Immunohistochemical Study of Natural Killer Cells in Patients with Bladder Cancer

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Abstract

Bladder cancer is the ninth most common malignancy all over the world. Immune picture is important in predicting clinical outcome with bladder cancer, so the **aim** of the present study was to investigate the number of killer cells in the tissue of the patients with the malignant and benign bladder cancer before treatment by using an antigen retrieval immunohistochemical method on archived bladder tumors tissue. In our study, the number of the patients tissue with malignant cancer that staining by immunohistochemistry of NK cells (20%) was lower than in benign (80%) ($p \leq 0.01$). The results of the present study were indicated that the lower level of the NK cells in the malignant tissues may be leads to impaired anti-tumor immune response toward the tumor cells and is subsequently unable to suppress progression of the tumor.

Keywords: Immunohistochemistry staining, NK cells, Bladder cancer

دراسه مناعيه نسيجيه كيميائيه للخلايا اللمفاويه القاتله في سرطان المثانه

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الخلاصة

يأتي سرطان المثانه بألمرتبه التاسعه من حيث الانتشار عالميا ، حيث ان الصوره المناعيه لمرضى سرطان المثانه هامه في ما يتعلق بالواقع السريري للمرضى وتطوره ولهذا هدفت الدراسه الحاليه الى التحري عن اعداد الخلايا القاتلات الطبيعية في انسجة مرضى سرطان المثانه الخبيث والحميد قبل العلاج. وقد بينت نتائج الدراسه الحاليه 23.3% من الانسجة السرطانيه من النوع الخبيث فقط ايجابيتها الى الصبغه الخاصه بخلايا القاتلات الطبيعيه بينما كانت النسبه الاكبر 80%الى الانسجه من النوع الحميد . استنتج من الدراسه الحاليه ان النسبه المئويه القليله لخلايا القاتلات الطبيعيه في انسجة السرطان من النوع الخبيث تعكس ضعف المناعه الطبيعيه في المرضى وقد تكون السبب في نشوء وتطور سرطان المثانه .

Introduction:

Many studies have shown that the infiltration of lymphocytes significantly correlates with a prolonged survival time of patients, at least in certain types of cancer [1, 2]. Typically, NK cells are not found in large numbers in advanced human neoplasms, indicating that they do not normally efficiently to malignant tissues. For instance, a low prevalence of gastric and colorectal (CRC) tumor-infiltrating CD56 + cells in livers with multiple metastases was detected, Moreover the percentage of intrahepatic NK (CD56 +) cells was also decreased in patients with metastases, compared to those without.

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This suggests that low NK cell number could be a reason for the escape of metastatic cells from the mechanisms of liver immune control [3]. In another study, the CD56 + /CD16 + cell ratio in renal cell carcinoma microenvironment was found generally lower than 1% , suggesting that a predominant number of CD16 + cells were CD56− macrophages, and a low NK cell infiltration [4].

Materials and Methods:

Subjects:

The study included 106 subjects (71 male and 35 female) with an average age of 53 years and a range of (26 to 80 years). Patients under suspicion for bladder cancer who were to undergo cystoscopy over the period of study from May-2012 to May-2013 were eligible for this study. They were diagnosed clinically by consultant urologists. Based on a clinical evaluation and a histopathological examination .The samples were get from Ghazi AL-Harery Hospital / Medicine city / Baghdad

Eighty six patients with 20 healthy subjects were classified into two groups:

Group 1: 86 Urinary bladder carcinoma (UBC) patients (57 males and 29 females with an average age of 53 years and a range of 26 to 80 years).

Group 2: 20 patients with benign urinary bladder diseases (UBD) other than cancer were considered as control group (10 males and 10 females with an average age of 52 years and a range of 32 to 78 years).

Tissue Biopsies:

Tissue biopsies from cases of bladder cancer, and cases of pathological urothelium control were fixed in 10 % buffered formalin and embedded in paraffin wax, and stained with hematoxylin-eosin stain.   Urothelium samples were taken from patients who do not suffer from bladder carcinoma in the past during cystoscopy (without treatment), and they were taken as control.

Preparation of Tissue Sections:

Paraffin embedded sections of bladder cancer tumor tissues were cut into 4 µm thicknesses using a microtome. The sections were applied on Fisher-brand positively charged slides and left overnight to dry at room temperature.

Principle of the assay:

This part of the study was performed at institute of Liver studies in King's College Hospital /London /UK. The immunohistochemical staining techniques is used for visualization of tissues antigens by sequential reaction of a specific antibody (primary antibody) to its corresponding antigen (CD56+) tissue samples; then a secondary antibody was added to the primary antibody and an enzyme complex with a chromogenic substrate, interposed by washing steps. The enzymatic activation of the chromogene results in a visible reaction product at the antigen site. The specimen counterstained and cover slipped, results are interpreted using light microscope [5].The Statistical Analysis System- SAS (2012) was used to shown the effect of difference factors in IHC study parameters. Chi-square test was used to significant compare between percentage this study.

Results and Discussion:

NK (CD56+) infiltrating cells were detected in the bladder of patients with cancer. The pattern of positive staining of NK cells was cytoplasmic. The result of the positive staining of NK cells was confirmed by using control positive staining in tonsils when we applied the technique (avidin-biotin technique). NK(CD56+) cells identified by positive anti-CD56 reaction which is demonstrated at the lower part of the panel Figure-1.The score of the positivity of the NK(CD56) staining cells ranged between 1-3 score. Various of score positivity staining were show in Table-1. The percentage of the benign tissue with immunohistochemistry staining of CD56+ cells was (80%) was higher than that of UBC patients (20%) Table-2.
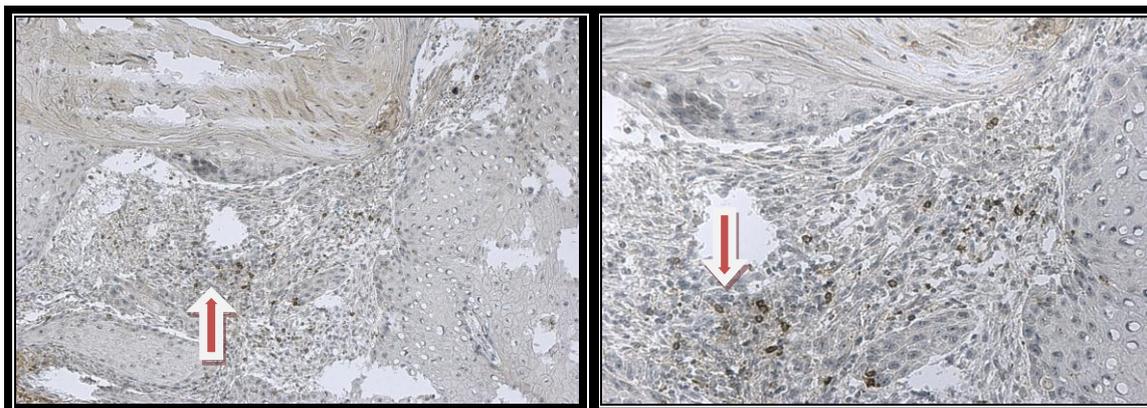
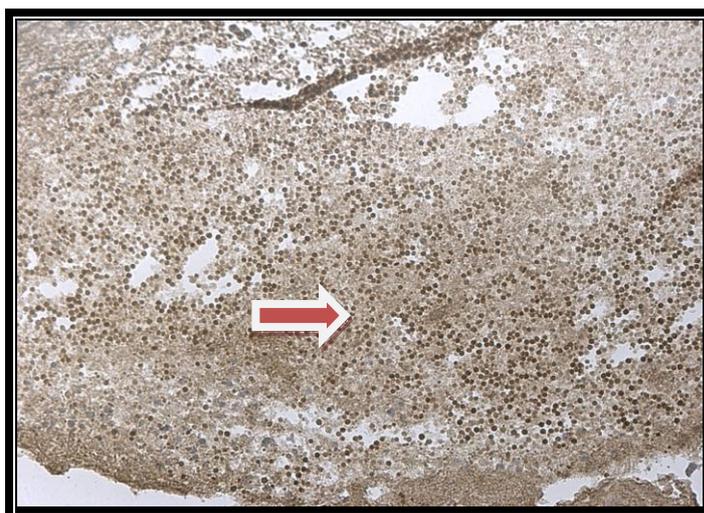**Figure 1- A, B** (Invasive transitional cell carcinoma, showing one scores of CD56+ immunostaining 10X, 20X)

Figure 1- C (Benign, showing two scores of CD56+ immunostaining)

Table 1- Frequency of NK cells (CD56+) IHC scores in bladder patients groups

NK	Malignant		Benin (Control)		Chi-square value- χ^2
	No.	Percentage (%)	No.	Percentage (%)	
*0 (N)	66	76.3	4	20	8.615 **
**1 (#)	15	17.5	13	65	6.937 **
***2 (##)	5	5.8	3	15	4.509 *
****3 (###)	0	0.00	0	0.00	0.00 NS
Chi-square value- χ^2	---	10.261 **	---	13.737 **	---
* (P≤0.05), ** (P≤0.01), NS: Non-significant.					

*0 (n) : Negative: No stained cells
**1 (#): The positive cells (stained with brown color) represented more than 10% of total cells.
***2(##): The positive cells (stained with brown color) represented more than 30% to 50% of total cells.
****3 (###): The positive cells (stained) represented more than 50% of total cells.

Table 2- NK cells (CD56+) production in bladder patients groups

Group	+ve		-ve	
	No.	Percentage (%)	No.	Percentage (%)
Malignant *UBC	20	23.3	66	76.7
Benin (Control)	16	80.00	4	20.00
Chi-square value- χ^2	------	9.157 **	------	------
** (P≤0.01).				

P<0.01 high significance
*UBC: urinary bladder cancer

Results of the present study are in agreement with the other study on 34 patients with gastric or colorectal cancer, they assessed by Immunohistochemical staining for detection CD56+ and they found number of CD56+ cells were decreased in metastatic livers (20.1 cells∕mm2) as compared with those of non metastatic liver(23.3 cells∕mm2), also confirmed their result by used FACS technique and reported decreased CD56+ in patients with metastases compared to those without (10.1± 11.6 vs 16.6 ±8.9%) , and suggest that low NK cell number could be a reason for the escape of metastatic cells from the mechanisms of liver immune control [6]. Several studies have shown that the infiltration of lymphocytes significantly correlates with a prolonged survival time of patients, at least in certain types of cancer [7, 8]. Typically, NK cells are not found in large numbers of advanced human neoplasm, indicating that they do not normally home efficiently to malignant tissues. In other study, the CD56 + /CD16 + cell ratio in renal cell carcinoma microenvironment was found generally lower than 1 , suggesting that a predominant number of CD16 + cells were CD56− macrophages, and a low NK cell infiltration [4]. These results were compatible with another study, which found that both the number and the cytotoxic function of natural killer (NK) cells were decreased in patients with superficial transitional cell carcinoma (TCC), and suggested, elevated concentration of serum TGF-b1 in loss of NK cytotoxicity in superficial TCC patients [9]. Another study on the head and neck tumors demonstrated by using flow cytometric as well as immunohistochemical methods, that the population of circulating immunoregulatory CD56 bright NK cells is lower in the peripheral blood of patients with HNSCC (5 ± 3.2) as compared with healthy donors (7.5 ± 2.5), regardless of the individual tumor stage or tumor type [10] Moreover, a local study on lung cancer in Iraqi patients it was recorded a decreased percentage of NK cells in peripheral blood of patients (6.250 ±0.30204 %) as compared with healthy control subject (10.500±0.84764) by using flow cytometry [11] . Other studies found that the cytolytic potential of NK cells isolated from lung cancer tissues was lower than that of NK cells from peripheral blood or normal lung tissue [12]. However, novel studies of tumor-associated NK cells demonstrated a striking phenotype, supporting the notion that tumor-induced alterations of activating NK cell receptor expression may hamper immune surveillance and promote tumor progression [12]. The results of the present study indicated that the lower percentages of NK cells may by lead to impaired anti-tumor immune response toward the tumor cells and is subsequently unable to suppress progression of the tumor .

References:
1. Naito, Y., Saito, K. and Shiiba, K. **1998**. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer, *Cancer Research*, 58(16), pp:3491–3494.
2. Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., Tosolini, M., Camus, M., Berger, A., and Wind, P. **2006**. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science,* 313, pp: 1960–1964.
3. Estrella, M. L., Mar´ıa, P. R., and Jos´e, M. **2011**. Natural Killer Cells in Human Cancer. *Biomedicine and Biotechnology*, 2011, Article ID 676198, 11 Pages.
4. Schoenborn J.R., and Wilson C.B. **2007**. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv. Immunol.* 96, pp: 41–101.
5. Kumar, G. L. and Rudbeck, L. **2009**. *Education Guide, Immunohistochemical Staining Methods* .Fifth Edition. Dako North America, Carpinteria, California. USA. pp:56-60
6. Gulubova, M., Manolova, I., Kyurkchiev, D., Julianov, A. and Altunkova, I .**2009**. Decrease in intrahepatic CD56+lymphocytes in gastric and colorectal cancer patients with liver metastases. *APMIS*, 117, pp: 870–879.
7. Naito, Y., Saito, K. and Shiiba, K. **1998**. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer, *Cancer Research*, 58(16), pp: 3491–3494.
8. Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., Tosolini, M., Camus, M., Berger, A., and Wind, P. **2006**. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science,* 313, pp: 1960–1964.
9. Chang-Te, L., Min-Tsung Y., Chin, L., Yun-Che, H., Cheng-Huang, S., Dai-Wei, L., De-Ching, C. and Shu-Fen, W. **2010**. Dysfunction of natural killer cells in patients with transitional cell carcinoma. *Cancer Letters*, 291, pp:39–45.
10. Wulff, S., Ralph, P., Kirsten, B., Thomas, T. and Barbara, W. **2009**. Decreased levels of circulating regulatory nk cells in patients with head and neck cancer throughout all tumor stages. *Anticancer Research*, 29, pp: 3053-3058.

11. Ibrahim, A. I., Saleh, D.S. and Majeed, A. **2014**. Reduced Natural Killer Cells Percentage in Patients with Lung Cancer. *Iraqi Journal of Science*. 55(4A), pp: 1496-1500.

12. Carrega, P., Morandi, B., Costa, R., Guido, F., Giuseppe, F., Giuseppe, A., Giovanni, B. R., Maria, C. M., Lorenzo, M. and Guido, F. **2008**. Natural Killer Cells Infiltrating Human Nonsmall-Cell Lung Cancer Are Enriched in CD56 Bright CD16 2 Cells and Display an Impaired Capability to Kill Tumor Cells. *American Cancer Society*, 112(4), pp: 863-875.