THE IMPACT OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 6 (STAT6) IN PREDICTION OF PROGNOSIS OF BLADDER CANCER

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Abstract

Background: Bladder cancer (BC) is the most common malignancy of the urinary system and represents the 11th most common cancer worldwide. Due to its aggressiveness, early prediction of its progression is recommended. Therefore, this work aimed to investigate the gene expression of STAT6 in muscle invasive bladder cancer (MIBC) as an indicator for progression free survival (PFS).

Methodology: This non-concurrent cohort clinical study that included 50 patients with MIBC who were treated at our centre between January 2015 to November 2020. The study included patients >18 years old, both sexes, having urothelial MIBC who were followed up for at least 2 years. The paraffin-embedded tissues of these patients were obtained from the corresponding units of pathology. Samples from cancerous and non-cancerous tissues were obtained and divided into parts: a small fresh part was frozen at -80 ̊c for gene expression, and a large portion was fixed in 10% buffered formalin for routine histopathology. The gene expression of STAT6 was estimated in all the studied cases using using the qRT-PCR technique.

Results: Most of cases were in grade III 90%. The majority of cases were T3 (46%), N0(64%), and M0 (82%). 36% of cases showed distant metastasis. The mean gene expression value of STAT 6 in cancerous tissues was 4.6±2.1 folds, and 0.99±0.11 folds in non-cancerous tissues. STAT6 gene expression was significantly higher in cancerous tissues compared to the non-cancerous tissues (P<0.001). There was a statistically significant positive correlation between STAT6 and tumor grade (r=0.437, P<.001), tumour stage (r=0.674, P<.001) and metastasis (r=0.204, p=0.043), and. STAT6 could significantly predict tumor progression at cutoff 2.1 with a sensitivity 100% and specificity 70%, AUC 0.688.

Conclusion: In MIBC cases, assessment of STAT6 gene expression could significantly predict tumor progression in BC cases and is correlated to tumor metastasis.

Keywords: STAT6, bladder cancer, prediction, survival

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INTRODUCTION:

The most frequent kind of urologic cancer and one with the highest recurrence rate is bladder cancer. The bladder cancer clinical course has a wide range of risk and aggressiveness. While high-grade non-muscle invasive cancers usually advance and muscle-invasive cancers are frequently fatal, low-grade, superficial bladder cancers have a low probability of death [1].

Like other members of the signal STAT (transcriptional activation by signal transducer and activator of transcription) family of proteins, STAT6 has a dual role as a signaling molecule and transcription factor. STAT6 is activated in response to interleukin (IL)-4 and IL-13 stimulation and plays a key role in Th2 polarization of the immune system [2]. Recently, the STAT6 signaling pathway was found to be highly activated in some tumors, such as prostate cancer, mammary carcinoma, and lymphoma [3].

In addition, a close correlation between IL-4/STAT6 activities and apoptosis and metastasis of colon cancer was reported by Chen wt al. [4]in a study using STAT6-high and STAT6-null colon cancer cell lines to analyze anti-apoptotic and pro-metastatic gene expression. Nevertheless, no studies have investigated the clinicopathological features and prognosis of CRC in relation to STAT6 expression.

STAT proteins are a family of latent cytoplasmic transcription factors including STATs 1–4, STAT5a, STAT5b, and STAT6 [5]. STAT proteins can participate in normal cellular responses via activation by several actors, such as growth factors and cytokines. Also, ample evidence has shown that

proteins such as STAT1, STAT3, and STAT5 play a critical role in a variety of tumors [6].

STAT6 is ubiquitously expressed in most cell types and contributes to the pathological process of allergic diseases and tumors. In the literature, STAT6 has been implicated in the development of lymphoma, prostate, lung, colorectal, and other cancers [7].

Moreover, evidence has suggested that STAT6 is involved in the HCC process and may predict a worse prognosis in patients with HCC [8]. However, the specific role of STAT6 in HCC and its mechanism remain unclear.

This work aimed to investigate the gene expression of STAT6 in MIBC as an indicator for Progression Free Survival (PFS).

PATIENTS AND METHODS

- **Study design and participants**

This non-concurrent cohort clinical study that included 50 patients with bladder cancer (BC) who were treated at our centre between January 2015 to November 2020.

The study included patients >18 years old, both sexes, having urothelial MIBC who were followed up for at least 2 years.

Patients with non-urethral BC, non-muscle invasive bladder cancer (NMIBC), patients with other malignancies, insufficient clinical data, and specimens with no control para tumoral sites were excluded from our study.

Samples from cancerous and non-cancerous tissues were obtained and divided into parts: a small fresh part was frozen at -80 ̊c for gene expression, and a large portion was fixed in 10% buffered formalin for routine histopathology. The tumor stage and grade were based on according to eighth edition of American Joint Committee on Cancer staging system (AJCC 8th edition) and the 2004 World Health Organization system respectively.

The patients were treated according to the detection of Helsinki informed consent were taken from all patients, the institutional Review Board (IRB) of Faculty of Medicine, Mansoura University, Egypt (ID: MDP 20.06.40).

All BC patients were subjected to history taking (clinical and family history), clinical examination, tumor staging, complete laboratory tests (coagulation profile, liver enzymes, kidney function tests (KFT), and complete blood count (CBC)), radiological studies including (Computed tomography or magnetic resonance imaging to assess regional and distant spread, bone scan) and measurement of STAT6 levels.

- **Gene expression of STAT6**

The mRNA level for STAT6 was extracted from using the RNeasy Mini Kit (Qiagen, 74104, Germany) according to the manufacturer's protocol. cDNA was obtained by cDNA Reverse Transcription Kit (Applied Biosystem, USA) according to the manufacturer's protocol. Quantitative RT-PCR analysis was performed using SYBER Green PCR Master Mix (Applied Biosystems, USA) and primers. The cycling parameters were carried out [9]. Data analysis was done by the 2-ΔΔCt method.

STATISTICAL ANALYSIS

Data for categorical variables were expressed as frequencies and percentages, and data for continuous variables were expressed as mean Standard Deviation or median (IQR). Mann-Whitney U tests were performed for continuous variables, while the Chi-square test was utilized to examine the significance between categorical variables. Receiving operating curve (ROC) was conducted to determine the best cutoff value. A correlation between the gene expression of the STAT6 and the pathological features was carried out using Pearson's correlation coefficient. A strong positive correlation was ® between 0.5 to 1.00, strong negative (r) was between -1.00 to -0.5. Kaplan Meier curve was used for plotting survival curve with log. Rank test that was used to detect the statistical significance. The threshold for statistical significance was set at $P<0.05$.

RESULTS

- **Demographic and clinical characteristics**

The mean age was 67.7±8.2years and mean BMI was 27.16±5.6. The male sex was more prevalent (84%). 22% of patients were DM and 28% were hypertensive cases and 14% of them were smokers. 74% of cases were not associated with other disease. The mean creatinine level was 1.39±1.38 mg/dl, the mean albumin level was 3.4±.4 mg/dl, and the mean hemoglobin level 10.67±1.2 mg/dl. 16% of cases underwent neoadjuvant chemotherapy and 20% of cases underwent adjuvant chemotherapy. **Table 1**

- **Clinicopathological features**

Most of cases were in grade III 90%. The majority of cases were T3 (46%), N0(64%), and M0 (82%). 36% of cases showed distant metastasis. **Table 2**

The gene expression of STAT6

The mean gene expression value of STAT 6 in cancerous tissues was 4.6±2.1 folds, and 0.99±0.11 folds in non-cancerous tissues. STAT6 gene expression was significantly higher in cancerous tissues compared to the non-cancerous tissues ($P<0.001$).

- **Correlation between STAT 6 gene expression, and Clinicopathological findings**

There was a statistically significant positive correlation between STAT6 and tumor stage ($r=0.674$, $P<.001$), tumor metastasis ($r=0.204$, $p=0.043$), and tumor grade ($r=0.437$, $P<.001$). **Table 3**

- **Cutoff of STAT6 to predict MIBC progression and specific survival**

STAT6 could significantly predict tumor progression at cutoff 2.1 with sensitivity 100% and specificity 70%, AUC 0.688 (p= 0.05). **Figure 1-2**

Table 1: patients characteristics among the studied cases.

		All N=50
Age(years) (Mean ±SD)		67.7±8.2
BMI (Mean ±SD)		27.16±5.6
Sex	**Male**	42(84%)
	Female	8(16%)
DM	**Yes**	9(22%)
	No	41(82%)
HT	**Yes**	14(28%)
	No	36(72%)
Smoking	**Yes**	7(14%)
	No	43(86%)
Others	**No**	37(74%)
	incidental prostate cancer	8(16%)
	Schistomiasis	5(10%)
Symptom	**No**	4(8%)
	Hematuria	37(74%)
	Intestinal obstruction	1(2%)
	irritative LUTS	5(10%)
	Necroturia	1(2%)
	loin pain	1(2%)
	obstructive LUTS	1(2%)
Create mg/dl		1.39±1.38
Albumin mg/dl		3.4±.4
Hemoglobin mg/dl		10.67±1.2
Neoadjuvant chemotherapy N (%)		8(16%)
Adjuvant chemotherapy		10(20%)

Data are presented as frequency (%) or mean ± SD. BMI: body mass index, DM: diabetes mellitus, HT: hypertension, LUTS: Lower urinary tract symptoms

Table 1: Clinicopathological features of bladder cancer patients

			All N=50 N(%)
Grade	**GI**		2(4%)
	GII		3(6%)
	GIII		45(90%)
Stage	**T2**		21(42%)
	T3		23(46%)
	T4		6(12%)
N	**N0**		32(64%)
	N1		5(10%)
	N2		13(26%)
M	**M0**		41(82%)
	M1		8(16%)
	MX		1(2%)
Distant metastasis	**Yes (from operation till metastasises)**	**Yes**	18(36%)
		No	32(64%)
	No (from operation till last follow-up)	**Yes**	32(64%)
		No	18(36%)

Data are presented as frequency (%). T: stage, M: metastasis.

Table 3: Correlation between the expression of STAT6 and clinical findings in bladder cancer patients (n=50)

	STAT6
T	r=.674
	P<.001*
M	r=.204
	P=.043*
Grade	r=.437
	P<.001*

r: spearman correlation, T: stage, M: metastasis. *: significant as P value ≤0.05

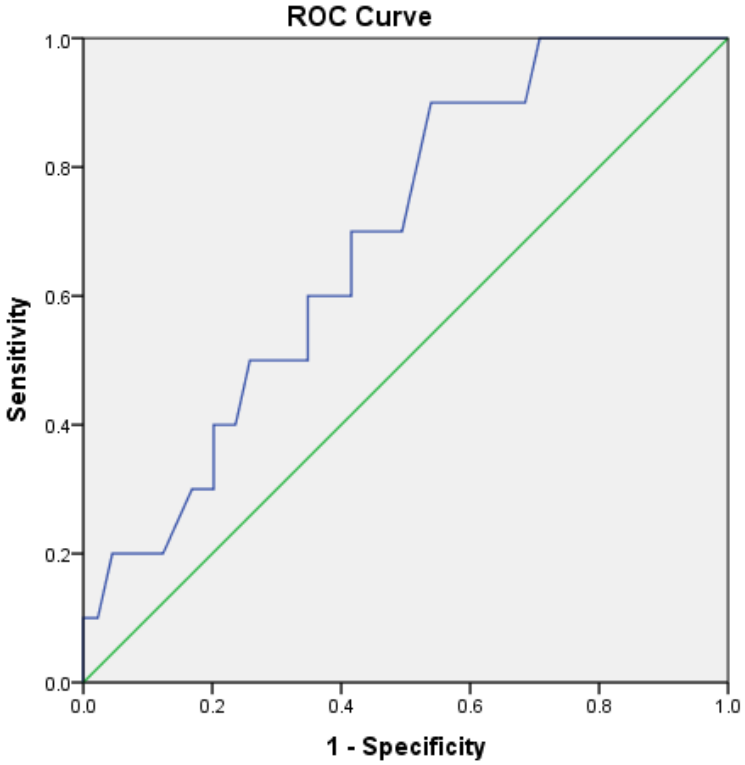

Figure1: The cutoff value of STAT6 for prediction of the tumor progression

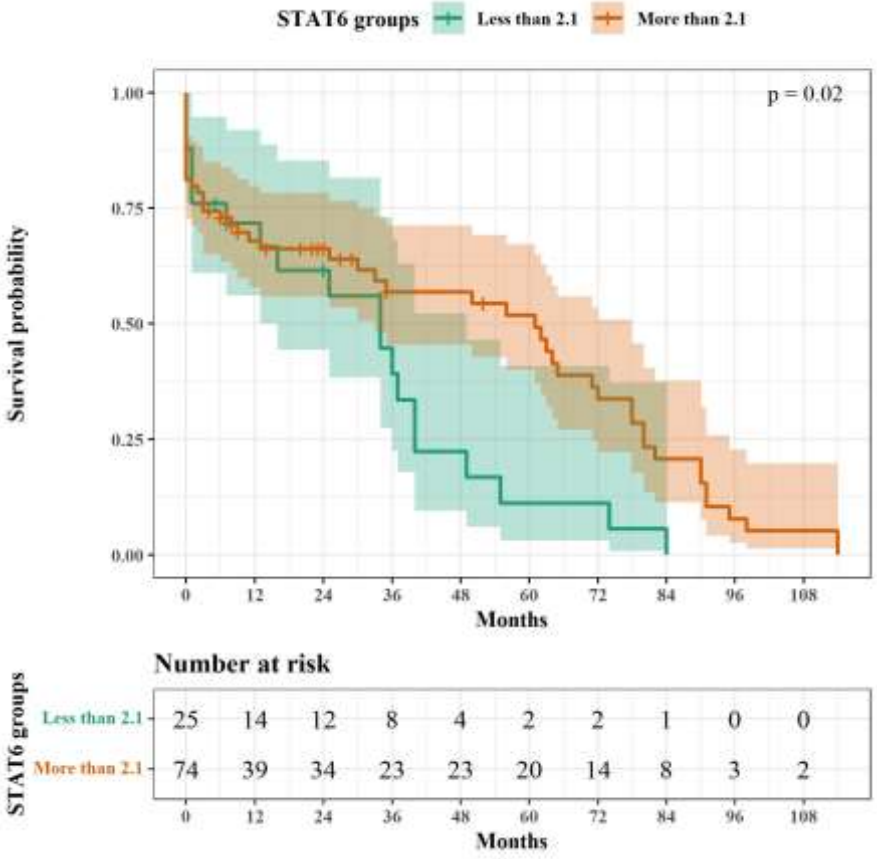

Figure 2: The effect of STAT6 on cancer specific survival in patients with muscle invasive bladder cancer

DISCUSSION

Bladder cancer (BC), the most common malignancy of the urinary system, is also the 11th most common cancer worldwide [10]. STAT6 is ubiquitously expressed in most cell types and contributes to the pathological process of allergic diseases and tumors. In the literature, STAT6 has been implicated in the development of lymphoma, prostate, lung, colorectal, and other cancers [7].

Cytokines serve as a means of communication among immune, cancer and non-transformed stromal cells in the tumor microenvironment. The signal transducer and activator of transcription (STAT) family of proteins serve an important role in the initiation of malignant transformation and in tumor establishment, and have been widely studied in experimental models and in patients with cancer [11].

Upon cytokine binding to its receptor, Janus kinases (JAKs) mediate STAT phosphorylation. In the nucleus, STAT binds to specific DNA sequences that result in the transcription of target genes. Interleukin (IL)-6, IL-10 and IL-23 signaling is mediated by JAK1 and recruits STAT3. Conversely, STAT6 functions as a transcription factor in the nucleus in response to IL-4 and IL-13 receptor binding after the activation of JAK1 and JAK3 [2].

STAT6 orchestrates numerous processes beyond immune response, including cancer cell proliferation, apoptosis resistance, metastasis, epithelial cell function, chromatin compaction, and DNA damage and repair. STAT6 signaling is frequently activated in malignant cells and regulates several genes crucial for the immune response, inflammation and proliferation. Persistent activation of STAT6 in different types of cancer results in proliferation, survival and metastasis, as well as in decreased antitumor immunity [2].

STAT6 polymorphisms have been identified in a subgroup of Malaysian patients suffering from Crohn's disease (CD) and in a patient cohort in Germany, demonstrating the importance of this gene in inflammatory processes in the colon. [12]

The major finding in our study was that urothelial carcinoma was the most prevalent among studied cases. Most of cases were in grade III 85.9%, T3 (62.1%), N0 (74.7%), and M0 (88.9%). The mean value of gene expression for STAT 6 was 4.24±2 folds. There was an insignificant positive correlation between STAT6 and the histological findings of the bladder cancer patients with a statistically significant positive correlation with tumor stage, tumor metastasis, and tumor grade. STAT6 could significantly predict tumor progression at cutoff 2.1 with sensitivity 100% and specificity 70%, AUC 0.688.

In this regard, Tawfik et al. [13] showed that 52.1% of cases were grade II and 47.1% of cases were grade III, nodal status was positive in 38.5% of cases and the majority of cases were T2 stage and 35.3% were T3 stage. Regarding infiltration to lymph node, 35.3% of cases showed N1 disease, 17.6% of cases showed N0 while 47.1% of cases showed no infiltration.

Signal transducers and activators of transcription (STATs) are a family of transcription factors involved in several biological processes such as immune response, cell survival, and cell growth. However, they have also been implicated in developing and progressing several cancers, including prostate cancer (PCa). Although the members of the STAT protein family are structurally similar, they convey different functions in different type of solid cancers. Especially STAT6 that highly associated to cancer progression [14].

Supporting our results**,** Xu et al. [15] who found that STAT6 was highly expressed in prostate cancer cases. Also, Calò et al., [16] reported that STAT6 may be involved in oncogenesis in several breast cell lines.

Regarding the expression of STAT6, wang et al. [17] reported that upregulation of STAT6 was positively correlated to tumor metastasis in prostate cancer cases.

This was also reported in previous studies where, STAT6 exhibited prognostic significance in cases with HCC ($P \leq 0.05$) and STAT6 genes showed higher diagnostic ability (AUC >0.7) [18].

Moreover, Yoshikawa et al. [19] suggested that STAT6 was involved in HCC and may be a poor predictor for HCC prognosis.

Existing evidence also indicates that STAT6 is involved in the development of HCC and could serve as a predictor of poor prognosis for patients with HCC [20].

Limitations: the study was performed in a retrospective manner with the possibility of missing data. The sample size was relatively small. The follow-up period was relatively short. To fully explain genetic alterations in MIBC, additional studies are needed, including whole exome sequencing.

CONCLUSION

In MIBC cases, assessment of STAT6 gene expression could significantly predict tumor progression in bladder cancer cases and correlated to the tumor metastasis.

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