Nucleotide Oligomerization Domain-like Receptor 4 (*NLR4*) Gene Expression and Interleukin 1-β (IL 1-β) Level in Urine Samples Before and After Intravesical BCG Therapy for Treatment of Bladder Cancer

Thesis

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Samar Ahmed Abd Elmoaty Eissa

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List of Abbreviations

Abb.	Full term
AIM	*Proteins absent in melanoma*
ASC	*Apoptosis-Associated Speck-Like Protein Containing CARD*
BCG	*Bacillus Calmette-Guérin*
CAPS	*Cryopyrin-associated periodic syndromes*
EAPC	*European Association for Palliative Care*
IFI16	*Interferon-γ ible protein 16*
IFNγ	*Interferon-γ*
LRR	*Leucine rich repeats*
MAPK	*Mitogen-activated protein kinase*
MMC	*Mitomycin C*
NACHT	*Neuronal apoptosis inhibitor protein*
NOD	*Nucleotide binding oligomerization domain*
RIG-I	*Retinoic acid-inducible gene I*
SNPs	*Single nucleotide polymorphism*
TCC	*Transitional cell carcinoma*
TNM	*Tumor-node-metastasis staging system*
TURBT	*Transurethral resection of bladder tumor*
VUR	*Vesico ureteral regurg*

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Abstract

Inflammasomes are cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses e.g. secretion of pro-inflammatory cytokines including interleukin 1β (IL-1β). Inflammasomes have been linked to different types of tumors including bladder cancer. The treatment of bladder cancer depends on how deeply the tumor invades into the bladder wall with non-muscle invasive bladder cancer (NMIBC) treated mostly by transurethral resection of bladder tumor (TURBT) with or without intavesical chemotherapy or immunotherapy. Bacillus Calmette-Guerin (BCG) instillation is the most common immunotherapy used in treating and preventing the recurrence of NMIBC, however it may fail and expose the patient to several side effects. In this study, we aimed to assess the expression of nucleotide oligomerization domain-like receptor 4 (NLR4) gene side by side with IL-1β level in urine as a predictor of BCG treatment failure in case of NMIBC, as well as a primary marker for muscle invasive bladder cancer (MIBC). The study was performed on 47 patients divided into 30 cases of NMIBC and 17 cases of MIBC. From NMIBC cases, five urine samples were obtained at different times of treatment, while pre-surgical sample was obtained from MIBC cases. NLRP4 gene expression and IL1-β levels were estimated by RT- PCR and ELISA technique respectively.

Pre-surgical NLRP4 gene expression level together with urinary IL-1β levels were significantly higher in case of NMIBC than encountered in MIBC, which may serve as primary non-invasive diagnostic method to differentiate between both types. Both levels were higher in pre-surgical samples among cases that showed recurrence within the following 6 months after surgery than in non-recurrent cases. After BCG treatment, both levels were increased in patients who developed tumor recurrence within the following six months after surgery than in non-recurrent cases. The present study suggests that NLR$ gene expression and IL-1 β levels in urine may serve as a predictive indicator for BCG treatment failure as well as an indicator of tumor invasiveness being higher in NMIBC.

Keywords: Bladder Cancer, BCG, IL1-β, Intravesical BCG Therapy, Nod-like Receptors, Tumor Immunity.

INTRODUCTION

Inflammasomes are part of the innate immunity essential in maturation of inflammatory cytokines such as interleukin (IL) -1β and IL-18 in response to infection or autogenous danger signals ***(Man et al., 2016)***. Inflammasome is a multi-molecular complex which consists of a nucleotide oligomerization domain like receptor (NLR) protein, the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain, and caspase-1. NLRs are major components of pattern-recognition receptors that recognize bacterial or viral pathogen-associated molecular patterns in order to initiate innate immune response ***(Iannello et al., 2016).***

Pattern-recognition receptors are found on the membrane surface e.g., Toll like receptors and C-type lectin receptors, or intra-cellularly e.g. NLRs. Like Toll like receptors, NLR proteins can interact with endogenous ligands or damage-associated molecular patterns from normal host tissues or tumor cells to induce autoimmune diseases or an antitumor response ***(Broz et al., 2016)***.

Tumor initiation, growth, invasion, and metastasis can be largely affected by chronic inflammation. Various innate immune pathways may engage with cellular components released from dead tumor cells due to hypoxia, chemotherapy and radiotherapy as a part immune surveillance system (***Gonzalez et al., 2016)***. Innate immune cells activated by a tumor or its components share

either in developing antitumor immunity by recruitment of effector cells or promoting tumor development by providing a pro-inflammatory environment. The role of inflammasomes in tumor development is not well known despite numerous studies in this field ***(Berraondo et al., 2017)***.

Bladder cancer is the 7th most commonly diagnosed cancer in the male population worldwide and the 11th when both genders are considered. Seventy five per cent of bladder cancer cases are non-muscle invasive bladder cancer (NMIBC). Transurethral resection of bladder tumor (TURBT) is the gold standard initial diagnostic intervention for bladder cancer and provides both therapeutic and prognostic roles in NMIBC. Adjuvant intravesical therapy with either immunotherapy or chemotherapy is used together with TURBT in order to reduce recurrence and/or progression through immune-stimulation or direct cell ablation ***(Babjuk et al., 2017).***

Bacillus Calmette–Guérin (BCG) immunotherapy remains the standard first line intravesical agent for NMIBC. The exact mechanism by which BCG prevents recurrence is unknown. However, it has been shown that the bacteria are taken up by the cancer cells. The infection of these cells in the bladder may trigger a localized immune reaction which clears residual cancer cells ***(Alhunaidi and Zlotta, 2019).*** BCG is taken in by urothelial and inflammatory cells after binding to urothelial cells via a fibronectin-dependent pathway, which

triggers a substantial inflammatory and immunologic response ***(Williams et al., 2017).***

Early and prominent recruitment of polymorph nuclear leukocytes is a unique characteristic of BCG-induced inflammation. They are found in large numbers (75% of immune cells) in urine after intravesical BCG instillation. A typical inflammatory response to bacterial infections, the cytokine profile of IL-2, IL-12, and Interferon γ is seen after BCG exposure due to a T-helper 1 response ***(Redelman et al., 2014).***

The production of cytokines from tumor cells has been demonstrated in urothelial tumor cell lines, in contrast to the initial hypothesis where macrophages and lymphocytes infiltrating the bladder wall were the primary cellular source of cytokines after instillation of BCG. In this scenario, NLRs are responsible for the release of several cytokines, particularly IL-1β and IL-18 ***(Poli et al., 2015).***

BCG is delivered as an induction course, consists of 6-weeks course of intravesical BCG instillation, followed by a maintenance course. Side effects of BCG therapy include cystitis, prostatitis, epididymo-orchitis, balanitis, ureteral obstruction, bladder contraction, myco-bacterial osteomyelitis, reactive arthritis, mycobacterial pneumonia, granulomatous hepatitis, granulomatous nephritis, interstitial nephritis, infectious vasculitis and disseminated infection ***(Green et al., 2019; Rezvani and Collins, 2019).***

Despite its efficiency, BCG treatment failure may occur. BCG treatment failure can be classified into 3 groups; BCG relapse, BCG-refractoriness and BCG-intolerance. In BCG relapse, tumor reoccurs after a disease free period. BCG-refractory tumors are the ones which do not respond to induction and maintenance doses of BCG or which progress during therapy. In BCG-intolerance, tumor reoccurs due to incomplete treatment as the person receiving it is unable to tolerate induction course of BCG. Around 50% of the cases fail BCG treatment and would require further treatment options ***(Grossman et al., 2019; Werntz et al., 2019).***

Because of the mentioned probable side effects together with the probable occurrence of BCG failure and the fact that those relapsed cases are difficult to be treated, it is mandatory to discover a prognostic marker that can efficiently predict failure, securing the patient from passing through BCG treatment schedule with a lot of possible burdens on his health and to early deciding radical surgery.