Cytogenetic Study of the Effect of *Schistosoma mansoni* Infection on Human Peripheral Blood Lymphocytes and the Role of β-Carotene and Vitamin E in Modulating this Effect.

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Abstract: Aim: This study has been made to determine the potential genotoxicity of *Schistosoma mansoni* on lymphocytes of infected patients using different mutagenic end points. The protective role of antioxidants pro vitamin β-carotene and vitamin E in minimizing these genotoxic effect was also studied. The study focused on the effect of schistosomiasis on the induction of sister chromatid exchange (SCEs) and other chromosomal aberrations. Patients and Methods: This work was conducted on 24 *Schistosoma mansoni* infected patients and 10 healthy adults as a control group. Lymphocytes from peripheral blood of patients and control group were used for culture and subsequent cytogenetic studies. Results: The results indicated that schistosomiasis was genotoxic in all examined tests. It induced a significant increase in the percentage of structural chromosomal aberrations and the frequency of SCEs. It also inhibited cell division and caused cell cycle delay. Lymphocyte cultures of *S. mansoni* patients treated with 10 µg/ml β-carotene or 20 mg/ml vitamin E showed a significant decrease in the percentage of structural chromosomal aberrations and the frequency of SCEs. Conclusion: Schistosomiasis has a genotoxic effect on peripheral blood lymphocytes. The use of the antioxidants β-carotene and vitamin E can be considered a promising approach not only toward inhibiting the genetic damage of schistosomiasis but also as prophylactic agents against infection with *S mansoni*. Furthermore, higher doses of antioxidant drugs, β-carotene and vitamin E, should be tried as an adjuvants to conventional therapy in a trial to improve treatment of schistosomiasis. [Journal of American Science. 2010;6(11):191-202]. (ISSN: 1545-1003).

Key words: Schistosomiasis, β-carotene, vitamin E, chromosomal aberration

1. Introduction

Schistosomiasis is a common parasitic disease, affecting millions of people, mostly in tropical and developing countries. One of the causative agents of the disease is a trematode worm, *Schistosoma mansoni* [1]. *Schistosoma mansoni* infects over 83 million people in Africa and the Middle East [2]. Egypt represents one of the most highly infected populations with schistosomes in the world [3] with an estimated prevalence of (33.7%-57.7%) in Upper Egypt [4].

Schistosomiasis has been suspected as a risk factor for various types of cancers e.g., bladder cancer, colorectal cancer and hepatic cancer. However, the mechanisms of the carcinogenesis are still unclear [5]. The fact that Schistosomiasis is found to have a mutagenic effect [6] and a co-mutagenic effect [7] may be one of those mechanisms.

The mechanisms of chromosomal aberrations involve the concepts of clastogens directly acting on DNA to produce strand breaks, and subsequently, the survival of these directly caused DNA strand breaks - or misrepairs of them -up to metaphase when they appear as chromosomal breaks or translocations [8]. Enzymes that continually repair DNA damage – frequently cannot counteract all of the oxidative attack, and the resulting damage may lead to genetic mutations that could contribute to carcinogenesis [9].

Many diseases are associated with oxidative stress; this is why the use of antioxidant rich food or antioxidant food supplements has become immensely popular. These antioxidants include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals such as selenium, manganese, copper, zinc and vitamins such as vitamins A, C and E beside compounds such as glutathione, uric

acid and flavonoids. These antioxidants protect, prevent or reduce the extent of oxidative destruction of cellular tissues. Elevated levels of lipid peroxidation products and the simultaneous decline of antioxidant defense mechanism has been suggested to be harmful through disruption of membrane lipid and damage of cellular organelles resulting in oxidative stress [10].

Carotenoids (provitamin A) and tocopherols (vitamin E) are lipid soluble antioxidants associated with decreased risk of several degenerative diseases [11].

Alpha tocopherol is the main form of vitamin E and this has been the most commonly studied dietary antioxidant supplement in clinical trials [12].

Vitamin E supplementation has been shown to take part in immunoregulation, antibody protection and resistance in planted tumor probably through increased tumourolytic effect of natural killer cells [13]. Also, it was reported that vitamin E protects against lipid peroxidation and prevents skin cancer [14, 15].

Dietary carotenoids play important roles in the promotion of human health as pro vitamin A, antioxidants and chemopreventive agents against certain types of cancers [16] and β-carotene have gained prominence for their role against reactive oxygen species (ROS), protecting the organism against oxidative stress and consequently preventing damages and tissue lesions [17].

Sister chromatid exchange (SCEs) represents the interchange of DNA replication products at apparently homologous chromosomal loci. These exchanges involve DNA breakage and reunion. SCE technique affords the opportunity for cytological detection of DNA interchange. This technique is used as a sensitive means of monitoring DNA damage. It is useful for assessing the cytogenic impact of clastogenic agents on chromosomes. The increased resolution of SCE detection afforded by fluorescence or Giemsa technique has permitted localization of SCEs relative to chromosome-banding patterns. In human chromosomes, SCEs occur preferentially in Q-negative bands or at the junction of Q-positive and Q-negative regions [18].

Double stranded breaks (DSBs) are dangerous DNA lesions as they can lead to massive loss of genetic information and to chromosomal rearrangements [19]. In the past ten years, researchers in genome stability have observed that many kinds of cancer are associated with areas where human chromosomes break [20].

Chromosome fragmentation represents an efficient means of induced cell death and is a clinically relevant biomarker of mitotic cell death. Chromosome fragmentation serves as a method to eliminate genomically unstable cells. Paradoxically, this process could result in genome aberrations common in cancer [21].

Centric fission results when a metacentric or submetacentric chromosome splits at the centromere, giving rise to two stable telocentric products, isochromosomes, or ring chromosomes [22].

The aim of the present study was to investigate chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes of Schistosoma infected patients. The roles of β-carotene and vitamin E as antimutagenic agents modulating the frequency of these chromosomal aberrations and sister chromatid exchange induced by schistosomiasis were also studied.

2. Materials and Methods

This study was conducted on 24 *schistosoma mansoni* infected patients (21 males and 3 females) with a mean age of 28.7 years who were submitted to Theodor Bilharz Research Institute, tropical medicine department. Lymphocytes from peripheral blood of patients were used for culture and subsequent cytogenetic studies. The results were compared to those of 10 healthy adults acting as a control group.

Sample collection:

Ten ml of blood were collected, five of which were collected in a Na-heparin vacutainer for the performance of lymphocyte cultures and the other five ml were collected in gel containing vacutainer for IgG antischistosomal antibody titer detection and liver functions (ALT and AST).

Stool analysis was also done.

For each culture, 25 metaphases were examined for chromosomal aberrations, 25 metaphases for sister chromatid exchanges, 100 metaphases for cell cycle kinetic and 1000 cells for mitotic index

Cytogenetic parameters:

Chromosome preparation for human peripheral blood:

For each sample, six lymphocyte cultures were set up, two of them were treated with 10 μg/ml β-carotene [13], another two were treated with 20 mg/ml vitamin E and the last two

cultures were used as controls. The two antioxidants were prepared as a suspension in 2% Cremophore-El under sterile conditions. Cultures were set up using whole heparinized venous blood.

One ml of blood was incubated in a culture medium consisting of 4 ml RPMI 1640 with L-glutamine + 1 ml fetal calf serum + 0.1 ml (penicillin + streptomycin) + 0.2 ml of phytoheamagglutinin. The medium was also supplemented with 10µg/ml 5-bromodeoxyuridin (DrdU). Cultures were set up in 9 ml culture tubes for 72 hrs. The culture tubes were wrapped in aluminum foil, transferred to a thermostatically controlled incubator at 37oC, kept in a tilted position and shaken daily. All the materials used were sterile. Two hours prior to termination of the culture i.e. 70 hrs after initiation of culture, Colcemid (0.4 µg/ml) was added and mixed thoroughly by shaking the tubes, then they were returned back to the incubator.

Harvesting and fixation of cells:

Each culture tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and cell pellet disrupted by flicking the base of the tube. A hypotonic solution 0.075 M KCl (37oC) was added to give a light cloudy solution about (5-6 ml) and left to stand for (20-30) min. Half ml of fresh fixative (Methanol: glacial acetic acid (3:1) was added to each tube. The tube was centrifuged again at about 1000 rpm for 10 min. The supernatant was discarded and cell pellet was disrupted. The cells were then fixed in 4 ml methanol/glacial acetic acid (3:1) fixative for 10 minutes at room temperature. The fixative was changed twice and the cells were finally suspended in a small amount of fixative (0.5-1 ml). Two or three drops of the cell suspension were dropped onto a clean slide dipped in cold 70 % ethanol. The slides were flame dried.

Slide staining:

The principle of the fluorescent plus Giemsa (FPG) technique [23] with some modifications was followed for scoring SCEs. After complete drying, the slides were divided and marked into 2 parts; part stained in 50 µg/ml of Hoechst 33258 dye for 15 minutes (protected from light). Slides were rinsed in distilled water, layered with 2 x SSC buffer (PH 7), cover slipped, immersed in clean wide Petri dishes full of 2 x SSC buffer (PH 7) and subjected to UV light e.g. predominantly 365 nm Hg line, 400 nm dichromic mirror for 90-120 minutes in a closed cabinet. Slides were rinsed in distilled water and immersed in 4 % Giemsa dye for 7 minutes. The second part was stained directly in 4% Giemsa stain for seven minutes as well as the 1st slides without staining in Hochest or exposed to U.V light for scoring of chromosomal aberrations.

Scoring of chromosomal aberrations:

For each patient or normal control, 25 metaphases were examined microscopically for chromosomal aberrations in β-carotene, vitamin E treated tubes as well as in the untreated tubes. Only cells having well spread chromosomes with minimal overlapping were selected for scoring. Photographs were made of all types of abnormal metaphase Figures. Structural aberrations such as gaps, breaks, deletions, end to end association, centric fusion and centromeric attenuation were recorded. Also, metaphase spreads were examined for evident numerical aberrations including euploidy (endomitosis).

Scoring of sister chromatid exchanges:

For each patient or normal control the frequency of SCEs was recorded in 25 metaphase spreads in second division cells in β-carotene, vitamin E treated tubes and untreated tubes. In some patients and because of the very low mitotic indices, we could not complete 25 metaphases and we calculated the mean of all found metaphases.

Mitotic activity:

The mitotic index was counted as the ratio of mitoses to interphase nuclei in 1000 cells.

Cell cycle kinetics:

Cell cycle analysis can be studied by calculating the replicative index (RI) [24], a derived index that reflects the relative contribution of each cell cycle to the sample population. In 100 consecutive metaphase cells, at each dose level, the number of first (M1), second (M2) and third or subsequent (M3) divisions were determined. The RI was calculated as follows: RI = 1M1 +2M2 + 3M3 /100.

Stool examination and measurement of Schistosomal antibodies, ALT and AST:

Preparation and examination of patients stool specimens by saline stool smear was done according to (Haridy, 1979), and measurement of Schistosomal antibodies in human sera was done by ELISA technique using (Schistosoma IgG (EIA-3512) from DRG international inc., U.S.A.). ALT and AST were evaluated coloremitrically in sera of patients and control by commercially available kits according to manufacturer's guidelines.

Statistical analysis:

Statistical analysis was performed by using SPSS software for windows II version. Student (t) test was used to compare means of SCE, cell cycle kinetics while Chi square test was used for chromosomal changes and mitotic indices.

3. Results

The biochemical data (ALT level, AST level and antischistosomal antibody titer) of normal controls and patients are represented in Table 1.

Table 1: Biochemical data (ALT level, AST level and antischistosomal antibody titer) of normal controls and patients.

	Control group	Patient group
ALT	2.9+/- 0.57	6.1±1.26
AST	6.4+/- 1.19	13.85±1.99*
Serum level of specific IgG		2.77±0.34

*P<0.05

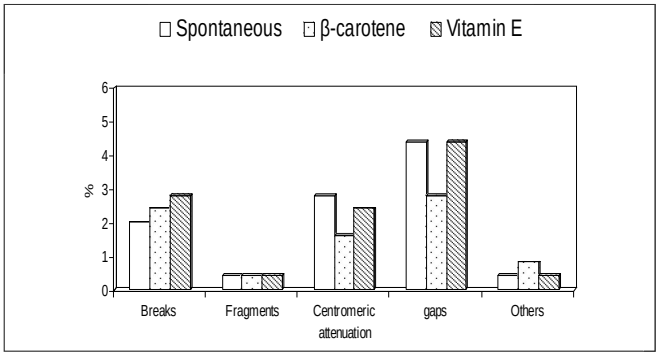


Figure (1): Effect of β-carotene and vitamin E on the percentage of different types of structural chromosomal aberrations in peripheral blood lymphocytes of normal controls

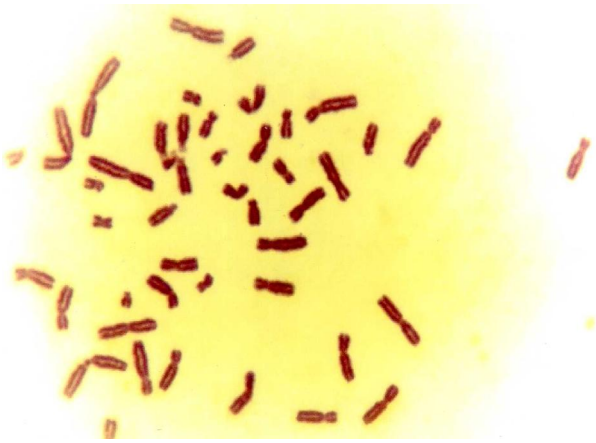


Figure (2): illustrates a normal metaphase from peripheral blood lymphocytes.

Chromosomal aberrations in peripheral blood lymphocytes:

Table 2 shows the effect of β-carotene and vitamin E on the number and percentage of metaphases of different types of structural chromosomal aberrations, frequency of SCEs cell cycle kinetic (RI) and mitotic indices (MI) in peripheral blood lymphocytes of both normal controls and *Schistosoma mansoni*-infected patients.

The data of breaks and deletions were combined together as the two are related. Features of centric fusion, end to end association and ring chromosomes are combined together in one parameter and grouped under "other aberrations" category.

Only a chromosomal aberration of the structural type was found in patients' cultures (Figs 3, 4,5,6)

The results point to the fact that infection with Schistosomiasis may induce structural chromosomal aberrations in peripheral blood lymphocytes. The number and percentage of different types of the induced chromosomal aberrations are recorded in Table 2.

Table 2: Effect of β-carotene and vitamin E on the number and percentage of metaphases of different types of structural chromosomal aberrations, frequency of SCEs cell cycle kinetic (RI) and mitotic indices (MI) in peripheral blood lymphocytes of both normal controls and *Schistosoma mansoni*-infected patients.

	Control			Patients		
	Non treated N=10 (250cells) N (%)	B carotene treated N=10 (250 cells) N (%)	Vitamin E treated N=10 (250cells) N (%)	Non treated N=20 (500cells) N (%)	B carotene treated N=11 (275cells) N (%)	Vitamin E treated N=11 (275cells) N (%)
structural chromosomal aberrations with gaps	24 (9.6)	19 (7.6)	28 (11.8)	99(19.8)**	47(17.1)*	34(12.4)●
structural chromosomal aberrations without gaps	14 (5.6)	13(5.2)	17 (6.8)	73(14.6)**	40(14.5)**	23(8.4) ●
Breaks	5 (2)	6 (2.4)	7 (2.8)	45 (9) **	20 (7.3) *	14 (5.1)
Fragments	1 (0.4)	1 (0.4)	1 (0.4)	15 (3)	5 (1.8)	2 (0.7)
Centromeric attenuation	7 (2.8)	4 (1.6)	6 (2.4)	23 (4.6)	15 (5.5)	8 (2.9)
Gaps	11 (4.4)	7 (2.8)	11 (4.4)	30 (16)	9 (3.3)	13 (4.7)
others	1 (0.4)	2 (0.8)	1 (0.4)	1 (0.2)	0 (0)	1 (0.4)
Total aberrations with gaps	25 (10)	20 (8)	26 (10.4)	114(22.8)***	49(17.8) *	38(13.8)●
Total aberrations without gaps	19 (5.6)	13 (5.2)	15 (6)	84 (16.8) ***	40(14.5)**	25(9.1) ●
SCE/cells	3.92±0.38	4.51±0.2	3.48±0.4	6.37±0.6**	5.91±0.6*	6.24±1.2*
RI	1.43±0.06	1.36±0.07	1.39±0.4	1.22±0.4**	1.31±0.06	1.36±0.11
MI	20.5±5.55	18.5±3.08	19.7±4.13	10.4±1.94***	11.45±2.31***	9.45±2.13***

* P< 0.005, ** P< 0.01, *** P < 0.001 in relation to spontaneous cultures of normal controls.
● P< 0.005, ●● P< 0.01, ●●● P < 0.001 in relation to spontaneous cultures of patients.
SCE: sister chromatid exchange. MI: mitotic index. RI: replicative index

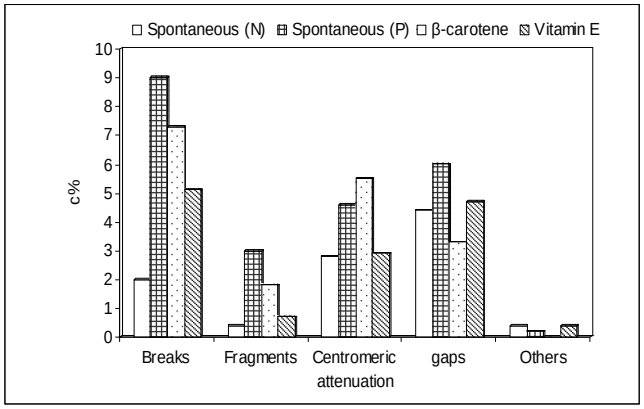
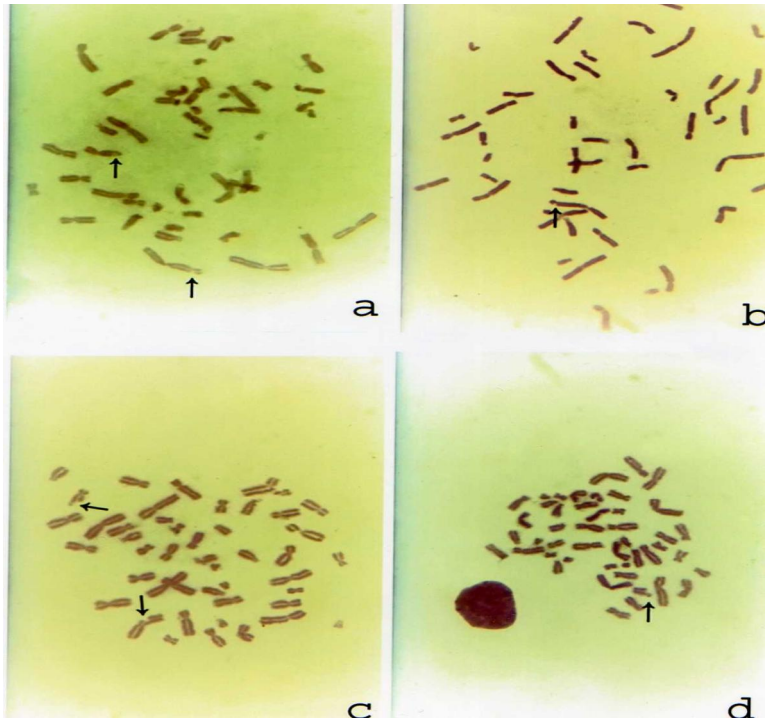
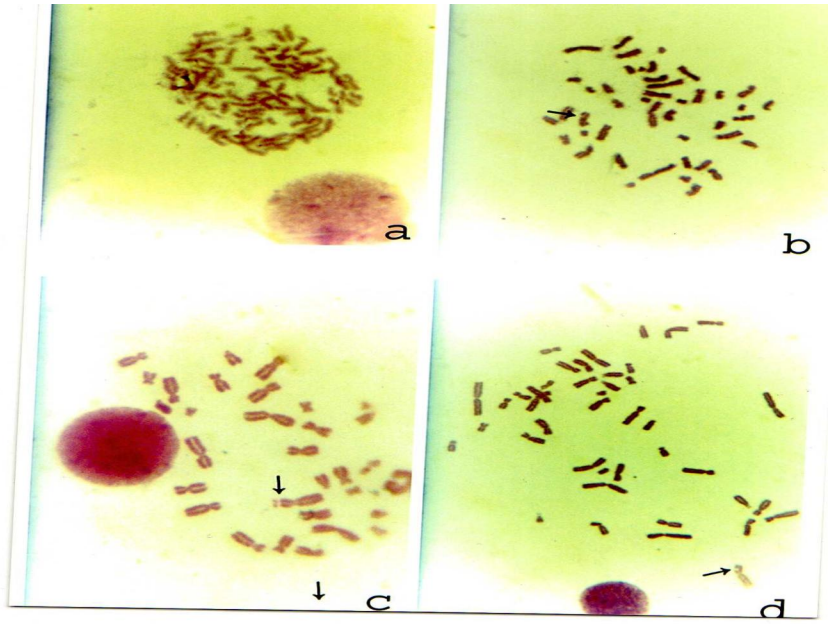


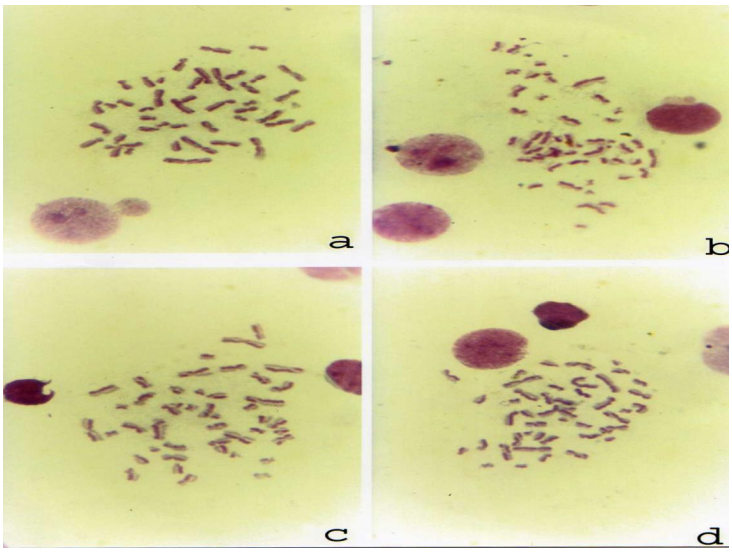
Figure (3): Effect of β-carotene and vitamin E on the percentage of different types of structural chromosomal aberrations in peripheral blood lymphocytes of *S. mansoni*-infected patients.



Figure(4):Metaphase plates from human peripheral blood lymphocytes showing a)Chromatid gaps b)& c)Chromatid break d) Fragments.



Figure(5): Metaphase plates from human peripheral blood lymphocytes showing a)Centromeric attenuation b) Chromatid gaps c) Chromosome gaps d)Chromatid gap



Figure(6) : Metaphases with sister chromatid exchange from human peripheral blood lymphocytes.

4. Discussion

Schistosomiasis is considered the most important of the human helminthiases in terms of both morbidity and mortality. Advances in molecular genetics and immunology hold the promise to control the spread of schistosomiasis and to guide development of new tools to combat this tropical disease [25].

In peripheral blood lymphocyte cultures of *S. mansoni* patients, spontaneous human lymphocyte cultures showed a significant increase in the number of metaphases with chromosomal aberrations even after excluding gaps ($p<0.01$) and the total number of aberrations with and without gaps showed significant increase ($p<0.001$). Breaks and deletions constituted the major part of these aberrations. The total number of breaks and deletions was significant at $P<0.01$. These findings run in

parallel with other studies [6, 26, 27, 28, 29, and 30].

Although some studies reported a significant increase in the number of gaps in *S. mansoni* infected mice [27, 29 and 6], the present study did not reveal any significant increase in the number of gaps. This may be due to the fact that there is not a clear idea about the nature of gaps. While some authors consider gaps to be chromosome fragile sites that are especially prone to forming non-staining gaps, constrictions or breaks in one or both of the chromatids on metaphase chromosomes either spontaneously or following partial inhibition of DNA synthesis [31], others considered the scoring of gaps to be highly subjective and therefore unsuitable indicator for mutagenic potential [32, 33, 34]. It was reported that chromatid gaps occur as two morphologically indistinguishable types; the clastogenic (DNA damage) and the tubagenic (non DNA damage) types. Thus the importance of recording gaps in assessing the mutagenic potential of a compound has been controversial [34].

Four to five sister chromatid exchange is considered within the normal distribution, 14-100 exchanges is not normal and presents a danger to the organism. SCE may be related to tumors [35].

Spontaneous lymphocyte cultures of patients showed a significant increase in the mean frequency of SCEs $p<0.01$. This finding was similar to what was reported previously in *S.mansoni* patients [26]. Data presented in this study demonstrate that a significant decrease in the mitotic index ($p<0.001$) was observed in spontaneous lymphocytic cultures of *S. mansoni* patients. These results are in agreement with other findings [26, 7]. Also, spontaneous lymphocyte cultures of patients showed a significant decrease in the replicative index (RI) ($p<0.01$) results which run in agreement with previous studies [26, 36].

How may schistosomiasis cause this genotoxic effect? In fact, the results of this study and the previous studies showed that schistosomiasis causes increase in the induction of chromosomal aberrations and the frequency of SCEs. Also, schistosomiasis causes a decrease in the mitotic index and a cell cycle delay in human somatic cells but the mechanism involved in this abnormalities is not totally clear. Suggested explanation is that disordered tryptophan metabolism occurring in schistosomal infection leads to an increase in the production of carcinogenic metabolites known to be genotoxic [36 and 37]. A second explanation is that schistosomiasis is accompanied by elevated enzymatic activities in serum, such as β-glucuronidase (βg) [38], an enzyme known to enhance the metabolic activation of procarcinogens such as 2-aminoanthracin [36] and 3, 3 –dichlorobenzidine [39]. It was reported that the active forms of these chemicals are able to induce SCEs in human blood lymphocytes [40]. A third explanation is the possible role of the immunization process in changing the type of sample cells [36]. The cause of the variability in the results of patients is unknown, however many factors such as disease duration, severity and patient immunity could be implicated in chromosomal aberrations and SCEs elevation of this type of infection [36]. Another explanation is the presence of Schistosoma toxins in the blood of infected patients and these toxins may cause a reduction in the cell growth and proliferation [41]. The last and most important explanation is that oxidation of DNA may lead to mutation (and hence to carcinogenesis); Free radicals can also damage DNA and result in mutations, altered capacity of cells to produce critical factors and derangement of the capacity to proliferate [42].

Oxidative stress may contribute to the development of fibrosis in the liver either through direct stimulation or by promoting the production of profibrotic cytokines. Furthermore, oxidative stress may also promote polarization of T-cell differentiation toward T helper 2 phenotype. Using experimental models of the disease, it has been shown that the granulomatous inflammatory response to *S. mansoni* eggs entrapped in the liver induces hepatic oxidative stress, with production of reactive oxygen species (ROS) and reduced anti-oxidant status of the organ. The ultimate result of ROS generation is killing of the parasite eggs; however, the process is potentially harmful for the host as production of ROS may initiate a fibrogenesis cascade in the liver or modulate tissue and cellular events responsible for progression of liver fibrosis. Thus, the pathophysiologic effect of ROS production associated with inflammatory response depend on a balance between opposing mechanisms that can either terminate the oxidative process or lead to increased generation of potentially harmful oxidants. The latter condition may promote the development of liver fibrosis, particularly in subjects with suboptimal antioxidant micronutrients status. The finding of an inverse relationship between serum retinol and intensity of S. mansoni infection and the finding of high levels of periportal fibrosis occurring with low antioxidant micronutrient concentrations suggest that micronutrients may have important roles in the differential morbidity patterns observed among communities who, otherwise, have comparable levels and intensities of S. mansoni infection [43]. The metamorphosis of normal

liver tissue to fibrotic tissue might give the chance for these mutagenic changes.

Evidence is accumulating in support of a role for ROS in the etiology of cancer. Inflammatory cells, such as neutrophils, macrophages, and eosinophils, are an important endogenous source of oxygen radicals. Stimulation of these cells by tumor promoters or by foreign bodies (parasites, bacteria, etc.) causes the release of ROS. In this context, it was found that H2O2/ myeloperoxidase system, which is the corner stone of the anti-microbial defense associated with inflammation, is activated in close contact with parasite eggs. The process although contributes to egg killing in vivo, yet, it causes accumulation of H2O2, superoxide anions and hydroxyl radicals in the host's tissues. It was reported that schistosome infection might have had suppressive effects on the host glutathione peroxidase (GPX) and glutathione S- transferase (GST) thereby minimizing the consumption of GSH either in eliminating H2O2, a reaction catalyzed by GPX, or in the conjugation of schistosomal toxins, a reaction catalyzed by GST. Therefore, the accumulation of free radicals and toxins is augmented with *S. mansoni* infection. Under such condition, the need for antioxidants increase and their presence may be crucial to eliminate the products of oxidative reactions and keep the ongoing immunological operations leading to destruction of eggs [44].

Another question is raised: How do these genotoxic changes lead to carcinogenesis? Zalata et al, (2005) suggested that the genotoxic agents produced endogenously through the course of schistosomiasis mansoni may play a role in colorectal cancer associated with schistosoma mansoni pathogenesis through the dysregulation of apoptosis by altering the expression pattern of Bcl-2 protein differently from non schistosoma associated colorectal carcinoma suggesting a different biological behavior [45].

The second part of our study included the antimutagenic effect of β-carotene and vitamin E against the genotoxic hazards occurred by schistosomiasis. The treatment of cultures of peripheral blood lymphocytes of normal controls did not induce any remarkable changes from spontaneous (non-treated) cultures in normal controls in any parameter.

Also, β-carotene did not decrease the aberrations and SCEs significantly when incubated with cultures of peripheral blood lymphocytes of Schistosoma-infected patients. The total numbers of metaphases with structural chromosomal aberrations with or without gaps or with SCEs were still higher in comparison to the spontaneous cultures of normal controls.

On the contrary, vitamin E decreased the aberrations caused by schistosomiasis when incubated with spontaneous blood cultures of *S. mansoni*-infected patients. The total number of metaphases with structural chromosomal aberrations with or without gaps decreased significantly ($p<0.05$) compared with spontaneous cultures of infected patients. The frequency of SCEs was not influenced by the presence of vitamin E.

The improvement in cultures after vitamin E treatment compared to β-carotene could be explained by the fact that while β-carotene can enter the cell and protect against strand breaks but not against oxidized DNA bases [46], vitamin E is effective in preventing chromosomal damage [47], reduction of DNA fragmentation [48] and micronuclei formation in blood lymphocytes [49].

The replicative indices in the case of the two vitamins revealed an improvement in cell cycle kinetic but the increase was not significant compared with either spontaneous cultures in normal controls or patients.

Neither β-carotene nor vitamin E improved the mitotic indices. The mitotic indices decreased significantly at $p<0.001$ in all patient cultures whether incubated with either of the vitamins or not.

In our experiments, although an improvement in the cell cycle kinetics had occurred, the mitotic indices were still low. This may be due to the elimination of highly aberrated cells.

A possible interpretation of the role of β-carotene or vitamin E against schistosome worms may be attributed to the antioxidative properties of these antioxidants, their metabolic modulator effect on some oxidases and the ability of these antioxidants to modulate the DNA repair mechanism by elimination of highly aberrated cells [48] or by gap junction-enhanced intracellular communication [50].

The use of β--carotene or vitamin E may inactivate or reduce the schistosome glutathione peroxidase (GPX) enzyme activity, which is an important antioxidant enzyme protecting S. mansoni by reducing hydrogen peroxide (H2O2) and peroxidized lipid [51, 52] thus damaging one key survival mechanism of the schistosomes.

As a conclusion to our study, β-carotene and vitamin E have anti mutagenic effect. This anti mutagenic effect may be due to the antioxidant protection by scavenging DNA damaging free radicals or by acting as a modulator of the metabolism selectively inhibiting certain forms of

mixed function oxidases or lastly through modulation of DNA repair mechanism.

In conclusion, the present results indicates that schistosomiasis has a potential mutagenic effect, increases the level of chromosomal aberrations and SCEs, decreases the level of mitotic indices and causes cell cycle delay. The present results indicate that the investigated antioxidants pro-vitamin β-carotene and vitamin E could improve the genetic damage induced by schistosomal pathogenesis.

We are thus justified to recommend giving antioxidant drugs like β-carotene and vitamin E on regular basis to minimize the genotoxic hazards that may occur as a sequel of infection.

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