Determination of T cell lymphocytes population in urinary bladder biopsy material from schistosomiasis *haematobium* Egyptian patients

Thesis

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ABSTRAT

The aim of the present study was to investigate in situ- expression of different markers related to T cell population Th1 (STAT4), Th2 (GATA3), Treg.(FOXP3) and T cytotoxic (CD8) in Egyptian patients suffering from chronic complicated schistosomiasis *haematobium* infection, using real time quantitative photocytometric analysis. On the other hand, to spot on the dominating T cell upon which the subsequent events had been built. Due to ethical consideration, the present work was applied only on tissue biopsies of the selected cases after cystectomy. Therefore, the existing research was built-in 29 schistosomiasis patients complicated with bladder cance. Cases in the present study were exposed to more or less continuous stimulation of *Schistosoma* egg antigen, either due to lack of treatment, failure of treatment or repeated exposure to infection. The cases in the current work were reported to be poorly controlled by unbalanced Th1/Th2 in which Th2 was dominated as proved by the significant higher expression level of GATA3 (Th2 marker) over STAT4 (Th1marker). In attempt to regain the control, Treg. (FOXP3) level was increased significantly, however, failed to dowen-regulate Th2(GATA3) which continue to expand resulting in more down-regulation of Th1 (STAT4). Instead, the relation between Th1 (STAT4) and T cytotoxic (CD8) was forcibly limited by the high expression level of Treg. (FOXP3) resulting in loss of their power in defending the host against both parasite and carcinogenic changes. These correlations give more clarification for the immune evasion process played by the parasite and tumor cells under the supervision of Tregulatory cells. In addition to the critical role of FOXP3 in manipulating STAT4 and CD8 in favor of malignant progression.

Key Words: Schistosomiasis *haematobium*- T cell population - quantitative photocytometric.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
BAC	Bilharzia associated bladder cancer
B.C.	Before Christ
BCG	Bacillus Calmette–Guérin
BCL2	B-celllymphoma2
C57BL/6	Referred to as "C57 black 6", "C57" or "black 6" (standard abbreviation: B6), is a common inbred strain of laboratory mouse
CBA	CBA/J inbred mouse strain is used to study granulomatous experimental
C	Celsius
CBC	Complete blood picture
CCA	Circulating cathodic antigen .
CD	Cluster of differentiation
CMI	Cell mediated immunity
CT	Computed tomography.
DAB	3,3' Diaminobenzidine
DNA	Double stranded nucleic acid
E2	Estradiol
ECG	Electrocardiography
ELISA	Enzyme linked immunosorbant assay
ER	Estrogen receptor
FCR	fragment crystallizable region receptor
F	Fahrenheit
FOXP-3	Forkhead box Foxp-3
GATA	Family of transcription factors characterized by their ability to bind to the DNA sequence
H&E	Haematoxylin and Eosin
HIV	Human immune deficiency
IgE	Immunoglobulin E
IgG	Immunogloblin G
IgM	Immunogloblin M
IHC	Immunohistochemistry
IHA	Indirect haemagglutination test
IFN-γ	Interferon-gamma
IL	Interleukin
JAK	Janus kinase

KS	Katayama syndrome
KDa	Kilo Dalton
LOH	Loss of heterozygosity
LT-HSC	long term repopulating hematopoietic stem cells
MHC	Major histocompatibility complex
mAbs	Monoclonal antibodies
MPGN	Membranoproliferative glomerulonephritis
MRI	Magnetic resonance imaging
NSA-BC	Non Schistosoma associated-bladder cancer
P-value	Probability value
r	Pearson correlation
RBC	Red blood cells
ROS	Reactive oxygen species
RNOS	Reactive nitrogen oxide species
SA-BC	Schistosoma associated-bladder cancer
SCC	Squamous cell carcinoma
SEA	Soluble egg antigens
S.haematobium	Schistosoma haematobium
Sh-SEA	S. haematobium soluble egg antigen
Sh28GST	Schistosoma 28 kDa glutathione-S-transferase
SM22	Smooth Muscle-Specific Protein
Spp.	Species
STAT	Signal transducer and activator of transcription
TCC	Transitional cell carcinoma
TCR	Tcell receptor
Thelper lymphocyte	T helper lymphocytes
TIL	Tumor-infiltrating lymphocytes
TNF-β	Tumor necrosis factor beta
Treg	T regulatory cells
UTI	Urinary tract infection
µg/ml	Microgram per milliliter

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INTRODUCTION

Schistosomal infections affect more than 240 million people worldwide and *S. haematobium*, accounts for nearly half of that number. The ancient Egyptians, through settling in and cultivating the Nile valley, were among the first to contract the disease and the main symptom, hematuria, was mentioned in Egyptian papyri (1500-1800 B.C.) **(Moustafa *et al*., 1999)**.

Although the symptoms are varied, the bulk of the morbidity and mortality of urogenital schistosomiasis can be ultimately attributed to the host immune response against *Schistosoma* eggs deposited within the walls of the urinary tract. Subsequently, lead to urinary tract inflammation, fibrosis, bladder dysfunction, and increased susceptibility to urothelial carcinoma. In fact, the annual deaths are about 150,000 due to urogenital schistosomiasis-induced complications makes *S. haematobium* one of the most lethal worms worldwide **(Fu *et al*., 2012).**

Several immunological field studies supported the idea that individuals living in endemic areas have different immune responses, making them either resistant or susceptible to infection with different levels of complication. **(Mduluza *et al*., 2001).**

The granulomatous reactions in urinary schistosomiasis are T helper cells dependent and the T cytotoxic cells to parasites are activated by the T helper cells. Therefore, these cellular factors participate in the immune responses to urinary schistosomiasis. However, there is no evidence concerning the exact role of these immune cells in long standing complicated schistosomiasis *haematobium* and despite the global burden of urogenital schistosomiasis, there remains little known about the basic mechanisms underlying the immuno-pathophysiology of this

parasitic disease. This is primarily due to the lack of an experimentally tractable animal model and limited research on human cases **(Airfax *et al*., 2012)**.

AIM OF WORK

The present work aimed to study the expression level of different T cell populations in biopsy materials taken from Egyptian patients suffering from chronic complicated schistosomiasis *haematobium* using specific imunohistochemical markers.

Objectives

- Study the cellular and immunochemical patterns of T cell populations T-helper 1 (Th1), T-helper 2(Th2), T regulatory (Treg.) and T cytotoxic cells, using specific markers, GATA3, STAT4, FOXP3 and CD8 respectively.
- Recognize on the dominating T cell upon which the subsequent events had been built.
- Analysis of different patterns using digital real- time image morphocytometry.
- Compare the different patterns of different markers in relation to Parsitological findings in tissue specimes.
- Evaluation of the usefulness of T cell imunohistochemical markers in detecting susceptibility of different human cases for complication.

REVIEW OF LITERATURE

Historical note:

Schistosoma haematobium was discovered by a German physician **Theodore Maximilian Bilharz** (figure 1) in 1851 during autopsy at Kasr El Ainy hospital. *S. haematobium* was first diagnosed by Ruffer in 1910 who recovered calcified *schistosome* eggs from two Egyptian mummies **(El-Zayadi, 2004).**

Figure 1: Theodor Maximilian Bilharz, 1825-1862.

Haematuria, the main sign of urinary bilharziasis was recorded in the Kahun papyrus **1900 B.C.** as "â-a-â" disease **(Nmorsi *et al*., 2007).** In **1864, Harley** used the generic name Bilharzia for a blood-fluke occurring in South Africa. In **1864**, both **Harley and Cobold** held the view that a mollusk acted as intermediate host. All workers failed to discover the host until **Miyairi and Suzuki**, in **1913**, first found that a mollusk (*Katayama nosophora*) was the vector of *Schistosoma japonicum*. Few years later, **Miyagawa** verified their findings **(Sacko *et al*., 2011).**