Psychosocial Stress, and Cellular Immune Response in Iraqi Bladder cancer patients

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Abstract

_The study was designed to show any association between psychological stress and cellular immune response by determined the expression of CD56 NK and CD3 T cell marker in bladder cancer patients . Fifty paraffin-embedded tissue blocks of bladder tissue from a Al-yarmouk teaching hospital in Baghdad were selected, and assessed by using immunohistochemistry for detection the expressions of CD56 and CD3 . Ten benign and 40 malignant bladder tumors were includes in this study , monoclonal antibodies CD56 and CD3 were used to determine the expressions of them by using immunohistochemical staining . CD56 was high expressed in 70% of benign specimens and in only 47.5 % of malignant types without significant differences , while CD3 was highly significant expressed in 80% of benign specimens and in only 42.5 % of malignant types ($P \leq 0.05$) . Also, the results showed a low cellular immune response for both markers (CD56 and CD3) in BC patients suffering from negative psychological stress (47.5,34.3, respectively) .

Keywords: CD56 NK marker, CD3-TCR, bladder tumors

الضغوطات النفسية الاجتماعية والاستجابة المناعية الخلوية في مرضى سرطان المثانة العراقيين

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الخلاصة

صممت الدراسة الحالية لدراسة أي ارتباط بين الضغوطات النفسية والاستجابة المناعية الخلوية عن طريق تحديد التعبير المناعي الكيمونسيجي لمعلم الخلايا الطبيعية القاتلة CD56 ومعلم الخلايا التائية الفعالة CD3 في مرضى سرطان المثانة البولية. تضمنت الدراسة 50 عينة نسيج مثانة مطمورة في شمع البرافين من مستشفى اليرموك التعليمي في بغداد . 10 عينة نسيج لاورام المثانة الحميدة و 40 عينة نسيج من سرطان المثانة البولية. وقد وجدت الدراسة أن مستوى التعبير المناعي الكيمونسجي الموجب لمعلم CD56 كان مرتفع نسبيا في مرضى أورام المثانة البولية الحميدة 70% مقارنة مع مرضى سرطان المثانة البولي 47.5% مع عدم وجود فرق معنوي ,بينما مستوى التعبير المناعي الكيمونسجي الموجب لمعلم CD3 كان مرتفع نسبيا مع فرق معنوي احصائي في مرضى أورام المثانة البولية الحميدة 80% مقارنة مع مرضى سرطان المثانة البولي 42.5% . كما اظهرت الدراسة استجابة مناعية منخفضة لكلا المعلمين في مرضى سرطان المثانة الذين يعانون من ضغوطات نفسية سلبية .

Introduction

Urothelial carcinoma is the predominant histological type in the United States and Western Europe, where it accounts for approximately 90 percent of bladder cancers In the United States, almost 75,000 new cases and 16,000 deaths occur each year due to bladder cancer (Siegel ***et al.***,2014)

Among cancer patients, psychosocial factors are known to be associated with the properties of natural killer (NK) cells and T cells, both of which are important for tumor control (Shibuya *et al.*,2002) . NK cells are thought to play a significant role in inhibition and surveillance of tumor metastases (Cerwenka & Lanier , 2001) . NK cells recognize and kill target cells in the absence of prior sensitization and are able to defend the host from infection or prevent the progression of a disease. Human NK cells express CD56 or Neural cell adhesion molecule (NCAM) which are (massively) being used as a major hallmark for the NK cell . CD56 is the prototypic marker of NK cells (80-90%), but also present on subsets of CD4+ and CD8+ T cells (Gharehbaghian *et al.*,2006; Zecchini & Cavallaro,2010) .

Cytotoxic T cells are known to be one of the major effectors cells in tumor immunity following detection of tumor-specific antigen and T cell clone response to autologous tumor cells in vitro (van der Bruggen *et al.*,1991) .

CD3 is a multimeric protein complex, known historically as the T3 complex, and it is serves as a T cell co-receptor that associates non-covalently with the T cell receptor (TCR) (Smith-Garvin *et al.* , 2009) , the CD3 protein complex is an important T cell marker for the classification of malignant lymphomas and leukemias (T cell neoplasms). It can also be used for the identification of T cells in coeliac disease (Leon , 2011). Impaired or reduced CD3 zeta chain (CD3-ζ) expression in T cells has been identified in various cancers and may be associated with an ineffective immune response. This phenomenon is observed in various types of cancer and is regarded as a causative factor in immune suppressive conditions in cancer patients . CD3 impairment has been detected not only in peripheral blood lymphocytes (PBL), but also in tumor-infiltrating lymphocytes (TIL) in colorectal cancer (Deakin *et al.*,1999).

Stress can be caused both by daily responsibilities and routine events specially wars and terrorism in Iraq , as well as by more unusual events, such as a trauma or

illness in oneself or a close family member . When people feel that they are unable to manage or control changes caused by cancer , they are in distress. Distress has become increasingly recognized as a factor that can reduce the quality of life of cancer patients . The tumor microenvironment effects by stress and psychological processes, the response of these stress results in activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis (Figure:1) . Factors released from these pathways can have direct effects on the tumor microenvironment, resulting in a favorable environment for tumor growth and progression. These dynamics can also adversely affect patient quality of life. (Lutgendorf *et al.*,2010)

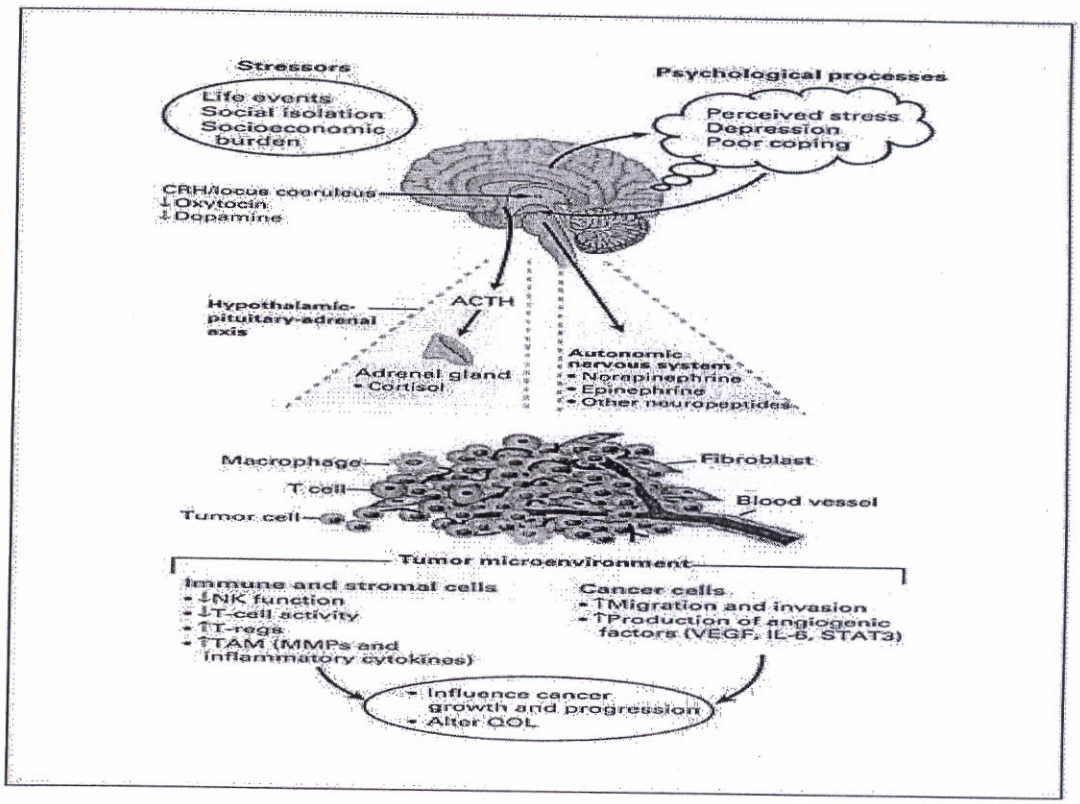

Figure (1): Host Factors and Cancer Progression: Biobehavioral Signaling Pathways and Interventions (Lutgendorf *et al.*,2010) .

So the aim of this study is to explore the role psychological stress in IHC expression of CD56 NK cells marker and CD3T cell marker for Iraqi patients with urinary bladder tumors.

Subjects , Materials and Methods

Forty paraffin-embedded tissue blocks from bladder carcinoma (BC) with different grades, 34 male and 6 female with an age ranged from 29 to 81 years, and ten benign bladder biopsies from benign bladder tumor (BT), 7 males and 3 females with an age range from 33-65 years used as control group were collected from Al-yarmook Teaching Hospital in Baghdad during the period from March to October 2014. Clinical data of the patients are presented in (Table- 1) . Patients were assessed for psychiatric disorders by direct interview . Psychological distress, past history of depression, several types of concerns, antidepressive medications and social support factors were investigated as psychosocial factors . The diagnosis of the tissue blocks were based on obtained histopathological reports. A consultant pathologist re evaluated all obtained tissue blocks to further confirm . One section was mounted on ordinary slide and stained with hematoxyline and eosin ,while another mounted on positive charged slide to be used for immunohistochemically (IHC) for detection NK cell marker CD56 and the T cell marker CD3 . Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from Novocastra, Newcastle, UK, RE7150-K , the slides were deparaffinized in xyline and rehydrated in a graded alcohols . Antigen retrieval was carried out using microwave at 600 Watt (2× 5 minutes) and then cooled down at room temperature for 20 min mouse anti-human-CD56, and anti-CD3 monoclonal antibodies were obtained from (Dako,Denemark) , IHC staining of individual marker was performed according to the manufacture's instructions . Finally, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin. Slides washed , dehydrated then mounting, and examining using Olympus microscope (Olympus ,Tokyo , Japan) at different magnification.

Table (1):Key Characteristics of The Bladder Cancer Patients

Variable	Number or value	Percentage%
Patients		
Male	34	85
Female	6	15
Age (years)		
Range	(29–81)	—
Mean	62.9	—
Median	65.5	—
Standard deviation	13.27	—
Histological type*		
TCC	39	97.5
AC	0	0
SCC	1	2.5
Grade		
Low grade	23	57.5
High grade	17	42.5
Stage		
Stage 1	24	60.0
Stage 2	14	35.0
Stage 3	2	5.0

* TCC: transitional cell carcinoma; AC: adenocarcinoma; SCC: squamous cell carcinoma.

Results and Discussion

Psychosocial stress has been related to impaired immunity in cancer patients. However, the extent to which these relationships exist in immune cells in the tumor microenvironment in humans has not been explored. We examined relationships among distress, social support, and natural killer and T cell activity (CD56, CD3 markers, respectively) in bladder cancer patients for tumor-infiltrating lymphocytes (TIL) .

Only five(12.5%) patients out of 40 with UBC had a good Psychological and social support while, the others 35 (88.5%) patients had Psychological distress .

Distress expressed in such emotions as depression or excessive anxiety have been identified in patients with advanced cancer (Jacobsen & Lesko, 1990) . Depression is experienced frequently by patients with bladder cancer, bladder cancer patients with shorter telomeres and high levels of depression symptoms have a threefold increased risk for mortality.

Also, the results of this study showed all women in this study had negative psychosocial stress. These results agreed with some investigators have suggested several possible risk factors for psychological distress in cancer patients, ie, female gender (Hagedoorn *et al.*,2008).

Women were more likely than men to focus on negative emotional aspects of stressful circumstances (Ge & Conger, 2003) . Hormonal regulation largely affects the rate of depression in women , also a woman's role as a wife, worker, mother, and caretaker contribute to the levels of everyday stress .

Immunochemistry: Both CD56 and CD3 were detected in the bladder tissue samples , the positive rate of CD56 expression was 47.5% (19/40) in bladder cancer patients and 70% (7/10) in benign bladder tumor . The results indicated low expression of CD56 NK marker in BC patients when compare to BT patients without significant value ($p > 0.05$) (Table :2) , also both strong (low- grade) and weak (high- grade) immunostaining intensity was demonstrated (Figure: 2). Our findings are consistent with previous work in ovarian cancer patients documenting impairments in NK cytotoxicity in ovarian cancer patients compared with patients with benign neoplasms (Lutgendorf *et al.*,2005), also the results agreed with Tezel *et al.* (2001) who suggested that the expression of NCAM(CD56) in tubular adenocarcinoma of the pancreas, particularly in moderately differentiated tumors, has a significant impact on the overall patient survival .

The positive rate of CD3 expression was 42.5% (17/40) in bladder cancer patients and 80% (8/10) in benign bladder tumor . The results indicated also low expression of CD3 T cell marker in BC patients when compare to BT patients with significant value ($p \leq 0.05$)(Table : 3) , also both strong (in low- grade) and weak (high -grade) immunostaining intensity was demonstrated (Figure : 3) . The results agreed with Ishigami *et al.*(2002) who suggested that reduced CD3 expression in TILs was strongly correlated with progressive disease in gastric carcinomas . Another study

showed down-regulation of CD3zeta-chain expression in T cells as a biomarker of prognosis in cancer (Whiteside , 2004) .

Cancer cells directly or indirectly affect CD3 expression in T cells and may interfere in their normal response to autologous tumor. Specifically, macrophages induced by cancer cells were considered to decrease or alter the function of CD3 in peripheral or TILs (Otsuji *et al.*,1996) . CD3- abnormalities were found in both intratumoral lymphocytes and peripheral blood lymphocytes in patients with various types of malignancies . These findings suggest that the reduced response of T cells to tumor growth provided a favorable environment for tumor extension.

Current evidence does not support the idea that stress causes cancer. Nevertheless, some animal studies suggest that over stress had negative effects on immune function and contributed to tumor growth. So we studied the relation between stress and cellular immune response marker in patients with bladder cancer.

Table (2): CD56 NK Cell Expression in Bladder Patients Groups

Study groups		CD56 expression		Total	Chi-square	P value
		positive	negative			
BC*	No	19	21	40	1.6226	0.20273NS
	Percentage%	47.5	52.5	100		
BT**	No	7	3	10		
	Percentage%	70	30	100		
Total	No	26	24	50		
	Percentage%	52	48	100		

BC* bladder cancer ,BT** benign bladder tumor, NS : non-significant

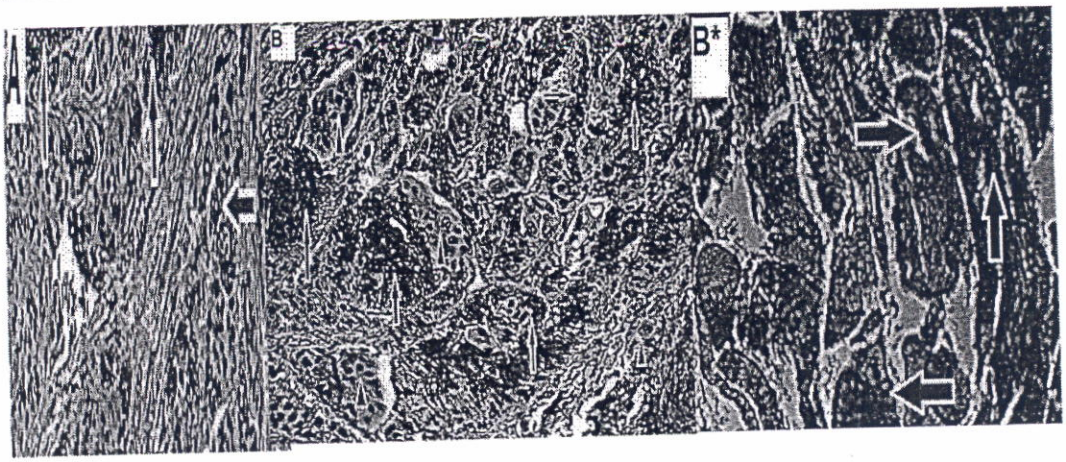

Figure (2:A,B,B*): A: Immunohistochemical Tissue Expression of CD56 in BC Patient Showing Reduce Expression of CD56 NK Marker in BC Patient with Stress Psychosocial Compare to Strong Positive Expression of CD56 in BC Patient have Support in B and Positive Expression of CD56 in BT patient B* . (brown)(arrow) (20X).

The results showed a bad cellular immune response for both markers (CD56 and CD3) in BC patients suffering from negative psychological stress Tables(4,5) indicated reduce expression of these cellular immune marker in BC patients with stress .The results agrees Lutgendorf with *et al.* (2005) who found that patients with greater social support had higher levels of NK cell activity both in PBMC and TIL, whereas patients with greater distress had more impaired NK cell activity in TIL .A multivariate model indicated that both social support and distress were independently associated with NK cell activity in TIL .

Stress might have an effect on our risk of disease by making us more likely to take part in unhealthy behaviors , such as smoking and drinking . So it is not possible to say how much smoking has played a part in bladder cancer. It is true that most bladder cancers are linked to smoking

Conclusion: This study has revealed the negative psychosocial factors, such as distress, are associated with reduction in the cellular immune response (reduce expression of CD56 and CD3 markers) in BC patients . These association may be relevant for bladder cancer control.

Table (4): The Relation between Stress and CD56 NK Marker Expression

Groups	CD56+ expression	
	CD56+	CD56-
Good support(5)	4/5(80 %)	1/5(20 %)
psychosocial Stress(35)	15/35(42.9 %)	20/35(57.1%)
Total	19/40(47.5%)	21/40(52.5%)
Chi-square & P value		2.420 p= 0.119769 NS*

NS: non-significant

Table (5) : The Relation between Stress and CD3 T cell Marker Expression .

Groups	CD3+ expression	
	CD3+	CD3-
Good support(5)	5/5(100 %)	0/5(0 %)
psychosocial Stress(35)	12/35(34.3 %)	23/35(65.7%)
Total	17/40(42.5%)	23/40(57.5%)
Chi-square &P value		7.7311 p=0.005428 **

* * High significant

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