ORIGINAL ARTICLE

CONTENTS

Immunohistochemical evaluation of GATA-3 in patients with urinary bladder cancer

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ABSTRACT

Aim: To analyze expression levels of GATA-3 in bladder tumor tissues and to prove a relation between expression of GATA-3 and clinicopathological characteristics of bladder tumors, including patient age, sex, tumor grade, and muscle invasion.

Materials and Methods: Forty formalin fixed paraffin embedded (FFPE) tissue blocks obtained from bladder tumor by transurethral resection are collected from teaching hospitals at Al-Najaf governorate. Those blocks are stained by using hematoxylin and eosin stain. Histopathological features were examined and then immunostained by GATA-3.

Results: Evaluation of GATA-3 expression in urothelial carcinoma, revealed GATA-3 test was (positive) in thirty-four samples of urothelial carcinoma, while GATA-3 test was (negative) in twenty-one samples of urothelial carcinoma. Correlation of GATA-3 with other variables (age, sex, and grade) was a statistically non-significant.

Conclusions: GATA-3 is an Immunohistochemical sensitive marker to diagnose urothelial carcinoma. GATA-3 expression showed non-significant relation with age, gender and histopathological parameters and its expression has been observed to be lost or reduced in substantial proportion in relation to urothelial carcinoma. This alteration or down regulation of GATA-3 is correlated with higher tumor grade and stage.

KEY WORDS: urothelial carcinoma, GATA-3, immunohistochemistry

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INTRODUCTION

Urinary bladder cancer (UBC) is a disturbing malignant disorder which participates about three percent to the recent development of cancer field [1]. In Iraq, urinary bladder cancer was considered the fifth cancer from the top ten cancers with percentage about 7.48% and the incidence rate was about 4.29 depending on Iraqi cancer registry 2021. The etiology and pathogenesis can be related to environmental and occupational agents. The heavy smoking habit is threating which induces cellular transformation through a proinflammatory effect [2]. Chronic inflammation is a strong agent in cancer of urinary bladder. It gives different of mediators like, cytokines and chemokines, which enhance proliferation and angiogenesis of tumor cell. In addition, it releases mutation generating reactive oxygen species to stimulate changes in microenvironment of tumor cell favouriting its metastasis [3]. The transformation of normal urothelial cell to a neoplastic one occurs through complicated sequences of molecular proceedings termed as epithelial plasticity. At early stages, these alterations are reversible. This process is started with epithelial mesenchymal transition (EMT) transcription factors. The differentiation of mesenchymal stem cells can be into a variety of cell types involving endothelial cells, and fibroblasts which induce growth of autocrine [4]. The carcinogenesis of urothelium is molecularly classified into two different pathways resulting in two variant prognostic and morphological subtypes, that are non-muscle invasive (papillary) and the muscle-invasive types [5]. The non-invasive papillary carcinoma drives from normal urothelial as the proliferative proceedings; beginning with hyperplasia, then neoplasia of papillary urothelium; bladder cancer with non-muscle-invasive (NMI-BC) with a little percentage (10–15%) to the part of non-invasive (high-grade) and urothelial carcinoma (invasive). Although there is a little tendency for muscle-invasive, it has potential of recurrence. The primary changes of genes influence tyrosine kinase receptor, fibroblast growth factor receptor-3 (FGFR-3) that stimulates growth of cell [6]. On the other hand, most

Table 1. Demographic and clinical characteristics of studied groups

Variable	Subgroup	Absolut (n)	%
Age group (years)	Less than 67	20	50
	Over 67	20	50
Sex	Male	31	77.5
	Female	9	22.5
Grade	Low	22	55
	High	18	45
Muscle invasion	Present	13	32.5
	Absent	27	67.5

Table 2. Relationship among studied groups according to immunohistochemistry of GATA-3

		GATA 3		Total	P-value
		Positive (n=34)	Negative (n=6)		
Age group (years)	Less than 67	18 (90%)	2 (10%)	20(100%)	0.7
	Over 67	16 (80%)	4 (20%)	20(100%)	
Sex	Male	27 (87.1%)	4 (12.9%)	31(100%)	0.6
	Female	7 (77.8%)	2 (22.2%)	9(100%)	
Grade	Low	18 (81.8%)	4 (18.2%)	22(100%)	0.8
	High	16 (88.9%)	2 (11.1%)	18(100%)	
Muscle invasion	Present	11 (84.6%)	2 (15.4%)	13(100%)	0.9
	Absent	23 (85.2%)	4 (14.8%)	27(100%)	

of invasive carcinoma drives from multiple genetic alterations of tumor suppressor genes involving p53, p16, and Rb that makes a basis for series of disordered growth of tissue to carcinoma in situ (CIS) followed by non-invasive (high grade) to muscle-invasive urothelial carcinoma. Both types have frequent mutations, and primitive missing of heterozygosis that locates on the chromosome 9, that acts a means to yield irregularities of genes which enhances a development of changes in gene [7]. GATA3 (GATA binding protein 3) is one of six types of family of a zinc finger transcription factor, and it has a critical function in activating, controlling cell growth, maturation, and differentiation of numerous cell and tissues types [8, 9] involving luminal part of glandular epithelial cells of the mammary gland, T lymphocytes, thymocytes, kidney, sympathic nervous system and hair follicles of the skin [10, 11]. GATA3, defined as precise marker of urothelial carcinoma of bladder, and it shows a high expression in works of Yuk et al. [12], and Wang et al. [13]. In spite of GATA3 is appeared more in bladder carcinoma, its high appearance may unfavorably suppress development of malignant cells in bladder carcinoma [14]. While, more expression of GATA3 indicates a low tumor grade and stage but a high recurrence survival rate (free) [15, 16]. A loss of balance between more expression of GATA3 in tissues of bladder cancer and suppressing role of GATA3 in cells of bladder cancer may interpreted with capability of GATA3 to make changes in bladder cancer basal cells to luminal cells [15, 16]. These days, GATA3 was documented to occupy an important function in controlling an immune response (anti-cancer function) in tumor microenvironment (TME) [17, 18]. During embryogenesis, GATA-3 is included during maturation of kidney and urothelium collecting system [19]. Immunohistochemistry of GATA3 is employed in the comparison between carcinomas of urothelium from other cancer types in surgical pathology [20, 21]. GATA3 is advised as a powerful tumor suppressor protein in breast cancer; its loss is connected with many tumor charectestics, in addition to poor prognosis [22]. The correlation of GATA3 knockdown and cell migration, cell invasion, epithelial to mesenchymal transformation has seen during in vitro studies [23, 24]. GATA3 expression in urothelial carcinomas was joined with molecular subgroup of the luminal cells [25].

AIM

The aim of this research is to analyze expression levels of GATA-3 in bladder tumor tissues and to prove a relation between expression of GATA-3 and clinicopathological characteristics of bladder tumors, including patient age, sex, tumor grade, and muscle invasion.

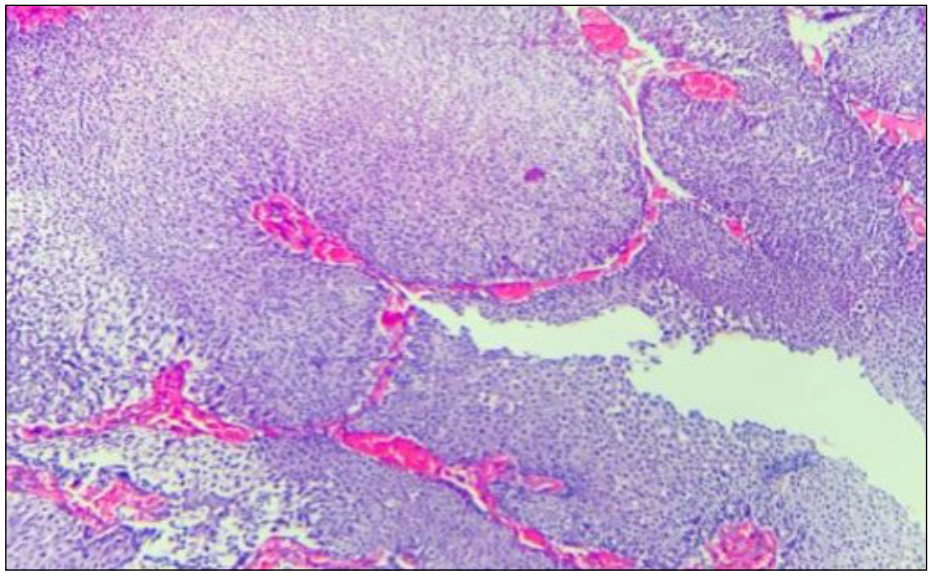

Fig. 1. Transurethral specimen resection shows low grade urothelial carcinoma; nuclei are smaller in size without invasion into lamina propria (H&E, Magnification power X100).

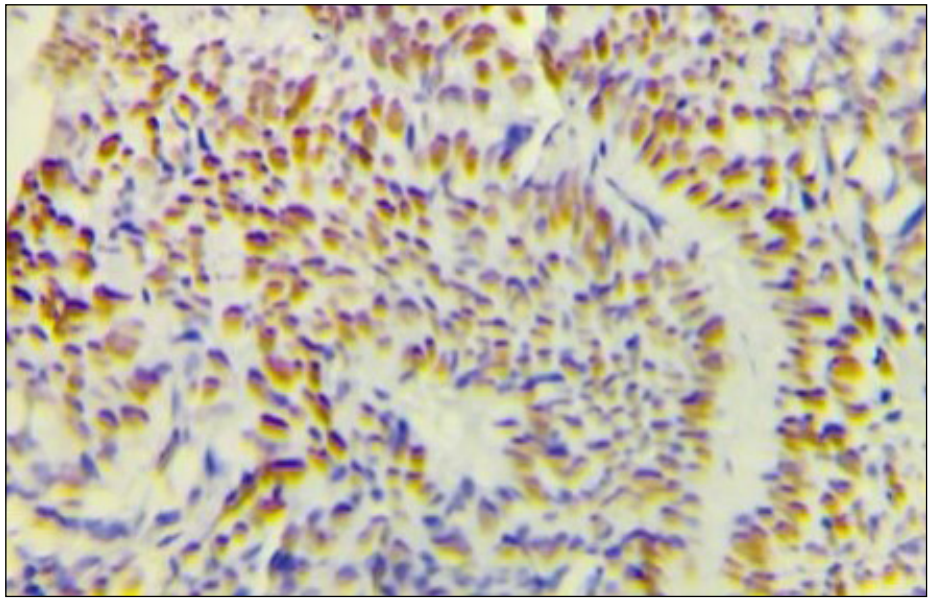

Fig. 2. Transurethral specimen resection of low grade urothelial carcinoma shows patchy nuclear staining for GATA-3 (immunohistochemistry) (Magnification power X400).

MATERIALS AND METHODS

A cross sectional study of 40 samples of Iraqi patients with urothelial carcinoma was completed at faculty of medicine- university of Kufa (middle Euphrates unit for cancer research). Formalin fixed paraffin embedded (FFPE) tissue blocks gathered by transurethral resection of bladder tumor are collected from teaching hospitals at Al-Najaf governorate, the diagnosis of urothelial carcinoma performed by histopathologist. The processing of immunohistochemistry (IHC) was performed by using positive charge slides. IHC staining protocol by labeled streptavidin biotin (LSAB) method was performed after de-paraffinization, heat induced antigen retrieval for 20 minutes, then blocking with peroxidase enzyme for 5 minutes, followed by primary antibody GATA-3 (Bio SB, clone: EP368). After incubation with primary antibody, the secondary antibody was added for 30 minutes followed by horse reddish peroxidase (HRP) for 30 minutes, and chromogen for 15 minutes. Each step washed twice with buffered solution. Finally, counter staining and mounting.

SCORING

Depending on the intensity of staining and the positive percentage, immunohistochemical evaluation of slides stained with GATA3 was scored. The score of intensity was determined as 0 (without stain), score one (simple stain), score two (moderate stain), score three (strong stain) divided by percentage score. Both scores will be assessed apart between the groups.

STATISTICAL ANALYSIS

To determine the relation between GATA-3 expression and urothelial carcinoma, a chi-square test was used along with

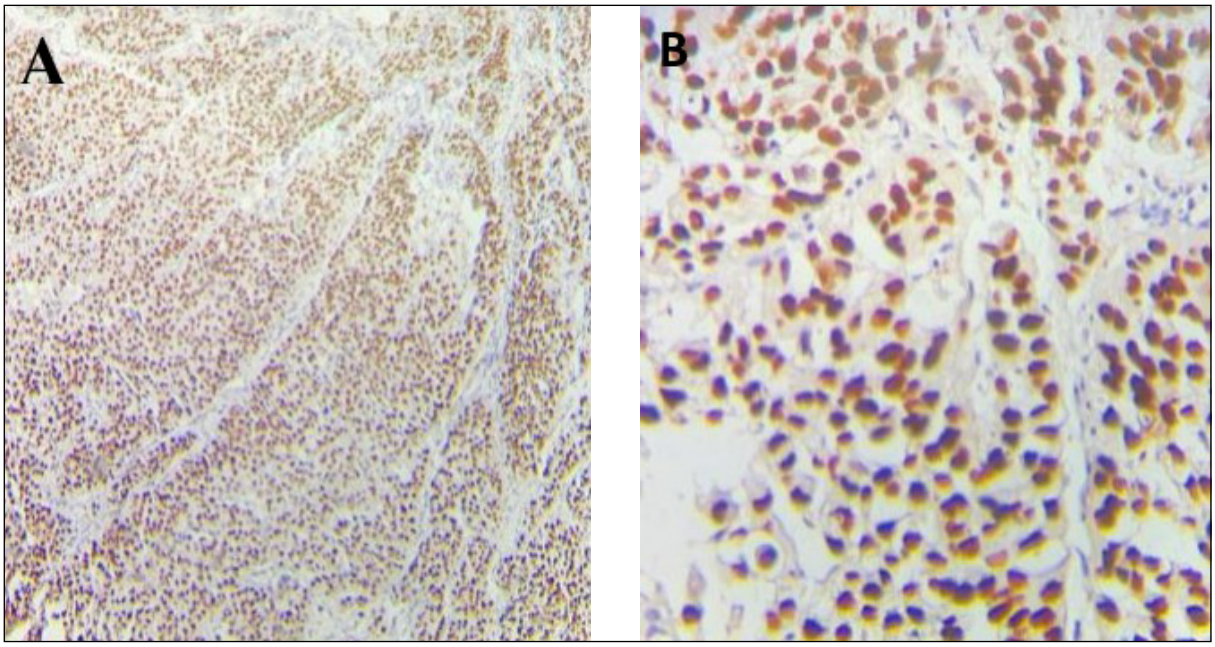

Fig. 3. Transurethral specimen resection of high grade urothelial carcinoma shows muscle invasion with diffuse strong nuclear staining (positive) for GATA-3 (immunohistochemistry) (Magnification power X100 (A), X400 (B)).

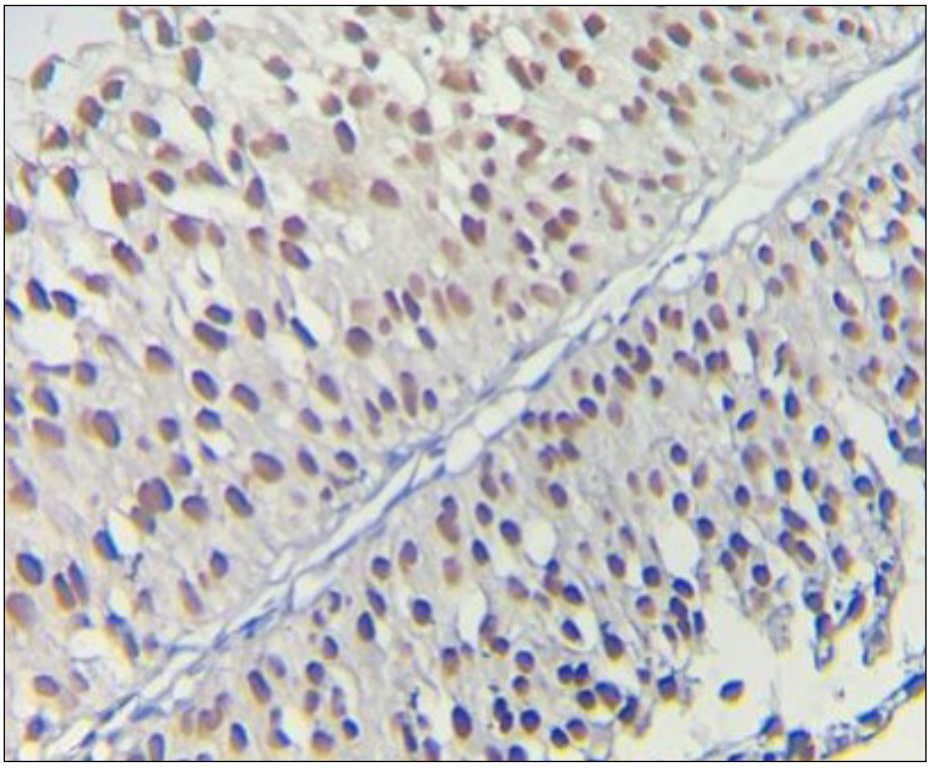

Fig. 4. Transurethral specimen resection of low grade urothelial carcinoma shows no muscle invasion with strongly positive expression for GATA-3 (immunohistochemistry) (Magnification power X100).

the SPSS program version 26, considering several variables: age, sex, histological grade, and muscle invasion. A P-value of 0.05 or less was considered statistically significant.

RESULTS

A forty FFPE tissue blocks of urothelial carcinoma involved in the present study, age categories of patients were less than 67 (50%) years and over 67 (50%) years. There was male predominance of 77. 5%. The microscopical study of these samples was made by using GATA-3 tumor marker test. GATA-3 test was positive in 34 samples of urothelial carcinoma, while GATA-3 test was negative in 21 samples of urothelial carcinoma, details of subject and their numbers are shown in table 1, table 2.

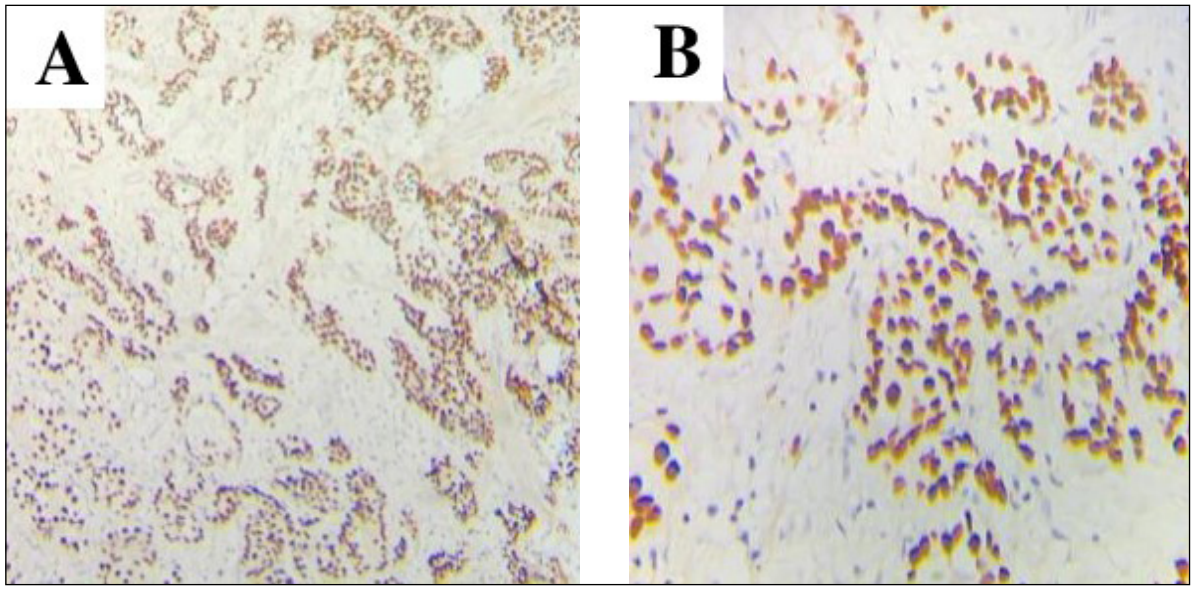

Fig. 5. Transurethral specimen resection of high grade urothelial carcinoma shows strong nuclear staining(positive) for GATA-3 (immunohistochemistry) (Magnification power X100 (A), X400 (B)).

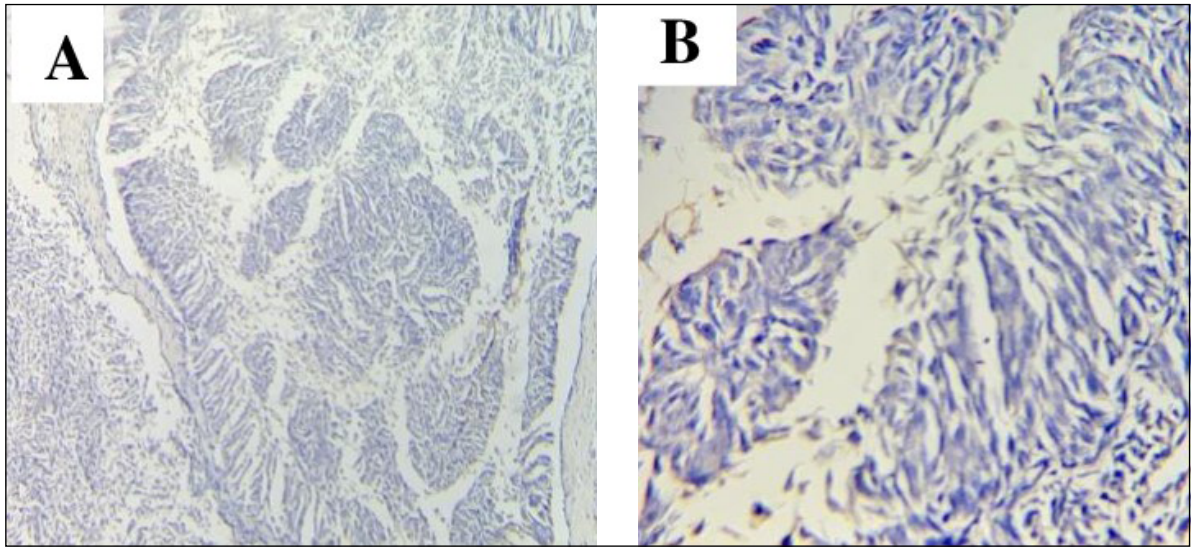

Fig. 6. Transurethral specimen resection of high grade urothelial carcinoma shows negative expression for GATA-3. (immunohistochemistry) (Magnification power X100 (A), X400 (B)).

Table 1 shows that the age category was less than 67 by about 50% and more than 67 by about 50%. The majority of gender group was at the male categories of 77.5% more than females of 22.5%.

Table 2 shows that most cases were positive for GATA-3 among patients suffering from urothelial carcinoma. Regarding to age, sex, grade, muscle invasion categories, GATA-3 was positive in age group less than 67 by about 90% (P-value=0.7), positive among males by about 87.1% more than females (P-value=0.6), positive in in high grade group by about 88.9% (P-value=0.8), and positive in the group where was absence of muscle invasion by about 85.2% (P-value= 0.9).

DISCUSSION

The present study was a fundamental step completed at the Faculty of Medicine, University of Kufa, Iraq, in the Middle Euphrates Unit for Cancer Research, to establish the relationship between GATA-3 and urothelial carcinoma. In the current study, the higher positivity of GATA-3 was at age categories less than 67 years of 90%, with male was more than female – 87.1% to 77.8% respectively (with no significant difference), and this variation of GATA-3 expression in gender category may reflect the characteristics of molecular and biology of bladder tumors, which was comparable with the observation made by Gupta et al [26] in the Indian subcontinent.

We also found clustering of patients over 60 years, like many other studies and four patients less than 40 years, however, no

significant relation of GATA-3 expression with demographic factors, including age, gender. Similar GATA-3 positivity (90%) was seen in approximation with other studies of Liu et al. [27], who found 86% of GATA-3 positivity and Leivo et al. [28] with 99% of positive GATA-3 expression. Regarding to grade of tumor, low grade urothelial carcinoma (Fig.1, Fig.2) showed high GATA-3 positivity of 81.8% in comparison with high grade tumor (Fig.3-5.) of 88,7% without significant difference.

This suggests that GATA-3 has an important role in maintenance and differentiation of normal tissue of urothelium. On the other hand, the same results were achieved by Taber et al. [29] and Kollberg et al. [30], who noted that the most of low grade tumors have moderate positivity. Relating to muscle invasion, GATA-3 expression rely on muscle invasion presence or absence. In our study, the difference between invasive urothelial carcinoma (84,6%) and non-invasive urothelial carcinoma (Fig.6.) (85.2%) was non-significant. Our results were agreed with study by Daeseon Yoo who proved that the relation between muscle invasion and non-muscle invasion urothelial carcinoma was non-significant [31, 32]. Lin et al. [33] analyzed GATA-3 expression changes in high and low grade urothelial carcinoma. They detected that GATA-3 expression downregulation in high grade (77.2%) in comparison with low grade (87.5%), which agreed with our results, GATA-3 downregulated in high grade (11.1%) compared to low grade (18.2%).

CONCLUSIONS

GATA-3 is an immunohistochemical sensitive marker to diagnose urothelial carcinoma. GATA-3 expression showed non-significant relation with age, gender and histopathological parameters and its expression has been observed to be lost or reduced in substantial proportion in relation to urothelial carcinoma. This alteration or down regulation of GATA-3 is correlated with higher tumor grade and stage.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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