Study of Hypoxia inducible factor -1α and CD34 as angiogenic factors in bladder cancer

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Abstract - HIF-1 has emerged as an attractive target for cancer therapy few publications advertising the hypoxic inducing factor -1 (HIF-1α).
A variety of markers for identification of microvessels in histological sections are available, CD34 is one of the most commonly used factors. Our study was designed to investigate HIF-1 in urine using Enzyme-linked immunosorbent assay (ELISA) technique and CD34 in tumor tissues by immunohistochemistry method. Methods: Samples were collected from thirty nine malignant patients, fifteen benign patients, and fifteen as controls. Urine samples, pathological samples from parafine embedded tissues were included. HIF-1 was detected using ELISA in urine pellete and CD34 was detected in immunohistochemistry in parafine sections. Results: There was a statistically significant difference between benign and malignant groups regarding HIF-1 level but there was no significant difference between stages or grades of malignant samples as regard HIF-1 activity and CD34 level. Conclusion: HIF-1 is expressed in all histopathological types of bladder cancer and Cd34 was expressed in different tumor tissues.

I. INTRODUCTION

Bladder cancer is the forth most incident cancer in males and ninth most incident in females, in The United States over 67,000 new cases are diagnosed per year (Jemal et al., 2007) and over 350,000 cases diagnosed worldwide (ferlay et al., 2007).
Bladder cancer is the most prevalent malignancy among Egyptian males (16%), producing more than 7900 deaths annually, which is strikingly higher than most other parts of the world (Khalid , 2005). In Egypt, where bilharziasis is endemic, bladder cancer is the commonest cancer in males and the 2nd in females (Zarzour et al., 2008). It is well known that the level of Schistosoma hematobiom infection prevalence in Egypt had decreased from 38% in 1980 to 3% in 2003 (Mohamed et al., 2008).
In Egypt over the past 26 years: The relatively frequency of Transitional Cell Carcinoma (TCC) increased from 22% in 1980 to 73% of bladder diagnoses in 2005, while Squamous Cell Carcinoma (SCC) decreased from 78% of diagnosed bladder tumors in 1980 to 27% of diagnosed bladder tumors (Ashley, et al., 2008).
Active smoking is now the strongest environmental risk for bladder cancer contributing to more than 50% of cases (James, 2008). The prevalence of cigarette smoking was reported to be increasing in the Egyptian males with time reaching up to 35% compared to 25.7% in the American males (Mohamed et al., 2008). People who smoke are estimated to have 2 to 4 fold greater risk of developing bladder cancer than non smokers. There are many studies of how tobacco carcinogens lead to genetic and molecular alterations. (Jian et al., 2008).
Various growth factors and molecules have been reported to be associated with tumor growth, progression, and survival in bladder cancer. Among these factors, tumor microvessel density (MVD) is thought to be one of the most useful prognostic markers for disease development, grade, recurrence-free survival, and overall survival (Miyata et al., 2004).
Angiogenesis is the formation of new blood vessels from the endothelium of the existing vasculature (Eichhorn et al., 2007). Angiogenesis is a complex process.
angiogenic switch occurs during proliferation of endothelial cells as they organize to form new blood vessels. What determines whether the switch is on or off are the levels of angiogenic inhibitors compared to the angiogenic activators (Frank, 2002). Blood vessels within a given tumor are heterogeneous and are subject to influences by the local microenvironment and cytokines (such as basic fibroblast growth factors, interleukin-8, and interferon) that are produced by tumor cells. However, distinct differences exist between blood vessels undergoing vasculogenesis versus sprouting angiogenesis, between blood vessels differentiating into arteries or veins, between blood vessels in different tissue beds, between the vasculature of different within a single tumor (Frank, 2002).

Two distinct pathways in bladder tumorigenesis are recognized, with one leading to the genesis of superficial tumors, and the other leading to more aggressive neoplasm. Although frequent recurrences in noninvasive tumors pose a therapeutic challenge, invasive tumors represent the major cause of morbidity and mortality. The last decade has witnessed the discovery and validation of a large number of markers involved in pathways of bladder tumorigenesis, many of which have shown to be important prognostic and therapeutic response indicators and potential therapeutic targets. Although the discovery and analysis of newer markers continue, analysis of several validated markers in combination is also required to devise molecular panels that can more faithfully predict Urithral Carcinoma prognosis (Mitra et al., 2006).

The angiogenic activity of tumor tissue is considered to have prognostic value and microvessel density (MVD), a measure of tumor angiogenesis, correlates with outcome in many human tumors. The angiogenic phenotype is considered to be a key step in early tumor progression, which allows the tumor to transform from a microscopic lesion to a rapidly expanding mass with metastasis thread. Oncogene-driven protein expression as well as a number of cellular stress factors, such as hypoxia, low PH, nutrient deprivation, or inducers of reactive oxygen species, are important stimuli of angiogenic signaling (Bergers and Benjamin, 2003). A variety of markers for identification of microvessels in histological sections are available, CD34 is one of the most commonly used factors (Wang et al., 1993).

Recent evidence indicates that low oxygen tension or hypoxia controls the differentiation of several cell types during development. Variations of oxygen tension are mediated through the hypoxia-inducible factor (HIF), a crucial mediator of the adaptive response of cells to hypoxia. (Heinis et al., 2010).

The presence of hypoxic lesions in solid tumors associated with a more aggressive tumor phenotype, resistance to radiation therapy and chemotherapy, and poor survival. (Pouyssegur et al., 2006). Hypoxia-inducible factor-1 (HIF-1) is a key transcription factor that regulates the cellular response to hypoxia. HIF-1 transactivates a large number of genes that are related to angiogenesis, erythropoisis, cell adhesion and glucose transport. (Junichi et al., 2008). HIF-1 has emerged as an attractive target for cancer therapy (Alexandros et al., 2008). A variety of markers for identification of microvessels in histological sections are available, CD34 is one of the most commonly used factors (Wang et al., 1993).

The aim of this work is to investigate its possible use of the urinary HIF-1 as a biological marker and quantitation of CD34 expression using immunohistochemistry as a means of assessing microvessel density (MVD) density in urinary bladder cancer patients, which may reflect the role as a prognostic factors in relation to different stages and grades.

Subject and Methods: This study was conducted at Early Cancer detection Unit, Ain Shams University Faculty of Medicine, during 2008-2009 and included 39 patients presenting with a Urinary bladder malignant tumors, 15 Benign bladder patients and 15 Controls. Controls were patients admitted for a wide spectrum of acute, non-neoplastic conditions to
the same network of hospitals and resident in
the same geographic area.
1- The study groups were subjected to
complete laboratory investigations.
2-Cystoscopic examinstion and biopsy was
taken from malignant and benign groups
suspected staged and examined by
histopathologist. Bilharzial antibodies were
measured in sera of malignant and benign
groups.
3-Urine investigation was done using the pellet
after discarding the supernatant into a separate
tube. The pellet was washed using phosphate
buffer solution PH 7.4 by adding 5 ml pBs
centrifuge at 4000 rpm minutes the supernatant
was discarded protease inhibitor was added to
the pellet then devided into four aliquots and
then preserved into the supernatant and were
stored at (-80 ºC).
Part of each pellet was applied on a slide before
adding the protease inhibitor, drove fixed with
95% ethanol, dried in air, stained by
papanicolaous stain, and sent to the pathologist
for cytological examination to detect the
presence of schistosomal ova and malignant
cells.
Determination of protein concentration into the
urine pellet:
4- Quantitative detection of HIF-1α in
urine pellets by R and D systems
immunoassay.
Principle of the assay: This survey
immunoassay employs a two site sandwich
ELISA to quantitate HIF-1 α in cellular
extracts. An antibody specific for HIF-1α has
been pre-coated onto a microplate. Standard
and samples are added and HIF-1α present in
bound by immobilized antibody. After washing
away unbound material, a biotinylated
detection antibody recognizing HIF-1α is used
to detect HIF-1α utilizing standard strept avidin
– HRP formed substrate solution is added to
the wells and color develops in proportion to
the amount of HIF-1α present at the initial step.
5- Urine cytology: Specimen selection is
important in cytology, hence, bladder wash is
recommended than voided urine, because of
better cell preservation and more numerous
neoplastic elements in the sample. Malignant
urothelial cells can be observed on microscopic
examination of the urinary sediment or bladder
wash.
6- Immunohistochemical staining: Enzymatic
digestion: Formalin fixation can mask antigen
and prevent its reaction with the antibody,
therefore application of pepsin partly restores
the antigen reactivity: pepsin was freshly
prepared for each run. It was applied to the
rehydrated sections (5 minutes digestion at
37ºC). Section were rinsed in tap water for 3
minutes after enzymatic digestion.
Preparation of DAB chromogen substrate
mixture: DAB (2,3 diaminobenzidine) forms
brownish and product that is insoluble in
alcohol and therefore, is suitable for permanent
mounting. It is prepared by adding 2 drops of
liquid DAB chromogen to one vial of 2.5 mll
ready-to-use substrate buffer, then adding, drop
of hydrogen peroxide (H2O2) substrate
solution and mix well.
Paraffin tumor sections of malignant samples
of patients who had undergone radical
cystectomy were assessed
immunohistochemically using mono-
and polyclonal antibodies for ET-1, ETAR, ETBR
and CD34 (MVD). Tumor samples were
obtained following either Cystoscope or
radical cystectomy. Tumor samples were
obtained following either Cystoscope 30
samples or radical cystectomy 9 samples.
7-Detection of bilharzial antibodies in sera :
Schistosomiasis fumouze is an indirect
haemagglutination test for the quantitative
detection of the antibodies present in sera from
patients suffering from bilharziasis with
Schistosoma mansoni (intestinal location), with
Schistosoma haematobium (urinary tract
location) and with Schistosoma intercalatum
(rectal location).
Schistosomiasis fumouze principle is based
on indirect haemagglutination. Sensitized red
cells are composed of sheep red blood
cells coated with a Schistosoma mansoni
antigen. Specific serum antibodies are revealed
by an agglutination of the sensitized red blood
cells: a reddish-brown film can be observed in
the well. In the absence of specific antibodies,
these red blood cells deposit, forming a ring in
well bottom. The unsensitized red blood cells
ensure the reaction specificity and allow the
II. RESULTS

Statistical analysis: The collected data were organized and tabulated and statistically analyzed using SPSS for windows version 13.0. Quantitative data was presented as mean ± standard deviation (SD), student t-test and Anova test for quantitation of three numerical groups were used for statistical analysis. For qualitative data, the number and percentage distribution was calculated and chi-square test was applied for comparison.

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Malignant(39)</th>
<th>Benign(15)</th>
<th>Normal(15)</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking:</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10(25.6%)</td>
<td>8(53.3%)</td>
<td>10(66.7%)</td>
<td></td>
<td>8.855 0.012</td>
</tr>
<tr>
<td>Positive</td>
<td>29(74.4%)</td>
<td>7(46.7%)</td>
<td>5(33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilharziasis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14(35.9%)</td>
<td>11(73.3%)</td>
<td>15(100%)</td>
<td></td>
<td>20.12 0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>25(64.1%)</td>
<td>4(26.7%)</td>
<td>0(0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary HIF-1:</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6(15.4%)</td>
<td>8(53.3%)</td>
<td>10(66.7%)</td>
<td></td>
<td>15.46 0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>33(84.6%)</td>
<td>7(46.7%)</td>
<td>5(33.3%)</td>
<td></td>
<td>17.58 0.001</td>
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<tr>
<td>Urine cytology:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18(46.2%)</td>
<td>13(86.7%)</td>
<td>15(100%)</td>
<td></td>
<td>17.58 0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>21(53.8%)</td>
<td>2(13.3%)</td>
<td>0(0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age(year)</td>
<td>61.74±10.52</td>
<td>56.27±18.1</td>
<td>41.20±9.58</td>
<td>F=1.889 0.03</td>
<td></td>
</tr>
<tr>
<td>Mean Rank of HIF-1</td>
<td>39.23±22.85</td>
<td>21.97±19.8</td>
<td>12.36±4.94</td>
<td>F=11.84 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table(1): Frequency distribution of different clinicopathological factors among studied groups

The mean rank of HIF-1 in malignant patients males group was 40.09 , in malignant females group was 34.54,and this difference was not statistically significant (P=0.585). The mean rank of HIF-1 was higher (40.26) in bilharzial positive patients than in bilharzial negative patients (37.37) and this difference was not statistically significant (P=710). Adenocarcinoma reported the highest induction of the HIF-1 (41.22) followed by both TCC (39.6) and SCC (37.38).
The mean rank of HIF-1 was nearly equal in grade I (45.81) and in grade II (45.46), but in grade III it was less (33.24) with no significant difference (P=0.250). There was no significant difference between stages of tumor regarding HIF level (P=0.959). There was a statistically significant association between levels of HIF-1 and CD34 (Table2), there was a positive correlation between these two variables ($r = 0.464, p < 0.001$) (data not present in tables).

<table>
<thead>
<tr>
<th>Clinicopathological Factors</th>
<th>No (%)</th>
<th>Mean rank of HIF-1* ±SD</th>
<th>Test</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>33 (84.6%)</td>
<td>40.09±23.35</td>
<td>t=0.552</td>
<td>0.585</td>
</tr>
<tr>
<td>Female</td>
<td>6 (15.4%)</td>
<td>34.45±21.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10 (25.6%)</td>
<td>40.60±22.67</td>
<td>t=0.218</td>
<td>0.828</td>
</tr>
<tr>
<td>Positive</td>
<td>29 (74.3%)</td>
<td>38.75±23.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilhariziasis:</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>14 (35.9%)</td>
<td>37.37±23.71</td>
<td>t=0.375</td>
<td>0.710</td>
</tr>
<tr>
<td>Positive</td>
<td>25 (64.1%)</td>
<td>40.26±22.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>9 (30%)</td>
<td>25.04±16.59</td>
<td>t=-2.459</td>
<td>0.018</td>
</tr>
<tr>
<td>High</td>
<td>21 (70%)</td>
<td>40.42±25.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathology:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transitional Cell Carcinoma</td>
<td>29 (74.3%)</td>
<td>39.60±22.31</td>
<td>F =0.036</td>
<td>0.965</td>
</tr>
<tr>
<td>Squamous Cell Carcinoma</td>
<td>8 (20.5%)</td>
<td>37.38±28.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2 (5.12%)</td>
<td>41.22±13.93</td>
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<td></td>
</tr>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 (10.2%)</td>
<td>45.81±30.18</td>
<td>F =1.443</td>
<td>0.250</td>
</tr>
<tr>
<td>II</td>
<td>15 (38.5%)</td>
<td>45.46±28.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>20(51.3%)</td>
<td>33.24±15.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stages:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14 (35.9%)</td>
<td>40.95±25.90</td>
<td>F =0.101</td>
<td>0.959</td>
</tr>
<tr>
<td>II</td>
<td>3 (7.7%)</td>
<td>34.09±3.378</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>8 (20.5%)</td>
<td>40.73±28.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>14 (35.9%)</td>
<td>37.74±20.26</td>
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</tr>
</tbody>
</table>

Table (2): Mean difference of HIF-1* among various clinicopathological factors in Malignant Group

HIF-1* Hypoxia inducible factor-1

The association between MVD and histopathological grade, tumor stage and prognosis was evaluated. MVD was identified by immunostaining of endothelial cells using anti-CD34 antibody. (Table3)
<table>
<thead>
<tr>
<th>Clinicopathological factors</th>
<th>CD34</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (1,2)</td>
<td>High (3,4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td></td>
</tr>
<tr>
<td>Groups: n=45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant (30)</td>
<td>9 (37.5%)</td>
<td>21 (100%)</td>
<td>19.688</td>
</tr>
<tr>
<td>Benign (15)</td>
<td>15 (62.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Pathology of malignant group: n=30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transitional Cell Carcinoma (21)</td>
<td>8 (88.9%)</td>
<td>13 (61.9%)</td>
<td>2.336</td>
</tr>
<tr>
<td>Adenocarcinoma (2)</td>
<td>0 (0%)</td>
<td>2 (9.5%)</td>
<td></td>
</tr>
<tr>
<td>Squamous Cell Carcinoma (7)</td>
<td>1 (11.1%)</td>
<td>6 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>Grades of malignant group: n=30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (14)</td>
<td>5 (55.6%)</td>
<td>9 (42.9%)</td>
<td>0.408</td>
</tr>
<tr>
<td>High (16)</td>
<td>4 (44.4%)</td>
<td>12 (57.1%)</td>
<td></td>
</tr>
<tr>
<td>Stages of malignant group: n=30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (13)</td>
<td>5 (55.6%)</td>
<td>8 (38.1%)</td>
<td>0.782</td>
</tr>
<tr>
<td>High (17)</td>
<td>4 (44.4%)</td>
<td>13 (61.9%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Association between CD34 score and different clinicopathological factors in malignant group
P value is highly significant at $p<0.001$

All blood vessels were highlighted by staining endothelial cells for CD34 related antigen, microvessel density was determined in area of invasive carcinoma in areas having the most intense neovascularization. Then it was subjectively graded on a scale of 1 to 4 (1=1-33, lowest, 2=34-66 intermediate, 3=67-99, intermediate, 4≥100highest). Tumors were grouped for statistical analysis in tables (3) into the following groups: low-stage (stage I and stage II) and high-stage (stage III and stage IV) and low-grade (grades I and II) and high-grade (grade III)

This factor detects the grade of angiogenesis at a different levels in tumors tissues. In different cancer types the high CD34 score was recorded in TCC, SCC and in adenocarcinoma and in adenocarcinoma. There was no significant difference between different stages of tumor as regard CD34 score. More than half of low-stage tumor (55.6%) had low level of CD34 and more than half of high -stage tumor (61.9%) had high level of CD34.

![Figure (1): ROC curve of Urinary HIF.](image)

The best cut off value which maximize the descrimation between malignant and benign.
cases was (21.7) ng/ml, and area under the positive = 74.4% and negative = 73.1

III. DISCUSSION

Tumor angiogenesis is crucial in maintaining the supply of oxygen and nutrients to the proliferating tumor cells, and is measured histologically by MVD estimations (Anirban et al., 2008).

Bedwani et al., (1998) reported that tobacco-smoking remains at present by far the major factor for bladder cancer in Egyptian men (Bedwani et al., 1998). The current study detected a significant difference between the studied groups as regard smoking history, nearly three quarter of the malignant group (74.4%) were smokers, this was in agreement with zarzour et al., (2008) who reported that incidence of bladder cancre was four fold higher in smokers than do people who have never smoked.

The finding of this study revealed that more than half of the malignant group (64.1%) had urinary schistosomiasis, these results agreed with Bedwani et al., (1998) who found a significant association between urinary schistosomiasis and bladder cancer.

The findings of the present study must be viewed against the backdrop of different methodological limitations of the study. A major-problem of the present, and of most previous case-control investigations, is recall bias. The use of hospital controls, moreover, represents an optimal design to reduce any information bias, as cases and controls are similarly sensitized towards reporting medical history. (Bedwani et al., 1998)

Other limitations and strengths of this study are common to most hospital-based case-control studies Hospital controls may differ from the general population in several respects, but we excluded from the control group all diagnoses potentially related to urinary tract conditions, and any potential risk factor for bladder cancer. The same catchments areas, the identical interview setting for cases and controls, and the almost complete participation are, moreover, reassuring, particularly as regards selection bias and differences in recall of clinical history.

The present study investigated the role of HIF-1 in voided urine in bladder cancer patients and its possible use as biological marker for early invasion detection of bladder tumors by ELISA. Herrmann et al., (2007) did not found any significant differences in the account of MVD in disease-free or overall survival. Masaki et al., (2009) reported that the tumor size may not be a determinant factor for HIF-1 activity.

The results of the current study revealed that there was a high level of HIF-1 in the malignant patients compared with the benign patients and controls. The results of the present study came in accordance with another with Adams et al. (2001) who discussed that the analysis of human primary tumors revealed greater HIF-1 protein expression in tumors compared with normal bladder specimens. Semenza (2002) reported that HIF-lalpha played a crucial role in tumor development and metastasis as well as in adjustment of tumor cells into hypoxic microenvironments. However, a variable degree of the cytoplasmic expression of HIF-1 is often recognized in the malignant tumors (Semenza, 2002). Masaki et al., (2009) suggested that therapeutic intervention of malignant tumors has been expected to be achieved by down –regulation of HIF-1α.
There were no statistically significant difference between smokers and non-smokers as regard urinary HIF-1. Shigeu et al., (2006) reported that it is possible that urine may supply oxygen to tumor cells. Bladder cancer arise frequently in patients who smoke, and chronic smoking decreases the oxygen tension of blood, which reflects that of urine. Therefore, in bladder cancer patients, low oxygen tension of urine may promote the invasion of superficial tumor into muscle layer by inducing angiogenesis.

The mean of HIF-1 was slightly higher in patients who were positive for bilharziasis test than in negative patients. Bedwani et al., (1998) reported a significant association between urinary schistosomiasis and bladder cancer. little number of cases who were non-smokers and did not report history of schistosomiasis, association between urinary schistosomiasis and bladder cancer may not distinguishing smokers and non-smokers. (Bedwani et al., 1998).

HIF-1activity highest level was recorded in Adenocarcinoma patients, the lowest level in SCC and TCC showed a moderate level of HIF-1activity, however all kinds of tumors showed no significant differences, this finding was in accordance with Masaki et al., (2009) that DNA binding HIF-1 activity is highest in clear cell Adenocarcinomas. Masaki et al., (2009), and Zhou et al., (2006) found that the protein expression of HIF-1alpha is strongly associated with the neoplastic progression of renal clear cell carcinoma, but its role in the development of bladder TCC is not clear yet. Consistent with the findings of the present study, Herrman et al., (2007) reported that TCC were better vascularized than SCC.

The result of this study showed higher levels of HIF-1 in grades I and II than grade III, this results was in agreement with those of Nadaoka et al.,(2008) who reported that HIF-1 alpha genotype did not significantly influence the incidence or disease status of bladder cancer. Contrarily, Theodoropoulos et al., (2004) reported a significant positive association between HIF-1 alpha immunoreactivity and histological grade.

There was a significant correlation between level of HIF-1 and CD34, Ioachim et al., (2006) reported that HIF-1alpha appear to play a role in bladder cancer, vascularization possibly and there was a trend of correlation between its expression and other angiogenic factors.

In the current study we used the immunohistochemistry CD34 to compare it in different clinicopathological factors, Awed et al., (2006) studied the use of CD34 for immunohistochemistry assessment of MVD and hence tumor angiogenesis and they reported that it gave the best scoring and is to a certain extent reliable. Korkopo et al., (2001) studied the MVD as an influential factor to overall survival of patients with invasive bladder cancer, they could not demonstrate MVD as a prognostic factor in superficial carcinoma.

In the present study the TCC showed high score of the CD34 than SCC and then comes the Adenocarcinoma, this result supports the postulate of Herman et al., (2007) that transitional cell carcinomas were better vascularized than squamous cell carcinomas.

CD34 expression level was high in high–grade tumors and vice versa. Canoglu et al., (2004) reported that MVD was correlated with tumor grades, stage and prognosis. More CD34 staining was also associated with advanced tumor stage. High MVD was correlated with the risk of clinical progression in both superficial and invasive bladder carcinoma.

IV. CONCLUSION

There was a statistically significant difference between benign and malignant groups regarding HIF-1 level but there was no significant difference between stages or grades of malignant samples as regard HIF-1 activity and CD34 level. This indicate that HIF-1 is expressed in all histopathological types of bladder cancer especially Adenocarcinoma. Cd34 was also expressed in different tumor tissues; this may reflect its possible role as a target in cancer therapy in the future.

REFERENCES


