RESEARCH ARTICLE

Inflammation, Oxidative Stress and L-Fucose as Indispensable Participants in Schistosomiasis-Associated Colonic Dysplasia

Nema Ali Soliman1*, Walaa Arafa Keshk1, Zeinab Salah Shoheib2, Dalia Salah Ashour2, Maha Moustafa Shamloula3

Abstract

Background: Schistosomiasis is a parasitic disease causing chronic ill health in humans with a serious consequences for socio-economic development in tropical and subtropical regions. There is also evidence linking Schistosoma mansoni to colonic carcinoma occurrence. The aim of this study was to evaluate some inflammatory and oxidative stress biomarkers, as well as L-fucose as linkers between intestinal schistosomiasis and colonic dysplasia development in mice. Materials and Methods: This study was conducted upon 80 mice that were divided into 7 sub-groups (10 mice each) according to the time of sacrifice in the post infection (p.i.) period, 10 mice being sacrificed every two weeks from 6 weeks p.i. to 18 weeks p.i. Tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS), and pentraxin 3 (PTX3) levels were estimated by immunoassay. The L-fucose level, and thioredoxin reductase (TrxR) and lactate dehydrogenase (LDH) activities were also evaluated in colonic tissue. Results: The current study revealed statistically significant elevation in the studied biochemical markers especially at 16 and 18 weeks p.i. The results were confirmed by histopathological examination that revealed atypical architectural and cytological changes in the form of epithelial surface serration and nuclear hyper-chromatizia at 14, 16 and 18 weeks p.i. Conclusions: inflammation, oxidative stress and L-fucose together may form an important link between Schistosomal mansoni infection and colonic dysplasia and they can be new tools for prediction of colonic dysplasia development in experimental schistosomiasis.

Keywords: Schistosoma mansoni - colonic dysplasia - TNFα - iNOS - PTX3 - L-fucose - TrxR - LDH

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Introduction

Schistosomiasis (Bilharziasis) heads the list of endemic parasitic diseases in Egypt, also in other 75 countries worldwide; it affects more than 200 million people, and putting more than 1 billion populations at great risk (Mostafa et al., 1999; Yosry, 2006). There are three major species: schistosoma mansoni (S. mansoni), schistosoma japonicum (S. japonicum) and schistosoma haematobium (S. haematobium) (Gryseels et al., 2006). Schistosoma mansoni eggs and worms deposition in colonic tissue cause chronic inflammatory reaction with production of reactive oxygen intermediates with subsequent oxidative stress, dysregulation in T cell response, and alterations in host epithelial carbohydrate expression (Hope et al., 2005). In endemic areas, schistosomiasis has been associated with various types of malignancy such as carcinoma of the intestine, liver, uterus and urinary bladder (Madbouly et al., 2007).

Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine that plays a key role in apoptosis and cell survival as well as in inflammation and immunity (van Horssen et al., 2006). Pentraxins are family of proteins divided into two groups: short pentraxins and long pentraxins. The long pentraxin group is pentraxin 3 (PTX3) which is produced in a variety of tissues during inflammation (Christersdottir Björklund et al., 2013).

Nitric oxide synthases (NOSs) are family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. The inducible isof orm of NOSs (iNOS) produces large amounts of NO as a defense mechanism of the body to attack parasites, bacteria and tumor growth (Yu et al., 2006).

Alpha-L-fucose (fucose) is a 6-carbon deoxyhexose found at the terminal positions of cell-surface oligosaccharide which have an important role in cell-cell adhesion, leukocytes adhesion, signal transduction, apoptosis and blood group recognition as well as, in pathologic processes include inflammation, infectious disease and cancer (Takeda et al., 2012). Thior edoxin (Trx) system exists in all living cells and has an evolutional history tied to DNA as a genetic material and as a defense against oxidative damage. Therefore, Trx reductase (TrxR) enzyme has been...
considered to play an important role in regulating cell growth and death and its dysregulation has been closely linked to tumorigenesis (Lincoln et al., 2003). Lactate dehydrogenase (LDH) is an essential enzyme involved in anaerobic glycolysis and is responsible for the anaerobic transformation of pyruvate to lactate. In recent years the relationship between neoplasia and LDH has been studied with increasing intensity (Vetraino et al., 2013).

Schistosoma plays a critical role in the development of malignant lesions in different organs. The pathogenesis of cancer is currently under intense investigation to identify reliable prognostic indices for disease detection. The objective of the present study was to evaluate the link between schistosomal infection and colonic dysplasia through inflammation, oxidative stress and L-fucose to find biomarkers that can be used for prediction of colonic dysplasia and subsequent cancer development.

Materials and Methods

Chemicals

Chemicals used unless otherwise described were purchased from Sigma (Sigma, St Louis, USA) and were of high analytical grade.

Induction of schistosoma mansoni infection

Laboratory bred Biomphalaria Alexandrina snails were purchased from the Schistosome Biological Supply Program, Theodore Bilharz Research Institute (TBRI), Giza, Egypt. The current work was carried out at Medical Biochemistry Department, in accordance to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) to minimize animal suffering and in accordance to the guidelines of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt. Mice were housed in appropriate cages and allowed ad-libitum for a commercial rodent chow and tap water (EL-Nasr Chemical Company, Cairo, Egypt) through the whole period of the experiment. The snails were placed in beakers containing dechlorinated water (1ml/snail) and exposed to direct light for 4h. at 28 °C to allow for cercarial shedding (Lewis et al., 1986). Cercarial concentration was adjusted as 40-50 cercariae/0.1ml dechlorinated water to be used for animal infection.

Experimental design

Eighty mice, laboratory-bred and parasite free male Swiss albino mice, 6 weeks old and 20-25g body weight, were used in this study. Mice were housed and infected in accordance with the institutional guidelines. Ten mice were left un-infected to serve as normal non-infected control (group I), they were sacrificed once at the end of the experiment. The remaining mice (group II), were infected sub-cutaneously with schistosoma mansoni cercariae (40-50 cercariae/mouse) according to Peters and Warren, 1969. The infected mice were divided into 7 sub-groups where 10 mice were sacrificed every two weeks interval from 6 to 18 weeks p.i.

Blood sampling

Animals were anaesthetized by ether, and while the heart was still beating, blood was collected on heparinized tubes, blood was removed for estimation of hemoglobin level using commercial kit supplied by Sigma scientific service, Egypt, the remaining part of the sample was centrifuged for 15 minutes at 1000xg for plasma separation which were divided in to small aliquots and frozen at -80°C until analysis, theuffy coat was aspirated; erythrocytes were washed three times with isotonic saline and haemolysed by diluting four fold with distilled water, centrifuged at 10,000xg for 15 min at 4°C. The supernatant (erythrocyte lysate) was collected and stored at -80°C until analysis of erythrocyte TrxR activity.

Tissue samples

After blood sampling, the animals were sacrificed, colonic tissues removed, washed with ice cold saline to remove extraneous materials then divided into pieces. One piece from each group was kept into in 10% neutral buffered formalin solution and fixed for histopathological examination for assessing morphological changes with hematoxylin and eosin (H.&E.) and the remaining pieces from all groups were homogenized in ice-cold 50 mM phosphate buffer (pH 7.4) at 7,700xg for 30 minutes at 4°C, and supernatant was divided in to small aliquots and frozen at -80°C until analysis. Protein content was measured by the method of Lowry et al. (1951).

Biochemical study

Assessment of inflammation: plasma/tissue homogenate were assayed for TNF-α level by ELISA kit (eBioscience, USA), Plasma PTX3 and tissue homogenate iNOS levels were determined using ELIZA kits (R&D Systems Inc., Minneapolis, MN, USA) and (Glory Science Co., Ltd) respectively. 

Assessment of redox status: erythrocytic/tissue homogenate TrxR activity was assayed according to the method of (Holmgren and Bjornstledt, 1995).

Assessment of L-fucose level and LDH activity: L-fucose level was estimated according to the method of (Dische, 1949) and LDH activity according to the method of (Babson and Babson 1973).

Statistical analysis

Statistical presentation and analysis of the present study results were conducted, using the mean, standard deviation by SPSS version 16. Analysis of variance [ANOVA] tests and Tukey’s test were used to determine the significance between more than 2 groups: according to the computer program SPSS for Windows. P value <0.05 was considered significant.

Results

Biochemical study

Assessment of inflammation: plasma/tissue TNF-α and plasma PTX3 levels showed statistically significant difference between all groups at different times p.i. with
Table 1. Non Enzymatic Biomarkers Associated Colonic Dysplasia in Schistosoma Mansoni Infected Mouse at (6, 8, 10, 12, 14, 16 & 18) Weeks Post Infection (p.i.) Compared to Non Infected Control Group

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>Group</th>
<th>6 weeks p.i.</th>
<th>8 weeks p.i.</th>
<th>10 weeks p.i.</th>
<th>12 weeks p.i.</th>
<th>14 weeks p.i.</th>
<th>16 weeks p.i.</th>
<th>18 weeks p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma tumor necrosis factor-α level (pg/ml)</td>
<td>Control</td>
<td>3.2±0.2*</td>
<td>6.3±0.1*</td>
<td>5.9±0.3*</td>
<td>5.9±0.3*</td>
<td>5.9±0.3*</td>
<td>5.9±0.3*</td>
<td>5.9±0.3*</td>
</tr>
<tr>
<td>Colonic tissue tumor necrosis factor-α level (pg/mg protein)</td>
<td>Control</td>
<td>10.1±0.5*</td>
<td>10.2±0.8*</td>
<td>8.5±0.8*</td>
<td>6.4±0.4*</td>
<td>6.4±0.4*</td>
<td>6.4±0.4*</td>
<td>6.4±0.4*</td>
</tr>
<tr>
<td>Colonic tissue iNOS level (ng/mg protein)</td>
<td>Control</td>
<td>11.2±0.8*</td>
<td>12.4±0.6*</td>
<td>13.2±1.2*</td>
<td>12.9±1.4*</td>
<td>12.9±1.4*</td>
<td>12.9±1.4*</td>
<td>12.9±1.4*</td>
</tr>
<tr>
<td>Colonic tissue L-fucose level (mg/100 mg wet tissue)</td>
<td>Control</td>
<td>1.4±0.2*</td>
<td>1.6±0.2*</td>
<td>1.8±0.3*</td>
<td>1.9±0.3*</td>
<td>1.9±0.3*</td>
<td>1.9±0.3*</td>
<td>1.9±0.3*</td>
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</table>

Table 2. Enzymatic Biomarkers Associated Colonic Dysplasia in Schistosoma Mansoni Infected Mouse at (6, 8, 10, 12, 14, 16 & 18) Weeks Post Infection (p.i.) Compared to Non Infected Control Group

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>Group</th>
<th>6 weeks p.i.</th>
<th>8 weeks p.i.</th>
<th>10 weeks p.i.</th>
<th>12 weeks p.i.</th>
<th>14 weeks p.i.</th>
<th>16 weeks p.i.</th>
<th>18 weeks p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma LDH activity (U/l)</td>
<td>Control</td>
<td>0.4±0.2*</td>
<td>0.6±0.3*</td>
<td>0.8±0.4*</td>
<td>0.9±0.5*</td>
<td>0.9±0.5*</td>
<td>0.9±0.5*</td>
<td>0.9±0.5*</td>
</tr>
<tr>
<td>Plasma TrxR activity (U/l)</td>
<td>Control</td>
<td>8.6±2.3*</td>
<td>6.3±0.4*</td>
<td>6.3±0.4*</td>
<td>6.3±0.4*</td>
<td>6.3±0.4*</td>
<td>6.3±0.4*</td>
<td>6.3±0.4*</td>
</tr>
<tr>
<td>Colonic tissue TrxR activity (U/gm wet tissue)</td>
<td>Control</td>
<td>10.1±0.5*</td>
<td>8.5±0.8*</td>
<td>6.4±0.4*</td>
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**L-fucose and LDH activity**

Plasma/tissue L-fucose levels and LDH activity showed statistically significant difference between all groups at different times p.i. with higher values for groups sacrificed at 10, 12, 14 p.i. and higher values for group sacrificed at 16, 18 weeks p.i.

**Histopathological findings**

Hyperplastic changes were detected 10 weeks p.i. onwards in nearly all the colonic specimens in the form of proliferation of the glands and increased mucosal thickness (Figure 1a). Some focal atypical changes were detected 14 weeks p.i. onwards in the form of focal mucin depletion (Figure 1b), mild nuclear hyperchromasia and crypt distortion and dilatation (Figure 1c). At 16, 18 weeks p.i. other atypical architectural and cytological changes lower values were for groups sacrificed at 10, 12, 14 p.i. and higher values were for group sacrificed at 16, 18 weeks p.i. as shown in Table 1. Colonic tissue iNOS level showed statistically significant differences between all groups at different times p.i. with higher values were for groups sacrificed at 14, 16 and 18 weeks p.i. as shown in Table 2.

Assessment of redox status: tissue TrxR activity showed statistically significant difference between all groups at different times p.i. with higher values for group sacrificed at 16 and 18 weeks p.i. Erythrocytic TrxR activity showed statistically significant difference between all groups at different times p.i. with lower values were for groups sacrificed at 14, 16 and 18 weeks p.i. than other groups but still higher than control. However, there were statistically significant difference between all groups and control as regards to both tissue and erythrocytic TrxR activity as shown in Table 2.
were detected in the form of epithelial surface serration (Figure 1d).

Discussion

Several explanations have been advanced for the possible role of schistosomiasis in colorectal dysplasia and tumorigenesis such as: the presence of endogenously produced carcinogens, chronic immunomodulation resulting in impairment of immunological surveillance, symbiotic action of other infective agents and the presence of schistosomal toxins (Schetter et al., 2010). While these factors may interact to induce carcinogenesis, chronic inflammation appears to play a central role, a phenomenon analogous to that described in the context of colitis-associated cancer (Medzhitov, 2008).

The great similarity and homology between the genomes of rodents and humans make them suitable model to study different pathological conditions. Since that mice model has a similar course to human schistosomiasis in addition that ten days in the life of a rodent are approximately one year in humans, the current study was established a mice schistosomiasis model through schistosoma mansoni cercariae infection (Abdul-Ghani and Hassan, 2010) to study the effect of intestinal schistosomiasis on the colon. From animal studies, it is estimated that the acute phase of infection for schistosomiasis occurs roughly 5-6 weeks after infection while the chronic phase begins greater than 12 weeks post infection with periportal liver fibrosis and extreme splenomegaly (H Salim et al., 2010).

Adenomatous polyps, chronic inflammatory bowel diseases and infectious diseases are the precursors of colorectal dysplasia and carcinoma. Colonic schistosomiasis is a specific acute or chronic inflammatory reaction in response to schistosoma ova that are deposited mainly in colorectal mucosa (Cao et al., 2010, H Salim et al., 2010). Among Egyptians, the incidence of colonic carcinoma is less than the incidence of bladder carcinoma. Colonic carcinoma occurs predominately in the Nile Delta region where the intermediate snail hosts of schistosomiasis are extremely abundant (Fedewa et al., 2009).

TNF-α is a potent mutagen due to its capacity to induce the generation of reactive oxygen species (ROS) and subsequent genetic instability in various types of cells (Yan et al., 2006). While PTX3 plasma levels are very low in normal condition but increase rapidly in several pathological conditions raising the possibility that PTX3 may have a diagnostic and prognostic role (Salustri et al., 2001). The present study showed significant increase in TNF-α and PTX3 levels in groups sacrificed at 16 and 18 weeks p.i. as compared to their allied control. Meanwhile, significant decrease in their levels was found at 10, 14 and 16 weeks p.i.

In consistence with our results Balkwill (2009); Mantawy et al. (2011) reported that TNF-α is a pro-inflammatory cytokine activates signal transduction pathways, leads to apoptosis, cell proliferation, angiogenesis, cellular senescence, DNA damage and increase the metastatic potential of tumors in animal models reflect its higher significant increase at 16 and 18 weeks p.i. In general, constitutive exposure to high levels of pro-inflammatory cytokines is thought to be pro-tumorigenic. Hamed et al. (2011) reported that blood level of TNF-α was higher in colonic carcinoma associated with schistosomal infection than colonic carcinoma without schistosomal infection. These data are in accordance with Talero et al. (2011) who had recorded the highest production of TNF-α in mice experimentally induced colorectal cancer.

PTX3 is over-expressed in some dysplastic lesions and malignancies and regulated by various cytokines, including TNF-α which reflects its higher significant increased level with TNF-α in 16 and 18 weeks p.i. groups (Willeke et al., 2005). In harmony of our results Okutani, 2006 suggested that PTX3 associated with inflammation and cancer cell apoptosis around the tumor microenvironment due to its over-expression by endothelial cells and macrophages in response to inflammatory signals, as well as its role in clearance of cells undergoing apoptosis, suggests that PTX3 may act as a biomarker for malignancy.

Collectively, Zhang et al. (2013) reported that inflammatory cells and their inflammatory cytokines that largely orchestrated the tumor microenvironment are indispensable participants in the neoplastic process, fostering proliferation, survival and migration.

In inflammatory process and carcinogenesis nitric oxide (NO) plays a multifaceted role, there is no doubt about the protective role of NO in physiological conditions. However, when the mucosa is threatened, the role of NO becomes multiple and the final effect will probably depend on the nature of the insult, the environment involved, and the interaction with other mediators (Calatayud et al., 2001). Recent literatures indicate that NO and iNOS can modulate cancer-related events including nitro-oxidative stress, apoptosis, cell cycle, angiogenesis, invasion, and metastasis (Yang et al., 2009).

The results of this current study showed a significantly increased in iNOS level in colonic tissue in all groups sacrificed p.i. than control non infected group with more higher value were for groups sacrificed at 14, 16, and 18 weeks p.i. To our knowledge no previous studies evaluate iNOS level as a biomarker of colonic dysplasia associated schistosoma mansoni infection in an experimental model. In harmony with results herein Ohshima et al. (2006); Salim et al. (2008) found that inflammatory response associated with infectious diseases cause iNOS-mediated oxidative stress during carcinogenesis and NO generated by iNOS in the schistosoma associated inflammatory process, causes damage to DNA and increase apoptosis.

Control mechanisms of ROS production play a critical role in tumor development as transformed cells generate more ROS than normal cells, importantly, ROS not only contribute to tumor progression by amplifying genomic instability but also transformed cells use ROS signals to drive proliferation (Yang et al., 2013). One of the strategies that neoplastic cells use to survive is to utilize the intracellular redox system such as the TrxR/Trx couple, glutathione (GSH)/oxidized glutathione (GSSG) couple and other related enzymes (Mukherjee and Martin, 2008).

TrxR are essential components of the thioredoxin system.
system and are therefore crucial for the control of cellular redox balance and maintain thioredoxins which are small proteins that catalyse redox reactions and contribute to many hallmarks of cancer, such as increased proliferation, inhibited apoptosis and angiogenesis (Arner and Holmgren, 2000). The results of this current study showed significant increase in its level in colonic tissue and erythrocyte in all groups sacrificed p.i. than control non infected group with 14, 16 and, 18 weeks p.i. groups showed the higher values in colonic tissue but these groups showed the lower values in erythrocyte TrxR activity. To our knowledge no previous studies to evaluate TrxR activity as a biomarker of colonic dysplasia associated schistosoma mansoni infection in an experimental model. 

However Lincoln et al. (2003); Smart et al. (2004); Biaglow and Miller (2005) found that TrxR was highly expressed in some tumors including gastric, non-small cell lung carcinoma and hepatocellular carcinomas and can be used as a potential target molecule for anti-cancer treatment.

Dysplastic and cancerous cells synthesize a wide variety of biochemical products that can be detected in body fluids, of predominance and with great significance are blood glycoproteins (Manjula et al., 2010). Alpha-L-fucose is commonly incorporated into human glycoproteins and glycolipids (Manjula et al., 2010). The results of this current study showed significant increase in L-fucose level in colonic tissue and plasma in groups sacrificed at 16 and 18 weeks p.i. only however, no statistically significant difference between control and the remaining p.i. groups. In harmony with this current study, Kossowska et al. (2005) showed that fucosylated oligosaccharides are specific markers for inflammation and tumorigenesis and previous clinico-pathological studies of colorectal and lung cancer patients as well as experimental models of these cancer cells indicated that increase in fucosylated residues promote metastatic potential and indicate poor prognosis which explained its higher level in 16 and 18 weeks p.i. groups.

Moreover Manjula et al. (2010) reported that L-fucose normally present in low concentrations in blood but it is increased in cancer and it has been documented that tumor cells modulate their surface by increasing fucosylation levels to escape recognition, which contributes to several abnormal characteristics of tumor cells, such as decreased adhesion and uncontrolled tumor growth. To our knowledge no previous studies evaluate L-fucose level as a biomarker of colonic dysplasia associated schistosoma mansoni infection in an experimental model. Collectively monitoring blood/tissue L-fucose levels could be a promising approach for the early detection, diagnosis and prognosis of colorectal cancer.

Many investigators have focused their work on the enzymatic reactions in tumor cells and consider most of these enzymes to be tumor markers as Shin et al. (2009) who reported that the activities of glycolytic enzymes as LDH which convert pyruvate into lactate are sharply intensified in colonic cancer, while key gluconeogenesis enzymes are sharply decreased. The results of this current study showed significant decrease in its level in colonic tissue homogenate in 6, 8, 10, 12, and 14 p.i. groups than control non infected group. Meanwhile, groups sacrificed at 16, 18 p.i. showed the highest values. However, significant increase in its plasma level in all p.i. groups than control non infected group with 16, and 18 weeks p.i. groups showed the highest values also.

The variation in plasma and tissue results can be explained by induced tissue damage in the infection period, led to the release of enzyme from the necrotic tissue to blood stream or due to the increased cell anoxia and irritation by toxic or metabolic products of the worm as reported by Ahmed and Gad (1995); Hamed et al. (2011). In accordance to results herein Thangaraju et al. (2009); Metwally et al. (2011) reported that elevation of LDH activity in malignant and bilharzial colonic carcinoma can be used as an indicator for the stimulation of anaerobic pathways in cases of abnormal growth of human cancer cells. Aly et al. (2004); Hamed et al. (2010) reported that, the increase in LDH activity in bilharziasis can be related to the fact that the schistosoma parasite is producing lactate as a sole metabolic end, enhancement of anaerobic glycolysis by schistosoma toxins and suppression of pyruvate oxidative metabolism in mitochondria.

Ross et al. (2002) reported that the sequestered eggs in the intestinal mucosa and submucosa caused severe inflammatory reaction with cellular infiltration and consequent granuloma formation. This in turn lead to mucosal ulceration, microabscess formation, polyposis, and neoplastic transformation.

Histopathological study results herein confirmed the biochemical results as it revealed the presence of atypical changes in the form of hyperplasia, nuclear hyperchromatias and epithelial serration in groups sacrificed at 14, 16 and 18 weeks p.i. These results were in accordance to Farid et al. (2006); Madbouly et al. (2007) who described the presence of pseudopolyps, multiple ulcers, and hyperplastic ectopic submucosal glands.

In conclusion, intestinal schistosomiasis should be considered as a precancerous condition for development of colonic dysplasia and cancer as a consequence of chronic inflammation that altered inflammatory, antioxidant and fucosylation status associated schistosomiasis. From another point of view, the problem of bilharziasis and its various aspects needs careful clinical studies to clarify its effect on the biochemical features of tumor development.

References


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