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## **Immunohistochemical expression of CD 14 in transitional cell carcinoma of the urinary bladder**

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**Abstract**--CD14 is a co-receptor for bacterial lipopolysaccharide (LPS) detection. It is found on myelomonocytic cells such as monocytes, macrophages, and Langerhans cells, CD14 expression in bladder cells is necessary for cytokine secretion and increased tumor growth. The goal of this study was to use immunohistochemistry (IHC) to assess CD14 expression in patients with transitional cell carcinoma of the urinary bladder in order to see if there was a link between CD14 marker expression in bladder cancer and cystitis. The immunoexpression of CD14 in paraffin sections from 30 bladder biopsy samples was separated into three groups: cystitis, low grade bladder cancer (L.G), and high grade bladder cancer (H.G), and studied using immunohistochemical assays (IHC). For bladder cancer (L.G & H.G), the percentage of samples that gave positive results for IHC/CD14 expression was 70% and 80%, respectively, compared to 30% for cystitis. The incidence of study samples appear in both sexes. Males had a higher percentage of bladder disease than females in all groups, with 80 % for L.G and 70 % for both cystitis and H.G. Bladder diseases manifested in different age group, (56-71) years showed highly frequent rate for both bladder cancer grade (L.G / H.G) whereas cystitis was shown to be more common in (40-55) years. According to the statistical analysis, there is a significant correlation in CD14 expression between study groups ( $p \leq 0.05$ ) as an inverse correlation in low grade bladder cancer to cystitis, as well as a non-significant correlation in high grade bladder cancer to cystitis. In comparison to previous study groups, the results of our study focuses on the

immunological role of CD14 highly expression in patients with low-grade bladder cancer, which appear highly significant difference (0.001 under  $p \leq 0.05$ ).

**Keywords**---CD14, urinary bladder, IHC, transitional epithelium.

## Introduction

The most frequent use of immunostaining for the identification of solid tumors is immunohistochemistry (IHC). CD14 tumor infiltrating lymphocytes can be detected immunohistochemically and utilized as a possible predictor factor in the diagnosis of urothelial cell carcinoma of the bladder tissue, It relies on both color and morphology to differentiate between benign and malignant cells in cytology specimens [1].

Bladder cancer is estimated to be tenth most prevalent cancer in worldwide according to GLOBOCAN statistics. In 2020,(573,278) new cases of bladder cancer were diagnosed around the world, causing (200,000) deaths [2,3]. Bladder cancer (BC) is a form of solid tumor marked by the presence of a substantial number of myeloid cells in the tumor [4,5]. Solid tumors represent a complex mass of cells that interfere with each other to stimulate a wide range of immunological markers that play a role in the diagnosis of microenvironment [6,7]. Bladder cancer occurs in both sexes and is not limited to males only, but the risk of males is three to four times higher than that of females. For women, bladder cancer related mortality has reduced, while for males, it has remained same. Bladder cancer claimed the lives of 16,400 people in 2016 [8].

Most bladder cancer is caused by unknown factors, according to researchers[9]. However, they have discovered certain risk factors and are beginning to understand how they drive bladder cells to become cancerous. Many biological and environmental factors interfere with the development of the cancerous condition, In addition to the role of several life style and nutritional factors [10]. Bladder cancer represents the next step in the case of cystitis, and the causes of inflammation are many, including bacteria, parasites, and sometimes viruses. In all cases, the tumor usually occurs after infection of the urinary system and the failure of the immune system to identify it [11]. One of the most prevalent urinary tract illnesses is cystitis. Cystitis is a condition caused by bacteria from the feces or vaginal flora invading the periurethral mucosa and ascending to the urine bladder. Uropathogens may have virulence properties that allow them to get past the host's defenses and into the tissues of the urinary system [12]. Inflammation recurs with the same pathological criteria and indicators when cystitis becomes out of control of the immune system and does not respond to antibiotics. It may lead to genetic and physiological changes that participate in the formation of a bladder tumor that can later develop into cancer [13,14] .

CD14 an antigen was first characterized as a membrane-associated glycosyl-phosphatidylinositol (GPI)-linked glycoprotein that act as co-receptor and has been demonstrated to play a crucial role in Toll-like receptor signaling pathways [15.16]. In tumor microenvironment, to an increased cytokine production and

tumor development need CD14 high expression in bladder cancer according to, CD14 expression in bladder cells may play a role in tumor cell functionality. To date, only a few studies have investigated CD14 expression in urothelial tissue with bladder problems "cystitis or cancer" [1].

## Materials and Methods

The study included 30 patients with different ages suffering from cystitis and bladder cancer (Low grade and High grade) at a period from October 2021 to March 2022 in Hilla province with recording information about patients. Biopsy samples from individuals with bladder cancer and cystitis were fixed in 10% buffered formalin and embedded in paraffin wax. CD14 expression is detected using an immunohistochemistry (IHC) approach based on CD14-specific antibodies, as directed by the manufacturer instructions. Positive expression was defined as staining of the cell cytoplasm. The following basic criteria were used to establish a semi-quantitative rating based on the intensity of positive cells: weak ( $\leq 25\%$ ); moderate (25-50%), and strong ( $\geq 50\%$ ) [17].

## Statistical Analysis

The statistical analysis was carried out with the help of the statistical package for social science (SPSS) version 26. Statistical the student's Chi-square test was used to compare the groups, and a P value of 0.05 was judged significant. In addition, Pearson correlation coefficients were performed to see if the analyzed parameters had any link [18].

## Results

Our study was conducted on 30 patients of all ages and sexes between October 2021 and March 2022, with 70 % of men in cystitis and high grade bladder cancer (H.G) samples and 80 % of males in low grade bladder cancer (L.G) samples (Figure 1). The study also revealed that bladder disease was classified into several age groups, ranging from 40 to 87 years old, with varied percentages (Figure 2).

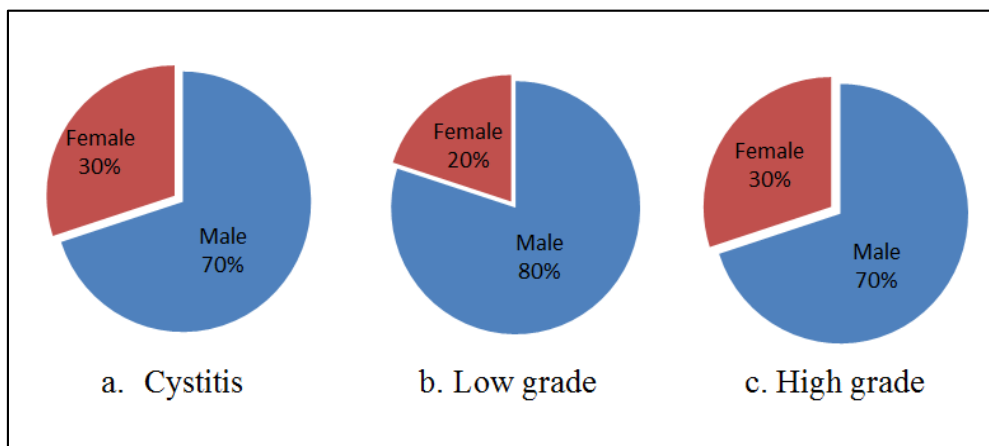


Fig. 1: Distribution of study samples according to sex

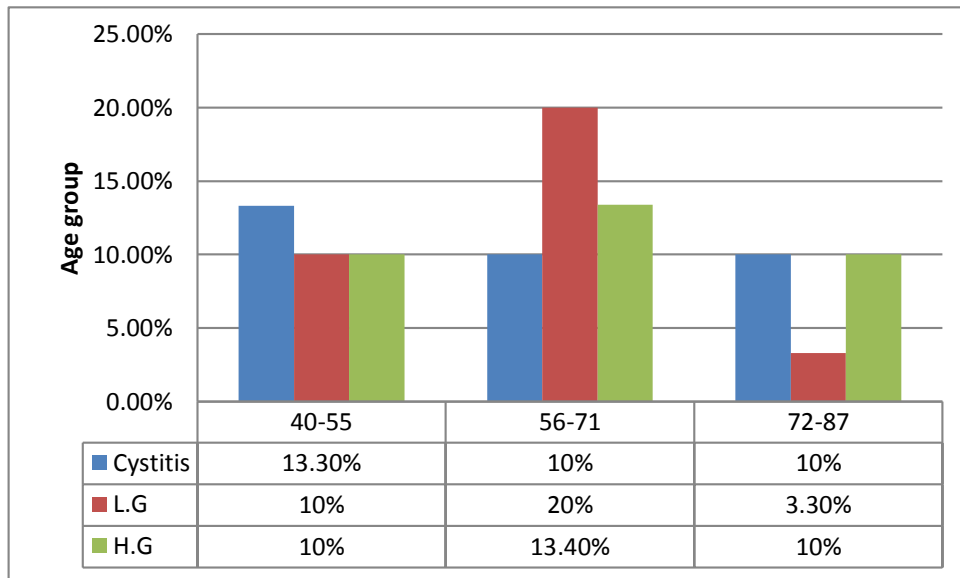


Fig.2: Distribution of study samples according to age group

Figure 3 showed a positive anti-CD14 response in a bladder biopsy revealed CD14 expression, as seen in the pointed brown region of the image, as compared to non-staining cells (negative CD14 expression). Additionally, positive CD14 expression appears in varying percentages amongst research samples, with cystitis accounting for around 30% of the positive CD14 expression, while L.G and H.G account for about 70% and 20% positive CD14 expression, respectively (Figure 4).

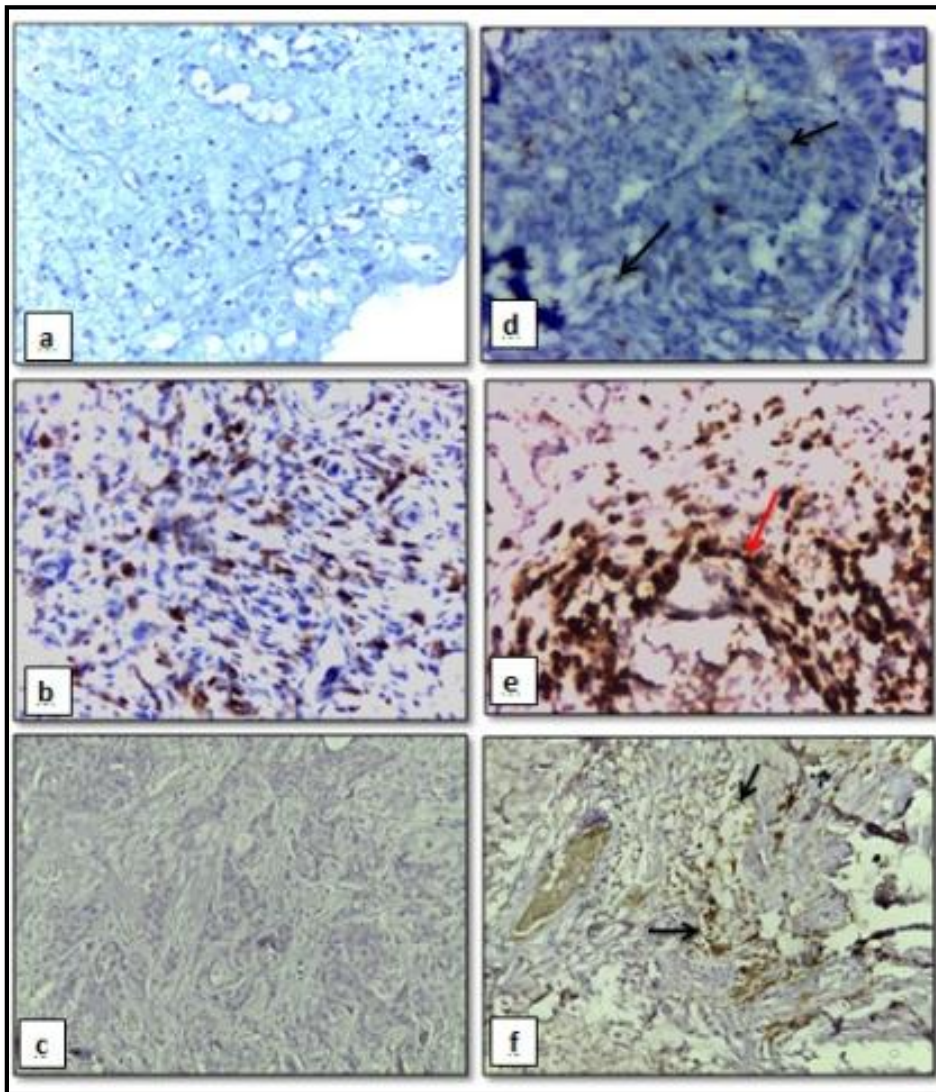


Fig. 3: Photomicrograph (a,b,c) show negative result for CD14 expression in biopsy of cystitis, Low grade and high grade bladder cancer, respectively. (d,e,f) show positive CD14 expression as pointed cytoplasmic brown stain for three cases of bladders biopsy ( Immunohistochemicalstaining for CD14 x 400)

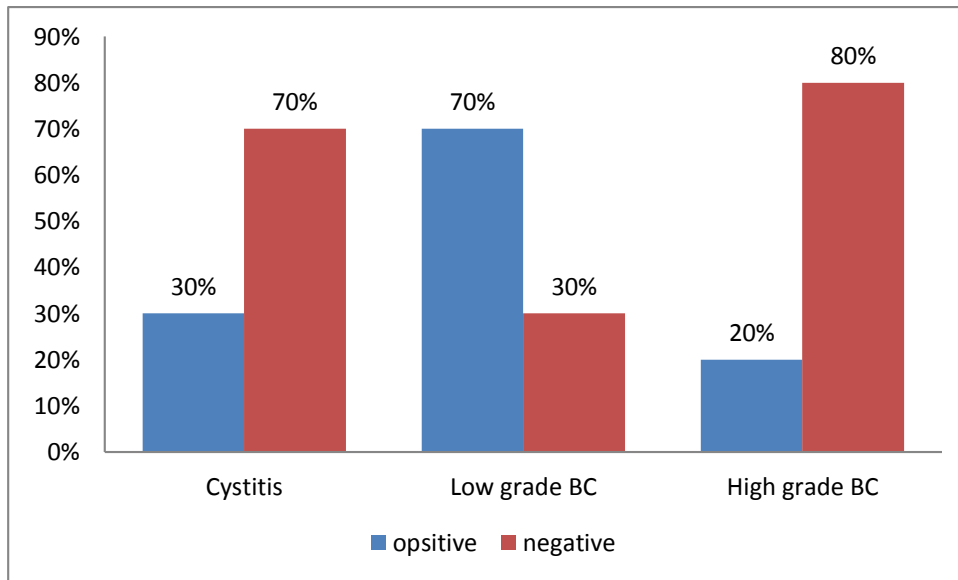


Fig. 4: Percentage of CD14 expression in cystitis and bladder cancer biopsy

Table 1 demonstrates the CD14 expression in bladder cancer (L.G / H.G) and cystitis correlations, demonstrating that there is a significant inverse correlation between low grade bladder cancer to cystitis, as well as a non-significant correlation in high grade bladder cancer to cystitis ( $p \leq 0.05$ ). As previously stated, there is a highly significant correlation substantial association in low grade samples with an inverse correlation to cystitis. Table 2 showed that all bladder biopsy groups have a high significant difference in CD14 expression (0.001 under  $p \leq 0.05$ ), although there is no significant variation in intensity across study samples that have positive CD14 immunostaining.

Table 1  
Correlations of CD14 expression in bladder cancer (L.G / H.G) to cystitis  
\* Significant correlation with an inverse relationship

Correlations				
		<b>cystitis/ CD14</b>	<b>L.G of BC /CD14</b>	<b>H.G of BC/ CD14</b>
cystitis/CD14	Pearson Correlation	1	-.048-	-.327-
	Sig. (2-tailed)		.896	.356
	N	10	10	10
L.G of BC /CD14	Pearson Correlation	*-.048-	1	-.218-
	Sig. (2-tailed)	.896		.545
	N	10	10	10
H.G of BC /CD14	Pearson Correlation	-.327-	-.218-	1
	Sig. (2-tailed)	.356	.545	
	N	10	10	10

Table 2  
Intensity of CD14 expression in study samples

Bladder diseases	CD14 expression		Intensity of CD14 expression			Total
	Result	No.	weak	moderate	strong	
Cystitis	positive	3	1	1	1	10
	Negative	7				
Low grade of bladder cancer	positive	7	2	2	3	10
	Negative	3				
High grade of bladder cancer	positive	2	2	0	0	10
	Negative	8				
P value (p≤0.05)	0.001 (highly significant)		0.1 (non-significant)			30
Chi-square	13.04		8.96			

## Discussion

Inflammatory diseases of the bladder are among the vital problems that should be taken care of, especially bladder cancer, which requires careful diagnosis and follow-up by focusing on the patient's immune system to prevent the inflammatory condition (cystitis) from progressing to malignant status. Our current study agrees with others have found that males are more likely than females to develop cystitis and bladder cancer of both sorts (low and high grade) [8,11]. The rising risk of cystitis and bladder cancer in males, as opposed to women, has been correlated to their physiological status and lifestyle, additional to cigarette use being more prevalent in men[19]. According to the present study, bladder cancer is more prevalent in people over 55 year, whereas cystitis is more common in people under 55 year, which is consistent with personal history and might be linked to other urinary system disorders or treatment [20].

Immunohistochemical technique for CD14 expression appear difference expression of CD14 between groups of research samples, highest percentage founded in low grade bladder cancer patient about (70%) (Fig.4). It can be explained by the role of the opposite side of immune activity, which occurs as a result of the formation of immune complexes from CD14 antigens/ receptors on the surfaces of urethral cells, which works to suppress apoptosis by reducing the vitality of the oncoprotein p53, leading to the genetic activation of oncogenic cytokines (TNF- $\alpha$ , IL-1,IL-2,IL-6,IL8 and IL-10 ) and all these chain stimuli, it suppresses immunity and encourages tumor cells to grow uncontrollably [21].

CD14 expression, on the other hand, reduced in cystitis samples, despite the fact that it is a co-receptor for bacterial component. This might be due to the capacity of antibiotics to regulate inflammation before immunological control and chemotherapy, which inhibits immunity [15]. The majority of high grade bladder cancer show lowest percentage for CD14 expression only 20%, this is supported by the fact that CD14 high cancer cells produce greater amounts of multiple inflammatory mediators and exhibit accelerated tumor development, resulting in bigger tumors, as compared to CD14 low cells in bladder cancer [22].

In this study, focused on the important correlation between research groups in terms of CD14 expression and discovered that there is a significant correlation

between low grade bladder cancer and cystitis with inverse significant correlation, while there is a non-significant correlation between high grade bladder cancer and cystitis with inverse correlation. (Table 1). That explained the fact of the degree of immune response in cystitis instances should be connected with the CD14 expression, which acts as a first step in reducing inflammation progressing to carcinoma and increasing cytokines and toll-like receptor secretion [23]. Moreover, the inverse correlation of CD14 expression between pathological bladder cases indicates the degree and strength of the patient's bladder tissue defense cells, which then work to redirect the type of immune response through the secretion of growth factors and specialized cytokines, which suppressor or strengthen anti-tumor function and control the inflammatory state [24]. The intensity of positive CD14 expression in research samples appears to non-significant difference, which is dependent on the amount of CD14 receptors present on cell surfaces, and hence may be deceptive due to immunostain, antibody, and chemical reagent inefficiency [25].

### Conclusion

A recent study found that low-grade bladder cancer cells overexpressed CD14, which was explained by the role of CD14 complexes with toll-like receptor signaling in tumor progression. Current data showed that CD14 expression is highly related with low grade and cystitis in an inverse correlation, but that there is no significant difference between CD14 expression intensity and bladder biopsies.

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