Chromosomal abnormalities in patients with active and chronic *Schistosoma haematobium* infection

Thesis

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Abstract

The aim of the present study was to explore the cellular kinetics, genomic instability and chromosomal abnormalities in Egyptian patients suffering from acute or chronic schistosomiasis *haematobium* infection.

This study was conducted on 46 patients, 22 of them were diagnosed as active cases while 24 patients suffered from chronic schistosomiasis *haemotobium* complicated by bladder cancer. Three different cytogenetic techniques were employed. These techniques included the nuclear morphocytometric analysis for whole chromosomal content using Feulgen stain, which was performed for all cases. In addition, the Fluorescent In Situ Hybridization (FISH) technique was applied on tissue specimens and the karyotyping technique was applied on peripheral blood monocytes obtained from eight selected cases.

All tissue specimens of chronic cases showed positive findings in nuclear morphocytometric analysis in the form of diploidy, tetraploidy and aneuploidy with high poliferative index. As for the ploidy analysis of urine derived epithelial cells from chronic patients, 5 samples showed aneuploid nuclei with high proliferatine index, while in acute cases, epithelial cells were succssefuly recovered and stained in only 4 urine samples, 3 of them were found to be diploid with a high proliferation index.

The 8 chronic patients were examined for the specific deletion of the p53 gene locus by FISH. Three samples (37.5%) were found to have a deletion of the p53 gene as evidenced by the presence of a single copy number of the gene. On the other hand, no numerical chromosomal aberrations were detected by karyotyping, where all samples showed a normal male karyotype (46, XY). However, one out of eight cases (12.5%) showed evidence of chromosomal fragmentations.

Key Words:

Schistosomiasis *haematobium*- chromosomal abnormalities- morphocytometry- FISH- Karyotyping.

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LIST OF ABBREVIATIONS

APCs	Antigen presenting cells
BAC	Bilharzia associated bladder cancer
CAA	Circulating anodal antigen
Cc	Cubic centimeter
CDA	Circulating cathodal antigen
CD	Cluster of Differentiation
CSEA	Circulating soluble egg antigen
CT	Computerized Tomography
DI	DNA index
DNA	Deoxyribonucleic acid
ECP	Eosinophil cationic protein
ELISA	Enzyme-linked immunosorbent assay
ES products	Excretory Secretory products
FFPE	Formalin fixed paraffin embedded
FISH	Fluorescent in situ hybridization
Gm	Gram
HPF	High power field
IFN	Interferon
Ig	Immunoglobulin
IHA	Indirect haemagglutination
IL	Interleukin
IOD	Integrated optical density
LOH	Loss of heterozygosity
Lyso-PS	Lysophosphatidyl-lecithin
Ml	Milliliter
NP- 40	Nonyl phenoxypolyethoxylethanol -40
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SCC	Squamous cell carcinoma
SD	Standard deviation
sh28GST antigen	*Schistosoma haematobium* 28kDa Glutathione S-transferase antigen
SSC	Saline Sodium Citrate

TCC	Transitional cell carcinoma
TGF	Transforming growth factor
TH cells	T helper cells
TNF	Tumour necrosis factor
T reg cells	T regulatory cells
µm	Micrometer

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Introduction

Schistosomiasis is a parasitic disease that dates back to antiquity. The ancient Egyptians, through settling in and cultivating the Nile valley, were among the first to contract the disease. The chief symptom, hematuria, was mentioned in the Egyptian papyri (1500-1800 B.C.). **Ferguson** in **1911** was the first investigator who reported a high frequency of bladder cancer in Egypt and suggested an etiological relation with urinary schistosomiasis **(Bolkainy and Chu, 1981)**. Furthermore, the consensus of available information strongly implicates an association between *S. haematobium* infection and the induction of bladder cancer **(Mostafa *et al.*, 1999)**.

In Egypt, bladder cancer has been the most common cancer during the past 50 years representing approximately 30,000 new cases each year **(Parkin *et al.*, 2005)**. This neoplasm accounts for 30.8% of the total cancer incidence and is ranked first among all types of cancer recorded in Egyptian males and second only to breast cancer in females **(Gouda *et al.*, 2007)**. The high-risk group included farmers aged 20 years and above, and they contributed to 19% of the total rural population **(Carmack and Soloway, 2006)**.

Despite the fact that urinary schistosomiasis constitutes a major global health burden, not only due to its high prevalence in particular foci, but also due it devastating complications, its causative agent, *Schistosoma haematobium*, has acquired the title of the neglected schistosome. There is a wide spectrum of chronic sequelae of urinary schistosomiasis ranging from chronic cystitis and hydronephrosis to the development of carcinoma of the bladder, where *S.*

haematobium is known to be a number one carcinogen in the eastern part of the globe **(Rinaldi *et al.*, 2011)**.

In addition, the number of persons infected with *S. haematobium* supersedes those infected with the other strains of the *Schistosoma* species altogether. The approximate number of human cases of schistosomiasis *haematobium* was estimated by **Rinaldi *et al.* (2011)** to be 112 million cases of human infections in Africa in contrast to 54 million human cases of *Schistosoma mansoni* in Africa and one million cases of *Schistosoma japonicum* in Asia.

The number of research papers investigating *Schistosoma haematobium*, however, seems to be the lowest among all schistosomes. The number of Pubmed citations over the last five years was reported to be 342 citations covering *Schistosoma haematobium* in Africa in contrast to 1377 citations covering *Schistosoma mansoni* in Africa and 644 citations about *Schistosoma japonicum* in Asia (**Rinaldi *et al.*, 2011)**. This relatively modest number of studies tackling urinary schistosomiasis reflects the unsatisfactory exploration of many aspects including the cytokinetic disturbances and subsequent genomic changes resulting from this serious parasitic agent.

Several attempts were made to evaluate the carcinogenic potential of experimentally induced schistosomiasis. It has been suggested that chronic inflammation and subsequent tissue injury attribute to the activation of bladder carcinogens that lead to gene mutations and DNA damage **(Badawi *et al.*, 1992 and Mostafa *et al.*, 1999)**. Mutations arising as a result of carcinogenic insults may lead to augmentation of the genetic instability and hence to malignant transformation **(Badawi *et al.*, 1992)**.

Healthy cellular growth and multiplication is essential to guard against neoplastic changes. Therefore, disturbances in cellular kinetics and cell cycle dynamics play a pivotal role in the genesis of chromosomal aberrations and consequently, the development of malignancy. Studying such abnormalities can be accomplished by different means and in different specimens such as circulating mononuclear cells in blood, tissue specimens and even in exfoliated cells in urine **(Carmack and Soloway, 2006)**.

DNA specific dyes, such as Feulgen stain, have been used to detect the total chromosomal abnormalities resulting from abnormal cell cycle e.g. aneuploidy or micronuclei. While Feulgen ploidy analysis interprets chromosomal content as a whole, karyotyping allows a closer look at each individual chromosome. It has been applied most popularly on cultured peripheral blood mononuclear cells.

Fluorescence in situ hybridization (FISH) is a cytogenetic technique employing nucleic acid probe technology and has been used to take a closer look at chromosomal abnormalities by detecting changes in a single locus or in multiple gene loci **(Lokeshwar *et al.*, 2005)**.