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RESEARCH ARTICLE

EXPRESSION OF P53 ONCOPROTEIN IN SCHISTOSOMA– ASSOCIATED SQUAMOUS CELL CARCINOMA OF THE URINARY BLADDER IN SUDANESE PATIENTS

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ABSTRACT

Background and Objectives: in Sudan, it is estimated that about 7 million people are infected with Schistosoma parasites, distributed all over the Sudan including even Khartoum state, the capital of Sudan. This retrospective hospital-based study aimed to examine immunohistochemical expression of p53 in urinary bladder mucosa associated with Schistosomiasis in Sudanese patients.
Material and Methods: Thirty-five paraffin-embedded formalin-fixed archival tissue blocks of bilharzia-associated bladder lesions were included in this study. Immunohistochemical staining was carried out using monoclonal antibody for P53.
Results: All the 30 malignant cases (86%) were of squamous cell carcinoma type. The P53 showed positive staining reaction in 25 cases (71%) while 10 cases (29%) were of negative staining reaction. Relation between tumor grades and P53 were as follows G I = 6, G II= 9, and G III= 10 cases.
Conclusion: immunohistochemical expression of p53 in squamous cell carcinoma of the urinary bladder is important for prognosis and better management

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INTRODUCTION

Bladder cancer is an important worldwide health problem with a global estimate of 386,300 new cases and 150,200 deaths in the year 2008. The majority of bladder cancers occur in males and there is a 14-fold variation in incidence internationally. The highest incidence rates are found in the countries of Europe, North America, and Northern Africa. It is estimated that one in 26 males and one in 87 females in USA will develop bladder cancer during their life time. Smoking and occupational exposure are the major risk factors in Western countries, while chronic infection with Schistosoma Haematobium (SH) in developing countries, particularly in Africa and the Middle East, accounts for the main total burden (Ferlay *et al*., 2010; World Health Organization Cancer Incidence in Five continents, 2002). According to the World Health Organization (WHO), about 779 million people are at risk of Schistosoma infection worldwide, 200 million are infected, and of whom three-quarters live in Africa. In Sudan, Schistosomiasis was introduced as early as 1909, in northern and Southeastern Sudan, but reached a large scale infectivity in Gezira population after establishment of the Gezira cotton irrigation scheme in 1925. It is estimated that 7 million people are infected, distributed all over the Sudan including even Khartoum state, the capital of Sudan (WHO, 2002; Thomson, 1911). Schistosomiasis was first linked to urinary bladder cancer by Furgesson in Egypt in 1911. There are several factors that may contribute to the oncological potential of Schistosoma infection. The bilharzia ova deposited in the bladder provoke an intense inflammatory and immunological reaction, associated with the production of granulomas and oxygen-derived free radicals, which may induce genetic mutations or promote the production of carcinogenic compounds such as N-nitrosamines and polycyclic aromatic hydrocarbons, leading to malignant transformation. Furthermore, infection by Schistosomiasis is often accompanied by chronic bacterial super-infection, which may in itself predispose to squamous cell metaplasia and neoplasia by promoting the formation of N-nitrosamines. International Agency for Research on Cancer (IARC) found that the intensity of infection is determined by urinary egg counts and compounded by smoking, and the combination was strongly considered.

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Positive association between bladder cancer and SH infection was detected, with odd ratios ranging from 2 to 14 (Zarzour *et al*., 2008; Chitsulo, 2000; Ferguson, 1911; Rosin, 1994).

Efforts have been made to study the specific genes involved in the induction of Schistosoma-associated bladder cancer (SA-BC). Cells exposed to SH cell total antigen (worm extract) were found to divide faster than those not exposed to the antigen (Shokeir, 2004; Vogelstein, 2004). The tumor-suppressor gene p53 encodes a 53 kDa nuclear phosphoprotein which in its wild-type conformation acts as a transcription factor and DNA-binding protein and functions as an inducer of apoptotic cell death when DNA damage becomes irreparable. Thus, p53 provides protection to normal cells and eliminates cells bearing significant genomic alterations. Loss of p53 function accelerates the process of tumor genesis and alters the response of cells to agents that damage DNA (Botelho *et al*., 2009; Levine, 1991; Strachan, 1999; Bell *et al*., 2002). Immunodetec Table p53 has been associated with aggressive behavior in cancers, including invasion, high grade and lymph node metastasis and has been reported in a range of bladder carcinomas. Furthermore, immune his to chemical positivity and specific mutations in p53 have recently been reported in Schistosomiasis-associated bladder cancers (Wu, 2005; Hollstein, 1991; Kamel, 1994; Warren *et al*., 1995). This study examines the immunohistochemical expression of p53 in urinary bladder tissues infected by Schistosomiasis. To our knowledge, this study is the first in Sudan examining the expression of this vital cell-cycle-associated protein in bladder tissues.

MATERIAL AND METHODS

This was a retrospective hospital-based descriptive study of thirty-five formalin-fixed paraffin-embedded tissue blocks of urinary bladder lesions obtained from the archives of Ibn-Sina Hospital, Sudan – Khartoum between January 2014 and December 2015. Clinical data of patients was obtained from the hospital medical records; these data included age, sex, histological diagnosis, and tumor grading. Ethical approval from Neelain University Ethical Committee (NUEC) and target laboratories were obtained before sampling and laboratory processing. Using a rotary microtome, 2-4 sections 3-5 µm thickness were cut from each block and put on two groups of slides, one for P53 immunostaining and the other for H&E staining to confirm the histopathological diagnosis obtained from the records. Data was collected and obtained from observation and analyzed by using (SPSS version 16).

Procedure was applied as follows: slides were placed into a 56-60 °C oven for 15 min, then transferred to a xylene bath and two changes of Xylene were performed for 5 minutes each. Excess liquid was shakenand slides were rehydrated in two changes of absolute ethanol, 90% ethanol, 80% ethanol, each for 3 minutes and then running tap water for 30 seconds. Antigen Retrieval: slides were washed with deionized H2O and placed in a heat-resistant plastic staining jar containing antigen retrieval solution. The water bath was operated for 20 minutes at 98 0 C, then let cool slowly at room temperature for at least 20 minutes. Inactivation of Endogenous Peroxidase: slides were placed on a flat level surface. Enough drops of 3% hydrogen peroxide were added to cover the whole section, incubated for 5 min at room temperature. Rinsed and washed with PBS for 2 minutes.

Primary Antibody Reaction: sample was pre-incubated with 5% BSA for 10 min. About 100 µl of primary antibody (Anti P53) solution was added, covering the tissue sections, incubated for at least 60 min at 37 °C in humidified chamber, and then rinsed and washed in PBS for 5 min. Secondary Antibody Reaction (Biotin/Extr Avidin Detection): slides were allowed to drain and carefully wiped. The biotinylated secondary antibody was diluted in a diluent to its optimal concentration. About 100 µl was applied to each slide, incubated in humidity chamber for at least 30 min at room temperature, and then rinsed and washed gently with PBS for 5 min. Substrate Preparation: slides were well drained and wiped, then enough drops of freshly prepared substrate mixture (50µL DAB chromogen +1ml substrate) to cover the tissue section, incubated for 5-10 min. or until desired color reaction was observed when monitored with the microscope. Reaction was terminated by rinsing gently with distilled water from a wash bottle. Counterstaining: slides were placed in a bath of Mayer's Hematoxylin, incubated for 3 min, rinsed gently with distilled water, and then rinsed under gently running tap water for 5 min. Finally, slides were dehydrated, cleared and mounted with D.P.X.

RESULTS

The patients' age ranged between 10 and 110 years with mean age of about 60 years. About halve the patients (45.7%) were into the age group 50 – 70 years (Table number 1). Males were 19 males (54.3%) and females were 16 (45.7%), 16 males and 14 females of them have carcinoma of bladder. The remaining five patients were negative for malignancy (3 males and 2females); they were chronic inflammation associated with Schistosomiasis (Granuloma). All the 30 malignant cases (86%) were of squamous cell carcinoma type. The frequency of histological cancer gradesis shown in Table number 2. The frequency of bladder cancer grades and benign lesions according to gender are shown in Table number 3. The P53 showed positive staining reaction in 25 cases (71%) while 10 cases (29%) were of negative staining reaction. Relation between tumor grades and P53were as follows G I = 6, G II= 9, and G III= 10 cases.

Table 1. Frequency of Bladder lesions according to age

Age	Frequencies	Percentage
10 – 30 year	3	8.6%
30 - 50	8	22.9%
50 – 70	16	45.7%
70 - 90	6	17%
90 - 110	2	5.7%
Total	35	100%

Table 2. The frequency of different histological grade of bladder cancer

Grade	Frequency
Grade I (Well Differentiated)	6
Grade II (Moderately Differentiated)	13
Grade III (Poorly Differentiated)	11
Total	30

Table No 3: The frequency of bladder cancer grades and benign lesions according to gender

Gender	Cancer	Benign	Total
Male	16 (3 G1, 7 G2, 6 G3)	3	19
Female	14 (3 G1, 5 G2, 4 G3)	2	16
Total	30	5	35
Percentage	86%	14%	100%

DISCUSSION

Research of P53 gene may be useful in early detection of aggressive forms of urinary bladder cancer particularly squamous cell type. In this study, about 40% of cancer cases showed positive reaction of p53 (10 out of 25 cases). This agrees with several studies worldwide (Fujimoto *et al*., 1992; Esrig, 1994; Esrig *et al*., 1993). The expression of the marker in different tumor grades had been studied, however, in only a limited number of studies it influenced the likelihood of patient's survival (Masliukova *et al*., 2006; Pfister *et al*., 1999).

Conclusion and Recommendation

It can be concluded that immunohistochemical expression of p53 in squamous cell carcinoma of the urinary bladder is important for prognosis and better management. It can be recommended that much larger population studies and research on bladder cancer are neededto test the P53 expression and obtain results with high statistical significance.

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