



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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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

سُبْحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

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List of Contents

Title	Page No.
List of Abbreviations.....	5
List of Tables.....	6
List of Figures	8
Abstract	11
Introduction	- 1 -
Aim of the Work	5
Review of Litertaure	
▪ Inflammasomes	6
▪ Bladder Cancer	45
▪ Bacillus Calmette-Guérin Immunotherapy for Bladder Cancer	76
Patients and Methods.....	88
Results.....	105
Discussion	138
Summary.....	150
Conclusion	154
Recommendations	155
References	156
Arabic Summary	

List of Abbreviations

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

List of Tables

Table No.	Title	Page No.
Tables of Review		
Table 1:	Summary of the main urinary tumor markers.....	56
Table 2:	Risk group stratification	60
Table 3:	Treatment recommendationin TaT1 according to risk stratification.....	65
Tables of Results		
Table 1R:	Demographic and clinical data of all studied cases.....	107
Table 2R:	Comparison between NLRP4 gene expression in pre-surgical samples in NMIBC and MIBC patients groups:.....	109
Table 3R:	Relation between NLRP4 gene expression in NMIBC patients group in pre-surgical samples in recurrent and non-recurrent cases:.....	111
Table 4R:	Relation between NLRP4 gene expressions in pre-surgical samples compared to after the 3 rd dose of BCG in NMIBC group:.....	113
Table 5R:	Relation between NLRP4 gene expression level after the 3 rd dose of BCG in recurrent and non-recurrent cases.....	115
Table 6R:	Relation between NLRP4 gene expression level after the 3 rd month post-surgically of the follow up cystoscopy in recurrent and non-recurrent cases.....	117
Table 7R:	Relation between NLRP4 gene expression level after the 6 rd month at the time of the follow up cystoscopy in recurrent and non-recurrent cases	119

List of Tables cont...

Table No.	Title	Page No.
Table 8R:	Relation between IL-1 β level in NMIBC and MIBC	121
Table 9R:	Relation between IL-1 β level before surgery and before the 3 rd dose BCG.	123
Table 10R:	Relation between IL-1 β level before surgery in recurrent and non-recurrent cases.....	125
Table 11R:	Relation between IL-1 β level before and after the 4 hours of the 3 rd dose BCG.....	127
Table 12R:	Relation between IL-1 β level before surgery and after the 3 rd month at the follow up cystoscopy in NMIBC group:	129
Table 13R:	Relation between IL-1 β level after the 3 rd dose BCG and after the 3 rd month at the follow up cystoscopy.	131
Table 14R:	Relation between IL-1 β level after the 3 rd dose BCG in recurrent and non recurrent cases.....	133
Table 15R:	Relation between IL-1 β level after the 3 rd month follow up in recurrent and non recurrent cases.	135

List of Figures

Fig. No.	Title	Page No.
Figures of Review		
Figure 1:	Recognition of the inflammatory ligand leads to sensor activation, oligomerization and the recruitment of adaptor protein that mediates the function	7
Figure 2:	Structure of inflammasome.....	8
Figure 3:	NO D-like receptor cellular pathways	11
Figure 4:	Canonical NLRP3 inflammasome activation by K ⁺ efflux	18
Figure 5:	Activation of NLRC4	20
Figure 6:	Noncanonical NLRP3 inflammasome activation	23
Figure 7:	Modulation of inflammasome pathways by bacterial and viral effectors	28
Figure 8:	Inflammasome-related inflammation-induced cancers	37
Figure 9:	Pathological grading of BC according to WHO 2016.....	57
Figure 10:	Classification TNM 2017.....	58
Figure 11:	Staging of bladder cancer.....	59
Figure 12:	Blue Light Cystoscopy enables cancerous tumors to fluoresce in a bright pink color	63
Figure 13:	Molecular cascade of immune response induced by intravesical BCG instillation	78
Figure 14:	Some pathological types of bladder cancer (a, b) papillary transitional cell carcinoma, (c, d) bladder squamous cell carcinoma.	93
Figure 15:	Preparing duplicate standard points by serially diluting the standard stock solution (4000 pg/L)	102

List of Figures *cont...*

Fig. No.	Title	Page No.
Figures of Results		
Figure 1R:	Relation between NLRP4 gene expression in pre-surgical samples in NMIBC and MIBC patients groups expressed as mean and SD.....	110
Figure 2R:	Relation between NLRP4 gene expression in pre-surgical samples in recurrent and non-recurrent cases of NMIBC group expressed as (mean + SD)	112
Figure 3R:	Relation between NLRP4 gene expression in pre-surgical samples compared to after the 3rd dose of BCG represented as (mean+ SD).....	114
Figure 4R:	Relation between NLRP4 gene expression level after the 3rd dose of BCG in recurrent and non-recurrent cases (represented as the mean+SD)	116
Figure 5R:	Relation between NLRP4 gene expression level after the 3rd month post-surgical in recurrent and non-recurrent cases (represented as the mean+SD)	118
Figure 6R:	Relation between NLRP4 gene expression level at 6th month follow up cystoscopy in recurrent and non-recurrent cases (represented as the mean+SD)	120
Figure 7R:	Relation between IL-1 β level in NMIBC and MIBC patients groups (represented by the mean+SD).....	122
Figure 8R:	Relation between IL-1 β level before surgery and before the 3rd dose BCG (pg/ml) (represented as the mean+SD).....	124

List of Figures *cont...*

Fig. No.	Title	Page No.
Figure 9R:	Relation between pre-surgical IL-1 β level recurrent and non-recurrent patients groups (represented by the mean+SD).....	126
Figure 10R:	Relation between IL-1 β level before and 4 hours after the 3rd dose BCG (represented by the mean+SD).....	128
Figure 11R:	Relation between IL-1 β level before surgery and after the 3rd month at the follow up cystoscopy in NMIBC group.....	130
Figure 12R:	Relation between IL-1 β level after 4 hours of the 3rd dose BCG instillation and after the 3rd month at the follow up cystoscopy (represented as mean+SD).....	132
Figure 13R:	Relation between IL-1 β level after the 3 rd dose BCG in recurrent and non recurrent cases (represented by the mean+SD).....	134
Figure 14R:	Relation between IL-1 β level after the 3rd month post-surgical in recurrent and non recurrent cases.	136
Figure 15R:	Changes in IL-1 β level and NLRP4 gene expression level (represented as normalized Ct) between 3 different samples (pre-surgical, after the 3 rd dose BCG and after 3 months) in both recurrent and non-recurrent cases.....	137

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 1 β (IL-1 β). 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 intravesical 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-1 β 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-1 β 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 NLRP4 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) -1 β 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-1 β 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.