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IMMUNOBIOCHEMICAL PARAMETERS AS DIFFERENTIAL FACTORS BETWEEN COLORECTAL CANCER AND ITS ASSOCIATION WITH INTESTINAL SCHISTOSOMIASIS

Samia A. Ahmed[1], Manal A. Hamed[1*], Omar S. Omar[2], Abdelbaset A. El-Aaser[3]

[1]Therapeutic Chemistry Department, National Research Center, Dokki, Cairo, Egypt.

[2] Breast Cancer Clinic, Department of Surgery, Cairo University Hospital, Cairo, Egypt.

[3] Cancer Biology Department, National Cancer Institute, Cairo University, Egypt.

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***Correspondence for Author:**

Dr. Manal A. Hamed
Therapeutic Chemistry Department, National Research Center, El-Tahrir St., Dokki, Cairo, Egypt.
manal_hamed@yahoo.com

ABSTRACT

Objective: Schistosomal infestation has been implicated in the etiology of several human malignancies including colorectal cancer (CRC). While sufficient evidence supports a relationship between *S. japonicum* species and CRC, the evidence linking *S. mansoni* to CRC occurrence is meager. Therefore, the object of the present study is to evaluate certain biochemical parameters as diagnostic tools to differentiate between colonic carcinoma and colonic carcinoma associated with *S. mansoni* infection among Egyptian patients. Design and Methods: Patients with intestinal shistosomiasis, colonic cancer and bilharzial colonic cancer were enrolled in this study. The parameters under investigation are intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VACM-1), endothelial leukocyte adhesion molecule-1 (E-selectin), fibronectin (FN), alpha fetoprotein, IL-10 and nitric oxide (NO). Results: The results revealed significant increase (P<0.0001) in all parameters under investigation in case of bilharzial colonic cancer patients than in colonic carcinoma or in schistosomal infection alone. Conclusions: These parameters succeeded in serving as biomarkers to differentiate between the two malignant types and elucidated their role in colorectal cancer secondary to shistosomiais.

Keywords: Colorectal cancer, shistosomiasis, adhesion molecules, tumor-biomarkers.

1. INTRODUCTION

Schistosomiasis is still a significant public health problem in developing countries [1]. Colonic schistosomiasis is a specific acute or chronic inflammatory reaction in response to Schistosoma ova that are deposited mainly in colorectal mucosa [2,3]. 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 [4, 5].

Colorectal cancer is responsible for more than 500,000 deaths worldwide every year [6]. In 70–80% of patients, the tumour appears to be localized at diagnosis. After resection of the tumour, 40-50% of patients will relapse, generally with distant metastases [7]. Tumour relapse and the formation of metastases is a multistep process involving complex interactions between tumour cells and the vascular endothelium [8]. These interactions are mediated by several cell-surface adhesion receptors including the selectins, integrins and immunoglobulin-like cell adhesion molecules [9]. Cancer cell adhesion to the extracellular matrix (ECM) is a crucial prerequisite for cell migration [10]. Although it is not clear how the ECM may regulate cancer cell migration, several reports have indicated that diverse ECM components located in the vicinity of cancer cells may induce various signals for cell motility and the regulation of cancer cell migration [10].

E-selectin is expressed on activated endothelial cells and may bind cells expressing specific ligands containing sialyl-Lewis residues [11]. Experimental studies have suggested that the efficiency of the E-selectin-mediated binding of colonic carcinoma cells to human endothelium correlates with tumour progression and the formation of haematogenous metastases [12].

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are both members of the immunoglobulin superfamily of adhesion molecules [11]. ICAM-1 is constitutively expressed by endothelial cells and by some leucocytes and serves as a ligand for the leucocyte b2 integrin receptors, lymphocyte function associated antigen-1 (LFA-1) and Mac-1 [13]. VCAM-1is found mainly on activated endothelial cells serving as a ligand for the a4b1 integrin receptor; VLA-4 [14]. Both ICAM-1 and VCAM-1, like E-selectin, are expressed on endothelial cells of small blood vessels adjacent to colorectal cancer cell nests [15]. Their expression is upregulated by cytokines produced by the tumour and soluble forms of all three adhesion molecules have been detected in the supernatants of

cytokine-activated endothelial cells and in the culture media of colonic carcinoma cell lines [16]. Elevated serum concentrations of E-selectin, ICAM-1 and VCAM-1 have only recently been described in patients with solid tumours, including colorectal cancer [17]. These serum levels correlate in many studies with tumour stage and the development of metastases, although their impact on patient survival is at present unclear [11].

Invasion into the normal surrounding tissue by the tumor is the first overt sign of its malignancy. Invasion occurs due to degradation of extracellular matrix components by the hydrolytic enzymes elaborated by the tumor or induced by it in the stromal cells [18]. The products resulting from the degradation of ECM components are released in the circulation, and subsequently cleared via the urine [19]. The appearance of the fragments of ECM proteins in blood plasma, if they normally do not occur, or the elevation in their level over the physiological concentrations, should indicate the existence of malignancy. With this view, the appearance of the cellular fibronectin (cFN) (fragments of the ECM protein) in blood plasma means that malignancies are initiated [20]. The major function of cFN is to provide binding sites to adhesion proteins and components of the extracellular matrix, but its role in blood plasma, if any, is not known. High plasma levels of total FN and cellular FN have been reported in patients with breast, ovarian, lung, ancreatic, colon and renal cell carcinoma [21].

Human alpha-fetoprotein (HAFP) is tumor-associated fetal protein, termed an oncofetal protein, consisting of 609 amino acids (AAs) including a 19 amino acid (AA) signal sequence [22]. HAFP has been shown to be a growth enhancing factor in its circulating, compact-folded, native full length configuration in studies of both fetal and tumor cell growth and proliferation studies [23]. Having served as a serum biomarker for cancers of the liver, gonads, and gastrointestinal tract, HAFP is now being investigated as an activator of cell surface receptors as well as a regulator of cytoplasmic transcription factors involved in signaling pathways [24]. When present in stress and shock environments, full-length HAFP undergoes a conformational change which temporarily converts the growth enhancing oncofetal protein to a growth inhibitory form referred to as-transformed AFP (tAFP) [25].

Nitric oxide (NO) is a pleiotropic regulator, critical to numerous biological processes, including vasodilatation, neurotransmission and macrophage-mediated immunity. NO has been detected in tumour cells of various histogenetic origins and has been associated with tumour grade, proliferation rate and expression of important signaling components associated with cancer development such as the oestrogen receptor [26]. Depending on the concentration

of NO within a cell and the cell's genetic background, NO is able to act in a dual mode, leading either to an induction of apoptosis or to a blunted execution of programmed cell death [27].

IL-10 is Th2-cytokine, which increases antibody synthesis, promotes the humoral immune response and suppresses the antitumor immunity [28]. IL-10 has been shown as immune suppressive cytokine, which aids the development of tumors through oppression of the anti-tumor cell mediated immune response. From another side, number of pre-clinical and clinical experiments with both animal models and human cell populations show regression of the tumor after IL-10 application. This data shows IL-10 as a cytokine with an anti-tumor effect [29].

The aim of the present study is to evaluate ICAM-1, VCAM-1, E-selectin, FN, alpha fetoprotein, IL-10 and NO as diagnostic biomarkers in differentiating between colorectal cancer and hilharzial colorectal cancer among Egyptian patients.

2. MATERIALS AND METHODS

2.1. Clinical cases

All cases were of Egyptian colonic carcinoma and *Schistosoma mansoni* infected patients attending in the Clinical and Medical Oncology Department, National Cancer Institute (NCI), Cairo University, Egypt. All cases were diagnosed by clinical, pathological and histological examinations at the Clinical Pathology Department, NCI. Colonic cancer complicating schistosomal infection cases were residents of rural areas while colonic carcinoma patients were residents of urban areas. The oriental cell microscopic slides were examined. The tumour grade and pathological differentiation are illustrated in Table I.

2.2. Patients groups

Group 1: 15 individuals (10 males and 5 females with ages between 18-58 years) served as the control for all groups. Group 2: 10 intestinal *S. mansoni*-infected patients. Their ages ranged from 29-65 years (7 males, ages 35-65 years, and 3 females, ages 29-50 years). Group 3: 15 colonic cancer patients with ages ranging from 23-64 years (11 males, ages 25-64 years, and 4 females, ages 23-52 years). Group 4: 21 schistosomal colonic carcinoma patients with a mean age of 45 years (16 males, ages 25-69 years, and 5 females, ages 21-52 years).

2.3. Ethics

Serum samples were subject to the ethical guidelines approved by the National Cancer Institute.

2.4. Blood samples

A fasting venous blood sample (5 ml) was withdrawn and centrifuged. Sera were separated and stored at -80ºC until used.

2.5. Biochemical assays

Intercellular adhesion molecule-1 (ICAM-1) was estimated using an enzyme linked immunosorbent assay (ELISA) kit (Invitrogen, Cat. No. KHS5411, Camarillo, Canada). The colored end product at 450 nm is proportional to the amount of soluble ICAM-1 in the sample.

Vascular adhesion molecule-1 (VCAM-1) at 450 nm was estimated using a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) Kit (Invitrogen, Cat. No. KHT0602/KHT0601, Camarillo, Canada).

Endothelial leukocyte adhesion molecule-1 (E-selectin) was estimated using an enzyme linked immunosorbent assay (ELISA) kit (Millipore, Cat. No. ECM330, Massachusetts, USA). The colored end product at 450 nm is proportional to the amount of E-selectin in the sample.

Nitric oxide was assayed by the method of Moshage et al. [30]. The assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Giess Reaction at 540 nm.

IL-10 (cat.no: KHC0101, Paisley, UK) was carried out by using the invitrogen human IL-10 kit. It is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA). The intensity of the end developed colour is directly proportional to the concentrations of IL-10 present at 450 nm.

Alpha-fetoprotein (AFP) (cat. no: 0500, San Antonio, USA) kit was estimated by the alpha diagnostic international human alpha-fetoprotein kit. It is a solid phase sandwich enzyme

linked-immuno-sorbent assay (ELISA). The intensity of the developed colour is directly proportional to the concentration of AFP present at 450 nm.

Fibronectin was estimated using an enzyme linked immunosorbent assay (ELISA) kit (Assaypro LLC, Missouri, US). The intensity of the developed colour is directly proportional to the concentration of fibronectin present at 450 nm.

2.6. Statistical analysis

Data were expressed as the mean ± SD of cases numbers in each group. The statistical analysis was performed using one way analysis of variance (ANOVA), CoStat Computer Program. The least significance value between groups was at $p < 0.05$.

3. RESULTS

3.1. Role of adhesion molecules in different cases

Regarding to the different adhesion molecules selected in the present study, ICAM-1 recorded significant increase ($p<0.05$) in colonic carcinoma and bilharzial colonic carcinoma cases by 13.43 and 20.97%, respectively. In case of intestinal bilharziasis cases, ICAM-1 showed insignificant increase by 6.25% (Table 1 and Fig.1). VCAM-1 showed significant increase in intestinal bilharziasis, colonic carcinoma and bilharzial colonic carcinoma cases by 19.74, 72.17 and 175.59%, respectively. The same pattern of change was recorded in E-selectin as it recorded increments by 41.10, 82.37 and 137.14% in intestinal bilharziasis, colonic carcinoma and bilharzial colonic carcinoma cases, respectively (Table 2 and Fig.1).

3.2. Potency of immuno- modulators in different cases

Significant increase in IL-10 was observed in intestinal bilharziasis (86.31%), colonic carcinoma (184.04%) and bilharzial colonic carcinoma (208.67%) cases (Table 3 and Fig.1). NO also showed significant increase in intestinal bilharziasis, colonic carcinoma and bilharzial colonic carcinoma cases by 62.30, 109.76 and 181.29%, respectively (Table 3 and Fig.1).

3.3. Role of glyco-proteins in different cases

Serum fibronectin recorded significant increase in intestinal bilharziasis, colonic carcinoma and bilharzial colonic carcinoma cases by 96.17, 195.18 and 368.17%, respectively. Alpha fetoprotein also recorded the same pattern of increment as it increased by 69.70, 107.81 and 160.59%, respectively (Table 4 and Fig.1).

Table 1: Clinical data of different cases.

Items		Control	Intestinal bilharziasis	Colonic carcinoma	Bilharzial colonic carcinoma
No of cases		15	10	15	21
Sex	Male	10	7	11	16
	Female	5	3	4	5
Age ranges	Male	23-58	35-65	25-64	25-69
	Female	18-38	29-50	23-52	21-52
Grade	I	-	-	3	6
	II	-	-	8	11
	III	-	-	4	4
Stage	P1a	-	-	3	3
	P2a	-	-	2	6
	P3a	-	-	2	3
	P5a	-	-	-	4
	P2b	-	-	5	3
	P3b	-	-	2	2
	P5b	-	-	2	-
Bilharzia	-ve	-	-	15	-
	+ve	-	10	-	21
Nods	-ve	-	-	6	9
	+ve	-	-	9	12
Cell type	A	-	-	6	10
	S	-	-	9	11

- A: adenocarcinoma; S: squamous cell carcinoma

Table 2: Adhesion molecules in shistosomiasis, bilharzial and non-bilhazial colonic carcinoma patients.

Parameters	Control	Intestinal bilharziasis	Colonic carcinoma	Bilharzial colonic carcinoma
ICAM-1	268.20 ± 5.54^{c}	285.69 ± 4.75^{c}	303.24 ± 7.81^{b}	324.46 ± 5.26^{a}
VCAM-1	597.38 ± 10.44^{d}	715.32 ± 17.72^{c}	1028.51 ± 32.74^{b}	1646.36 ± 30.62^{a}
E- Selectin	112.33 ± 23.18^{d}	158.50 ± 12.31^{c}	204.86 ± 31.59^{b}	266.38 ± 20.91^{a}

- Data are mean± SD of cases numbers of each group.
- Values are expressed as ng/ml for ICAM-1 and CACM-1 and n mole/ml for E- Selectin.
- Un-sheared super script letters are significant values between groups at $p<0.05$.
- Statistical analysis is carried out by ANOVA test, CoStat, Software Computer Program.

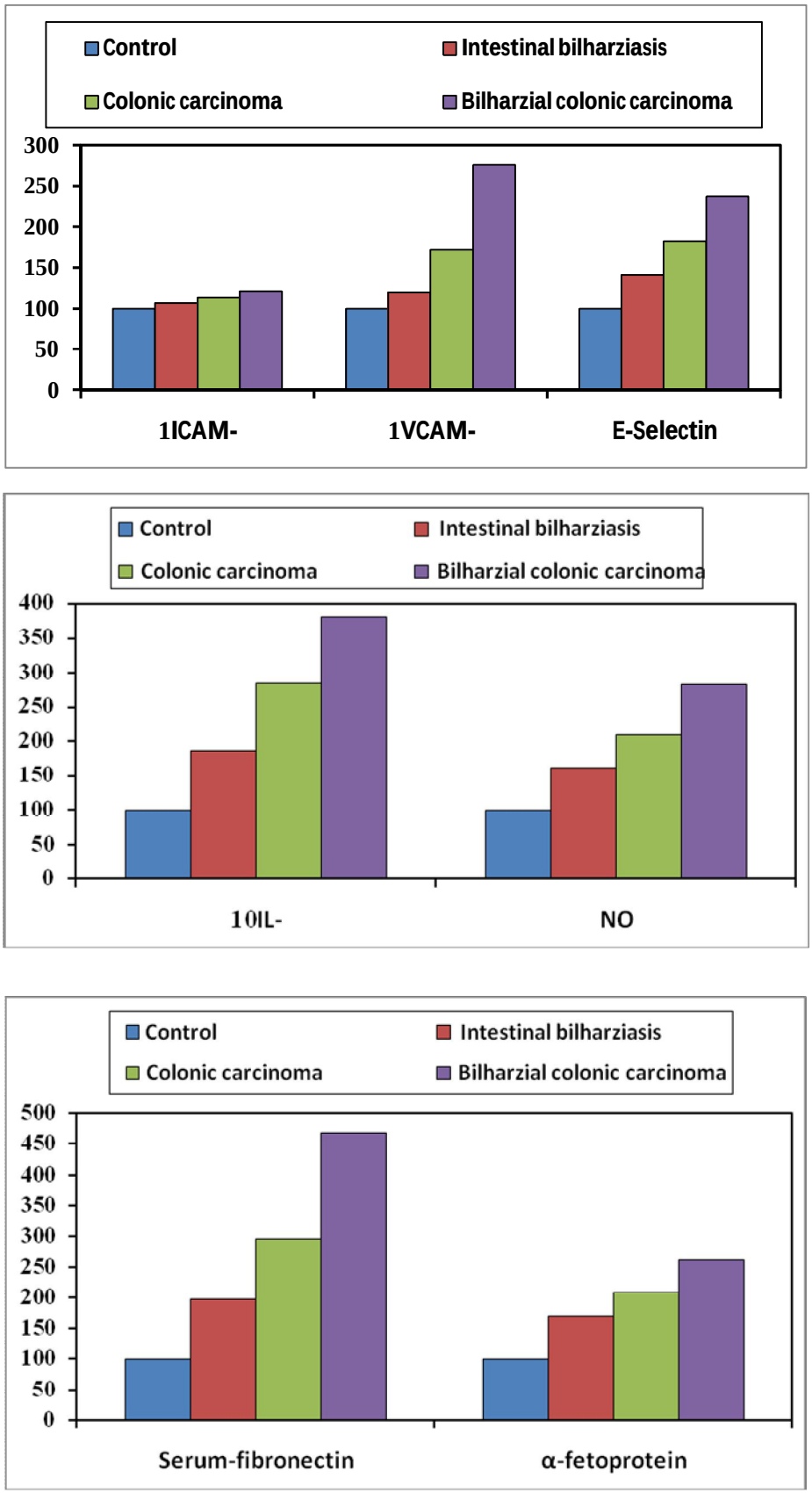

Fig. 1: % changes of different parameters over control group.

Table 3: Immuno-modulators in shistosomiasis, bilharzial and non-bilhazial colonic carcinoma patients.

Parameters	Control	Intestinal bilharziasis	Colonic carcinoma	Bilharzial colonic carcinoma
IL-10	28.20 ± 1.26^{d}	52.54 ± 1.57^{c}	80.10 ± 1.78^{b}	107.35 ± 22.588^{a}
NO	35.44 ± 6.91^{d}	57.52 ± 6.59^{c}	74.34 ± 5.17^{b}	99.65 ± 6.87^{a}

- Data are mean± SD of cases numbers of each group.
- Values are expressed as ng/ml for IL-10 and n mole/ml for NO.
- Un-sheared super script letters are significant values between groups at $p<0.05$.
- Statistical analysis is carried out by ANOVA test, CoStat, Software Computer Program.

Table 4: Glycoproteins in shistosomiasis, bilharzial and non-bilhazial colonic carcinoma patients.

Parameters	Control	Intestinal bilharziasis	Colonic carcinoma	Bilharzial colonic carcinoma
Serum-fibronectin	28.25 ± 0.75^{d}	55.42 ± 3.46^{c}	83.39 ± 3.17^{b}	132.26 ± 1.49^{a}
α-fetoprotein	9.34 ± 1.11^{d}	15.85 ± 3.26^{c}	19.41 ± 1.20^{b}	24.34 ± 2.15^{a}

- Data are mean± SD of cases numbers of each group.
- Values are expressed as ng/ml.
- Un-sheared super script letters are significant values between groups at $p<0.05$.

Statistical analysis is carried out by ANOVA test, CoStat, Software Computer Program.

4. DISCUSSION

In this study, bilharziasis, colonic carcinoma and bilharzial colonic cancer patients showed significantly higher serum levels of the cell adhesion molecules; E-selectin, ICAM-1 and VCAM-1 when compared with healthy controls. The bilharzial colonic cancer patients recorded the most potent higher level among groups. This increment was attributed to the highly inflammatory reactions due to garnuloma formation and egg deposition in case of schistosoma infection [5,31]. Previous studies have demonstrated elevated preoperative ICAM-1 and VCAM-1 levels in colorectal cancer patients [32], whereas results regarding E-selectin are more variable with some reports showing elevated levels of E-selectin in colorectal cancer patients, whilst others have failed to detect such a difference [33]. There was a strong association between the serum levels of all three adhesion molecules, disease stage and the presence of nods. This was in agreement with the results of Wittig et al. [34]

who recorded that soluble E-selectin upregulate ICAM-1 expression in a range of human tumour cell lines. The cellular source, mechanism of release and the structure of these soluble adhesion molecule isoforms are at present unknown [11]. It is likely that soluble products are derived from a range of sources including enzymatic cleavage from endothelial, leucocyte and tumour cell surfaces, perhaps influenced by the intra-tumoral cytokine environment [35].

In the present study, the association between the three adhesion molecules expressions and the elevation of IL-10 in colorectal cancer patients suggests an important role of these molecules in the host immune response. Stanilov et al. [28] suggested the role of IL-10 more a pro-tumor than as anti-tumor properties. The pro-tumor properties of IL-10 can be explained with the inhibitory effect on the Th1-cytokine production, the inhibitory effect on the engaging of apoptosis and stimulation of cell proliferation [36]. Contradictory, Mocellin et al. [37] added that IL-10 inhibits tumor-induced angiogenesis and enhance the production of tumor-toxic molecules (NO), which leads to tumor regression in some preclinical models.

In this work, serum fibronectin level in colonic and bilharzial- colonic cancer patients recorded highly significant increase than in shistosomal infection patients when compared with healthy subjects. The occurrence of cellular fibronectin in fragmented form in the plasma of cancer patients is consistent with the argument that hydrolytic enzyme-aided invasion would degrade ECM components and lead to their appearance in blood plasma in fragmented form [38]. In normal subjects it is likely that it arises as a consequence of tissue remodeling [39]. Bone remodeling, in particular, is an ongoing physiological process with continuous osteoclastic destruction of the tissue by matrix metallo- and serine proteinases [40]. From the blood plasma the fragments are likely to be eliminated in the urine.

We recorded association between the plasma levels of FN and the stage of the disease. Warawdekar et al. [38] attributed the presence of fragments of cellular-FN in the urine to the degradation products of cFN that cleared from the plasma into the urine. The same authors confirmed the presence of malignancy if cFN fragments had been found in urine. Grimaud et al. [41] added that the level of fibronectin increased significantly in colonic cancer patients associated with shistosomiasis due to the highly intragranulomatous deposition of connective tissue matrix.

Regarding to AFP, the present study revealed highly significant increase of AFP in bilharzial colonic cancer patients than in cancerous tissue. The increased level of AFP in schistosomal

infection patient is mainly due to the extensive granulomatous inflammatory reaction and to eggs deposition [42].The reported colorectal carcinomas have generally occurred in middle-aged to older men with the rectum most commonly affected, the serum AFP level is usually as high as several-thousand nanograms per milliliter [43]. AFP-producing colorectal carcinoma generally has a poor prognosis because of the frequent occurrence of blood-borne metastases. All the reported cases have extensive liver and/or lung metastases at the time of diagnosis and a very poor prognosis milliliter [43].

With respect to the nitric oxide level, it recorded significant increase in bilharzial colonic patients than the other groups. Hirata et al. [44] (2001) attributed this increase in NO level to the egg deposition that appear to be the major stimuli for NOS expression. Wenzel et al. [27] demonstrated that NO effectively inhibits apoptosis by scavenging superoxide anions generated in the mitochondria. In consequence, it prevents the down regulation of the anti-apoptotic factor bcl-XL, which controls the mitochondrial apoptosis pathway in HT-29 cells. The same authors added that NO as an antioxidant could markedly alter the response of colonic tumor cells to various anticancer drugs. Moreover, NO was function as an immunosuppressive cytokine that favors tumor escape from immune surveillance [37].

In conclusion, the selected biomarkers play a potential role in predicting colorectal cancer and its complications *via* schistosomal infection. An improved understanding of the mechanisms of association of adhesion mediators, their effect on the host immune response and the role of angiogenesis inhibitors on their expression is required to elucidate their place in colorectal cancer progression. Detailed studies must be done among colon cancer associated with schistosomiasis infection, especially in developing countries where *S. mansoni* is endemic, to establish new biomarkers for early diagnosis of the disease.

Conflict of interest

The authors declared no conflict of interest.

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