RESEARCH ARTICLE

Impact of Toll-Like Receptors 7 and 9 and Tumor Necrosis Factor-alpha in Iraqi Patients with Urinary Bladder Carcinoma

Manhal F. Ahmed*, Shahlaa M. Salih

College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

Received: 17th September, 2020; Revised: 09th October, 2020; Accepted: 24th November, 2020; Available Online: 25th March, 2021

ABSTRACT

Background: Toll-like receptors (TLRs) are key sensor molecules that are expressed together on immune cells and on tumor cells and trigger an immune response and cancer treatment. The study aimed to evaluate the relationship between TLR-7 and 9 and tumor necrosis factor-alpha (TNF-α) level, and progression of urinary bladder carcinoma (UBC).

Methods: This study included 57 UBC Iraqi patients with 50 healthy control subjects. Enzyme-linked immunosorbent assay kits estimated serum level of TLR-7, TLR-9, and TNF-α.

Results: Showed a significant increase in TLR-7 in muscle-invasive bladder cancer (MIBC) in comparison to none muscle-invasive bladder cancer (NMIBC) and control. Expression of TLR-9 and TNF-α were significantly higher in MIBC and NMIBC in comparison to control. Also, results revealed a significant positive correlation between TLR-7 and TLR-9 with TNF-α level.

Conclusions: Upregulation of serum circulating TLR-7 and TLR-9 may regulate TNF-α production and could be playing a role in UBC progression and development.

Keywords: TLR7, TLR9, TNF-α, Urinary bladder carcinoma.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.1.20

How to cite this article: Ahmed MF, Salih SM. Impact of Toll-Like Receptors 7 and 9 and Tumor Necrosis Factor-alpha in Iraqi Patients with Urinary Bladder Carcinoma. International Journal of Drug Delivery Technology. 2021;11(1):109-112.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Urinary bladder cancer (UBC) is a multi-factorial that generally established by chronic exposition to environmental risk factors and genomic instability caused by genetic variations.[1] Smoking which the dominant risk factor for bladder cancer (BC).[2,3] Other factors, including arsenic exposure, exposure to carcinogenic chemicals, and inflammation, are common risk factors for BC.[4] The UBC is a worldwide illness and graded globally as the seventh most predominant cancer in males than females in ratio of 4:1. It is considered the 4th most common cancer in males, while it is the 8th most popular cancer in females.[5] In Iraq, UBC is the 6th most common malignancy, and males are three times more prospective than females to develop the disease.[6] The genetic markers that have been examined are TLRs. They are complicated domestic of transmembrane receptors involved in identifying conserved molecular patterns of a microbial source and endogenic damaged molecules.

Additionally, their role in preserving tissue hemostasis due to the inflammation. The TLRs employee leukocytes as a response to microbial-infected tissues; therefore, the innate and adaptive immune responses are persuaded, and also, they provide necessary signals for the determination of inflammation. but also play crucial roles in cancer.[7,8] Ten different TLRs have been recognized in humans, and two of these receptors are TLR7 and TLR9, which play an essential role in the pathogenesis of different malignancies; among these is UBC and many researches demonstrate that bladder cancer cells express functional TLR7 and TLR9 with possible effects on cancer progression.[9,10] The TNF-α is an inflammatory cytokine that is mediated in wide variety of diseases, including cancer. It is mainly produced by macrophages that encourage cell proliferation and differentiation, cell death, and regeneration. The role of TNF-α related to all cellular steps complicated in tumorigenesis, including cellular survival, promotion, transformation, angiogenesis, invasion and metastasis.[11,12] There are substantial direct and indirect signals to implicate the TNF-α in UBC progression. In angiogenesis, TNF-α is known to significant role in the regulation of thymidine phosphorylase, which has been confirmed to be a major factor in UBC progression and metastasis.[13] This current study was intended to study the impact of TLR 7 and 9 in inflammation and UBC progression.

MATERIALS AND METHODS

Subjects

Venous blood samples were collected from 53 Iraqi patients with urinary bladder cancer. They were sub-grouped in to

*Author for Correspondence: manhal.farooq@gmail.com

48 males and 9 females; their ages was ranged from 35 to 85 years old, during the period from February to April 2019, from the Urology department of Al-Jaibachi private Hospital and Al-Yarmook Teaching Hospital in Baghdad. The tumors were subclassified into low grade and high grade according to the histological observations on the basis of WHO classification criteria. The TNM stage was classified as non-invasive (Ta-T1) and invasive (T2–T4) according to the American Joint Committee on Cancer guidelines (AJCC). Fifty blood samples of healthy volunteers who age-matched to the UBC patients were enrolled in this study.

Blood Collection

Five mL of blood samples were collected from each individual. The blood was left-hand to clot in plain tubes and centrifuged to collect the serum, and then stored at - 20 °C.

Serum Level of Toll-like Receptors (TLRs)

Sandwich enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, Canada) was used to measure serum level of TLR7 and TLR 9. The TNF-α was estimated by quantitative ELISA kit (R&D, USA).

Statistical Analysis

Serum level of TLR7, TLR 9, and TNF-α was statistically investigated via statistical package for the social sciences (SPSS) program version 24. The data were assumed as mean ± Standard error (SE) and variances between means were measured by analysis of variance (ANOVA) followed by least significant difference (LSD). The p values less than 0.05 were taken as significant.

RESULTS

The results suggested that there are no significant differences ($p > 0.05$) between the mean age of UBC patients (68 years) compared to control (55 years); in contrast, 86% of UBC patients' group were ≥ 50 years have the most frequent. The UBC male patients' incidence was higher (84.4%) than female (15.8 %). Most UBC patients were cigarette-smokers (80.7%), while non-smoker UBC patients presented at a lower frequency (19.3%). More than 50% of UBC patients were at the clinical stages I–II (44%) and 56.0% had an invasive tumor stage Table 1.

Serum TLRs

The results revealed that serum TLR-7 level was significantly decreased (1.73 ± .21 ng/mL) in control than UBC patients. The muscle-invasive UBC and none-muscle invasive UBC level revealed that TLR-7 level was (2.78 ± 0.19ng/mL) in MIBC followed by NMIBC (2.04 ± 0.17) with significant differences. The results of the TLR-9 level displayed a significant increase in level of TLR-9 in the invasive stage of bladder cancer patients (54.86 ± 3.11pg/mL) followed by the non-invasive UBC patients serum (27.13 ± 1.90pg/mL). In contrast, the healthy control presented a remarkable low level (16.53 ± 1.52pg/mL) Table 2.

Serum Tumour Necrosis Factor Alpha (TNF-α)

Comparison between the TNF-α levels in NMIBC and MIBC of BC and the healthy volunteer established that the MIBC BC had a notable high level of TNF-α (57.60 ± 2.78 pg/mL) than the NMIBC (26.13 ± 1.41pg/mL) with significant differences ($p < 0.05$), while the level was lower in healthy control (11.60 ± 0.85 pg/mL) with significant differences ($p < 0.05$) compared with NMIBC and MIBC Table 2.

Correlation of Serum TLRs with TNF-α in UBC

Pearson correlation tests used to depict the relationship between TLR-7 and TLR-9 with TNF-α result indicated a positive weak correlation between TLR-7 level and TNF-α ($r = 0.470$, $P < 0.01$) (Figure 1).

Figure 2: illustrated the correlation between TLR-9 and TNF in the UBC. The results indicated a positive correlation

Table 1: Characteristics of UBC patients and controls

Characteristic		*UBC*		*Healthy*		
	N	*%*	*N*	*%*		*p-value*
Gender	Male	48	84.2	33	66	Non-sig.
	Female	9	15.8	17	34	Non-sig.
Age group	≤50 years	8	14	10	20	Non-sig.
	≥50 years	49	86	40	80	Non-sig.
Smoking	Yes	46	80.7	22	44	Sig.
	No	11	19.3	28	56	Sig.
Tumor invasion	Non-invasive	25	44	-	-	Non-sig.
	Invasive	32	56	-	-	

Table 2: Serum levels of TLR-7, TLR-9 and TNF- α in UBC and control

		Serum conc. (mean ± SE)		
Groups	*N*	*TLR-7*	*TLR-9*	*TNF-α*
Healthy	50	1.73 ± .21 b	16.53 ± 1.52c	11.60 ± 0.85c
Non-invasive	25	2.04 ± 0.17 b	27.13 ± 1.90b	26.13 ± 1.41b
Invasive	32	2.78 ± 0.19a	54.86 ± 3.11a	57.60 ± 2.78a

Diverse letters represent a significant differences with probability $P < 0.05$ of the same column.

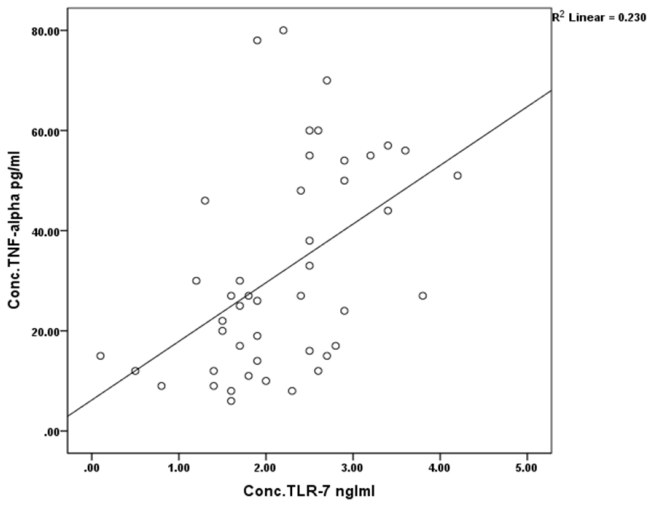

Figure 1: Correlation between TLR-7 and TNF-α in UBC patients

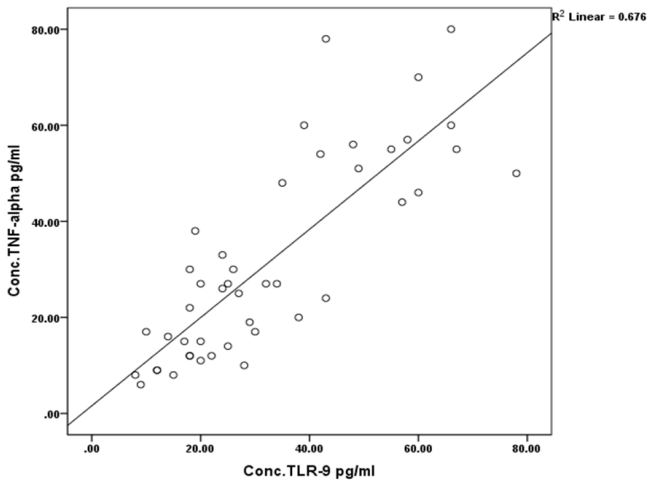

Figure 2: Correlation between TLR-9 and TNF-α in UBC patients

($r = 0.822$, $p < 0.01$). This result supports the fact that TLR-9 associated with an increased in the TNF-α level.

DISCUSSION

The current study revealed an association between activation of TLR7, TLR9 and TNF-α production respectively with the development of UBC.TLR7 play role in bladder cancer and there is some evidence that its pathway is crucial in anti-tumor immunity and the risk of UBC may be influenced by TLR7 gene SNPs in the first intron.[19,20] Downregulation of TLR7 is recommended by Al-Humairi *et al.*[21] to have a part in the etiology and UBC pathogenesis. Targeting TLR 7 can activate the innate immune cells, resulting in cellular and humoral immunity, thus initiating a sequence of anti-tumor events.[22] The R-837 advised as a TLR7 specific agonist that encourages pro-inflammatory immune response with the same mechanism of action as BCG acts. Several studies recorded the therapeutic potency and activity of R-837 in superficial bladder cancer.[23,24] Another study reported that targeting TLR-7 byTMX-101 and TMX-202 are inform action for treatment of non-muscle invasive BC in rat model.[25] The TLR9 expression was found in human bladder cancer cells and it signaling initiates the accumulation, development, and lymph nodes migration of antigen-loaded tumor dendritic cells. Inside the lymph nodes, these dendritic cells facilitate the activation of tumor-specific T cytotoxic lymphocytes, which pro-liferate and accumulate into the tumor site to control cancer growth.[10,14] A study exposed that the numerous incentives, such as LPS, had failed to encourage crucial immune response. In contrast, the CpG ODNs targeting TLR9 and order as a superior to BCG in the orthotopic murine model of BC.[18] The TLR9 targeting has received considerable attention aimed at their competence to initiates an anti-tumor immune response against BC.[15] CpG ODNs in reducing tumor growth in MB49 mouse model of bladder cancer was verified by Ohadian and Nowroozi.[16] It has generally fine tolerated and demonstrated the potential as a novel immune-therapeutic method for cancer treatment. The responses of tumor to TLR9 agonist mono-therapy had been experiential in numerous tumor types, including solid hematologic and tumor malignancies.[17] TNF-α is a pleio-tropic cytokine that has an essential role in immune homeostasis, inflammation, and host defense against different infections. It may stimulate many effects such as angiogenesis, cell differentiation, and cell migration, all of which directly affect tumor immune surveillance.[26] In accordance with the present results, several studies recorded that the TNF-α serum concentration was significantly higher in advanced invasive stage and has been implicated in tumor invasion and metastasis.[27,28] Also, these results are compatible with Metwally *et al.*[29] who found a significant increase in TNF-α level in bladder cancer patient's sera versus normal controls. Another study suggested that TNF- α can be used as a diagnostic biomarker for urinary bladder cancer.[30]

REFERENCES

1. Volanis D, Kadiyska T, Galanis A, Delakas D, Logotheti S and Zoumpourlis V. Environmental factors and genetic susceptibility promote urinary bladder cancer. Toxicol Lett. 2010;193: 131-137.
2. Zeegers MP, Goldbohm RA and van den Brandt PA. prospective study on active and environmental tobacco smoking and bladder cancer risk (The Netherlands). Cancer Causes Control. 2002;13: 83-90.
3. Samanic C, Kogevinas M, Dosemeci M, Malats N, Real FX, Garcia-Closas M, Serra C, Carrato A, García-Closas R, Sala M, Lloreta J, Tardón A, Rothman N and Silverman DT .Smoking and bladder cancer in Spain: effects of tobacco type, timing, environmental tobacco smoke, and gender. Cancer Epidemiol Biomarkers Prev .2006; 15: 1348-1354.
4. Wu CC, Huang YK, Chung CJ, Huang CY, Pu YS, Shiue HS, Lai LA, Lin YC, Su CT and Hsueh YM . Polymorphism of inflammatory genes and arsenic methylation capacity are associated with urothelial carcinoma. Toxicol Appl Pharmacol.2013; 272: 30-36.
5. Oeyen E, Hoekx L, De Wachter S, Baldewijns M, Ameye F, Mertens I. Bladder cancer diagnosis and follow-up: the current status and possible role of extracellular vesicles. Int J MolSci.2019; 20:821.

6. Iraqi Cancer Board 2018 Annual report: Iraqi cancer registry 2015. Ministry of Health\Environment, Baghdad.
7. Rakoff-Nahoum S, Medzhitov R.Toll-like receptors and cancer. Nat Rev Cancer.2009; 9:57–63.
8. Basith S, Manavalan B, Yoo T, Kim S, Choi S. Roles of Toll- like Receptors in Cancer: A Double-edged Sword for Defense and Offense. Arch Pharm Res.2012; 35(8): 1297-1316.
9. Lacagnina MJ, Watkins LR, Grace PM.Toll-like receptors and their role in persistent pain. Pharmacol. Ther.2018; 184:145–158.
10. Olbert P , Kesch C, Henrici M , Subtil F, Honacker A, Hegele A,Hofmann R, Hänze J.TLR4- and TLR9-dependent effects on cytokines, cell viability, and invasion in human bladder cancer cells. UrolOncol.2015; 33(3):19-27.
11. Aggarwal B, Shishodia B, Sandur S, Pandey M, Sethi G. Inflammation and cancer: How hot is the link? .Biochemical pharmacology.2006; 72: 1605–162.
12. Cai1 J, Yang M, Hou N, Li X. Association of tumor necrosis factor-α 308G/A polymorphism with urogenital cancer risk: a systematic review and meta-analysis. Genet. Mol. Res. 20015;14 (4): 16102-16112.
13. Marsh H, Haldar N, Bunce M, Marshall S, Monier K, Winsey S, Christodoulos K, Cranston D, Welsh K, Harris A. Polymorphisms in tumour necrosis factor (TNF) are associated with risk of bladder cancer and grade of tumour at presentation. British Journal of Cancer.2003; 89: 1096 – 1101.
14. Kang TH, Mao C, Kim YS, Kim TW, Yang A, Lam B, Tseng SH, Farmer E, Min Park Y, Fu Hung C.TLR9 acts as a sensor for tumor-released DNA to modulate anti-tumor immunity after chemotherapy. Immunotherapy cancer.2019; 7: 260.
15. Adams,S.Toll-like receptor agonists in cancer therapy. Immunotherapy.2009; 1(6):949-964.
16. Ohadian MS and Nowroozi MR.Toll-like receptors: The role in bladder cancer development, progression and immunotherapy. Scand J Immunol.2019; 90: e12818.
17. Krieg AM. Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. Oncogene.2008; 27: 161–167.
18. Mangsbo SM, Ninalga C, Essand M, Loskog A, Tötterman TH. CpG therapy is superior to BCG in an orthotopic bladder cancer model and generates CD4+ T-cell immunity. J Immunother.2008; 31(1):34-42.
19. Hemmi H, Kaisho T, Takeuchi O. Small anti-viral compounds activate immune cells via the TLR7 MyD88- dependent signaling pathway. Nat Immunol.2002; 3: 196.
20. Al-Humairi, RMA, Al-Musawi, MT, Ad'hiah, AH. Bidirectional expression of Toll-like receptor 7 gene in urinary bladder cancer and urinary tract infection of Iraqi patients. Gene Reports.2019; 17: 100491.
21. Al-Humairi, RMA, Al-Musawi, MT, Ad'hiah, AH. Serum level and single-nucleotide polymorphisms of toll-like receptor-7 among urinary bladder cancer Iraqi patients. Egypt J Med Hum Genet.2019; 20:11.
22. Urban-Wojciuk Z, Khan MM, Oyler BL, Fahraeus R, Marek-Trzonkowska N, Nita-Lazar A, Hupp TR, Goodlett DR. The Role of TLRs in Anti-cancer Immunity and Tumor Rejection. Front. Immunol.2019; 10:2388.
23. Itoh T, Celis E. Transcutaneous immunization with cytotoxic T-cell peptide epitopes provides effective anti-tumor immunity in mice. J Immunother.2005; 28:430-437.
24. Liu H, Schwartz MJ, Hwang DH, Scherr DS. Tumour growth inhibition by an imidazoquinoline is associated with c-Myc down-regulation in urothelial cell carcinoma. BJU Int.2008; 101:894–901.
25. Alke J, Hulsbergen-van de Kaa CA, Maj R, Oosterwijk E, Witjes J. A placebo-controlled efficacy study of the intravesicalimmunomodulators TMX-101 and TMX-202 in an orthotopic bladder cancer rat model. World J Urol. 2018; 36: 1719–1725.
26. Wajant H. The role of TNF in cancer. Results Probl Cell Differ.2009; 49:1-15.
27. Kakehi1 O, Hirao Y, Kim W, Ozono S, Masumori N, Miyanaga N, Nasu Y, Yokomizo A. Bladder Cancer Working Group Report. Jpn J ClinOncol. 2010; 40:57–64.
28. Chen Z, Zhou W, Dai M, Jin W. Association between the interaction polymorphisms of interleukin-10 and smoking on patients with bladder cancer risk from a Case Control Study. Zhonghua Liuxingbingxue Zazhi. 2013;34(2):183-186.
29. Metwally M, Ali A, Mohamed M, Khaled H, Ahmed S. Levels of certain tumor markers as differential factors between bilharzial and non-biharzial bladder cancer among Egyptian patients. Cancer Cell International.2011; 11(8):1-11.
30. Mahdi NK, Ghdbhan AF, Saleh MM. Pro-inflammatory Cytokines for Evaluation of the Diagnostic Performance for the Urinary Bladder Cancer. Austral - Asian Journal of Cancer. 2013; 12(3): 169-173.