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Is HPV Implicated in Bladder Cancer?

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Abstract: Bladder cancer is a common malignancy in Egypt. Human papilloma virus (HPV) could have a possible etiologic role in bladder carcinogenesis. This study aimed to estimate the prevalence of HPV16, 18, in cancer bladder as well as proliferative and preneoplastic bladder lesions and to study the correlation of HPV positive cases with different clinico-pathologic parameters. The present study was conducted on 64 cases obtained from the archival material, of the Pathology Department Cairo University, Kasr Al ainy Hospital, that were identified histopathologically and clinically as cancer bladder (group I) 39 cases, proliferative and preneoplastic lesions (group II) 10 cases and cystitis (group III) 15 cases. Samples were subjected to detection of HPV16 and 18 DNA by PCR. HPV was detected in a total number of 20 cases out of 64 cases, with a higher prevalence in group I (14 cases). A positive relationship between the stage of cancer and infection with both HPV16 and HPV18 was detected. A positive relationship was also noticed between the presence of squamous metaplasia and dysplasia and infectivity with both HPV16 and HPV18. No relationship with HPV and bilharziasis could be detected. This study confirms a significant association of HPV16 and HPV18, with both bladder cancer and proliferative and preneoplastic urinary bladder lesions in studied cohort, indicating a possible viral role in bladder carcinogenesis. Both HPV types were significantly associated with proliferative and preneoplastic lesions as well as tumors stage III; findings would suggest that HPV may play a significant role in the carcinogenic process as well as progression of cancer bladder toward higher stages.

Key words: HPV16 · HPV18 · Cancer Bladder

INTRODUCTION

In Egypt, bladder cancer has been recorded as the most common cancer throughout the past 50 years [1]. In 2002, Egypt's world-standardized bladder cancer incidence was 37/100,000, representing approximately 30,000 new cases each year [2]. The estimated incidence in males in rural areas in Egypt is about 32 per 100,000 [3].

Most scientific research is geared towards improving life expectancy and management policies and to date the actual causative agents & risk factor evaluation have had to take second place [4]. Of the many risk factors that have been implicated in the occurrence of bladder cancer some factors have been shown to vary according to their geographic location, for example in Western countries the leading etiologic factors are smoking and occupational exposures whereas, historically, squamous cell carcinoma was the predominant form of bladder cancer in Egypt constituting from 59% to 81% of reported bladder cancers between 1960 and 1980 and it was closely related to bilharzial infestation of the urinary bladder [5-7] lately transitional cell carcinoma has become the most frequent type. These results corroborate findings from a few small-scale hospital-based studies which conclude that the etiology of bladder cancer in Egypt has changed significantly over the past 26 years. The changing patterns of bladder cancer in Egypt over the past 26 years raises the suspicion that there are other possible risk factors other than bilharziasis that might play an important role in the pathogenesis of bladder cancer in Egypt and this requires serious investigation [8].

Human papilloma viruses (HPVs) are a group of more than 100 related viruses and, more than 30 types can be passed from one person to another through sexual contact [9]. Genital HPV thus, is an extremely common viral infection particularly in the West and approximately 5.5 million new genital HPV transmissions occur in the United States every year, representing about one-third of all new sexually transmitted disease (STD) infections and an estimated 20 million men and women are thought to

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have genital HPV at any given time [10]. In Egypt only about 10.3% of women in the Egyptian population are estimated to harbor cervical HPV infection at a given time [11].

Previous evaluation of HPVs have classified types 16 and 18 as carcinogenic to humans (Group I), types 31 and 33 as probably carcinogenic to humans (Group 2A) and some types other than types 6, 18, 31 and 33 as possibly carcinogenic to humans (Group 2B). Infections with high-risk HPV types (HR-HPV), such as 16, 18 and 33, have been demonstrated in a high percentage of patients with several cancers [12].

Human papillomavirus infection is also common in women and has been closely linked to the development of carcinoma of the cervix. It has been suggested that infection with HPV may also be an important factor in the subsequent development of bladder cancer [13]. The relationship between HPV and bladder cancer might be due to the fact that the micro-organism, which generates the infection incorporates its DNA into the cell nucleus resulting in malignant transformation of the infected cell [14]. A number of studies using various molecular techniques have looked at the relationship between HPV infection and bladder cancer, the results of which are somewhat conflicting [15].

The aim of the study is to estimate the prevalence of HPV16, 18, in cancer bladder as well as proliferative and preneoplastic bladder lesions and to study the correlation of HPV positive cases with different clinicopathologic parameters.

MATERIALS AND METHODS

Study Patients: This study was conducted from October 2010 to January 2015 on 64 formalin- fixed paraffin embedded tissue samples. The bladder tissue biopsies and clinical data collection were conducted on a retrospective manner as they were selected from the Department of Pathology, Faculty of Medicine and Cairo University.

Histopathological Evaluation: Serial sections were prepared from each tissue block and were cut at 5 microns thickness. Sections were stained by Hematoxylin and Eosin (H&E) for routine histopathology.

Slides were examined for lesions of cancer bladder and its type, premalignant lesions (Dysplasia, squamous metaplasia), presence of bilharzial and non bilharzial cystitis. Schistosomal affection was diagnosed based upon the presence of schistosomal eggs in examined tissue. All tumors were graded pathologically as high or low grade lesions for transitional cell carcinoma and grades well, moderately or poorly differentiated carcinoma for squamous cell carcinoma [16]. Tumor staging was done according to the degree of infiltration of the bladder wall, perivesical fat and adjacent structure [17].

The samples were divided into 3 groups. Samples diagnosed as cancer bladder (Group I), proliferative and premalignant lesions (Group II) and a third group which is considered as a control group negative for malignant or premalignant lesion of bladder samples diagnosed as cystitis (Group III).

Detection of HPV 16 AND 18 BY conventional PCR

DNA Extraction: DNA extraction was done using the kit supplied by Qiagen through the following 6 steps:

- **Removal of paraffin**; **Lysing:**
- **Heat application**:
- **Binding:**
- **Washing:**
- **Elute**:

Amplification of target HPV16& 18 gene by PCR: PCR was performed in a final volume of 50 µl, using the components of the mix produced by **Bio Basic INC**.

Sequence of HPV16 and HPV18 primer were as follows:

- p16F: (5'AAGGGCGTAACCGAAATCGGT3')
- p16R: (5'GTTTGCAGCTCTGTGCATA3')
- p18F: (5'AAGGGAGTAACCGAAAACGGT3')
- p18R: (5'GTGTTCAGTTCCGTGCACA3').

Gel electrophoresis for PCR- products: The DNA was visualized by placing the gel on an UV transilluminator. The EB intercalated into DNA and gave a bright pink band.

Statistical Analysis: Microsoft excel 2010 was used for data entry and the statistical package for social science (SPSS version 21) was used for data analysis. Simple descriptive statistics (Arithmetic mean and standard deviation) used for summary of quantitative data and frequencies used for qualitative data. Bivariate relationship was displayed in cross tabulations and Comparison of proportions was performed using the chi-square test. One-way Annova and post-hook tests were used to compare normally distributed quantitative data. Mann-Whitney test was used to compare non-normally distributed quantitative data.

RESULTS

64 cases were enrolled in the present study. All specimens were analyzed for the presence of HPV types 16 and 18 using conventional polymerase chain reaction method (PCR). The age of patients ranged from 12 years to 87 years (Mean of 58.73 years). The percentage of male (58 cases) to female (6 cases) was 90.6% and 9.4% respectively. The samples were divided into 3 groups; (Group I) included *cancer cases,* (Group II) included *proliferative and preneoplastic lesions* and a third group of cystitis samples control group (Group III).

Cancer Bladder (Group I): This group comprised 39 cases, 23 cases were diagnosed as *transitional cell carcinoma "TCC"* and 16 were *squamous cell carcinoma "SCC"*. The mean age of patients in this group was 61.6 years, most of them were males (89.7%) and 23% presented by haematuria. Distribution of cases according to type and grade of cancer is listed in Table 1.

The most frequent stage for all cancer cases observed was stage III, representing 61% of all cancer cases.HPV18 was positive in *14 cases* as listed in the Table 2.

All positive cases in the transitional cell carcinoma group were diagnosed as high grade invasive transitional cell carcinoma, while three out of five positive cases in the squamous cell carcinoma group were diagnosed as moderately differentiated squamous cell carcinoma and the remaining two were well differentiated squamous cell carcinoma.

There was a positive relationship between the stage of cancer and infection with both HPV16 or HPV18 with a "p value" equal 0.036 and 0.043 respectively, where 85.7% of *positive* cases in this group belonged to stage III, while the rest of positive cases belonged to stage II.

There was insignificant relationship between HPV16 & HPV18 with respect to age and sex of patients, giving "p values" more than 0.05. Although HPV18 and HPV16 were detected more in transitional cell carcinoma, yet there was an insignificant statistical relationship between HPV16 & HPV18 and the type of cancer. Also there was insignificant relationship between HPV16 & HPV18 with respect to grade of transitional cell carcinoma, in spite of the association between HPV positivity and high grade of cancer detected in both transitional and squamous cell carcinoma groups.

Bilharziasis was detected in 21 cases 53.8% of samples in this group. The distribution of HPV positive cases in relation to associated bilharziasis and type of cancer is listed in the table below. The relationship between HPV16 & HPV18 positivity and associated bilharzial infestation did not prove to be statistically significant.

Table 1: Distribution of cases according to type and grade of cancer

	Transitional cell carcinoma		Squamous cell carcinoma
Total no. of cases(39)	23		16
Grade:		*Grade (Differentiation):*	
		•Well	2
•Low grade papillary	5	•Moderate	10
•High grade invasive	18	•poor	4

Table 2: Distribution of HPV 18 and HPV16 positivity in relation to type of cancer:

Total number of cases	Positive cases		Negative cases
39 cases	*14 cases*		*25 cases*
	HPV 18	HPV 16	
Transitional cell carcinoma	9 cases	*3 cases*	*14 cases*
Squamous cell carcinoma	5 cases	*2 cases*	*11 cases*

Table 3: Distribution of HPV positive cases in relation to associated bilharziasis and type of cancer

	Bilharziasis positive cases	
	HPV18	HPV16
Transitional cell carcinoma	3 cases	one case
Squamous cell carcinoma	4 cases	one case
Total no. of positive cases	7 cases	2 cases

Table 4: Distribution of HPV positive cases according to type of lesion in group II:

	Positive cases 4 cases		
Total number of cases 10 cases	HPV18	HPV16	Negative cases 6 cases
Squamous metaplasia	3 cases	2 cases	3 cases
Dysplasia	One case	-	2cases
Papillary hyperplasia	-	-	One case

Table 5: Distribution of cases according to HPV 18 and HPV16 positivity in group III

	Positive cases 2 cases		
Control group (15 cases)	HPV 18	HPV 16	Negative cases 13 cases
Bilharzial cystitis	One case	One case	7 cases
Polypoid cystitis	One case		6 case

Table 6: Distribution of HPV16 and HPV18 positivity in different groups:

	Total number of cases positive for HPV (20 cases) (*31.25%of all cases)*					
	HPV18 (*31.25%of all cases)* *(100%of all positive cases)*			HPV16 (*12.5%of all cases)* *(40%of all positive cases)*		
Total number of cases in all groups (64 cases)	No. of cases	*% of all positive cases*	*% of all cases*	No. of cases	*% of all positive cases*	*% of all cases*
Group I	14	70%	21.87%	5	25%	7.81%
Group II	4	20%	6.25%	2	10%	3.12%
Group III	2	10%	3.12%	1	5%	1.5%

Group II Proliferative and Preneoplastic Lesions: This group included 10 cases of dysplasia, papillary hyperplasia, squamous metaplasia and squamous metaplasia with dysplastic changes. Age of patients in this group ranged from 12 years up to 83 years with a mean of 53.7 years. Nine were males and a single case was a female, Bilharzial infestation was detected in two out of the ten cases both cases were diagnosed as dysplasia.

HPV18 was detected in 4 samples with a concomitant HPV16 positivity in two of them. All of them were males and none of these cases showed an associated bilharzial infestation.

Statistical analysis of samples in this group revealed a positive relationship between the presence of squamous metaplasia and dysplasia with infectivity with both HPV16 and HPV18 with a “p value” equal 0.007 and 0.002 respectively. There was a statistically insignificant relationship between HPV16 & HPV18 with age and sex of patient in this group.

Group III Cystitis Group: This group included 15 cases of inflammatory urinary bladder lesions; with 7 cases diagnosed as polypoid cystitis and 8 cases of bilharzial cystitis. Age of patients in this group ranged from 17 years up to 80 years (Mean of 54.47 years). 14 cases were males and a single case was a female.

Statistical analysis of samples in this group revealed absence of a significant relationship between HPV16 and HPV18 and the type of lesion, whether bilharzial or polypoid cystitis giving P values of 0.921 and 0.919 respectively. There was also an insignificant relationship between HPV16 & HPV18 and age and sex of patients in this group.

Comparative results with regards to HPV positivity in the three studied groups revealed that the group with the highest number of positive cases was the *“Cancer group”* (Group I) (14 cases), followed by group II*”Proliferative and preneoplastic lesions”* (4 cases) and finally group III *“Control group”* with the least number of positive cases (2 cases). Most of the cases which showed HPV positivity belonged to patients in the sixth decade (40%) and the majority of positive cases were males (80%).

Polymerase Chain Reaction Results in Different Groups

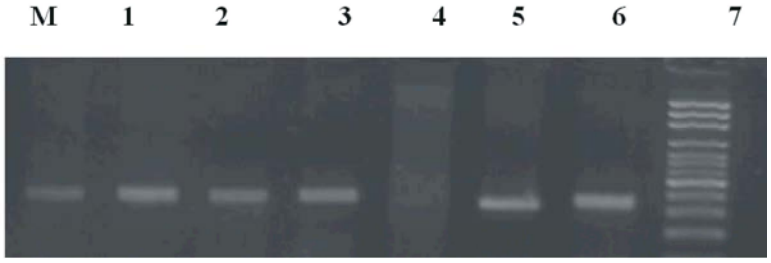

Fig. 1: "An agarose gel electrophoresis": An agarose gel electrophoresis show PCR products of HPV 16 gene expression in different studied groups.
Lane M: DNA marker with 100bp.
Lane 1&6: PCR products of HPV 16 gene in TCC group.
Lane 2: PCR products of HPV 16 gene in squamous cell carcinoma group.
Lane 3: negative case no PCR product of HPV 16 gene in polypoid cystitis group.
Lane 4&5: PCR products of HPV 16 gene in proliferative and preneoplastic group.
Lane 7: PCR products of HPV 16 gene in bilharzial cystitis group.

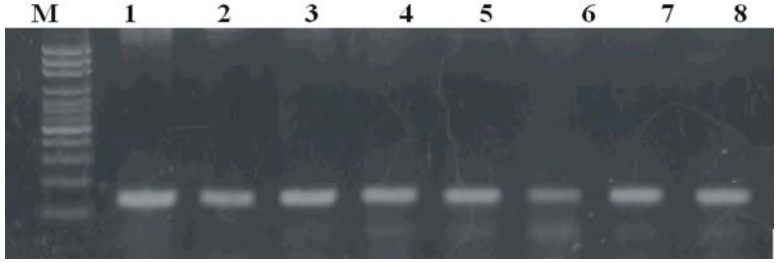

Fig. 2: "An agarose gel electrophoresis": show PCR products of HPV 18 gene in different studied groups.
Lane M: DNA marker with 100bp.
Lane 1&3: PCR products of HPV 18 gene TCC group.
Lane 2&5: PCR products of HPV 18 gene squamous cell carcinoma group.
Lane 4: PCR products of HPV 18 gene in polypoid cystitis group.
Lane 6&7: PCR products of HPV 18 gene proliferative and preneoplastic group.
Lane 8: PCR products of HPV 18 gene bilharzial cystitis group.

Group I

TCC +ve for both HPV18&16

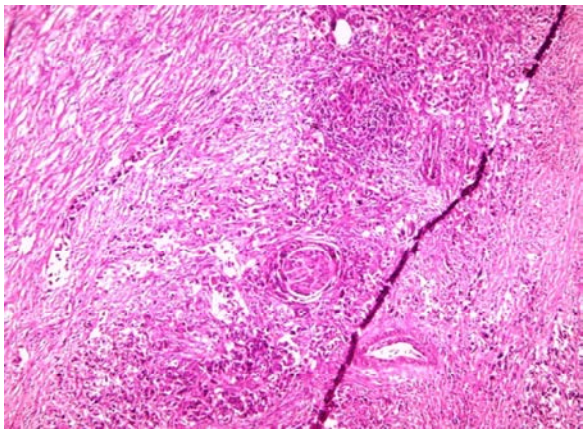

Fig. 1A: Invasive high grade TCC with perineural and muscular invasion (H&E x100)

TCC +ve for HPV18

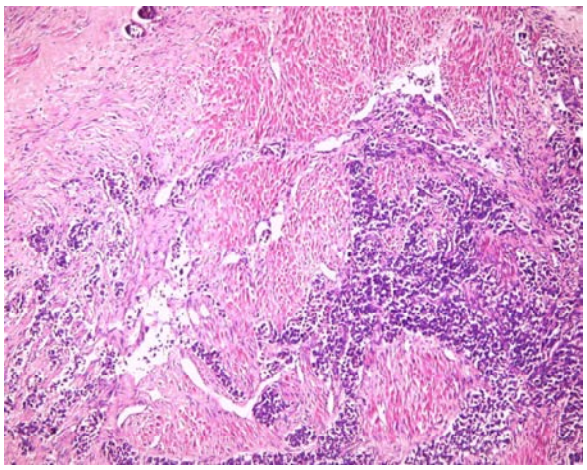

Fig. 1B: Invasive high grade TCC with muscle invasion& Bilharziasis (H&E x100)

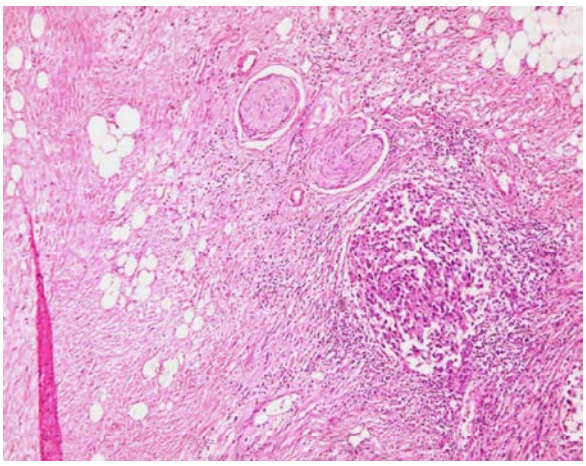

Fig. 1C: Invasive high grade TCC with perivesical fat invasion (H&E x100).

Sqcc +ve for both HPV18&16:

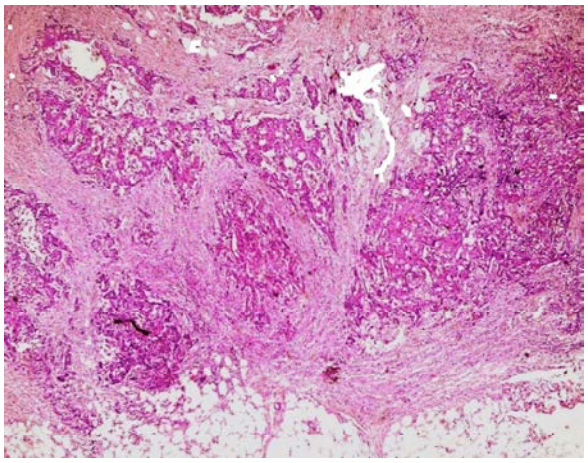

Fig. 1D: Poorly differentiated SCC with pervesical fat &muscle invasion (H&E x40).

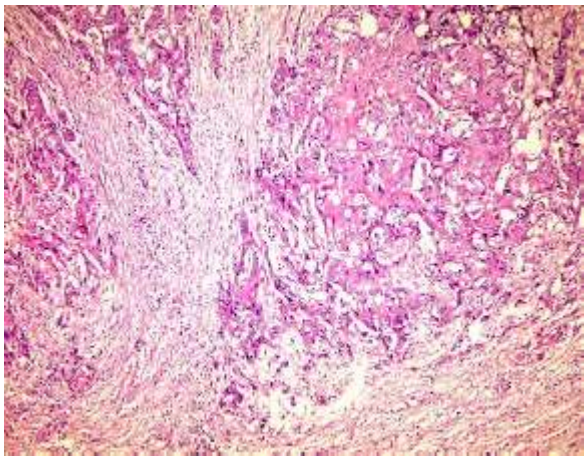

Fig. 1E: Poorly differentiated SCC with pervesical fat& muscle invasion (H&E x100).

Group II

Preneoplastic lesions +ve for both HPV18&16:

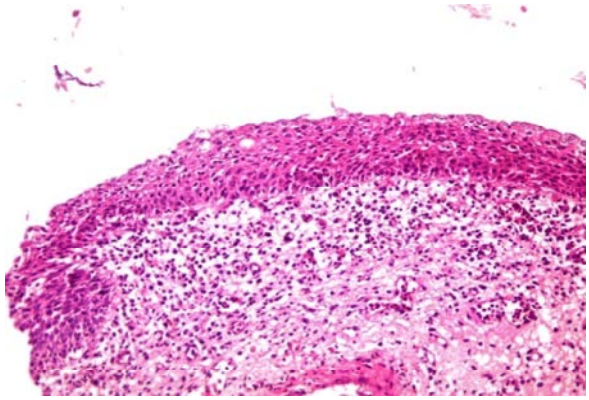

Fig. 2: Dysplasia (H&E x100).

Group III

Cystitis lesion +ve for both HPV18:

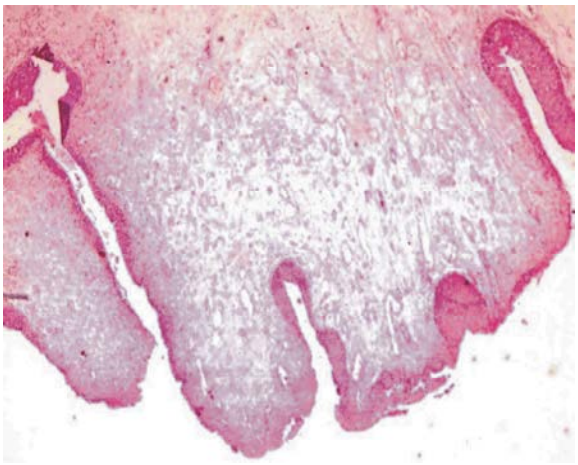

Fig. 3: Polypoid cystitis (H&E x40).

DISCUSSION

Cancer bladder is one of the common cancers which represent a growing health problem and its incidence varies widely throughout the whole world. In Egypt, bladder cancer has been recorded as the most common cancer throughout the past 50 years [7].

Historically, squamous cell carcinoma was the predominant form of bladder cancer in Egypt constituting from 59% to 81% of reported bladder cancers between 1960 and 1980, lately transitional cell carcinoma has become the most frequent type [5, 6, 7]. The changing patterns of bladder cancer in Egypt over the past 26 years raises the suspicion that there are other possible risk factors other than bilharziasis that might play an important role in the pathogenesis of bladder cancer in Egypt [8].

The present study was conducted on 64 cases. The conventional Polymerase Chain Reaction method (PCR) was performed to examine the possible role of HPV types 16 and 18 in bladder carcinogenesis as well as proliferative and preneoplastic and inflammatory urinary bladder lesions. The samples were divided into 3 groups; (Group I) includes *cancer cases* 23 cases of transitional cell carcinoma and 16 cases of squamous cell cancers. Most of the cancer bladder cases received at our department were of the transitional cell type a finding conforming with the obvious recent shift of the type of bladder cancer reported in Egypt from squamous to transitional cell carcinoma [8] (Group II) *proliferative and preneoplastic lesions* and a third group (Group III), *cystitis*. A comparative study was carried out for the prevalence of HPV 16 and 18 in both types of cancer (Transitional cell and squamous cell) as well as for each group (Malignant, proliferative and preneoplastic and the inflammatory lesions whether bilharzial or polypoid cystitis).

The prevalence of both HPV18 as well as HPV16 were noticed to be higher in the "*Cancer group* "(Group I) (70% of all positive case for HPV 18 and 25% for HPV16 respectively), followed by group II"*Proliferative and preneoplastic lesions"* (20% of all positive cases for HPV18 and 10% for HPV16 respectively) and finally group III *"control group"* with the lowest number of positive cases (10% of all positive cases for HPV18 and 5% for HPV16 respectively).

These findings were more or less similar to those obtained from Egypt [18] who detected HPV DNA in 48.9% out of 98 frozen specimens of bladder cancer.

In 1995, Kamel *et al.* [19] analyzed 47 bladder carcinomas for the presence of DNA-HPV subtypes 6, 11, 16, 18, 31 and 33 by nucleic acid in situ hybridization HPV DNA was found in 27/47 (57%) bladder carcinomas, with multiple subtypes in 20 cases. Of the 59 tissue specimens diagnosed as transitional cell carcinoma, HPV DNA was detected in 21 (35.6%) samples. A value, close to that in the present study which showed the prevalence of HPV 18 and 16 to be more in the transitional cell carcinoma group (64.28% of all positive cases for HPV18 and 21.4% for HPV16 respectively), than the squamous cell carcinoma group (35.72% of all positive cases for HPV18 and 14.28% for HPV16 respectively).

The prevalence of HPV-16 in cancer cases in the present study was 12.8% of all positive cases; whereas the prevalence of HPV-18 was 100%. In a comparison of these two carcinogenic types, HPV18 was the most prevalent; these findings were similar to those obtained by Barghi *et al*. [15]. Interestingly, although Chan *et al*. [20] used HPV type 6, 11, 16, 18, 31 and 33 type specific probes, all positive samples were found to contain HPV-18 DNA. Elsewhere two Japanese studies, Anwar *et al*. [21] and Furihata *et al.* [22] found incidences of HPV18 positivity to be 81% and 31%, respectively.

These findings are in contrast to those noticed by Badawi *et al*. [23] who observed that the prevalence of HPV16 in bladder cancer cases was higher than HPV18 with rates of 44.4% and 13.9% respectively, this could be attributed to different techniques used with varying specificities and sensitivities as well as to the use of different primers, which could also affect the experimental results.

Youshya *et al*. [24] suggested that HPV may play a significant role in the progression of TCCs toward higher stages and/or grades by inactivation of the tumor suppressors or other unknown mechanisms. With respect to grade and stage in the present study statistical analysis of samples in groupI "Cancer group" revealed a positive relationship between the stage of cancer and infection with both HPV16 or HPV18 with a "p value" equal 0.036 and 0.043 respectively, where 85.7% of *positive* cases in this group belonged to stage III, while the rest of positive cases belonged to stage II. On the other hand there was an insignificant relationship between HPV16 & HPV18 with respect to grade of cancer, in spite of the association between HPV positivity and high grade of cancer detected in both transitional and squamous cell carcinoma groups. This could be attributed to the limited number of cases in this study.

The high prevalence of HPV DNA in bladder tumor tissue in the present study and others emphasizes their role in bladder carcinogenesis. It seems that carcinoma development may be triggered by HPV infection. Inactivation of the tumor suppressor pRB by the human papillomavirus (HPV) oncoprotein E7 is a mechanism by which HPV promotes cell growth [25]. Human papillomavirus type 16 proteins, E6 and E7, have been shown to cause centrosome amplification and lagging chromosomes during mitosis, leading to chromosomal instability. Genomic instability is also thought to be an essential part of the conversion of a normal cell to a cancer cell [26].

Another group of researchers like, Helal *et al*. [27] from Egypt, reported HPV16 and 18 in one out of 114 samples using insitu hybridization method. Sur *et al*. [28] from South Africa, detected HPV DNA in only 1 out of 64 screened paraffin embedded TCCs. Youshya *et al*. [24] from the United Kingdom, went as far as to suggest

that HPV is unlikely to play any etiologic role in the development of bladder TCC. Aynaud *et al.* [29] also found no trace of HPV DNA in 58 bladder TCCs examined. Chetsanga *et al.* [30] similarly detected HPV DNA in only one of 44 TCCs using a degenerate PCR technique followed by dot blot analysis with type specific probes for six HPV types commonly detected in anogenital lesions. The dissimilarities in HPV prevalence reported by these investigators suggest that the association of HPV with bladder TCC may vary with different detection techniques and geographical locations.

One can assume from comparing the different results published in the literature, that several factors may be responsible for the discrepancies observed such as: 1) The different populations studied may have different risk factors, such as genetics, geographic location and life-style, any of which might affect carcinoma development in the bladder epithelium. 2) Different researchers have often used different techniques with varying specificities and sensitivities. 3) Technical errors made by the laboratory investigator or even the choice of the right primer could also affect the experimental results [31].

To our knowledge, so far very few studies have investigated the prevalence of HPV in proliferative and preneoplastic bladder lesions, most of these studies focused on the prevalence of such viruses in cancer bladder tissue and few included the proliferative and preneoplastic lesions in the studied group [15].

In the present study the prevalence of both HPV18 as well as HPV16 among the group II *"proliferative and preneoplastic group"* was found to be (40% and 20% respectively), statistical analysis of samples in this group revealed a positive relationship between the presence of squamous metaplasia as well as dysplasia and the infectivity with either HPV16 or HPV18 with a "p value" equals 0.007 and 0.002 respectively. Intrestingly none of the proliferative and preneoplastic positive cases showed an associated Bilharzial infestation which is thought to be a strong factor participating in the occurrence of keratinizing squamous metaplasia of the urinary bladder [32].

These findings signify that the association of HPV infectivity and urinary bladder proliferative and preneoplastic lesions as squamous metaplasia and dysplasia may be a very good point for future research and needs to be further investigated, in addition to the fact that HPV might not only be a good risk factor for cancer bladder occurrence, but also could play a role in the development of proliferative and preneoplastic lesions and share in the early steps of cancer development. The prevalence of HPV among the control group III (Bilharzial and polypoid cystitis) were found to be very low in comparison to both groups I and II, with only one case being positive for both types and belonging to the bilharzial cystitis group and one case positive to HPV18 and belonging to the polypoid cystitis group. These findings go in parallel with those observed by Barghi *et al*. [15], Badawi *et al*. [23], Agliano *et al*. [33], Eslami *et al*. [34], Chen *et al*. [35], Simoneau [36], Soulitzis *et al*. [37], Fioriti *et al*. [38], Wiwanitkit [39] who all found a significant difference between case and control groups for HPV positivity.

Bilharziasis was detected in 53.8% of cases in group I and 20% of cases in group II, being relatively higher in the malignant group, especially with squamous cell carcinoma (30.7%) followed by transitional cell carcinoma (23%).These findings go in hand with those obtained by El Bolkainy *et al*. [5] as well as Salem and Mahfouz [8] who found that the frequency of SCC was significantly greater in the cases presenting with schistosomal eggs, than in those without.

Out of the twenty cases found to be positive for HPV, eight, were positive for bilharzial infestation, yet statistical analysis was insignificant. The low frequency of HPV positivity in schistosomiasis-associated bladder carcinomas agrees with the findings of Helal *et al*. [27] and Cooper *et al.* [40] and who failed to demonstrate HPV in 25 schistosomal squamous cell carcinomas by using in situ hybridization technique.

In the present work, single HPV DNA type infection was significantly higher than co-infection with other HPV types, where infection with HPV18 alone was seen in 100% of total number of HPV positive cases. This is in agreement with Badawi *et al.* [23] and Matsumoto *et al*. [41] who could not detect multiple HPV types among either cases or control groups. On the other hand Ho *et al* [42] reported that concurrent or sequential detection of different HPV types confers an increased risk for intraepithelial neoplasia development.

CONCLUSION

In conclusion, our study confirms the significant association of HPV16 and HPV18, for both bladder cancer and proliferative and preneoplastic urinary bladder lesions in Egyptian patients. The interesting hypothesis of a viral synergistic action in bladder carcinogenesis is highly possible. In addition, such HPV types were significantly associated with proliferative and preneoplastic lesions as well as tumors stage III; findings that suggest that HPV may play a significant role in the carcinogenic process as well as progression of cancer bladder toward higher stages.

Abbreviations:
HPV: Human papilloma virus
PCR: Polymerase chain reaction
TCC: Transitional cell carcinoma
SCC: Squamous cell carcinoma

REFERENCES

1. El-Mawla, N.G., M.N. El-Bolkainy and H.M. Khaled, 2001. Bladder cancer in Africa: update. SeminOncol., 28: 174-178.
2. Parkin, D.M., F. Bray, J. Ferlay and P. Pisani, 2005. Global cancer statistics, 2002. Cancer J. Clin., 55: 74-108.
3. Amal, S.I. and I. Elsebai, 1983. Epidemiology of bladder cancer and ligand binding. Volume 1. Cancer bladder, Inc Florida press, 1983: 28-32.
4. Felix, A.S., A.S. Soliman, H. Khaled, M.S. Zaghloul, M. Banerjee, M. El-Baradie, M. El-Kalawy, A.A. Abd-Elsayed, K. Ismail, A. Hablas, I.A. Seifeldin, M. Ramadan and M.L. Wilson, 2008. The changing patterns of bladder cancer in Egypt over the past 26 years: Cancer Causes Control., 19: 421-429.
5. El-Bolkainy, M.N., N.M. Mokhtar, M.A. Ghoneim and M.H. Hussein, 1981. The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer, 48: 2643-2648.
6. El-Bolkainy, M.N., 1998. Topographic pathology of cancer, 1stedn. The National Cancer Institute, Cairo University, Cairo.
7. El-Mawla, N.G., M.N. El-Bolkainy and H.M. Khaled, 2001. Bladder cancer in Africa: update. SeminOncol, 28: 174-178.
8. Salem, H.K. and S. Mahfouz, 2011. Changing patterns (Age, incidence and pathologic types) of schistosoma-associated bladder cancer in Egypt in the past decade. Urology, 79(2): 379-83.
9. http://www.cancer.gov/cancertopics/factsheet/Risk/HPV
10. Bruni, L., M. Diaz, X. Castellsague, E. Ferrer, F.X. Bosch and S. De Sanjosé, 2010. Cervical human papillomavirus prevalence in 5 continents: metaanalysis of 1 million women with normal cytological findings. J. Infect. Dis., 202: 1789-1799.
11. Dailard, C., 2003. HPV in the United States and Developing Nations: A Problem of Public Health or Politics? The Guttmacher Report on Public Policy. August 2003, Volume 6, Number 3.
12. Muñoz, N., F.X. Bosch, S. De Sanjosé, R. Herrero, X. Castellsagué, K.V. Shah, P.J. Snijders and C.J. Meijer, 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl. J. Med., 348(6): 518-27.
13. De Villiers, E.M., C. Fauquet, T.R. Broker, H.U. Bernard and H. zur Hausen, 2004. Classification of papilloma viruses. Virology, 324(1): 17-27.
14. Zheng, Z.M. and C.C. Baker, 2006. Papilloma virus genome structure, expression and post-transcriptional regulation. Front Biosci, 11: 2286-2302.
15. Barghi, M.R., A. Haji mohammad mehdiarbab, S.M.M. Hosseini Moghaddam and B. Kazemi 2005. Correlation between human papillomavirus infection and bladder transitional cell carcinoma. BMC Infectious Diseases, 5: 102.
16. Mostofi, F.K., C.J. Davis and I.A. Sesterhenn, 1999. World Health Organization International Histological Classification of Tumors. Histological Typing of Urinary Bladder Tumors. 2nd Edition. Springer Verlag: Berlin Heidelberg.
17. Edge, S.B., D.R. Byrd, C.C. Compton, A.G. Fritz, F.L. Greene and A. Trotti, 2010. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer, pp: 347-76.
18. Khaled, H.M., A.A. Bahnassi and A.R. Zekri, 2003. Correlation between pp: 53 mutations and HPV in bilharzial bladder cancer. Urol Oncol, 21: 334-341.
19. Kamel, D., P. Paakko, R. Pollanen, K. Vahakangas, V.P. Lehto and Y. Soini, 1995. Human papillomavirus DNA and abnormal pp: 53 expression in carcinoma of the urinary bladder. APMIS, 103: 331-338.
20. Chan, K.W., K.Y. Wong and G. Srivastava, 1997. Prevalence of six types of human papillomavirus in inverted papilloma and papillary transitional cell carcinoma of the bladder: an evaluation by polymerase chain reaction. J. Clin Pathol., 50: 1018-21.
21. Anwar, K., K. Nakakuki, T. Shiraishi, H. Naiki, R. Yatani and M. Inuzuka, 1992. Presence of ras oncogene mutations and human papillomavirus DNA in human prostate carcinomas. Cancer Res., 52: 5991-6.
22. Furihata, M., Y. Ohtsuki, S. Ogoshi, A. Takahashi, T. Tamiya and T. Ogata, 1993. Prognostic significance of human papillomavirus genomes (Type-16, -18) and aberrant expression of p53 protein in human esophageal cancer. Int J Cancer, 54: 226-30.
23. Badawi, H., H. Ahmed, A. Ismail, M. Diab, M. Moubarak, A. Badawy and M. Saber, 2008. Role of human papillomavirus types 16, 18 and 52 in recurrent cystitis and urinary bladder cancer among Egyptian patients. Medscape J. Med., 10(10): 232.

24. Youshya, S., K. Purdie, J. Breuer, C. Proby, M.T. Seaf, R.T. Oliver and S. Baithun, 2005. Does human papillomavirus play a role in the development of bladder transitional cell carcinoma? A cmparison of PCR and immunohistochemical analysis. J. Clin. Pathol., 58: 207-210.
25. Fan, X., Y. Liu and J.J. Chen, 2003. Activation of c-Myc contributes to bovine papillomavirus type 1 E7-induced cell proliferation. J. Biol. Chem, 278: 43163-65.
26. Patel, D., A. Incassati, N. Wang and D.J. McCance, 2004. Human papilloma virus type 16 e6 and e7 cause polyploidy in human keratinocytes and upregulation of g(2)-m-phase proteins. Cancer Res., 64: 1299-306.
27. Helal Tel, A., M.T. Fadel and N.K. El-Sayed, 2006. Human papilloma virus and pp: 53 expression in bladder cancer in Egypt: relationship to schistosomiasis and clinicopathologic factors. Pathol Oncol. Res., 12(3): 173-8.
28. Sur, M., K. Cooper and U. Allard, 2001. Investigation of human papillomavirus in transitional cell carcinoma of the urinary bladder in South Africa. Pathology, 33: 17-30.
29. Aynaud, O., D. Piron, R. Barrasso and J.D. Poveda, 1998. Comparison of clinical, histological and virological symptoms of HPV in HIV-1 infected men and immunocompetent subjects. Sex Transm Infect., 74: 32-4.
30. Chetsanga, C., P.U. Malmstrom, U. Gyllensten, J. Moreno-Lopez, Z. Dinter and U. Pettersson, 1992. Low incidence of human papillomavirus type 16 DNA in bladder tumor detected by the polymerase chain reaction. Cancer, 69: 1208-11.
31. Gutiérrez, J., A. Jiménez, J. de Dios Luna, M.J. Soto and A. Sorlózano, 2006. Meta-analysis of studies analyzing the relationship between bladder cancer and infection by human papillomavirus. J. Urol., 176(6 Pt 1): 2474-8.
32. Khafagy, M.M., M.N. El Bolkainy and M.A. Mansour, 1972. Carcinoma of the Bilharzial urinary bladder: a study of the associated mucosal lesions in 86 cases. Cancer, 30: 150-9.
33. Agliano, A.M., A. Gradiloone, P . Gazzaniaga, M. Napolitano, R. Vercillo, L. Albonici, G. Naso, V. Manzari, L. Frati and A. Veggghione, 1994. High frequency of human papillomavirus detection in urinary bladder cancer. Urol. Int., 53: 125-129.
34. Eslami, G., M. Golshani, M. Rakhshn, F. Fallah and H. Goudarzi, 2008. The study on relation of Human Papillomavirus with bladder transitional cell carcinoma.Cancer Therapy, Vol 6, 355-360.25. Fan, X., Y. Liu and J.J. Chen, 2003. Activation of c-Myc contributes to bovine papillomavirus type 1 E7-induced cell proliferation. J. Biol. Chem., 278: 43163-65.
35. Chen, L., S. Ashe, M.C. Singhal, D.A. Galloway, I. Hellstrom and K.E. Hellstrom, 1993. Metastatic conversion of cells by expression of human papillomavirus type 16 E6 and E7 genes. Proc Natl Acad Sci U S A, 90: 6523-6527.
36. Simoneau, M., H. LaRue and Y. Fradet, 1999. Low frequency of human papillomavirus infection in initial papillary bladder tumors. Urol. Res., 27: 180-4.
37. Soulitzis, N., G. Sourvinos, D.N. Dokianakis and D.A. Spandidos, 2002. P53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett., 179(2): 175-83.
38. Fioriti, D., V. Pietropaolo, F.S. Dal, C. Laurenti, F. Chiarini and A.M. Degener, 2003.Urothelial bladder carcinoma and viral infections: different association with human polyomaviruses and papillomaviruses. Int. J. Immunopathol Pharmacol., 16: 283-8.
39. Wiwanitkit, V., 2005. Urinary Bladder Carcinoma and Human Papilloma Virus Infection, an Appraisal of Risk. Asian Pacific J. Cancer Prev., 6: 217-218.
40. Cooper, K., Z. Haffajee and L. Taylor, 1997. Human papillomavirus and schistosomiasis associated bladder cancer. Mol Pathol., 50: 145- 148.
41. Matsumoto, K., H. Yoshikawa, T. Yasugi, S. Nakagawa, K. Kawana, A. Takeoka, N. Yaegashi, T. Iwasaka, K. Kanazawa, Y. Taketani and T. Kanda, 2003. IgG antibodies to human papillomavirus 16, 52, 58 and 6 L1 capsids: casecontrol study of cervical interaepithelial neoplasia in Japan. J. Med. Virol., 69: 441-446.
42. Ho, G.Y.F., R. Bierman, L. Beardsley, C.J. Chang and R.D. Burk, 1998. Natural history of cervicovaginal papillomavirus infection in young women. N Engl. J. Med., 338: 423-428.