CD14 and Bladder Cancer: is there any correlation.

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Abstract

Background: Epithelial cells have evolved a variety of cell-and tissue-specific mechanisms for bacterial detection to enable cells to modulate the inflammatory response depending on the particular situation in a specific organ. These mechanisms provide a means of maintaining a proper balance between defense, tissue injury and their combined effects on organ function, and the moleculeCD14may have a role to play.

Objective: to find any correlation between CD14 marker expression and bladder cancer.

Material and methods: The immunoexpression of CD14 in paraffin sections from 96- bladder cancer tissues and 36-bladder tissues from patients with other bladder disease rather than cancer was investigated using immunohistochemical assay (IHC). The patients were divided into three groups: Group-1: Newly diagnosed bladder cancer patients, 69(43.9%), Group-2:Post-chemotherapy patients, 27(17.2%), Group-3: Other bladder disorders rather than bladder cancer 36(22.9%). The final diagnosis of patients with bladder cancer was established by clinical examination confirmed by cystoscopy and histopathological examination for bladder tissue specimens.

Urinary tract infections were studied for all groups by culturing urine samples using specific culture media.

Results: The results showed that CD14 protein was over expressed in 68.57% of the patients with approximately equal frequent IHC score among patients(23.8%) for each of weak and intense immunoreactions, and (21.0%) for moderate one , and there was no significant difference in the scores of positive IHC CD14 expression in bladder tissue of the cancer patients when compared with non-cancer patients, but there was significant difference between cancerous patients in correlation to the tumor grades.

Positive urine cultures were detected in 28(40.6%) of group-1, 13(48.1%) of group-2, and 12(33.3%) of group-3, while all healthy subjects were free of infection, and a significant difference between bacterial infected patients with and without bladder cancer, in which there was highly CD14 immunoexpression in bladder tissue in Gram-negative bacterial infected patients .

Conclusion: CD14 expression correlated significantly with Gram-negative bacterial infection, but not with cancer.

Key words: Immunohistochemistry, CD14**,** bladder cancer

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Introduction

CD14, is an LPS receptor [(1)], and plays an important role in the signal transduction causing endotoxic shock and is anchored to the monocyte / macrophage cell membrane via glycosylophatidyl inositol [(2, 3)].

Recent reports have described the presence of mCD14 in epithelial cells, endothelial cells, and fibroblasts [(4-6)]. Bladder epithelial cells express CD14 on their surfaces. This fact was evaluated by several investigators. The assessment of CD14 expression was based on using different bladder carcinoma cell lines that were analyzed by flow cytometry using the well-characterized anti-CD14 monoclonal My4 antibodies [(7)]. They improved that

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the bladder carcinoma epithelial cells express mCD14

A soluble form of CD14 is secreted into the urine of superficial bladder cancer patients who receive intravesical BCG. The measurement of soluble urinary CD14 could be of prognostic significance for the response to immunotherapy [8].

In order to limit and control bacterial infections, the host relies on innate immune defense mechanisms, which initially engage epithelial cells of the mucosal surfaces. These cells are the first to encounter luminally localized bacteria and, they have evolved a variety of cell-and tissue-specific mechanisms for bacterial detection [9]. The early recognition of pathogens by cells of the innate immune system is critical to the survival of the host. Attachment of bacteria to epithelial target cells as well as the action of a secreted toxin triggers mucosal chemokine responses in the urinary tract [10]. However, the predominant mechanism prevents bacteria from gaining access to the sensitive upper urinary tract does not require the presence of bacteria per user; it rather recognizes the presence of bacterial LPS [11]. **For the above mentioned data, we intend to investigate, is there any correlation between bladder cancer and CD14 expression.**

Materials and methods

Subjects: patients groups which enrolled Tissue samples from cases of bladder cancer patients as group 1 (96 newly diagnosed cases and 16 cases who had received chemotherapy), and 10 cases of chronic non-specific cystitis, 10 cases of mild non-specific cystitis, 3 cases of chronic bilharizial cystitis, 7 cases of dysplastic urothelium and 6 normal urothelium as group 2 which represent patients with diseases other than cancer and this group represent group 2.

Normal urothelium samples were taken from patients who did not suffer from bladder carcinoma in the past and had a macroscopically normal bladder mucosa during cystoscopy, and they were taken as control (group3). Tissue were fixed in 10 % buffered formalin and embedded in paraffin wax, were stained with hematoxylin-eosin.

Tumor grade was characterized by 2 independent pathologists

Voided urine samples were collected before cystoscopy or surgery for patients of groups 1-3 and for healthy subjects, as aseptically as possible, in sterile containers. The collected mid-stream specimens were transported to the laboratory within 30 minutes of the collection and cultured on a specific media for bacterial isolation.

Immunohistochemical Detection of CD14 Proteins Expression in Paraffin Embedded Sections: According to procedure mentioned by Celis et *al*., 2005 [12]**.**

The antibodies used were: Biotinylated Link: which is F (ab') rabbit anti-mouse IgG HRP-STAR13B (Serotec, UK), and Sheep anti-rabbit IgG: HRP-STAR54 (Serotec, UK), or secondary Anti-mouse Antibody conjugated with peroxidase enzyme. (Sigma).Mouse anti-human CD14 (Serotec,UK) (MCA596F): Batch No.0798.

Slides were examined and stained cells were counted with the assistance of an experienced histopathologist by light microscope at X400 magnification .Immunostaining was scored according to cut-off value. This cut-off for

positivity was 10% positive cells for CD14 [7].Quantitative IHC scoring was evaluated by counting the number of positive and negative cell nuclei in several randomly selected fields in each section .Tumor reactivity was expressed as the marker percent (i.e., the number of stained tumor cells per 1000 cells in each section).More than 1000 cells evaluated under 40 X high power field and the percentage of positive cells was calculated as follows:

For CD14, the intensity of positivity scored as [7]:

a) 0: No reaction.
b) 5-10 %: Weak reaction.
c) 10-25 %: Moderate reaction.
d) 50-80 %: Intense reaction.

Statistical analysis

Student's *t* test, the chi-square (*x2)* test. Correlation coefficient was used. Probability values of $p<0.05$, and $p<0.01$ were considered statistically significant.

Results

CD14 protein was overexpressed in 72 patients (68.6%) ,with approximately equal frequent IHC score among patients(23.8%) for weak and intense immunoreaction ,and (21.0%) for moderate one . Table 1shows the frequency distribution of CD14 overexpression scores in group subjects.

Positive immunoreaction of CD14 was found in bladder tissue samples of 41 out of 55 newly diagnosed patients with bladder carcinoma, 10 bladder tissue samples of previously diagnosed bladder carcinoma and had received chemotherapy ,and in 21 bladder tissue samples of patients without bladder cancer, including 3 normal urothelium biopsies ,with intense immunostaining reaction being the most frequent score among patients of group-1,and group-2.while weak immunostaining reaction observed in group-3 as the most frequent score.(Table 2) .

Frequency Of CD14 In Patients With & Without Bladder cancer

The CD14 IHC results tissue samples taking together all patients with bladder cancer(Group-1 and Group-2) ,and considering patients without bladder cancer and harboring other urological diseases,were correlated to each other and summarized in table 3, in which 51 out of 71(48.57%) bladder cancer patients showed positive results, while 21 of non-cancerous cases, showed positive results(Figure 1A).Chi-Square test showed that there was no statistical difference between bladder cancer and other urological disease for CD14 IHC Scores in tissue sample taken from each case .

Frequency of CD14 IHC Scores In Bladder Cancer Patients In Correlation To Tumor Grade Of TCC:

CD14 protein immunostaining was evaluated in 105 paraffin-embedded bladder tumor specimens. CD14 identified by positive anti-CD14 reaction which is demonstrated at the lower part of the panel; and the luminal endothelial cells.

Regarding the tumor grade of transitional cell carcinoma, CD14 was detected in 15 out of 25of grade-1, 15 out of 19 of grade-2, and 12 out of 13 of grade-3. There was no CD14 detected in bladder tumor with grade-4.

Chi-square was used to compare the results of frequency distribution of CD14 scores among tumor grades of TCC and it showed a significant correlation between each score and tumor grade ($P<0.05$) , Figure 1 .

Frequency of CD14 Overexpression In Relation To the Bacterial Infection:

Forty out of 105 patients presented with Gram-negative bacterial infection,

in which immunohistochemistry staining of CD14 protein, showed that 33 patients were positive with intense reaction being the most frequent score among them (19.0%) (Table 4).

The results showed highly significant correlation (P<0.01) in CD14 expression with bacterial infection criterion.

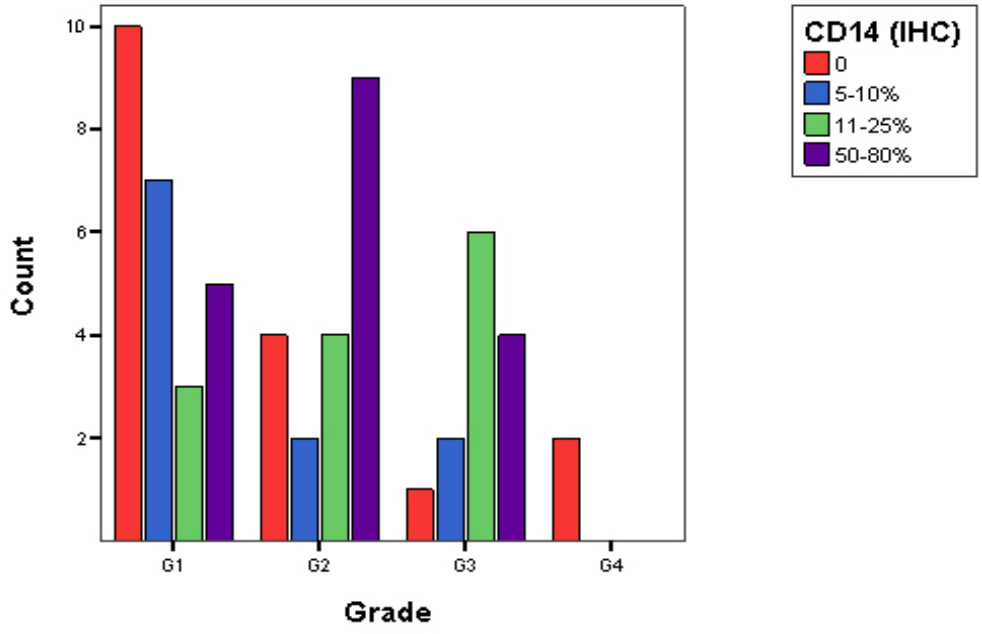

Figure 1: The percentage of CD14 overexpression in bladder carcinoma patients in relation to the tumor grade.

Table 1: Frequency distribution of CD14 Overexpression

CD14 Overexpression	*Patients (%)*
Positive	***72 (68.6)***
_ Weak reaction	25 (23.8)
_ Moderate reaction	22 (21.0)
_ Intense reaction	25 (23.8)
Negative	***33 (31.4)***
Total	**105(100)**

Table 2: Frequency table of CD14 IHC scores in study groups .

CD14 IHC Scores		Groups			
		Group-1	**Group-2**	**Group-3**	**Total**
0	**No.**	14	6	14	34
	%of Total	(25.5)	(37.5)	(40.0)	(31.4)
5-10 %	**No.**	11	3	11	25
	%of Total	(20.0)	(18.8)	(31.4)	(23.8)
10-25%	**No.**	12	3	7	22
	%of Total	(21.8)	(18.8)	(20.0)	(21.0)
50-80 %	**No.**	18	4	3	25
	%of Total	(32.7)	(25.0)	(8.6)	(23.8)
Total	**No.**	55	16	35	106
	%of Total	(52.4)	(15.2)	(33.3)	(100.0)

0: Negative .5-10 % : Weak reaction .10-25 %: Moderate reaction .50-80 %: Intense reaction.

Table 3: Frequency of CD14 IHC scores in patients with & without bladder cancer.

CD14 IHC Scores		Type-CA		Total	Chi-square	Sig.
		Cancer	Non-Cancer			
0	**No.**	20	13	33		
	%of Total	(28.2)	(38.2)	(31.4)		
5-10%	**No.**	14	11	25	7.092	0.071
	%of Total	(19.7)	(32.4)	(23.8)		
10-25%	**No.**	15	7	22		
	%of Total	(21.1)	(20.6)	(21.0)		
50-80%	**No.**	22	3	25		
	%of Total	(31.0)	(8.8)	(23.8)		
Total No.(%ofTotal)		71(67.6)	34(32.4)	**105(100%)**		

Table 4: The percentage of CD14 expression in relation to the bacterial infection

		Infection			Total
		No growth	**Gram-negative**	**Gram-Positive**	
0.0%	**No.**	24	7	2	33
	%of Total	(22.9)	(6.7)	(1.9)	31.4
5-10 %	**No.**	21	4	0	25
	%of Total	(20.0)	(3.8)	(0.0)	23.8
10-25%	**No.**	13	9	0	22
	%of Total	(12.4)	(8.6)	(0.0)	21.0
50-80 %	**No.**	5	20	0	25
	%of Total	(4.8)	(19.0)	(0.0)	23.8
Total	**No.**	63	40	2	105
	%of Total	(60.0)	(38.1)	(1.9)	100.0
Chi-square		31.567			
Sig.		<0.01* *			

** Highly significant correlation.

Discussion

We have used the bladder tissue biopsies to study IHC expression of CD14 in patients with and without bladder cancer.

In the present investigation, we have characterized the immunoexpression of CD14 in bladder tissue of both cancer and non-cancer patients, without significant difference, but its expression in cancer tissue was with significant correlation to the tumor grade. Variation in CD14 expression levels among individuals should correlate with variation in the ability to mount an inflammatory reaction. The factors that contribute to the variable expression level of mCD14 in bladder epithelial cell line, has not been identified [(13)].Variation in mCD14 expression levels among individuals should correlate with variation in the ability to mount an inflammatory reaction.

High mCD14 expression levels were found in grade-1 and grade-2 then in grade-3, while the small size of grade-4 sample showed no CD14 expression .A possible explanation for the down-regulation is that during chronic inflammation, inhibitory feedback mechanisms would decrease the expression of CD14. An alternative explanation is that the down-regulation is a consequence of death and shed of the urothelial cells.

In our study high CD14 IHC expression levels were found in Gram-negative bacterial infected bladder tissue of both cancerous and non-cancerous patients, and it's expression was significantly correlated with this type of bacterial infection (data are not shown) .This is compatible with the fact that CD14, is a cell surface protein involved in LPS binding [(1)], in which LPS is the major constituent of gram-negative bacterial cell wall. It is one member of a group of molecules, called pathogen-associated molecular pattern molecules that are recognized by host

tissue that express pattern recognition receptors. Recognition of microorganisms by this mechanism forms part of the primitive form of defense called innate immunity [(14)]. LPS stimulated an overall increase in mCD14 but specifically induced mCD14 in low mCD14 expression cells [(5)].It has been previously reported that tissue-specific CD14 expression is regulated at the level of transcription, and an 80-kb genomic fragment containing the critical regulatory elements that enhance the tissue-specific expression of CD14 [(15)].LPS specifically up-regulated several genes included CD14 genes [(16)], furthermore, LPS significantly induced CD14 mRNA expression [(17)].

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