Expression of HLA Class 1 In Iraqi Bladder Cancer Patients.

Kareem Hamed Ghali*

الخلاصة

تضمنت الدراسة 60 مريضا مصابا بسرطان المثانة و80 فردا من الاصحاء للمقارنة وبصورة متطابقة (تقريبا) من حيث الجنس والعمر والقومية . اوضحت الدراسة وجود فروق معنوية في اربعة انتيجينات هي -Cw7,-A2, -B5, -B51 عند المقارنة بين العينتن المرضية والقياسية , حيث اظهر الانتيجين -Cw7 ارتفاعا معنويا في العينة المرضية بينما اظهرت الانتيجينات -B51, -B5,-A2انخفاضا معنويا في عينة المرضى . كما لوحظ وجود علاقات اخرى بين العينتين عند دراسة طور المرض ودرجته.

Abstract

HLA types studied in sixty bladder cancer patients. A control sample of 80 apparently disease free individuals was included for comparisons, matched for ethinical background (Iraqi Arabs), sex and age.

The study demonstrated significant deviations in four antigens, HLA -A2, -B5, -B51 and -Cw7, when comparisons was made between patients and controls. The frequency of antigen Cw7 was increased in the patients, while antigens A2, B5, and B51 showed decreased frequencies. Other associations were also observed when the tumour staging and grading were considered.

Introduction

In Iraq, Bc is the one of the commonest ten cancers in males after bronchogenic cancer, according to the results of Iraqi Cancer Registry (1,2). The total number of Bc cases in Iraq during the last 23 years was 11732 cases. The incidence was markedly increased in recent years (3). Although the reasons for this increase are not fully elucidated, the epidemiological data suggested that environmental as well as genetic factors as major causes in the Bc etiology (3,4).

The successful of cancer prevention strategies is facilitated by the understanding of the etiology of the disease, knowledge of immunity, enzymes, biochemistry, tumour markers and cytogenetic of Bc. In Iraq little is known about these factors.

*University of Wassit, College of Medicine.

Human leukocyte antigen (HLA) are proteins located on the surface of the white blood cells (WBC) and other tissues in the body, they are controlled by genes on the short arm of chromosome 6. The HLA loci are part of the genetic region known as the major histocompatibility complex (MHC)

MHC class I downregulation is an important mechanism of tumour escape from T cell-mediated immune responses. Approximately 40-90% of human tumours derived from various MHC class I tissues were reported to be MHC class I deficient(5)

Materials and Methods

A total of sixty Iraqi patients with Bc attending the Hospital of specialized surgical in Baghdad, from different areas, Four ml of blood were collected in tubes containing glass beads for HLA typing.

Principle

Serological method is the simplest and fastest procedure for HLA testing that is using blood serum which contains antibodies to HLA.

The lymphocytotoxicity test is the most common assay. It is based on the reaction of HLA antisera with membrane antigen on lymphocytes. However this test has a complement dependent reaction. Lymphocytes are used in this procedure.

Preparation of plates

Terasaki plates contain 60 wells are used for this purpose. One μl of HLA antisera is placed in each well, then the plates is covered and stored in freezer for requistem.

Procedure

1-One μl of cells (2000-3000 cells) is added to each well of a tissue typing plate, which contains antisera, then incubated at room temp. (20-25C°) for 30 min.

2- Five μl of rabbit complement are dispensed in each well and incubated for 60 min. at room temp.

3-One μl of (5% w/v) eosin dye solution is added to each well and allowed for about 5 min., then the reading is performed under a phase contrast

Results

As demonstrated in tables (1,2,3) a decrease of HLA antigens was observed in the expression of HLA antigen class I,(A and B) which involved HLA-A2, B5, B51 in total patients when compared with controls (20% vs. 42.5; 13.3% vs. 36.3%; 8.3%vs. 25% respectively).These differences were significant (for HLA-A_2 P< 0.01, RR= 0.34, PF= 0.28, X^2y= 6.881; for HLA-B5 P< 0.01, RR=0.27, PF=0.26, X^2y = 8.583; for HLA-B51 P< 0.05, RR = 0.27, PF = 0.18 X^2y = 5.406).

While the HLA-Cw7 showed a significant increase in the expression in patients when compared with controls (P< 0.01, RR= 3.5, EF=0.24, X^2y = 7.644). Moreover, some antigens of class I such as A28 and Cw4, showed frequent decrease in some patients as compared with controls, but by using chi-square with Yates no significant differences were obtained.

HLA Antigens in Different Stages and Grades of Bc.

The total BC patients (60) comprising 46 with low grade (I-II) and 14 of high grade (III-IV) (Tables 4,5,6,7), significant differences were observed when compared between two stages, or grades, or with total controls. Low grade show a significant decrease in HLA-B5 when compared with controls (p< 0.01 X^2y = 8.304) (Table5), while it showed significant increase in HLA-Cw7 vs. controls (P< 0.01, X^2y = 10.492) (Table6), but no significant differences were observed when compared between high grade vs. low grade and controls.

Concerning stages of the disease, superficial cases showed a significant increase of HLA-Cw7 vs. controls (P< 0.01, X^2y = 7.643). While HLA-A2 antigen showed a significant decrease in invasive stage vs. controls (P< 0.05, X^2y = 4.437) (Table 4), but no significant differences were observed between superficial and invasive stages(Table 7).

Table-1:Observed numbers and percentage frequencies of HLA- class 1 (A) antigens in bladder cancer patients and controls.

Antigens	Patients (60)		Controls (80)	
	No	%	No	%
1	14	23.3	19	23.8
2	12	20	34	42.5
3	12	20	14	17.5
9 (23+24)	18	30	22	27.5
10 (25+26+34+66)	6	10	4	5
11	2	3.3	6	7.5
19	2	3.3	6	7.5
23	2	3.3	8	10
24	12	20	12	15.0
25	0	0	0	0
26	3	5	2	2.5
28 (68+69)	7	11.7	17	21.3
29 (19)	2	3.3	6	7.5
30	1	1.6	1	1.3
34	0	0	1	1.3
66	0	0	0	0
68	0	0	0	0
69	1	1.6	0	0

Table-2:Observed numbers and percentage frequencies of HLA- class 1 (B) antigens in bladder cancer patients and controls.

Antigens	Patients (60)		Controls (80)	
	No	%	No	%
4	18	30	26	32.5
5 (51+52)+53	8	13.3	29	36.3
6	32	53.3	23	28.8
7	4	6.6	8	10
8	6	10	2	2.5
12 (44+45)	7	11.6	10	12.5
13	5	8.3	5	6.3
14	0	0	2	2.5
15 (62+63)	3	5	1	1.3
16 (38+39)	2	3.3	7	8.8
17	1	1.7	4	5
18	3	5	6	7.5
21 (49+50)	12	20	16	20
22 (54+55+56)+42	3	5	0	0
27	1	1.7	1	1.3
35	6	10	17	21.3
37	1	1.7	0	0
38	1	1.7	7	8.8
39	0	0	0	0
40 (60+61)	3	5	0	0
41	1	1.7	2	2.5
42	0	0	0	0
44	7	11.6	8	10
45	0	0	2	2.5
49	4	6.7	5	6.3
50	4	6.7	9	11.3
51	5	8.3	20	25
52	0	0	1	1.3
53	2	3.3	6	7.5
55	1	1.7	0	0
60	3	5	0	0
62	1	1.7	0	0
65	0	0	0	0
73	2	3.3	1	1.3

Table-3:Observed numbers and percentage frequencies of HLA- class 1 (C) antigens in bladder cancer patients and controls.

Antigens	Patients (60)		Controls (80)	
	No	%	No	%
1	2	3.3	2	2.5
2	0	0	0	0
3	3	5	2	2.5
4	7	11.6	20	25
5	0	0	2	2.5
6	3	5	3	3.8
7	20	33.3	10	12.5
8	0	0	2	2.5

Table-4: Distribution of HLA- class 1 (A) antigens in bladder cancer patients according to grading and staging.

Antigens	Grading				Staging			
	Low grade (I-II) N= 46		High grade (III-IV) N= 14		Superficial N=25		Invasive N=35	
	No.	%	No.	%	No.	%	No.	%
1	11	23.9	3	21.4	7	28	7	20
2	11	23.9	1	7.1	5	20	7	20
3	9	19.7	3	21.4	5	20	7	20
9	12	26.0	6	42.9	8	32	10	28.6
10	5	10.7	1	7.1	3	12	3	8.6
11	2	4.3	0	0	2	8	0	0
19	1	2.2	1	7.1	0	0	2	5.7
23	1	2.2	1	7.1	1	4	1	2.9
24	9	19.7	3	21.4	6	24	6	17.1
25	0	0	0	0	0	0	0	0
26	1	2.2	2	14.2	1	4	2	5.7
28	4	8.7	3	21.4	0	0	7	20
29	1	2.2	1	7.1	0	0	2	5.7
30	1	2.2	0	0	0	0	1	2.9
34	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0
69	1	2.2	0	0	0	0	0	2.9

Table-5: **Distribution of HLA- class 1 (B) antigens in bladder cancer patients according to grading and staging.**

Antigens	Grading				Staging			
	Low grade (I-II) N= 46		High grade (III-IV) N= 14		Superficial N=25		Invasive N=35	
	No.	%	No.	%	No.	%	No.	%
42	0	0	0	0	0	0	0	0
44	7	15.2	0	0	6	24	1	2.9
45	0	0	0	0	0	0	0	0
49	3	6.5	1	7.1	1	4	3	8.6
50	2	4.3	2	14.3	2	8	2	5.7
51	3	6.5	2	14.3	0	0	5	14.3
52	0	0	0	0	0	0	0	0
53	1	2.2	1	7.1	0	0	2	5.7
55	1	2.2	0	0	0	0	1	2.9
60	3	6.5	0	0	2	8	1	2.9
62	1	2.2	0	0	1	4	0	0
65	0	0	0	0	0	0	0	0
73	2	4.3	0	0	0	0	2	5.7

Table-6: **Distribution of HLA- class 1 (C) antigens in bladder cancer patients according to grading and staging.**

Antigens	Grading				Staging			
	Low grade (I-II) N= 46		High grade (III-IV) N= 14		Superficial N=25		Invasive N=35	
	No.	%	No.	%	No.	%	No.	%
1	1	2.2	1	7.1	0	0	2	5.7
2	0	0	0	0	0	0	0	0
3	3	6.5	0	0	2	8	1	2.9
4	7	15.2	0	0	4	16	3	8.6
5	0	0	0	0	0	0	0	0
6	0	0	3	21.4	0	0	3	8.6
7	18	39.1	2	14.3	10	40	10	28.6
8	0	0	0	0	0	0	0	0

Table -7: Associations of HLA antigens showing in bladder cancer patients (total and subgroup).

Type compartion	Antigen	RR	EF	PF	X^2y	P≤
Total patients						
Vs. controls	HLA 2	0.34	—	0.28	6.881	0.01
	5	0.27	—	0.26	8.583	0.01
	51	0.27	—	0.18	5.406	0.05
	7	3.5	0.24	—	7.644	0.01
Low grade	5				8.304	0.01
Vs. controls	7				10.492	0.01
Superficial Vs. controls	-7				7.643	0.01
Invasive Vs. controls	2				4.437	0.05

RR: Relative risk
EF: Etiological fraction
PF: Preventive Fraction
X^2y: Chi square with Yates
P: propility
Vs.: versus

Discussion

HLA- class I antigens are highly polymorphic cell surface glycoproteins. The importance of these molecules resides on the fact that cytotxic T-lymphocytes (CTLs) can only kill cells expressing a foreign antigen if these cells express MHC class 1 antigen. HLA-antigens in the present study involved detection of HLA-class 1 (A, B, C) in 60 patients and 80 healthy controls (Tables 1,2,3).The patients were divided according to stages and grades as shown in (tables 4,5,6). There were highly significant decrease in HLA-A2, B5 and B51 in the patients compared with healthy controls (P<0.01; P<0.01; P<0.05, respectively). The expression of HLA-class I antigens in Bc has been investigated by several authors (6,7, 8). It is postulated that tumour cells which have lost or have reduced class 1 antigen have an advantage to escape this cytotoxic mechanism (9).

Similar observations were noticed by Nouri *et al*., (10) and Amirghofran *et al*. (11). These observations provide the background of HLA antigen therapy in Bc patients who are candidates for salvage cystectomy due to localized recurrences resistant to conventional treatment. However only HLA-Cw7 among other HLA-class I molecules, showed significant increase ($P<0.01$) in patients compared with healthy controls. Moreover, when stages and grades are considered the results indicate that HLA-Cw7 antigens was relatively well preserved in low grade cases, while it had reduced B5 in low grades. In comparison, according to stage, there was a significant increase in HLA-Cw7 superficial cases, but invasive tumours show markedly reduced HLA-A2 antigen. Cordon Cardo *et al*., (12) observed an association between the decrease in HLA- class I expression and advanced tumour stage. Similar observations were noticed by Tomlinson and Bodmer (13) and Bicknell *et al*., (14). On the other hand, Nouri *et al*. (10) found an association between HLA - class I antigens loss and the occurrence of grade III tumours.

It is important to emphasize in these findings that no HLA allels are associated with disease in 100% of cases. Hence, the effect of MHC proteins is likely to be one of the several genetic and environmental factors, that determine susceptibility to disease (15). In general, this study suggests that there are two groups of HLA-class I antigens in iraq Bc cases. The first group works as a protective antigens like HLA-A2, B5 and B51. The second group works as susceptibilitive antigen to disease such as HLA-Cw7.

References

1. **Iraqi Cancer Registry (ICR) Results: Ministry of Health, 1976-1985. Central Public Health Laboratory, Baghdad, Iraq, 1987.**
2. **Iraqi Cancer Registry (ICR) Results: Ministry of Health, 1995-1997. Iraqi Cancer Board, Baghdad, Iraq, 1999.**
3. **Ad'haih, A.H.; Ghali, K.H. and El-Hassani, M.B.: An epidemiological approach to bladder cancer in Iraq from 1976 to 1998. Al- Mustansiriyah J-Sci. 2000; 11 (3): 25-30.**
4. **Marouf, B.A.: Cancer induction due to environmental contamination with DU in Iraq. 14 th Iraqi cancer conference, saddam medical city, Baghdad- Iraq. 2000; p 9.**
5. **Bubeník J :Tumour MHC class I downregulation and immunotherapy .Oncol Rep. 2003 Nov-Dec;10(6):2005-8. Irn. J-Med. Sci. 1997; 22 (3 and 4): 145.**

6. **Oliver, R.T.D.; Nouri, A.M.E.; Crosby, D. and et al.,: Biological significance of beta hCG, HLA and other membrane antigen expression on bladder tumours and their relationship to tumour infiltrating lymphocytes (TIL). J. Immunogenetics. 1989; 16 : 381-90 (Cited by Oliver and Coptcoat, 1998).**
7. **Nouri, A.; Smith, M. et al.,: Selective and non selective loss of immunoregulatory molecules (HLA-A, B, C antigens and LFA-3) in transitional cell carcinoma. Br. J. Cancer. 1990; 62: 603-606.**
8. **Nouri, A.M.; Hussain, R.F. et al., Induction of MHC antigens by tumour cell lines in respones to interferons. Eur. J. cancer. 1992; 28 (A): 1110-1115.**
9. **Schmidt, W. and Fesen, S. H.: Resistance to cell- mediated cytotoxicity is correlated with reduction of H2K gene products in AKR Leukemia. Immunojenetic .1982; 16: 257-264**
10. **Nouri, A.M.E.; Oliver, R.T.D. and Nargund, Y.H.: Clonal development of bladder cancer and it's relevance to the clinical potential of HLA antigen and TP 53 based gene therapy. Cancer Surveys Volume 31: Bladder Cancer, 1998.**
11. **Amirghofran, Z.; Khezri, A.A. and Mohammadi, S.: Expression of HLA- class I and II, ICAM-I and CD_{44} antigens in T.C.C. of bladder.**
12. **Cordon- Cardo, C.; Fuks, Z. et al: Expression of HLA-A, B,C antigens on primary and metstatic tumour cell populations of human carcinomas. Cancer Res.1991; 51: 6372-6380.**
13. **Tomliuson, I.P. and Bodmer, W.F.: The HLA system and the analysis of multifactorial genetic disease. Trends in Genetics. 1995; 11: 493-498 (Cited by Oliver and Coptcoat, 1998)**
14. **Bicknell, D.; Kaklamanis, L.; Hampson, R.; Bodmer, W. and Karran, P.: Selection for beta (2)- microglobulin mutilation in mismatch repair- defective colorectal carcinoma. Current Bio. 1996; 6: 1695-97.**
15. **Brodsky, F.M.: "Antigen presentation and the major histocompatibilitycomplex in Stites, DP.; Terr, AL. And Parslow, TG., (ed): medical Immunology, 4 th ed., prentice- Hall International Inc. U.S., 1997; pp. 83-94.**