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The role of vitamin D3 in the recurrence of non-muscle invasive bladder cancer

Hazim Hadi Muzaail[1], Ahmed El-Assmy[3], Ahmed M. Harraz[3], Amira Awadalla[2],Ahmed A. Shokeir[2] and A.F. Abdel-Aziz[1]*

[1] Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt.

[2] Center of Excellence for Genome and Cancer Research, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

[3]Urology and Nephrology Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

**** Correspondence to:** afaziz2012@hotmail.com, 01008764445*

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Abstract: Aim: To investigate the exact role of vitamin D3, CD68 and CD1-alpha in non-muscle invasive bladder cancer (NMIBC).

Subjects and Methods: This study including (99) male with non-muscle invasive bladder cancer NMIBC (Ta-T1) who were subjected to transurethral resection (TURBT). As well as control group consists of 50 male individuals. We determined the gene expression level for CD68, CD1-alpha and the protein concentration of vitamin D3 level in blood samples in patients and controls.

Results: Compared to the control group, vitamin D3 levels were considerably lower in the study group. There was no significant correlation between vitamin D3 with either grade or stage. In comparing the recurrence and non-recurrence groups, vitamin D was significantly increased in non-recurrence group. CD68 was highly expressed in recurrence more than nonrecurrence and control group patient but in case of CD1-alpha gene expression; it was significantly decrease in case of recurrence in a comparison to nonrecurrence and control patients.

Conclusions: The results suggested the possible effect of vitamin D3, CD68 and CD1-alpha in reducing the recurrence status, as presented, CD1-alpha and vitamin D3 were significantly increased in non-recurrence cases; but CD68 was significantly decrease in non- recurrence.

Key words: non-muscle invasive bladder cancer (NMIBC); vitamin D3, CD68, CD1-alpha.

1. Introduction

Bladder cancer is the sixth most prevalent cancer in both men and women globally, and the fourth most frequent cancer in males **(1).** Males are four times as likely than females to develop bladder cancer **(2).** Given the fact that smoking and exposure have been identified as contributing causes to the gender discrepancy, males continue to be at a larger risk of acquiring bladder cancer than women, even when lifestyle and environmental variables are included **(3).**

The most well-known kind of bladder cancer was urothelial carcinoma, which accounts for 90% of all cases **(4)**. Transitional cell carcinoma (TCC) is the most frequent kind of urothelial carcinoma of the bladder and therefore is classified into two types: non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). NMIBC (pTa, the cancer was only in the innermost layer of the bladder lining) and (pT1, the cancer has begun to grow

into the connective tissue beneath the bladder lining) could be effectively treated with transurethral resection and intravesical This study including 99 male with NMIBC (Ta-T1) who were subjected to transuretheral resection

(TURBT). As well as control group consists of 50 male individuals.

The local Ethical Committee approved of this study . The procedures were performed according to the ethical isssues of the Helsinki Declaration. Informed consent was taken from all patients.

Methodology

Molecular studies

Gene expression of CD68, CD1_alpha using Real-time PCR

The CD68 and CD1-a mRNA level was detected by RT-qPCR technique.RNA was extracted from the all blood samples and converted to c-DNA.The relative quantification of the CD68 and CD1-a were examined by real-time qPCR. All data was correlated with GAPDH. The relative amount of genes was calculated by $2^{-\Delta\Delta CT}$ method.

Determination of vitamin D3 in blood cell samples using ELISA

MYBIOSOURCE (cat no. MBS3042311 offers a ready-to-use microwell ELISA plate to detect vitamin D3).

Statistical analysis

Whenever possible, continuous data were presented using the mean (SD). The significance was determined using the T- test, and the percentages of categorical values were determined using Chi Square tests. P-values of less than 0.05 were considered to be statistically significant.

3. Results and Discussion

Clinical characteristics of study groups

This study included 99 TCC male patients divided into recurrence and non-recurrence groups. The recurrence group consists of 58 patients with mean age 62.5±11.2 years. In this group, 3 patients (5.4%) were GI, 46 patients (82.1%) were GII and 7 patients (12.5%) were GIII. While the non-recurrence group contains 41 patients with mean age 58.7±12.2 years.

One patient (2.4%) was GI, 26 patients (36.4%) were GII and 14 patients (34.1%) were GIII.

The control group consists of 50 health individuals with mean age 59.7±7.1 years (**Table 1**).

Table 1: Clinical characteristics of study groups

	Recurrence	**Non-recurrence**	**Control**
N	58	41	50
Age (Mean ± SD)	62.5±11.2	58.7±12.2	59.7±7.1
Grading n (%) GI GII GIII	3 (5.4%) 46 (82.1%) 7 (12.5%)	1 (2.4%) 26 (36.4%) 14 (34.1%)	-

Relation between vitamin D3 and recurrence status

the relation between vitamin D and recurrence status, Table (2) shows significant increase in vitamin D in control group compared with recurrence and non-recurrence groups (p<0.05). In comparing the recurrence and non-recurrence groups, vitamin D was markedly increased in non-recurrence group versus the recurrence group (p<0.05).

Table 2: Vitamin D between groups

	Mean ±SD	***P*1 value**	***P*2 value**	***P*3 value**
Control (Mean ± SD)	24.15 ± 2.98	0.000	0.000	-
Recurrence (Mean ± SD)	8.88 ± 2.61	0.000	-	0.000
Non-recurrence (Mean ± SD)	13.77 ± 3.54	-	0.000	0.000

*P*1: value: control vs. recurrence

*P*2: value: control vs. non-recurrence

*P*3: value: recurrence vs. non-recurrence

Relation between CD68 and CD1_a gene expression and recurrence status

The relation between CD68, CD1-a and recurrence was determined; the results showed a significant decrease in CD68 in control group compared with recurrence and non-recurrence groups (p<0.05). In comparing the recurrence and non-recurrence groups, CD68 significantly increased in non-recurrence group compared with recurrence group (p<0.05) as shown in (table 3). But in case of CD1-a by comparing the expression between groups; the expression in control group was significantly increased more than recurrence and nonrecurrence (p<0.05), and the expression of recurrence was highly expressed more than non-recurrence (p<0.05) as shown in (table 4).

Table 3: CD68 between groups

	Mean ±SD	***P*1 value**	***P*2 value**	***P*3 value**
Control (Mean ± SD)	0.98±0.08	0.000	0.000	-
Recurrence (Mean ± SD)	4.127±0.2	0.000	-	0.000
Non-recurrence (Mean ± SD)	2.08±0.17	-	0.000	0.000

Table4: CD1_alpha between groups

	Mean ±SD	***P*1 value**	***P*2 value**	***P*3 value**
Control (Mean ± SD)	0.99±0.17	0.000	0.012	
Recurrence (Mean ± SD)	0.48±0.21	0.000		0.000
Non-recurrence (Mean ± SD)	0.8±0.53		0.012	0.000

Discussion

Previous studies had discussed the link between vitamin D and cancer progression, but it was still ambiguous. As a result, the purpose of this study was to assess the association between vitamin D levels and the risk of bladder cancer.

Several studies proved the crucial role of CD68 in tumor cells; its expression by tumour cells was unsurprising, given that metastatic tumor cells frequently express immunological markers to avoid macrophage-mediated destruction. phagocytosis and cell damage caused by cytotoxic CD8+ T cells T lymphocytes by invading a healthy, non-tumor tissue environment. The molecular characteristics of macrophages appear to be the most important. CD68 Indeed, Antigens expressed by macrophages in tumour tissue may be over expressed which reflected a prometastatic state and could be linked to bad health prognosis **(15)**.

This study proved that CD68 was markedly increased in non- recurrence patients in comparing to recurrence group. Various studies have examined BC incidence and serum level of CD68 and our results was compatible to **(16)** study which stated that higher levels of CD68 and other macrophage markers in the tumour stroma have been linked to higher tumour grade, lymph node metastasis, and other malignant characteristics that characterise tumor development and aggressive behaviors. **(16)**.

Previous research has shown that CD1a+ DC infiltration and its connection with clinicopathological variables in patients with advanced laryngeal cancer who had a complete laryngectomy as an initial therapy. Infiltration of CD1a+ DCs was substantially related with more advanced (T4 and Stage IV) patients. Unexpectedly, the CD1a-high group had poor clinical outcomes, and it was a predictive factor in multivariate analyses using TMN staging **(17)**.

These data revealed that CD1-a levels in non-recurrence patients were significantly lower than in recurrence and control groups (p0.05). According to multivariable analysis, CD1a+ DC infiltration was associated with a poor clinical outcome and was an independent predictive factor in this research. The mechanism by which CD1a+ DCs induce negative consequences is currently unclear **(17)**.

Numerous studies have found that vitamin D can aid in the prevention of colorectal and breast cancer **(18, 19).** The most frequent form of vitamin D in the human body is 25-hydroxy vitamin D. Other studies have discovered that 25(OH) D has anticancer characteristics by

promoting cell differentiation and death while inhibiting angiogenesis and metastasis **(19).** Previous research has also demonstrated that vitamin D can decrease development and induce death in human bladder tumor cells in vitro, indicating that it could be utilized to treat bladder cancer **(20, 21)**. Numerous epidemiological studies have been conducted to assess the association between vitamin D level and the risk of bladder cancer, but no consistent results have been identified **(18, 22, 25)**.

In our study, vitamin D levels were significantly higher in the non-recurrence group when compared to the recurrence group (p0.05). Numerous studies have been conducted to investigate the prevalence of bladder cancer and the degree of vit D. Other research, on the other hand, do not establish the link between Vit D level and Bladder cancer **(26).** In another study, Afzal et al. revealed that reduced plasma VD was associated with an increased risk of tobacco-related malignancies, including bladder cancer. Mondul et al. observed that men with lower Vit D 3 serum concentrations were more likely to develop bladder cancer than men with greater serum levels **(23)**. A recent meta-analysis confirmed these finding **(27)**.

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