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AN INITIAL INDICATION OF PREDISPOSING RISK OF *SCHISTOSOMA MANSONI* INFECTION FOR HEPATOCELLULAR CARCINOMA

By

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Abstract

Estimated 500,000 - 1 million cases of hepatocellular carcinoma (HCC) are reported to occur yearly worldwide, with a mean annual incidence of around 3 – 4% of global population. HCC is rapidly fatal in most patients; that makes its incidence and mortality rates almost equal. In the last 5–10 years there were many alarming reports of sharply increased incidence of HCC. In Egypt, HCC reported to account for about 4.7% of chronic liver disease (CLD) patients, which has tremendous impact on socio-economic development in the country. Available data suggests indirect evidence of an association between *Schistosoma mansoni* and hepatocellular carcinoma, possibly through potentiation of hepatitis infections. The present study was conducted case control analysis of 60 HCC patients. Chronic schistosomiasis cases were confirmed by finding Anti-*Schistosoma mansoni* antibodies IgG by ELISA. Hepatitis C viral infection was proved by detection of viral load by quantitative Real time PCR. Among the study group 56.6% (34/60) were dweller in rural in Al-Fayoum governorate. Within hepatocellular carcinoma cases 26.7% (16/60) and 33.3% (20/60) suffered mono chronic schistosomiasis and mono hepatitis C (HCV) infections respectively, with no statistically significant differences ($p=0.37$), indicating comparable risk value of both infections in predisposing directly to HCC. Additionally; frequency of HCC patients with assumed potentiated HCV infection by chronic *Schistosoma mansoni* 6.7% (4/60) were statistically significant ($p<0.05$) less among total HCC patients included in this study, when compared to HCC patients preceded by either pure chronic schistosomiasis 26.7% (16/60) or pure HCV infection 33.3% (20/60). Our present study is one of few, addressing the possibility of direct relation between *S. mansoni* & hepatic carcinoma, concluding an initial indication of equal risk value of both human chronic *S. mansoni* infection and hepatitis C viral infections in precipitating hepatocellular carcinoma among Egyptian patients.

Key words: Patients, *Schistosoma mansoni*, hepatitis C, hepatocellular carcinoma.

Introduction

Schistosomiasis is a water-borne trematode infection, which is endemic in 76 countries mainly (46 countries) in Africa. About 207 million people were infected with 120 million people showing symptoms and 20 million are severely ill (Leonardo *et al,* 2012). Sixty percent of the Egyptian population is at risk of infection, especially school aged children in rural areas were at risk because of their daily contact with infected water (Sayed *et al,* 2014).

Hepatocellular carcinoma (HCC) was the 5th most frequent neoplasm and the third most common cause of cancer related death, accounting for approximately one million deaths per year worldwide (Pleguezuelo *et al,* 2010). Regardless the limited data about the association between liver cancer and *Schistosoma* infection, a number of human cases of HCC have been reported in association with *S. mansoni.* Egypt had the highest prevalence rates of hepatitis C virus (HCV) infection in the world; however, the risk and attribution related to HCV in Egyptian patients with the hepatocellular carcinoma (HCC) remained unknown (Hassan *et al*, 2001). The burden of HCC is increasing

with a doubling in the incidence rate in the past 10 years. This alarming raise in the incidence rate has augmented the need of investigating the contribution of the emerging risk factors to its development (Anwar *et al,* 2008).

This study aimed to clarify the risk association between chronic schistosomiasis *mansoni* and hepatocellular carcinoma.

Subjects, Material and Methods

A case control study was carried out on patients attending outpatient clinic and inpatients of medical oncology department, health insurance hospital in Fayoum governorate during the period from December 2010 to May 2012. The work was carried out on 60 HCC patients and 20 healthy persons, aged 45 to 70 years old. They were classified into three groups; GI: HCC cases with history of chronic schistosomiasis *mansoni* or acute on top of chronic S. *mansoni*, GII: HCC cases without schistosomiasis *mansoni* and GIII: Apparently healthy cross marched persons**.** Children and adults less than 45 years old and patients with acute infection without a history of chronic schistosomiasis were excluded as HCV and HBV have a reciprocal inhibitory effect on each other's replication levels (Crespo *et al,* 1997), so these patients were excluded as well.

About 2 ml of blood was collected from each selected individual in this study by venipuncture. Serum was then divided into 2 eppendorf tubes stored in -20^{o}C to be tested serologically later on. Also, fresh fecal specimens were collected from all cases and control group individuals in dry, clean, plastic containers with tight-fitting lids. Each container was labeled with the patient`s name, date of collection and a coding number. All patients were subjected to clinical, abdominal ultrasonography examinations and data was recorded. For all patients with hepatocellular carcinoma, tumor size and site were reported as well. All specimens were obtained prior to radiation- and chemotherapy.

Schistosoma mansoni ova detection was done using Kato-Katz smears (Abo-Madyan *et al,* 2004) and formol-ether concentration method. Hepatitis infection was confirmed by quantitative Real Time PCR (q-PCR) reaction by detecting hepatitis C viral genomic template (Seif El-Nasr *et al*, 2006). ELISA for anti *Schistosoma* serum IgG antibody level using commercially available kits was performed (Othman, 2013).

Ethical issues (considerations): The study was started after approval of the ethical committee, Faculty of Medicine, Cairo University. Data and sample collection were started after acquiring all approval & permissions of authorities at the chosen hospital. Participation in the study was voluntarily and participants signed an informed consent according to institutional guidelines. Also all procedures done in this study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later modifications, and with approval of the institutional research & ethics committee. All acute patients were treated and those proved to be HCC were managed according to the current protocol and monitored regularly in the outpatient clinic of medical oncology department in health insurance hospital in Fayoum governorate.

Statistical analysis: Data were described in terms of range mean±standard deviation (M±SD), for quantitative data and frequencies (number of cases) and percentages with categorical data. Comparison of categorical variables between groups was done using Chi square (χ^2) test. Fisher exact test was used instead when expected frequency was less than 5. Comparison of between groups for quantitative variables student t test was done for parametric data and Mann-Whitney U test was employed to compare nonparametric data, Anova (F test) in comparing more than two groups in cases of normally distributed variables. A probability value ($p < 0.05$) was considered statistically significant. All calculations & graphs were done

by Graph-pad Prism version 6, Graph-pad Software, La Jolla California, USA.

Results

The HCC ages ranged from 45-70 years old with a median 55 years old, and mean age of patients was 56.17, so HCC patients were older with significant difference when compared to healthy control. The residence HCC didn't showed any significant difference versus healthy group, however residence distribution within HCC infected with *Schistosoma* showed that all of cases were from rural areas (20/20) (100%), when compared to HCC patients without *Schistosoma* infection. Details are given (tables 1 & 2 and figures 1, 2 & 3).

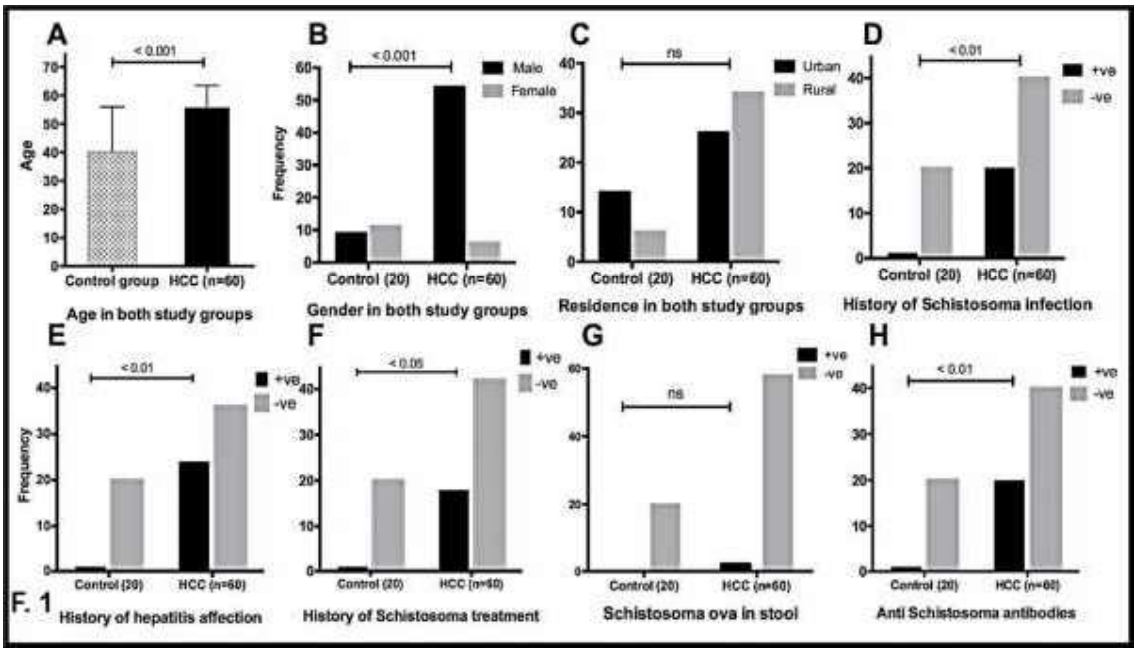

Figure 1: Age (A), gender (B), residence (C), history of schistosomoasis (D) and hepatitis C (E), history of schistosomiasis treatment, and anti-*Schistosoma* antibodies revealed significant differences in all between HCC in patients and controls except for residence and ova detection in stool analyzed by Fishers' exact test, P<0.05 significant, P<0.01 highly significant and P<0.001 extremely significant.

According to anti-*Schistosoma* antibodies; 60 HCC cases were subdivided into: G1: *S. mansoni* +ve, HCC +ve = 20 patients. G2: *S. mansoni* -ve, HCC +ve = 40 patients. Different variables between both were given (tab. 2 & figs. 2 A, B, C, D, E, F, G & H).

Table 1: Different variables of 60 HCC cases in relation to control group.

Variables		HCC cases (G1n=60)		Control group (G2 n=20)		*P* value
		No.	Percentage	No.	Percentage	
Age (M±SD)		56.17± 7.37		40.05± 15.6		< 0.001*
Sex	Male	54	90.0	9	45.0	0.001*
	Female	6	10.0	11	55.0	
Residence	Urban	26	43.3	14	70.0	0.06
	Rural	34	56.7	6	30.0	
Stool analysis	-ve	58	96.7	20	100	0.6
	+ve	2	3.3	0	0	
Bilharzias history	Yes	20	33.3	0	0	0.001*
	No	40	66.7	20	100	
Hepatitis history	Yes	24	40.0	0	0.0	0.001*
	No	36	60.0	20	100.0	
Anti-*Schistosoma*- TTT	Yes	18	30.0	0	0	0.003
	No	42	70.0	20	100	
Anti *Schistosoma* Ab's	-ve	40	66.7	20	100	0.003
	+ve	20	33.3	0	0	

Table 2: Variables in (G1) *S. mansoni* +ve, HCC +ve in relation (G2) *S. mansoni*-ve, HCC +ve.

Variables		G1 *Schistosoma*+ve/ HCC+ve (n=20)		G2 *Schistosoma*-ve/ HCC+ve (n=40)		P value
		No.	Percentage	No.	Percentage	
Sex	Male	18	90	36	90	0.99
	Female	2	10	4	10	
Residence	Urban	0	0	26	65	0.000*
	Rural	20	100	14	35	
Stool analysis	-ve	18	90	40	100	0.11
	+ve	2	10	0	0	
Bilharzias history	Yes	20	100	0	0	0.000*
	No	0	0	40	100	
Hepatitis history	Yes	4	20	20	50	0.058
	No	16	80	20	50	
Anti-*Schistosoma*- ttt	Yes	19	95	0	0	0.000*
	No	1	5	40	100	
Anti *Schistosoma* Ab's	-ve	0	0	40	100	0.000*
	+ve	20	100	0	0	

*= Statistically significant (P <0.05)

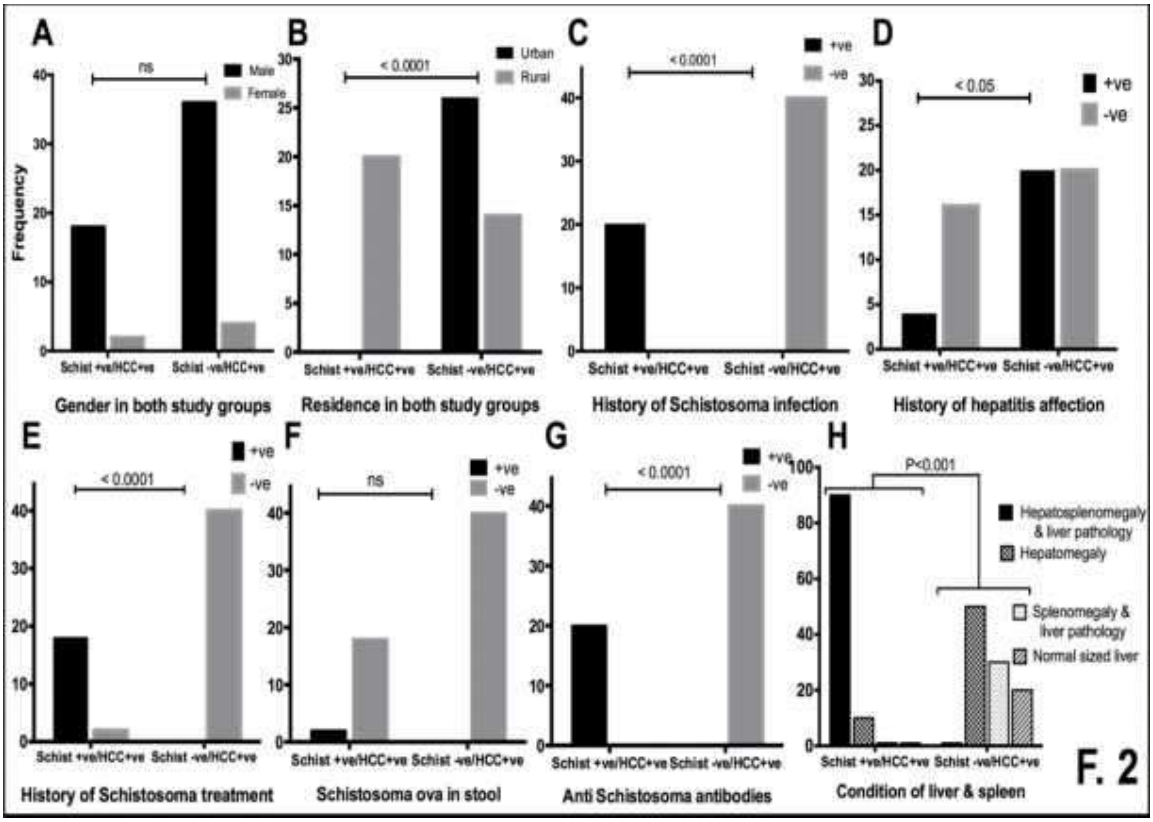

Figure 2: A gender, (B) residence, (C) history of schistosomiasis and (D) hepatitis C, (E) history of *Schistosoma* treatment, (F) detection of *Schistosoma* ova in stool, (G) anti-*Schistosoma* antibodies and (H) liver and spleen condition revealing significant differences in all between HCC patients with schistosomiasis with or without, except for gender and ova detection in stool, analyzed by Fisher's exact test (P<0.05 significant).

Within HCC cases (16/60 or 26.7%) and (20/60 or 33.3%) suffered from either chronic schistosomiasis or HCV mono infection respectively, without significant (P=0.37) differences. Also, four cases out of 60 HCC patients (4/60 or 6.7%) suffered from com-

bined HCV and chronic schistosomiasis, which were less ($p<0.05$), when compared to HCC patients with either mono chronic schistosomiasis (16/60 or 26.7%), or mono HCV (20/60 or 33.3%). Analysis revealed that HCC patients with HCV (24/60 or 40%) whether mono or combined infections were comparable to HCC patients with schistosomiasis (20/60 or 33.4%) whether mono or combined infections without ($P=0.07$) significant difference (Fig. 3).

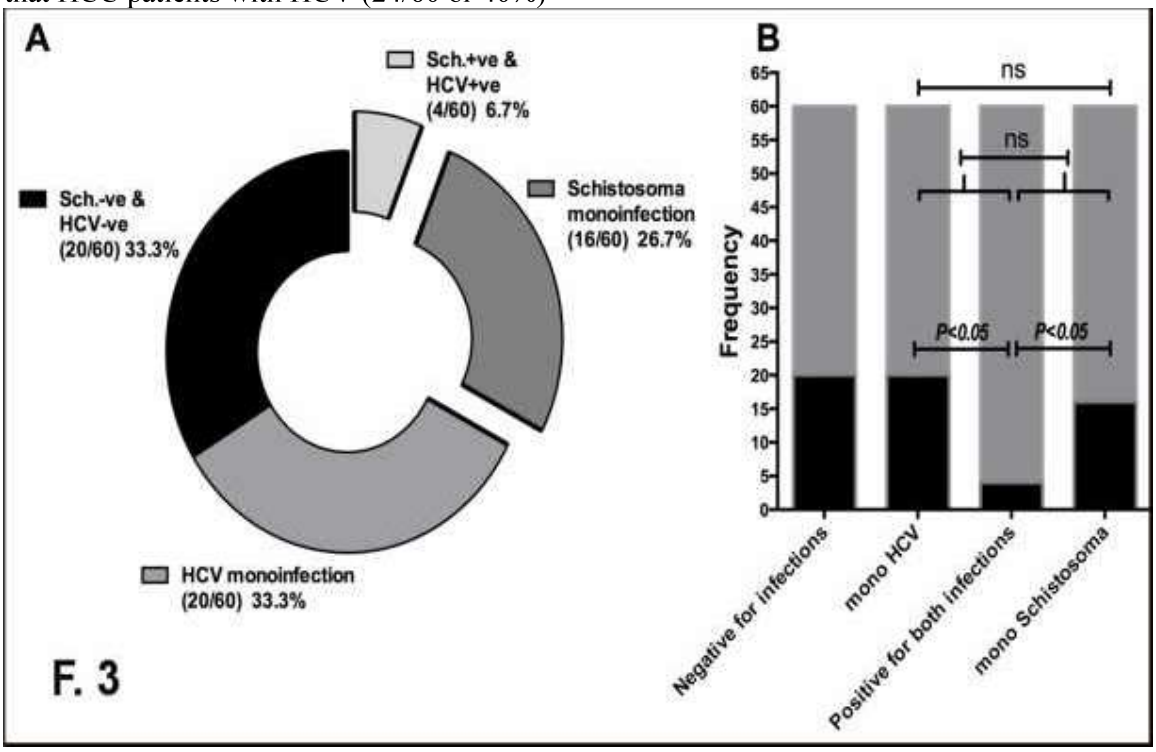

Figure 3: A-diagrammatic presentation of *S. mansoni* and hepatitis C among hepatocellular carcinoma, B- column graph showing same predisposing risk factor of both schistosomiasis and HCV without significant difference between them, analyzed by Fisher's exact test ($P>0.05$ not significant).

Upper abdominal ultrsonographic examination showed fibrotic pattern in 20 cases (100%) with history of schistosomiasis. As to schistosomiasis complications, HCC cases (18/20, or 90%) suffered from evident chronic complicated schistosomiasis presented by hepatosplenomegaly and liver pathology, and 10% (2/20) only presented by hepatomegaly. Among 40 HCC cases without schistosomiasis, 20 (50%) were presented by hepatomegaly, 12 (30%) with splenomegaly and liver pathology and eight (20%) had normal sized liver in spite of focal malignancy, with significant difference between both groups (Fig. 2).

Discussion

Hepatocellular carcinoma (HCC) represents an international public health concern as one of the most common and deadly cancers worldwide (Liu *et al*, 2015). In Egypt, the role of mono *S. mansoni* infection in development of HCC has not well evaluated (El-Tonsy *et al*, 2013).

In the present study the age distribution among the HCC studied group ranged from 45-70 years old with mean age of patients was 56.23, significantly older than healthy control ($P<0.001$). These results agreed with Abdel-Wahab *et al*. (2007) who concluded that the number of newly diagnosed patients with HCC increases annually, and that mean age of HCC among those patients was 54.26 ± 9.2 with a high prevalence between 51 and 60 years old.

Regarding sex distribution, the results showed 90% of HCC cases were males. This

agreed with El-Zayadi *et al*. (2005), where the calculated risk of development of HCC was three times higher in males than in females, which may be explained by differences in exposure to risk factors.

Literature had demonstrated that the association between *S. mansoni* and HCC was probably an indirect one, through potentiating the effect of hepatitis virus on the liver. In fact, patients infected by *S. mansoni* have higher rates of HBV and HCV infection compared with non-infected controls (Khurana *et al*, 2005; Bacelar *et al,* 2007).

Nevertheless, schistosomiasis as a parasitic affection was not the main direct cause leading to HCV infection (Kamal *et al*, 2004). Mudawi *et al*. (2007) reported that HCV was of low seroprevalence in the study population and that parenteral antischistosomal therapy was not a significant risk factor in transmission of infection in the Sudan. This relationship does not appear to exist for hepatitis B virus (HBV) Eltoum *et al.* (1991) found no association between schistosomiasis and HBV infection in, Sudan. Also, Yates *et al.* (1999) reported that HCC in the Egyptians with and without a history of hepatitis B virus infection there was neither associated with HCV infection nor HCV/ RNA level. In the present study, the risk value of *S. mansoni* infection as predisposing to HCC in Egyptian patients, showed in Egyptian HCC patients, HCV was believed as the most important hepatocellular risk factor with $P<0.01$, and that HBV infection presented only marginal tendency of increased risk of HCC $P<0.06$. This agreed with Schiefelbein *et al.* (2012).

In the present study, within HCC cases 26.7% (16/60), and 33.3% (20/60) suffered from either chronic schistosomiasis or HCV mono infections respectively, but without significant differences ($p=0.37$) that indicated a comparable risk value of both mono infections as predisposing factors to HCC in Egyptian patients. All the twenty HCC cases with schistosomiasis suffered from evident chronic complicated schistosomiasis as hepatosplenomegaly and liver pathology (90%) and only hepatomegaly (10%). In the forty HCC patients without schistosomiasis; 50% were presented with hepatomegaly, 30% with splenomegaly and liver pathology. The remaining 20% had normal sized liver, which may indicate that *S. mansoni* induced liver pathology and splenomegaly possibly participate greatly in the development of the HCC.

In the present study, *S. mansoni* ova were detected in only 2 cases (10%), while the rest 90% of anti-*Schistosoma* antibodies positive cases were negative by microscopic examination. The anti-*Schistosoma* antibodies positive cases suffered from chronic infection, which Kato-Katz technique was less sensitive, due to rectal and sigmoidal tissue fibrosis that trapped *Schistosoma* ova and prevent its excretion in stool (Berhe *et al*, 2004). Also, this technique was inadequate, because of parasite day-to-day fluctuation of egg output (Lodh *et al*, 2013).

In the present study, as combined risk factors of hepatitis and schistosomiasis, there was only 6.7% compared to either mono-schistosomiasis *mansoni* (26.7%) or mono HCV (33.3%). The result didn´t go with Angelico et al. (1997), who reported that co-infection with *S. mansoni* and HCV were highly prevalent among Egyptian patients with chronic liver disease and that association might have mutual interaction, increasing the severity of liver pathology and the risk of HCC. In the present study, the two main risk factors that may predispose to HCC in the Egyptian patients were either *S. mansoni* and/or HCV, their single assumed role in the development of HCC exceeded the combined effect of both infectious diseases together.

Fattovich (1988) suggested that fibrosis was the common pathway by which several risk factors exert their carcinogenic effect in chronic schistosomiasis *mansoni*. Also, Takermura *et al.* (1998) stated that the chronic effect of the schistosomiasis *japonicum* was studied by many authors who believed that

chronic schistosomiasis was the major effect of liver cirrhosis. Thorgeirsson and Grisham (2002) stated that chronic infection with either *S. japonicum* or *S. mansoni* led to hepatic fibrosis that may cause in increasinh the risk of HCC.

Others showed that reactive oxygen species (ROS) were formed in the immediate surrounding area of eggs deposited in the liver tissue, and led to the destruction of the eggs (Abdallahi *et al*, 1999) with strong association between the establishment of the oxidative stress in situ (in hepatic tissue) and the load of *S. mansoni* (Cunha *et al,* 2012). Besides, there was backing evidence for a mechanistic linkage between oxidative stress and lipid peroxidation-induced DNA damage, seeing that oxygen radicals can injure other cellular macromolecules such as lipids to generate reactive intermediates that destructively combine to DNA bases initiating a precancerous micro-environment status (Verlecar *et al*, 2008). In Egypt, Shawki *et al*. (2014) studied the DNA damage in peripheral blood lymphocytes from patients with hepatocellular carcinoma (HCC) and those with HCV infection with and without associated cirrhosis and normal controls. They concluded that the accumulation of DNA damage was important in HCC evolution, and that DNA damage indicating intracellular oxidative and nitrative stress may lead to mutagenesis and consequently malignant transformation, which emphasizes the need to optimize the therapy for reducing the degree of genomic damage.

Conclusion

The outcome results addressed the possibility of direct relation between *Schistosoma mansoni* and HCC, and that an initial implication of equal risk value of both human chronic *S. mansoni* infection and hepatitis C viral infections in precipitating HCC among Egyptian patients.

The query is still open that drives strongly for further investigations to reveal the mechanisms through which this parasitic infection possibly predispose to HCC such a study is ongoing as will be published in due time.

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