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Prognostic value of FOXA1 expression in urinary bladder cancer

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Abstract:

The aim of currentstudy was to determine the expression of the FOXA1 protein in human bladder cancer, and demonstration of correlations between this parameters and clinical pathologic variables as grade and stage of tumour,non muscle invasive tumour and also by using this marker can classify bladder cancer in to basal and luminal type. The Current study is designed to detect the role of FOXA1expression in bladder carcinoma as a possible marker for detecting the biological behaviour of malignancy and its correlation with grade and muscle invasiveness for both diagnostic and prognostic purposes. The Current study focuses on a technique of immunohistochemistry for detection FOXA1expression in bladder cancer. The samples are collected randomly in southern Iraq in AL-Nasiriya city from AL Hussein teaching hospital. Number of samples is (100) of bladder tissue, (70)of bladder cancer tissue and (30) controls benign tissue. Results of this study reveal that FOXA1 expression is positive in(29) out of (70) sample. The study approves that FOXA1 expressed was increased in low grade bladder cancer(63.6%), FOXA1 expression high in the early tumour stage as (Ta) stage (94.7%), FOXA1 expression was high in non-muscle invasive (85.7%) ,FOXA1 has also shown an increase in male with bladder cancer represent (51.1).

So FOXA1can be used as a marker for assessment of bladder cancer aggressiveness. This study represents an important first step in Thi-qar because there are absence of a studies about this topic which to highlights this tumour and divides in to two types luminal and basal .

KeywordsBladder cancer, FOXA1, Immunohistochemistry**:**

Introduction

One of the most prevalent cancers of the urinary tract, bladder cancer (BCa) is expected to cause (17,980) deaths and(80,000) new diagnoses globally in 2020, invasive bladder cancer has mutation histological variants . The conventional urothelial carcinoma also known as transitional cell carcinoma accounts for the majority of invasive bladder malignancies [1, 2]. Bladder cancer is a disease in which cells lining the bladder lose the ability to regulate their growth and begin divideun controlled this

abnormal growth results in a mass of cells that forms tumor [3]. Bladder cancer also called to urological cancer has been defined increasing occurrence particularly in advanced countries [4].

Bladder cancer is the most common pathological conditions among tumors of the urinary system that require a combination of several techniques and immunological tests to reach the type of tumor or stage tumor and despite the development of pioneering techniques in the field of medicine the exact diagnosis of the case has not been made without relying on taking a biopsy from bladder tissue [5]. Types of bladder cancer included Transitional cell carcinoma (TCC) is the most common type about (97%) followed by squamous cell carcinoma (SCC) about (2%) and the lowest frequency is adenocarcinoma as (1%) [6].In Iraq, bladder cancer is the fifth of ten cancers, the third cancer in men and the eighth in women. 866 men were diagnosed with bladder cancer, compared to (297) women, according to the Iraqi Cancer Registry[7]. As for the year 2021, it will be ranked fifth among other most common cancers, with an infection rate of 1,769, as the number of male infections is 1,360, while females are 409, and this indicates that male infections are higher than females[8].

Generally urothelial carcinoma was classified in to (NMIBC) non-muscle-invasive bladder cancer and (MINBC) muscle-invasive bladder cancer according to the invasion of bladder wall [9]. While(NMIBC) has a low risk of distant metastasis, (MIBC) it is more aggressive and metastasis of cancer cells to other organs . (MIBC) requiring radical cystectomy with chemotherapy [10,11]. Despite of complete surgery and adjuvant treatment the five years of survival in MIBC is nearly 36% [12]. The FOXA1protein (fork head box protein A1), known also as (hepatocyte nuclear factor 3α)is transcription factor that play an important role in gene regulation , it has role in controlling the functions of the liver ,renal and pancreas. In addition to regulating a variety of tissues through experiments in embryonic development, have revealed a specific role for FOXA1 in the development of the gland mammary and prostate activity of the nuclear hormone receptors ERα and AR, the FOXA1 modulates estrogen receptors and androgen receptors function in breast and prostate carcinoma in addition to the function of FOXA1protine in various aspects of cancer cells development, FoxA1 is important in regulating the formation and development of low tumors, a type of tumor that grows slowly and it has no ability to mutate and metastasize, FoxA1 regulates the expression of growth and cell division genes in cancer cells and foxa1 reacts with sex hormones and other growth factors to increase the process of cell division and the proliferation of cancer cells in the bladder, So it has a role in the formation and development of carcinoma tumors and it involved in gene regulation processes by association with DNA [13]. FOXA1 pioneer factor that stimulates the conformation of chromatin opening to allow binding of other transcription factors through direct interactions withandrogen receptors (AR), and helps to form androgen receptor signals that stimulate the growth and survival of prostate cancer cells and normal prostate . In addition, it has an (AR) independent role of regulate epithelial to mesenchymel transition . In prostate cancer, The mutations converge with the coding sequence and binding regulation elements of FOXA1, which leads to functional alterations . In addition, the activity of FOXA1 in prostate cancer can be modified post translationally through various mechanisms such as protein demethylation by LSD1, Thus , FOXA1 have an important role in the development of prostate cancer [14]. The FOXA1 protein is expressed in the liver, pancreas, lung, colon, bladder and is able to bind more stimuli than a thousand genes involved in regulating signal transmission within cells and the cell cycle [15].

Materials and methods

Collection of Samples

Samplesinclude (100)of bladder tissue formalin fixed,parafen embedded blocks, it divided into two groups: (70) samples (patients group) of bladder cancer tissues and (30) samples (control group) of benign bladder lesion .The age ranges is between (20-80) years old. All samples were collected from Al-Hussein educational hospital in Thi-Qar province during the period between Juluy 2022 and February 2023.Samples were fixed in (10%) formalin when starting of collection and room temperature for histopathological and immunohistochemically analysis. The Patient data are collected either immediate from the patients as a questioner or from the histopathological data. Patients samples were divided according to T stages tumour (Ta- T1-T2-T3-T4) and tumour grade to high grade and low grade as in Figures (1,2).

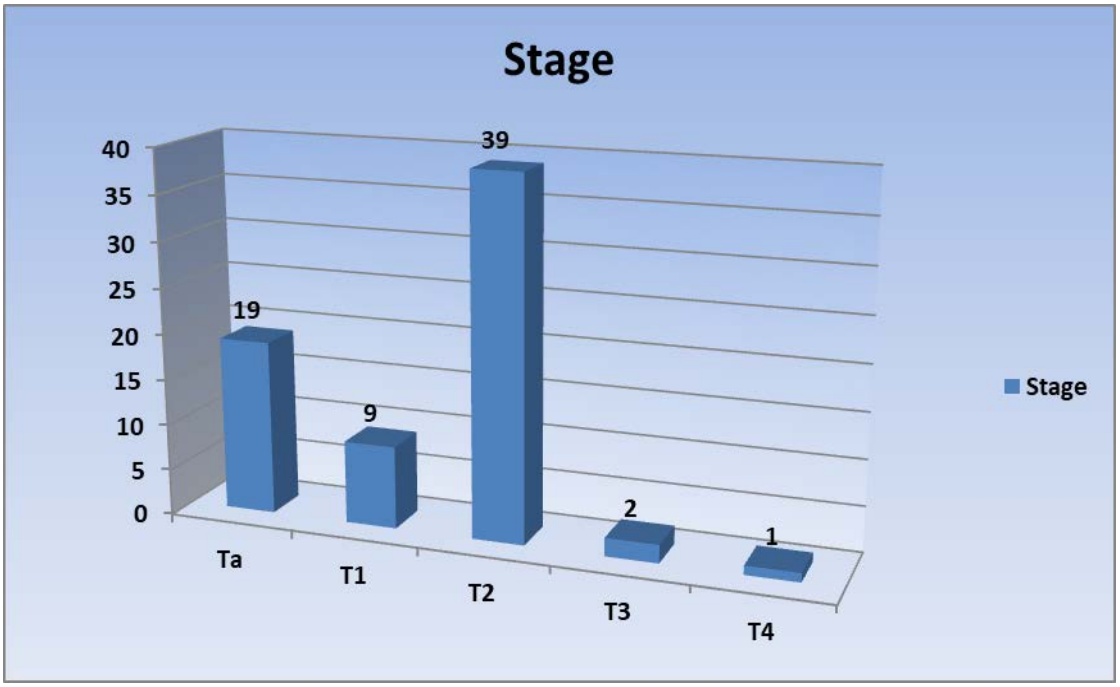
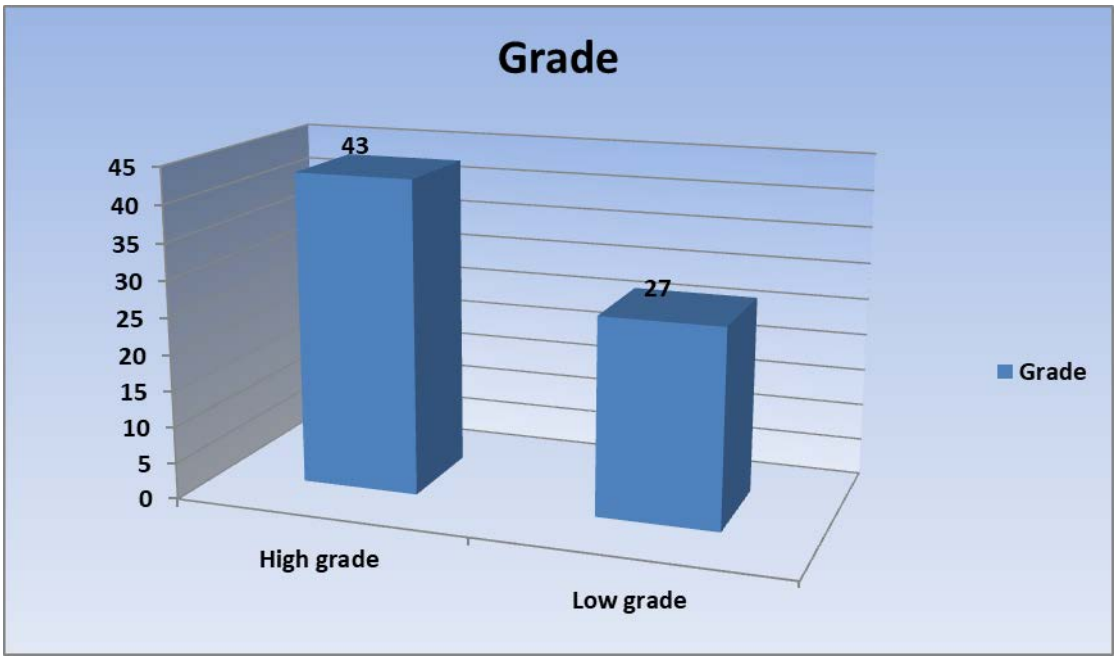


Figure (1)patient samples according to T stages of tumour .



Figure(2) patient samples according totumour grades .

Immunohistochemistry assay (IHC) :

The immunohistochimical technique has been performed on(FoxA1) biomarker rendering .For are samples, sections of tissues were 4micron thickness paraffin embedded that arranged on a slides positive charge then exposed to(IHC)technique.

The first step is the Paraffin wax was removed by immersing tissue slides in xylene solution twice for (5) minutes each time, and then is rehydratedto the tissue using a series of ethanol alcohol in a descending concentration (100%-90%-70%), respectively, for two minutes for each.

Antigen retrieval solution with a (pH6) was used so that the antibodies could bind with the antigens present in the tissue cells for 30 minutes , and then the slides were washed with a buffer solution twice for (5) minutes each time. Peroxidase blocking drops were added to the slides to remove the activity of the internal peroxide of the tissue. for five minutes at room temperature, it washed twice with a buffer solution for (5) minutes each, so that the slides are ready for staining using the immunohistochemistry staining kit IHC, also the F0XA1 primary antibody was added to the slides and left for a (1 hour) at room temperature, then the slides were washed using buffer solution twice for 5 minutes each. Poly excel target binder solution was added to the slides and left for 15 minutes, then they were washed with buffer solution twice for 5 minutes. Each time, Poly excel HRP secondary antibody solution was added to the slides for (15) minutes, then washed with a buffer solution twice for (5) minutes each time, DAB (substrate chromogen solution) was added to the slides and left for 5 minutes and then the slides were washed with distilled water for (5) minutes, the slides were placed in a haematoxylin dye solution for one minute and then washed with running water for three minutes, the fluids were withdrawn from the tissues using a series of ascending alcohol concentrations (70%-90%-100%) for a minute for each concentration, then the slides were placed in xylene for two minutes, and

finally drops of DPX are added to the tissue slides and covered with a cover slide[16] with some changes .

Statistical analysis

The data has been analyzed by the statistical package for available from SPSS percentages are used to display the data. The Pearson Chi-square test is used to determine the significance of the differences between significant qualitative data.Whenever the P-value < 0.05,is considered significance .

Results

The results of the current study in table(1) showed the positive expression of FoxA1 that (29) cases out of (70) patients with bladder cancer have an increased in the FOXA1expression (41.4%.). positive FOXA1 expression is high associated with the stage (Ta) represents (18)case out of (29)positive expression of FOXA1 , and also showed decrease expression of FOXA1 in other tumour stages such as (7) cases in the T1stage,(3) in T2 stage, (1)case in T3 stage, while FOXA1expression was loss in advanced stage T4. The results of our study in table (2)showedThat FOXA1positive expression increased in low-grade urothelail carcinoma in (21) out of (29) positive FOXA1, whereas FoxA1 expression showed a decreases in high grade in (8) cases out of (29)positive expression FOXA1.The results explained in table (3)FOXA1 expression was high in non-muscle-invasive in (24) out of(28) positive FOXA1, while expression was decreased in muscle-invasive(4) cases.The study showed that positive FOXA1 expression was high in males with urothelial carcinoma represent (23) out of (29), in females a decrease in FOXA1 expression was observed (6) cases.

The finding of the study shows the positive FoxA1 expression is for luminal subtype of bladder cancer represent (29),The negative FOXA1 expression is for basal subtype (41) as in Figure(3).

Table [1]: Correlation between FOXA1 expression and T stages of bladder cancer.

Clinic pathological variables Tumor Stages	Positive expression of FOXA1	%	Negative expression of FOXA1	%	Total
Ta	18	74.2	1	25.8	19
T1	7	23.8	2	76.2	9
T2	3	25.0	36	75.0	39
T3	1	25.0	1	75.0	2
T4	0	0.0	1	100.0	1
Total	29	41.4%	41	58.6%	70

Cal.X^2: 18.64 df: 4 P-value 0.001

Table [2] : Correlation between FOXA1 expression and tumor grades.

Clinic pathologic varibles.tumour grades	Positive expression of FOXA1	%	Negative expression of FOXA1	%	Total
High	8	21.6	29	78.4	37
Low	21	63.6	12	36.4	33
Total	29	41.4	41	58.6	70

Cal.X^2: 12.68 df: 1 P-value 0.001

Table[3]correlation between FOXA1 expression and non-muscle invasive type .

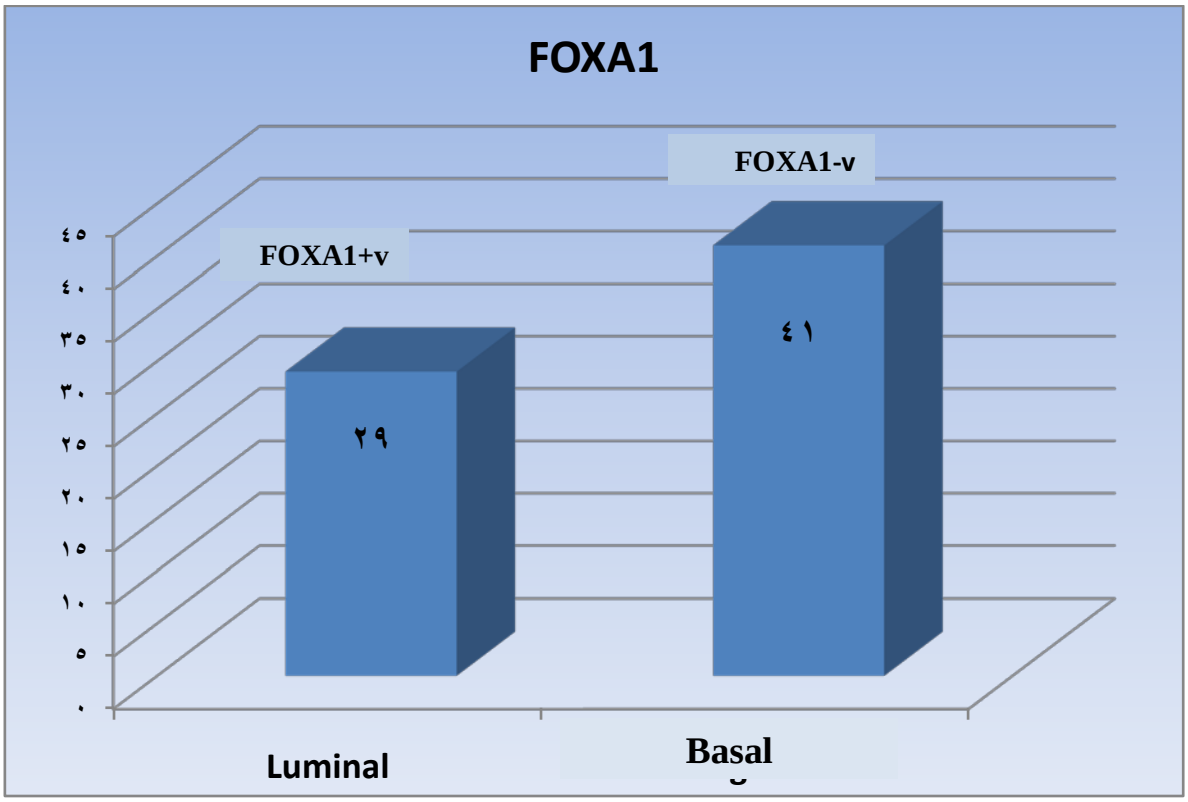
Clinic pathologic variables. Type tumor	FOXA1 expression positive	%	FOXA1 expression negative	%	Total
Muscle-invasive bladder cancer	4	9.5%	38	90.5%	42
Non-muscle invasive bladder cancer	24	85.7%	4	14.8%	28
Total	29	41.4%	41	58.6%	70

Cal.X^2:40.63 df: 1 P-value 0.02

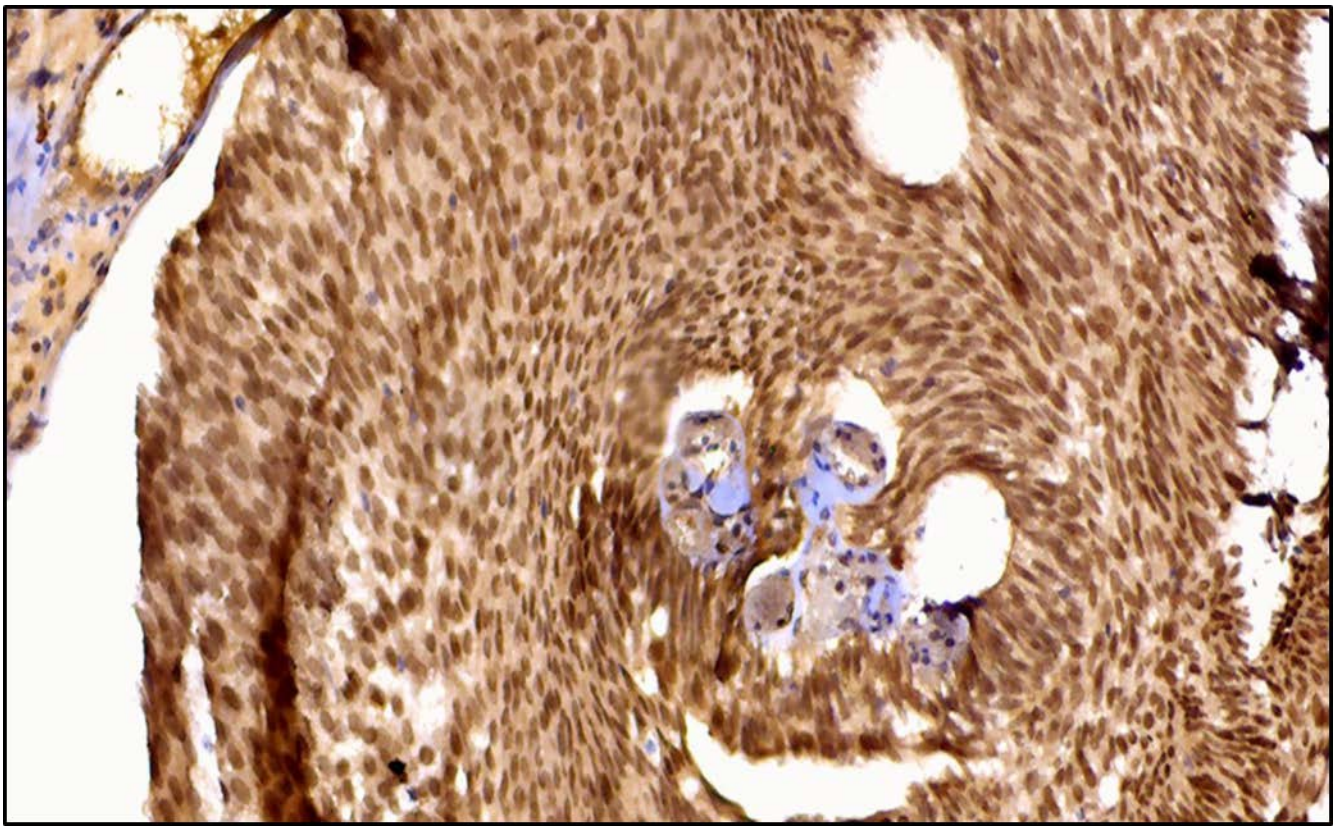
Table [4]: **correlation between FOXA1 expression and sex**

Sex	Positive expression of FOXA1	%	Negative expression of FOXA1	%	Total
Male	23	51.1%	22	48.9%	45
Female	6	48.0%	19	52.0%	25
Total	29	41.4%	41	58.6%	70

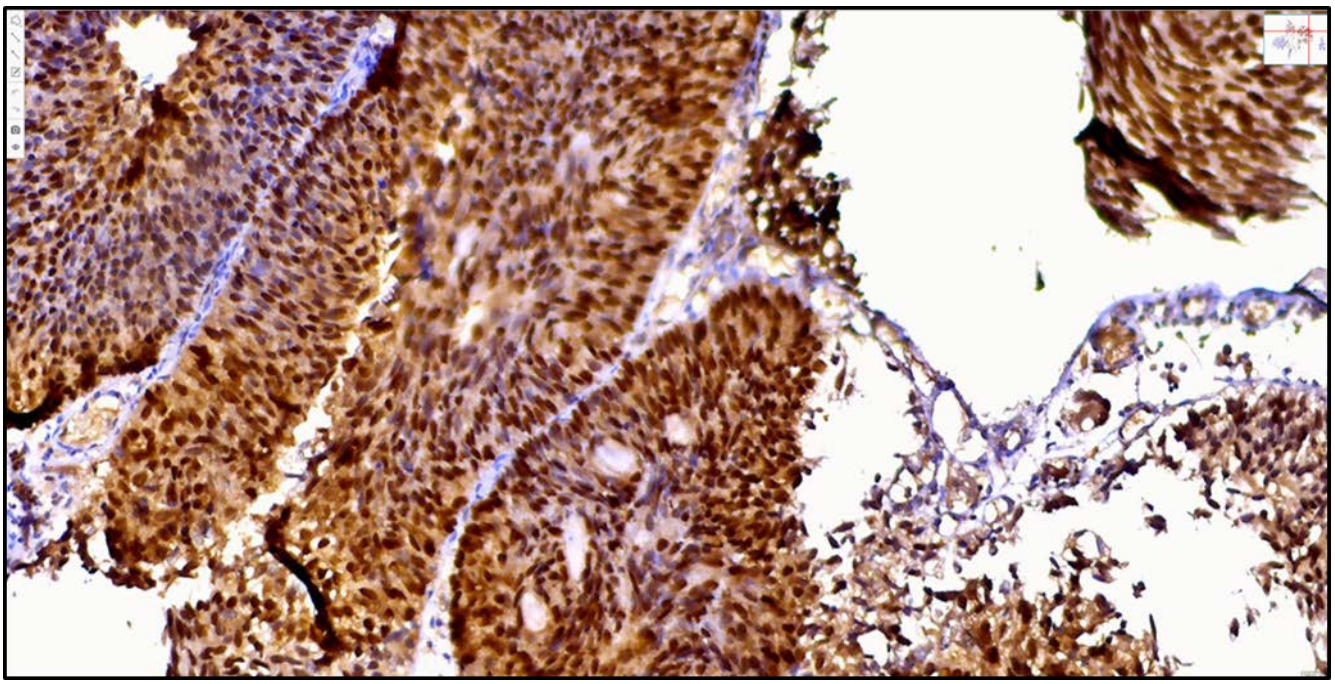
Cal.X^2: 0.06 df: 1 P- value 0.001



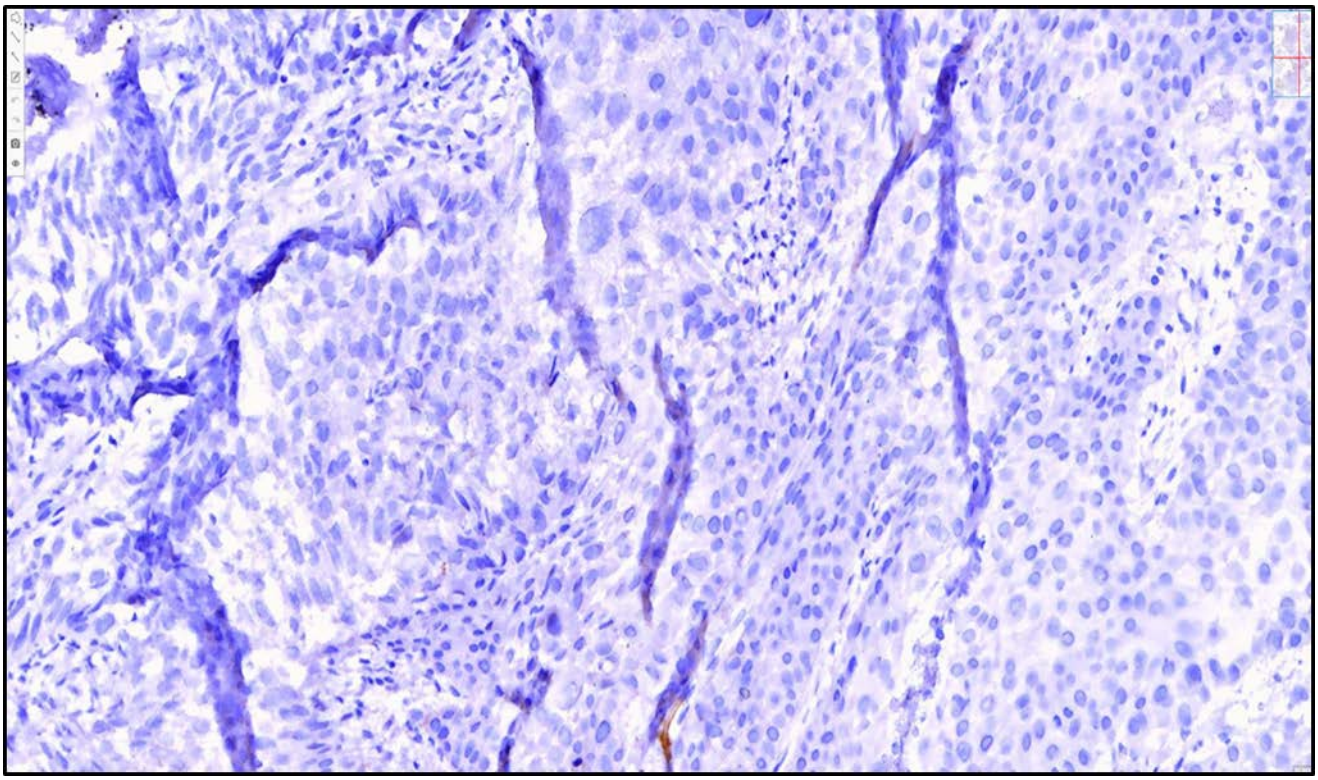
Figure(3) subtypes of bladder cancer



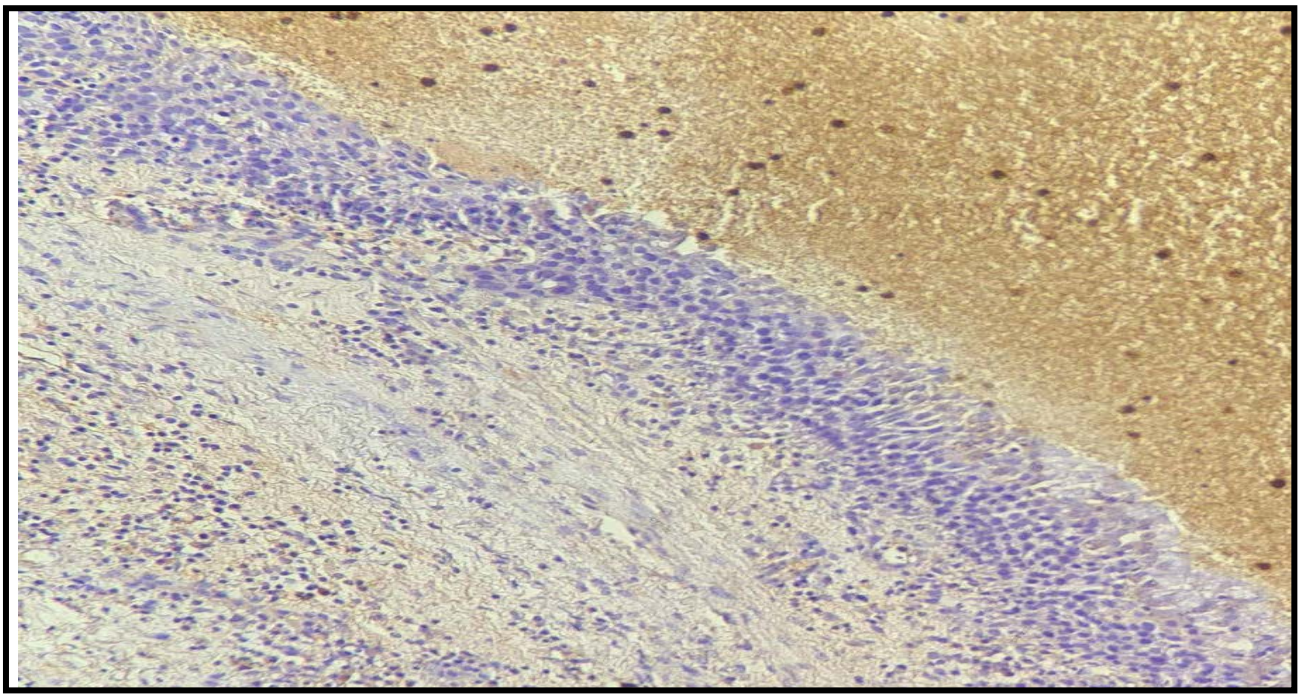
Figure(1):high grade invasive urothelial carcinoma shows positive nucleus staining for FOXA1 (400X)(H& DAP) .



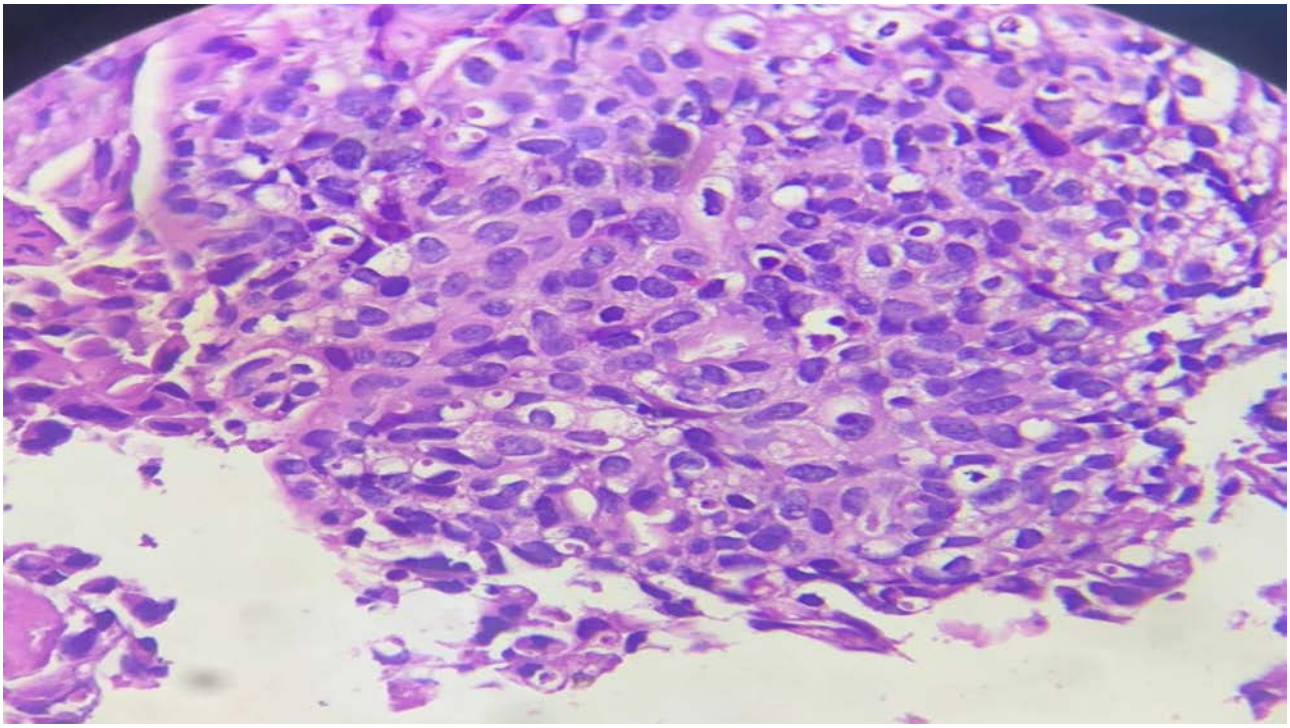
Figure(5) Low grade non invasive papillary urothelial carcinoma show positive nuclear staining for FOXA1power(200X)) stain (H & DAP)



Figure(6) Low grade invasive squmaus cells carcinoma showednegativestaining for FOXA1(400X)(H & DAP)



Figure(7) normal bladder mucosa (400X)(H & DAP)



Figure(8) High grade urothelial carcinoma (400X)(H &E)

Discussion

The results of the current study showed that bladder tumors with a high expression of FoxA1 are associated with the tumour stage Ta and have lowe progression rates compared to lowe expression of FoxA1 in advanced stages,As the tumour stage increases the expression FoxA1 decreases, the expression FoxA1 is lost in most advanced tumour such as T4.The reason for the association of FOXA1 in Ta stage, that it expresses the presence of a carcinoma tumour that does non invasion the muscles of the bladder wall to the role of FOXA1 in regulating the process of cancer cell proliferation and .,This marker has a role in regulating the activity of genes associated with bladder cancer, and increased expression of FOXA1 in the Ta stage, in which cancer cells are non-aggressive in their behavior, and the cancer has not metastasis to the bladder wall, divides slowly compared to other advanced stages , their more aggressive in invading the bladder muscles it divides quickly, increases the possibility of the tumour metastatic to other parts of the body. Therefore, FOXA1 is used as a prognostic factor for differentiating cancers of low severity. The results of our study agreed with the study of [17] Showed the high expression of FOXA1 was associated with the Ta stage(93%) and the low expression of FOXA1 with increase in the T1,T2,T3 stages and the loss of expression of FOXA1 was also in the advanced T4 stage.

The results of present study showed FOXA1 expression increased in low-grade (63.6) while FOXA1 expression showed a decrease in high-grade (21.6), It is possible the reason for the high expression FOXA1 associated with low grade bladder cancer because FOXA1 is considered one of the most important genetic factors for the development of bladder cancer, as it a role in the process of gene activation and controlling the growth of cells,thinks its contribute to inhibiting the growth of cancer cells , FOXA1 can stimulate differentiation of cancer cells into normal cells which reduces tumour grade, and high expression of FOXA1 in cancer cells limits thecancerability of to metastasise and proliferate

leading to a low grade and may be important for development of new treatment strategies for bladder cancer. Our results are consistent with the study[18] Showed that FOXA1 expression significant high with low grade in (87%) of patients with bladder cancer.

The results showed that FOXA1 expression was high in non-muscle-invasive bladder cancer represent (85.7%), while expression was decreased in muscle-invasive bladder cancer (9.5). maybe that high expression of FOXA1 has an important role in regulation of genes associated with cellular differentiation , When a stop occurs in the FOXA1 expression it can lead to a disorder in the cellular differentiation of cancer cells, thereby increasing of the possibility invasive of the bladder muscles, and thus can contribute to the metastasize and development of bladder cancer, as for muscle invasive bladder cancer, as it quickly invading the bladder muscles, the reason for the decrease in FOXA1 expression in this type of bladder cancer may be due to the role of FOXA1 in inhibiting the growth and division of cancer cells, and therefore when the expression of FOXA1 protein decreases cancer cells can grow and divide more rapidly, thus growth of bladder cancer occurs.

The current study explained that positive FOXA1 expression was high in males with urothelial carcinoma by (51.1%), in females a decrease in FOXA1 expression was observed. In this study identified some causes that increase the risk of bladder cancer and involves the gender of the patient , found that bladder cancer is more common in male than females . The reason of high FOXA1 expression was association with bladder cancer in male may be attributed to the fact the hormonal difference between male and female, FOXAI it reacts directly to testosterone in cancer cells, and its high in male, and studies revealed an important relation between FOXA1 and gender due that FOXA1 may be correlated with hormones responsible for the growth and development of cancer cells in the bladder,So it is a signaling factor for nuclear steroid receptors, which are among the most important hormones in the regulation and growth of body cells FOXA1 contributes significantly to bladder cancer phenotypes.[19]. And the reason for the association of high FOXA1 expression with affected males may not be fully known, but it may be attributed to the percentage of males exposure to risk factors such as smoking and exposure to toxic chemicals which may increase risk of bladder cancer. The incidence of bladder cancer between males and females is due to differences in the activity of sex steroid hormones levels, study is similar to the study conducted by the researcher [17] The study showed that determine the functional importance of FOXA1 in bladder cancer. that high FOXA1 expression was associated with tumours in males with a rate of (54%), and a decrease in the expression of FOXA1 was also observed in cases of female by (36%).

Conclusions:

1- We can benefit fromthe excessive expression of FoxA1 to expect tumorbehaviour,progressionorprogrossionofdiseas.

2- it is also may be of benefit to determine the type of treatment that used to treat bladder cancer .

3-Marker is generally used to classify bladder cancer in to luminal and basal type .

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