	1748 \pm 147	2660 \pm 345
t	9.21	8.47	4.34	2.58	2.95	11.92	7.86
P ⁰ <	<0.001**	<0.001**	<0.001**	<0.01	<0.005**	<0.001**	<0.001**
Group II							
M \pm SE	10735 \pm 1396	5739 \pm 729	1198 \pm 221	255 \pm 139	763 \pm 177	2780 \pm 369	4996 \pm 733
t	7.21	6.99	5.42	1.84	4.32	7.53	6.82
P ⁰ <	<0.001**	<0.001**	<0.001**	<0.05*	<0.001**	<0.001**	<0.001**
t	2.22	2.00	2.90	0.40	1.28	1.95	1.82
P ¹ <	<0.025*	<0.05*	<0.005**	>0.05	>0.05	<0.05*	<0.05
Group III							
M \pm SE							
t	17065 \pm 2061	8842 \pm 909	1588 \pm 624	925 \pm 450	1018 \pm 330	4655 \pm 721	8186 \pm 1192
P ⁰ <	7.94	8.91	2.54	2.06	3.08	6.45	6.98
t	<0.001**	<0.001**	<0.025*	<0.05*	<0.005**	<0.001**	<0.001**
P ¹ <	4.04	4.20	1.79	1.46	1.40	3.32	3.21
t	<0.001**	<0.001**	<0.05*	>0.05	>0.05	<0.005**	<0.005**
P ¹¹ <	1.83	1.89	0.46	1.14	0.50	1.72	1.67
	<0.05*	<0.05*	<0.05*	>0.05	>0.05	<0.05*	>0.05

P⁰ = Comparison of the groups with control
 P¹ = Comparison between group I and II
 P¹¹ = Comparison between group II and III

Group I: Squamous cell carcinoma
 Group II: Transitional cell carcinoma
 Group III: Adenocarcinoma

ns: Non-Significant (P>0.05)
 * : Significant (P<0.05)
 **: Highly significant (P<0.005)

Table 2Urinary immunoglobulins, Beta-2-microglobulin and light chain proteins (κ and λ) in different groups of bladder cancer patients as compared to normal subjects.

	IgA	IgM mg/gm Creatinine	IgG	B ₂ M	Kappa	Lambda Unit/gm Creatinine	κ/λ
Control							
M \pm SE	0.00	0.00	0.00	0.06 \pm 0.009	0.00	0.00	0.00
Group I							
M \pm SE	446 \pm 43	81 \pm 13	1150 \pm 68	2.31 \pm 0.33	39 \pm 3.53	21 \pm 3	2.35 \pm 0.21
t	10.13	6.07	16.71	6.00	11.05	7.65	11.09
P ⁰ <	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
Group II							
M \pm SE	499 \pm 86	118 \pm 30	1735 \pm 220	3.65 \pm 0.72	57 \pm 8.52	27 \pm 3.24	2.30 \pm 0.28
t	5.66	3.83	7.72	4.92	6.63	7.94	8.25
P ⁰ <	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
t	0.41	0.84	1.99	0.94	1.48	0.98	0.08
P ¹ <	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
Group III							
M \pm SE							
t	696 \pm 192	417 \pm 186	3615 \pm 383	8.47 \pm 1.47	103 \pm 14	72 \pm 15	1.73 \pm 0.38
P ⁰ <	3.63	2.25	9.44	5.67	7.43	4.88	4.58
t	<0.001**	<0.025	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
P ¹ <	1.06	1.69	5.46	3.35	3.68	2.91	1.03
t	>0.05	>0.05	<0.001**	<0.005**	<0.001**	<0.005**	>0.05
P ¹¹ <	0.70	1.38	3.09	2.20	2.05	2.48	0.86
	>0.05	>0.05	<0.005	<0.025*	<0.025*	<0.01*	>0.05

DISCUSSION

The aim of the present work is to study the variation of low molecular weight proteins, immunoprotein-patterns present in urine of different groups of bladder cancer patients in an attempt for predicting and differentiating between the early and late stages of bladder carcinoma.

The observation in urinary excretion of high and low molecular weight protein components especially in group II (grade III) and group III of our patients with bladder carcinoma may be caused by the admixture of proteins to urine from the tumour surface or by glomerular lesion due to deposition of tumour derived antigen-antibody complexes.

Despite the demonstration of a significant relationship between the clinical tumour staging and the urinary protein excretion, Peterson et al[13] indicated that the predominant part of protein components in urine arises from a process of glomerular filtration followed by tubular dysfunction.

In agreement with our results, El-Aaser et al.[14] found increased levels of urinary IgA and IgG in Egyptian patients with urinary bladder cancer. In our results IgG level showed a gradual increasing levels with progression from low grade of carcinoma to high grades among the three studied groups of bladder cancer patients. Johansson and Kistner[15] demonstrated that the level of urinary immunoglobulins in bladder cancer patients were related to the tumour size and not to the malignancy grade.

Since, immunoglobulins are synthesized in lymphoid tissues, lymphocytes and plasma cells, it would appear that, the synthesis of light chains and heavy chains is closely balanced on the separate polyribosomes. In malignant cells, control of the synthesis of the polypeptide chains is frequently disturbed, one chain may be produced in excess or to the exclusion of the others i.e. there is excessive formation of light chains frequently excreted in urine[16]. In diseases of bladder cancer, it is yet not clear whether the secretion of light chain proteins in urine is produced by the tumour cells or by simultaneously proliferating plasma cells[17].

Since cytological examination unfortunately has limited value in the prediction of malignancy, the detection of monoclonal light chains gives strong evidence of malignancy[18].

In normal subjects of the present study, both kappa and lambda light chain proteins have been never detected in urine. In comparison to the normal subjects, a highly significant increased amount of urinary light chain protein (kappa) has been demonstrated in the three groups of bladder cancer patients, while in group (III) of patients with adenocarcinoma, the amount of the light chain proteins were highly significant increased as compared to group (I) of squamous cell carcinoma and significantly increased as compared to group (II) of transitional cell carcinoma.

In addition, the increased urinary excretion of light chain protein of IgG type kappa in patients with residual or recurrent advanced grades of tumours had been also observed[19]. Moreover, Berggard and Peterson[20] suggested that patients associated with tubular proteinuria excrete increased quantities of free light immunoglobulin chains. Kyle[21] claimed that, the detection and quantitation of monoclonal light chain proteins in urine proved to possess an important diagnostic and prognostic values. The light chain type has been also appeared to affect the response to treatment, since the kappa type has a better prognosis than lambda type[22].

The use of β_2 -microglobulin as a marker has been studied previously in patients with urological malignancies[23]. The amount of β_2 -microglobulin in the urine of the present bladder cancer patients showed a highly significant increased value in the three groups of bladder cancer patients as well as in their related grades as compared to normal subjects. However, in comparison, between the three groups, only group(III) of adenocarcinoma showed a highly significant increased values as compared to group(I) of squamous cell carcinoma and a significantly increased values as compared to group (II) of transitional cell carcinoma.

Although, the values of the urinary β_2 -microglobulin showed increased levels with progression from low grades to high grades of carcinoma, the data showed insignificant values between each other. The increase of low molecular weight proteins such as B₂-microglobulin in urine of patients with uroepithelium bladder tumours was also reported[12]. Decenzo et al[24] suggested that the β_2 -microglobulin content appears to discriminate among patients with tumours of different grades and the absence of

antigen on the original tumour was found to correlate with the development of invasive cancer.

Thus it can be concluded that the detection of monoclonal light chain protein of IgG type kappa and β_2 -microglobulin in urine of patients with bladder cancer can be used for diagnosis and predicting the early and late stages of the disease and its different pathological types.

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