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Cytokines, Lipid Peroxide and Nitric Oxide in Egyptian Hepatocellular Carcinoma on Top of HCV and HBV Infection

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Abstract:

Background and Aims: Many studies have shown the relative roles of hepatitis B and C viruses in hepato-carcinogenesis. The aim of this study is to define the independent and interactive roles of some cytokines namely, TNF α, IL-6, IL-1 β together with NO and TEARS in the genesis of HCC following the infection with such viruses.

Patients and methods: Blood samples were taken from 58 patients with hepatocellular carcinoma and were divided into four groups: a) 28 patients with HCV, b) 10 patients with HBV, c) 11 patients with B+C, d) 9 patients without viral infection. In addition, 20 healthy subjects served as control group for each, TNF α, IL-6, and IL-1 β were measured using ELISA technique, in addition to NO and TBARs using chemical methods.

Results: Patients with coinfection B-C viral infection showed the highest levels in studied parameters. Patients with HCV and HBV separately showed more or less similar results. However, patients without viral infection showed the least higher levels comparing to the control group.

Conclusion: Cytokines in addition to NO and TEARS have a definite role in hepatic carcinogenesis. Coinfection with the two viruses carries a synergistic risk factor of hepatocellular carcinoma development. Depending on the results of the studied parameters HCV did not show predominancy on HBV. Further studies are needed to clarify the exact mechanism of carcinogenesis especially in HCV patients.

Key Words: HCC, HCV, HBV, IL-1 β, IL-16 TNF α, NO. TBARs

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumours in developing countries (Laurent Purg *et al*., 2001), HCC risk factors, such as infection by hepatitis B or C viruses (HBV and HCV), cirrhosis of various etiology, primary hemo chromatosis, and prolonged exposure to Afatoxin BI, are well proven (Kato, 2001). However, the carcinogensis mechanisms are still poorly understood and seem to differ according to the risk factor involved (Arbuthnot and Kew, 2001) and (Umeda and Hino, 2002). Epidemiological and experimental evidences have established that chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) are the major risk factors for hepatocellular carcinomas (HCC) in humans (Kew *et al*., 1997, Kondo *et al*, 2001). The epidemiological studies also indicate that the relative role of these two viruses in hepatocellular carcinogenesis vary considerably among different populations. Coinfection with the two viruses carries a synergistic risk of hepatocellular carcinoma formation.

Cytokines are synthesized and secreted in the liver mostly by kupffer cells and play a key role in inflammatory processes and immunological responses related to liver diseases, which are initiated by hepatocytes damage (Flisiak, 1999). Peter *et al*. (2000) emphasized a central role for interleukin 6 (IL-6) and soluble interleukin 6 receptor (sIL-6R) in liver regeneration. They added that, a possible

therapeutic potential for the designer cytokine type IL-6 in clinical situations associated with liver regeneration. However, *Liorent et al. (1996)* reported high degree of cytokine gene expressions namely transforming growth factor one beta (TGRFIB), interleukin one beta (IL-1β) and Interleukins 2, 6, 8 and 10 as well as tumour necrosis factor alpha, in post hepatitis C liver cirrhosis. The authors concluded that these cytokines appear to participate in the pathogenesis of the mild to severe liver damage and liver carcinogenesis.

Cellular oxidative respiration results in the generation of a number of reactive oxygen species (ROS) including super oxide (O_2) hydroxyl radical (OH") and other free radicals that rapidly dismutase to form H_2O_2. Accumulations of ROS contribute to cell injury through effects on gene and protein expression, DNA damage and lipid peroxidation. (*Schlenker et al, 2000*). In this regard, hepatocyte expression of inducible nitric oxide synthase (iNOS) and synthesis of nitric oxide (NO) posses protective anti oxidant functions in models reperfusion injury (*Kuo et al., 2000*). This effect is independent of both the oxidant species and the specific proinflammatory roles of cytokine that characterize these pathophysiological states. However, *Kuo et al. (2000)* reported that super oxide enhances interleukin-1β and mediate CI transcription of the hepatocyte-inducible nitric oxide synthase gene.

Moreover IL-1β followed by TNF-α and IL-6, and other cytokines were the most effective cytokines to induce MN^{2+}-superoxide dismutase activity in human hepatoma cells (*Pontisso et al., 1998*).

It is concluded from the pervious short review that IL-1β, IL-6 and TNF-α together with oxygen species mainly in the form of lipid peroxide and nitric oxide, are very dynamic effectors of the normal and the pathological behavior of the liver cells. Moreover, these effects are modulated in the complicated situation with infection by HCV or HBV. Therefore, investigating the nature of the expression of these proinflammatory effectors at protein level in addition to the oxidant/antioxidant mechanism in the blood of patients with hepatocellular carcinoma with or without viral infection could elucidate their role in protection and/or pathogenetic causation of this complicated condition.

Aim of the Study:

1. To investigate the role of three cytokines namely TNF-α, IL-1β and IL-6 together with lipid peroxide and nitric oxide in cases of hepatocellular carcinoma with or without previous viral infection among Egyptian patients.
2. To highlight the effect of bilharzial infection on hepatic carcinogensis.
3. To find any relation between the previous parameters and the type of viral infection.
4. To report any correlation between these parameters.
5. To find a correlation between these parameters and the grading of the hepatocellular carcinoma.
6. To correlate these parameters with the liver functions and its enzymatic status.
7. To discover a new regimen for using such cytokines as follow up parameters and determining a possible role for using anticytokines in the treatment of hepatocellular carcinoma.

Material and Methods

The present study include 58 subjects divided into the following groups:

Group 1: included 28 patients (26 males and 2 females) suffering from hepatitis C virus infection with hepatocellular carcinoma. Their ages ranged from 33-70 years with mean ±SD of (52-5+10.6) out of the 28, 14 patients had a past history of Bilharziasis.

Group 2: included 10 patients (all are males) with hepatocellular carcinoma and hepatitis B virus infection. Their ages ranged from 31-68 years with mean ± SD of (52.8±9.2) Two out of the 11 patients had past history of Bilharziasis, and three had history of previous operations.

Group 3: included 11 patients (all males were diagnosed with hepatocellular carcinoma and co-infection by both virus B and C. their ages ranged from (28-71) years with mean ± SD is (47.3+11.3) Five out of

them had past history of Bilharziasis and three had history of blood transfusion.

Group 4: included 9 patients (all were males) with hepatocellular carcinoma without co-infection with viral infection, but two of them had past history of Biharziasis. Their ages ranged from 50-78 with mean $\pm$ SD of (58.5$\pm$13.1)

The patients were recruited among the:

1. Attendants of internal medicine department Al-Zahraa University Hospital (Al-Azhar University).
2. Attendants of Tropical medicine department, El-Minia University Hospital and El-Minia Institute of Oncology.
3. Attendants of Internal medicine department, Assiut University Hospital and Assiut Institute of Oncology.
4. Attendants of Internal medicine department, Sohage University Hospital.

Smokers in addition to Patients with diabetes mellitus, heart failure, hypertension, renal failure, Cancer, and Fever were excluded from this study.

Group 5: Included 20 healthy non-smoker male subjects, their ages and socio-economic status matched with the previous groups, serving as a control group.

For all patients and healthy subjects the following parameters were done:

1. Full history and thorough clinical examination.
2. Chest X-ray.
3. Abdominal ultrasonography.
4. Routine liver function tests including, total bilirubin, total protein albumin, liver enzymes: Alanine amino transferase, (ALT), and alkaline phosphatase, in addition to prothrombin time and concentration.
5. Antigens and antibodies for hepatitis B virus (HBV) and hepatitis C virus antibodies (HCVJ by ELISA technique.).
6. HCV / RNA detection by polymerase chain reaction, (PCR).
7. Liver biopsy was carried out only on patients with HCC provided that the prothrombin time and concentrations are favourable. The grading was carried out according to Comparing the cytokines among patients with different grades of HCC was only carried out in the group of HCC on top of HCV as this group comprised the highest number of patients allowing statistical comparison of results.
8. Five-millmeter blood had been taken from each patient and control subject under complete aseptic conditions using suction tubes. Sera had been separated and deeply frozen until the time of usage to estimate the following:
   i. Tumour Necrosis factor-Alpha (TNF-α). By ELISA technique.
   ii. Interleukin-one Beta (IL-1β). By ELISA technique.
   iii. Interleukin six (IL-6). By ELISA technique.
   iv. Alpha fetoprotein (α-FP). By ELISA technique.
   v. Lipid peroxide (LPS) in the form of TEARS MDA equivalents. By Satoh (1978).
   vi. Nitric oxide (NO). By Van Bezooijeen *et al*, (1998).

Results

The pathological study of liver biopsies of 28 patients suffering from hepatocelluler carcinoma with HCV co-infection revealed that *15* are grade 11 and *13* are grade III.

Symptoms and signs of Egyptian HCC patients are shown in table (1). Table (2) shows risk factors of HCC patients.

Patients with HCC and both B and C viral co-infection recorded the highest serum level of TNF α compared to the other four groups ($p < 0.001$, while no statistical difference was recorded between HCC with B infection and HCC with C infection (the two pathological grades). On the other hand patients with HCC without viral infection recorded the least level, but still statistically higher than control $p<0.001$ (table 3).

IL-6 was the highest in the sera of HCC B+C patients $P<0.001$ compared to all groups. HCC-C patients showed significant increased levels compared to HCC-B patients ($p<0.01$) Patients with HCC non-B non-C showed the least levels, but still higher compared to the control $p<0.001$ (Table 3).

Serum level of IL-1β was significantly higher in patients with HCC-B+C compared to all studied groups

p<0.001. No significant difference was recorded between patients with HCC-B and HCC-C, while patients with HCC non-B non-C recorded the least levels, but still higher than control p<0.01 (Table 3).

Serum levels of TEARS, MDA equivalent and NO showed the highest levels in patients with HCC B+C compared to all studied groups (p < 0.001) patients with HCC-B showed no significant difference compared to HCC-C patients (table 3).

On the other hand, no significant difference was recorded in HCC-C patients with Bilharziasis compared to non-Bilharzial patients in all studied parameters (table 4).

Patients with HCC associated with HCV infection showed positive correlation between TNF-α and IL-1β, IL-6, α-FP, serum bilirubin, alkaline phosphatase, NO and tumor grading and between each other, while negative correlation between prothrombin concentration and IL-6, s.bilirubin, alkaline phosphatase, total protein, NO and tumour grading.

Table (1): Symptoms and signs of all included patients (NO. 58)

Symptoms and signs	Frequency of + ve cases	
	NO.	Percentage
Fever	3	5.1
Malaise	41	70.6
Weight loss	13	22.4
Jaundice	41	70.6
Abd. Pain	58	100
Epistaxis	40	68.9
Bleeding gums	39	67.2
Itching	3	5.1
Haematemsis	16	27.5
Melena	7	12.1
Foeter hepaticus	8	13.7
Gynacomastia	7	12.1
Spider navei	5	8.6
Flapping tremors	8	13.7
Palmer erythema	17	29.3
Hepatomegaly	45	77.5
Splenomegaly	51	87.9
Ascites	42	72.4
Oedma L.L	24	41.3
Easy Fatigability	51	88

Table (2): Risk factors for all patients (NO. 58)

Risk factors	NO.	Percentage
None	17	29.3
Blood transfusion	8	13.7
Surgical op.	1	1.7
Tattooing	1	1.7
Dental proced.	1	1.7
Oral Bilh. Ttt	3	5.1
Iniect Bilh. Ttt	17	29.3
Combined Bilh. ttt	4	6.8
Smoking	6	10.3
No of cases	58	100

Table (3): Biochemical parameters in all studied groups mean±SD.

	Control	HCC/non BC	HCC/B	HCC/C	HCC/CII	HCC/CIII	HCC/BC
TNF- pg/m1	6.34	30.43	53.74	66.12	43.51	68.37	154.3
	0.83	4.03	8.74	3.90	3.28	5.15	31.4
11-6- pg/ml	2.82	26.57	38.06	55.99	36.62	78.17	133.5
	0.35	3.39	3.69	4.72	4.48	2.001	13.33
IL-1B- pg/ml	5.23	34.40	55.21	58.38	43.73	75.29	204.1
	0.62	3.20	6.20	3.83	2.56	4.2	23.01
A f.p. – ng/ml	3.35	453.8	539.4	582.4	359.9	797.5	661
	0.43	39.7	48.48	49.55	53.04	30.17	54.50
T.P. g/dL	7.57	6.46	6.91	6.9	7.1	6.7	7.03
	0.087	0.21	0.22	0.16	0.24	0.19	0.32
A/b. – g/dL	4.32	2.74	2.75	2.8	2.64	2.99	2.69
	0.06	0.22	0.14	0.10	0.13	0.14	0.195
Proth. %	94.5	68.78	64.6	66.7	74.8	57.5	56.0
	0.45	4.89	4.9	2.9	4.27	1.88	2.098
T.Bil. –mg/dL	0.75	2.3	2.8	2.9	1.8	4.3	4.2
	0.018	0.29	0.31	0.28	0.19	0.20	0.26
SCPT-IU	13.6	104.8	115.5	83.82	69.0	100.9	107.9
	1.09	14.5	18.09	9.93	9.93	17.3	10.44
Al.ph-IU	30.74	117.3	139.0	189.1	126.6	261.3	240.5
	1.15	11.9	15.97	19.9	18.86	25.24	12.98
TBARS- M/L	1.79	2.72	3.27	3.08	2.87	3.29	4.86
	0.09	0.32	0.27	0.25	0.29	0.41	0.27
NO-nM/ml	24.28	78.17	155.7	148.6	69.03	240.4	343.5
	1.43	6.29	14.91	19.29	5.42	21.29	43.65

Table (4) : Comparison between HCC-C associated with Bilharziasis (No.=14) and without Bilharziasis (No.=14) in all parameters studied. (M±SD).

	Bilharziasis	Non-Bilharziasis	P-Value
TNF-a (pg/ml)	59.56 +5.369	52.69+5.706	0.3880
Il-Ib (pg/ml)	58.83+4.855	57.94+6.102	0.9097
Il-6 (pg/ml)	63.71+6.061	48.09+60813	0.0987
α - FP (ng/ml)	651.7+55.47	513.1+79.89	0.1659
Prothrombin (%)	67.14+3.361	66.43+4.912	0.9054
Albumin (g/dl)	2.807+0.1714	2.800+0.1177	0.9729
Total protein (g/ml)	6.686+0.2383	7.243+0.1603	0.0633
SGPT (IU)	87.50+16.64	80.14+11.45	0.7186
Total Bilirubin (mg/ml)	3.048+0.4174	2.791+0.3665	0.6473
ALK-Phosphatase (IU)	177.0+21.11	260.4+60.71	0.2057
NO (nM/ml)	32.83+5.277	26.61+5.701	0.4305
TBARS (μ ML)	2.466+0.21.57	3.043+0.3256	0.3256

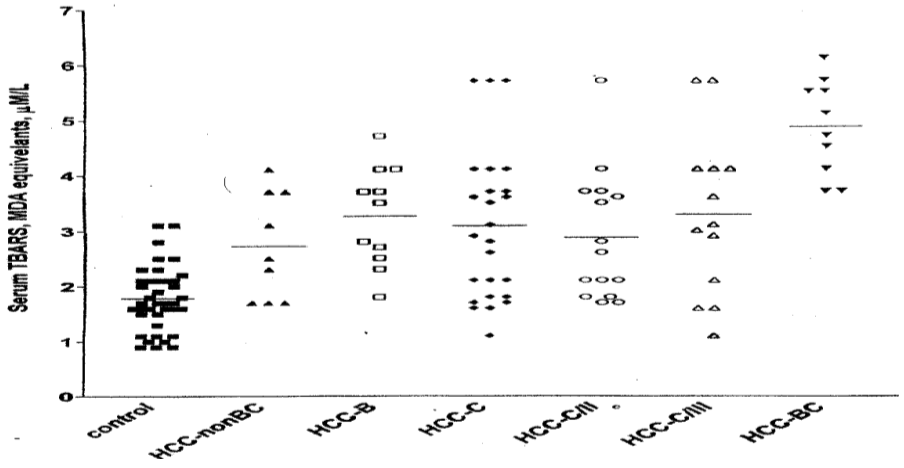

Fig. (1): Scaterogram for the individual data of serum TEARS in all studied groups.

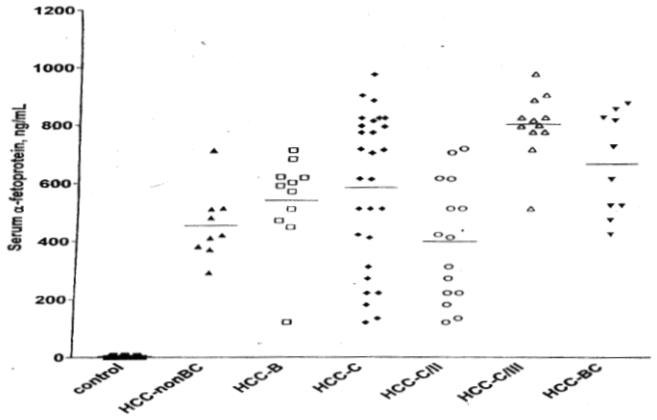

Fig. (2): Scaterogram for the individual data of α-fetoprotein, in all studied groups.

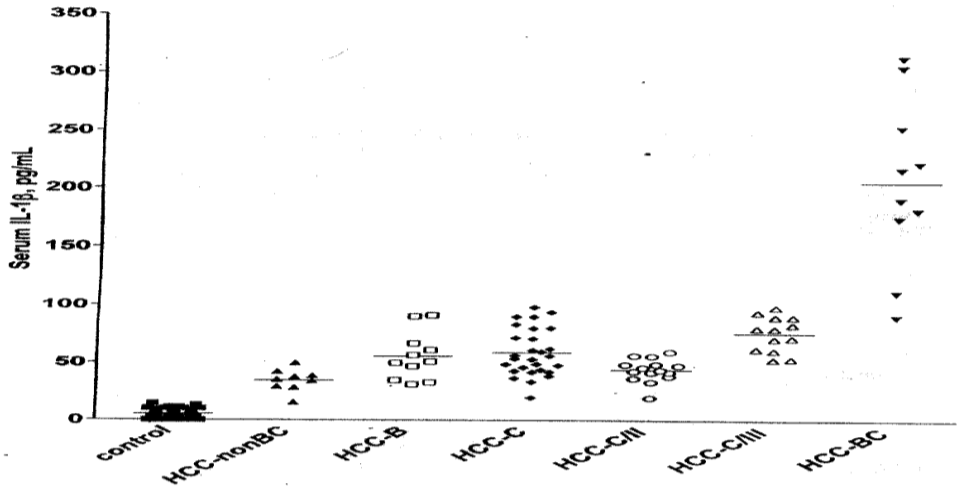

Fig. (3): Scaterogram for the individual data of IL-1B in all studied groups.

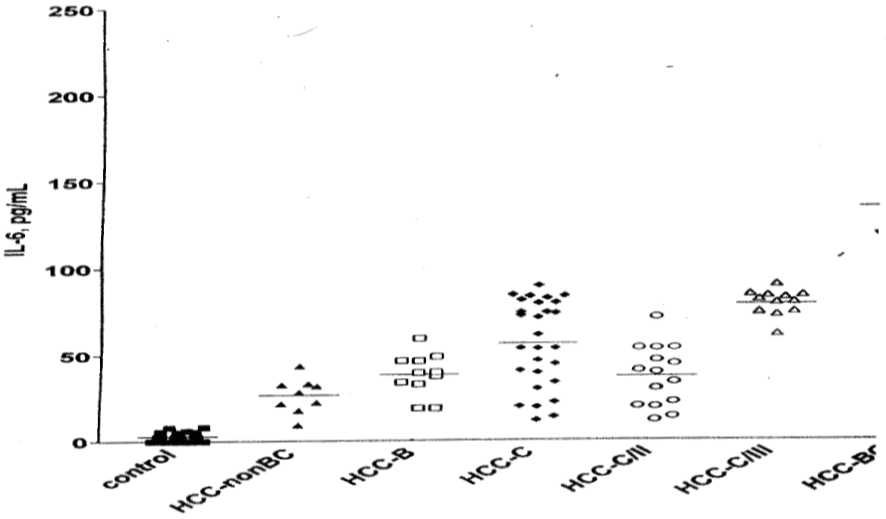

Fig. (4): Scaterogram for the individual data of serum IL-6 in all studied groups.

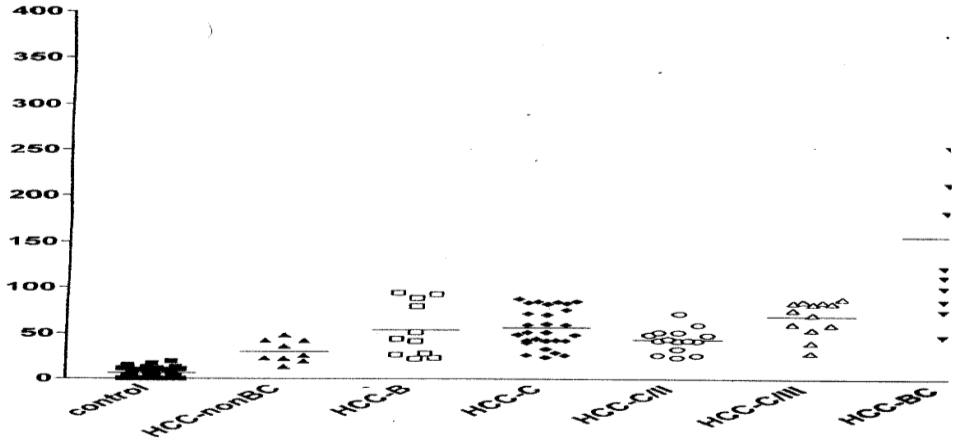

Fig. (5): Scaterogram for the individual data of serum TNF-α in all studied groups.

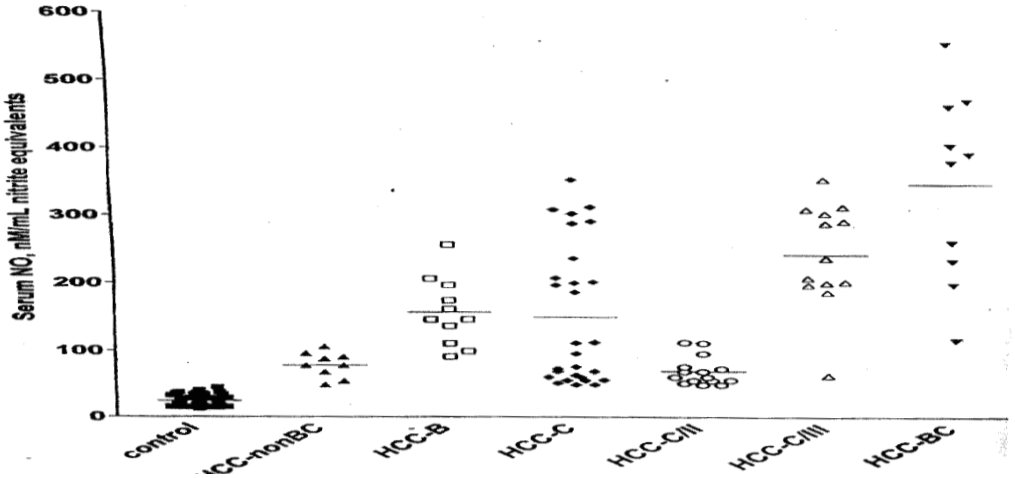

Fig. (6): Scaterogram for the individual data of serum NO in all studied groups.

Discussion

Cytokines are synthesized and secreted in the liver mostly by Kupffer cells and play a key role in inflammatory processes and immunological responses related to liver diseases, which are initiated by hepatocytes damage. This type of signaling between different types of liver cells can produce opposing reactions; for instance, transforming growth factors beta (TGF-B) as well as interleukins (IL-1β), (IL4) and (IL-6) induce fibrosis. In contrast, transforming growth factor alpha (TGF-α) interleukin, one alpha (IL-1α) and interferons (IFN) are inhibitory to fibrosis. Loss of the balance between these stimul-ations seems to be responsible for activ-ation of non parenchymal cells, that result in an accumulation of extracellular matrix proteins including collagens with liver cirrhosis as a clinical effect (Flisiak, 1999).

Our results are in agreement with many authors. *Loginov et al. (2001)* reported high levels of TNF-α IL-4, IL-1β and IL-6 in sera of patients suffering from post hepatitis C cirrhosis. *Lee et al. (1998)* reported high serum levels of IL-6 in HCC patients due to viral infection. This finding could be explained by upregulation of IL-6 production, which can eventually lead to Liver cirrhosis and HCC.

Kobsel and Ramadori (1997) reported that lysozyme synthesis and secretion were found to be augmented by IL-1β, TNF-α and IL-6 in human hepatoma cells. The authors concluded that these cytokines might have a role in modulation and production of protein molecules participate in the mechanism of carcinogenesis.

Liorent et al. (1996) reported high degree of cytokine gene expression namely transforming growth factor (TGF) interleukin 1 beta (IL-1β), Interleukins 2, 6, 8 and 10 as well as tomour necrosis factor-alpha (TNF-α) in post hepatitis C liver cirrhosis and cirrhotic specimens. The authors concluded that these cytokines appear to participate in the pathogenesis of the mild to sever liver damage.

Osman et al, (2000) reported that IL-1β increased significantly in chronic hepatitis C than normal controls. In addition, serum concentrations of this cytokine correlated with both indices of hepatic dysfunction and parameters of hepatic inflammation. The result of present study showed higher levels of cytokines of either of Th1 origin (TNFα) or of Th2 origin (IL-6). Also, IL-1β showed significant increase. All these increased cytokines showed highest level in Egyptian HCC patients with HCV and HBV co-infection followed by either HCV or HBV with no significant difference. HCC with no viral infection showed lowest levels of all HCC patients but still higher than normal controls.

This significant rise in all cytokines could be:

- Direct release of these cytokines from tumor cells but chronic HCV showed significant higher levels than normal (***Osman et al., 2000***).
- Upregulation of the genes corresponding to these cytokines.
- Host defense mechanism to guard against the disease.
- As a part of angiogenesis especially TNF-α
- As a part of antiapoptotic mechanism
- Chronic hepatitis (HBV) even HBs Ag negative, anti-HBs positive cases. This is mostly probably related to persistence of low levels of hepatic HBV DNA, which can also be isolated from tumor tissue.

The HBV encoded x protein, which is known to regulate both proliferation and apoptosis (***Sheu et al., 1997***).

Chun – Chieh Chen et al. (2005) added that induces HCC mainly by causing dose dependent association between the number of putative high-risk genotypes in the IL-1β, TNFα and HCC. Genetic variations in cytokines and DNA repair genes contributes to susceptibility to HBV related HCC.

The present study showed that serum levels of TEARS-MDA equivalent NO showed the highest levels in patients with HCC B+C compared to all studied groups.

Cytokines such as IL-1β and TNF-α activate the vascular smooth muscle cells to produce nitric oxide.

Moreover, Majamo *et al*. (1998) and Ahn *et al*. (1999) reported that hepatotropic viral infections are able to upregulate the number of gene expressions in human hepatocytes. So, the authors suggested that NO may mediate important pathogenic events in the course of chronic viral hepatitis.

Laskin et al. (2001) reported that in many models of liver damage nitric oxide and its oxidation products, such as peroxynitric, contribute to the injury process by directly damaging the tissue or by initiating additional immunologic reactions that result in damage.

Balance between protooncogene and suppressor genes is disturbed in HCC. Loginov *et al*. (2001) reported that protooncogene C-fos was high in HCC tumor tissue.

Osman *et al*. (2005) had reported that that Egyptian HCC patients on top of HCV showed significant higher level of serum P53 immunohistochemical staining of tumor tissue and insulin like growth factor than negative HCV, HCC patients and both had higher levels than normal controls. They explained these results by mutation of P53 in HCC patients especially those with positive HCV thus leading to sharp increase in P53 protein levels, but abolishes function of P53 in tumor cells.

So HCC may result from step use process involving different preneoplastic lesion that reflect multiple genetic events such as tumour suppressor gene inactivation and growth factors over or reexpression.

Thus increased protooncogenes, decreased or distrurbed function of suppressor genes, growth factor genes, virological factors via cytokines and oxidative stress have been implicated in hepatocarcinogenesis, which is subsequently multifactorial pathology.

The pathological study of the liver biopsies of 28 patients suffering from hepatocellular carcinoma with HCV infection revealed that *15* are grade II and *13* are grade III.

Patients with HCC associated with HCV infection showed, positive correlation between TNF-α and IL-1β, IL-6, alpha fetoprotein, serum bilirubin, alkaline phosphatase and tumour grading, and between each others, while negative correlation was noted between prothrombin concentration and serum albumin in one side and IL-6, S. bilirubin, alkaline phosphatase, and tumour grading on the other side. This means that the more advanced the HCC is, the more the reflection by cytokines, the pathological grading, and effect on the synthetic function of the liver.

However, no correlations were found between the studied parameters, the ages of the patients and the tumor sizes.

Bilharziasis per se is not a documented risk factor for increasing the serum levels of any of the previous studied parameters.

Also, regarding the pathological grading of HCC on top of HCV, there was no evident statistical difference between the group of patients with concomitant bilharzial infection and the group without bilharzial infection. Thus, Egyptian bilharziasis does not seem to play a role in hepatic carcinogenesis.

However, the levels of studied parameters in hepatocellular carcinoma associated with HCV infection, does not show any significant differences compared to their levels in hepatocellular carcinoma with HBV, which may be attributed to the same role of the two viruses in induction of such molecules.

Conclusion

Guarding against HCC development on top of HBV must require vaccination against HBV infection.

Also, further work must be done to reach an effective vaccine against HCV.

Patients with HCV or HBV infection should provided with anti-inflammatory drugs or cytokine antagonists aiming to guard against HCC. Moreover, our work supports that antioxidants have a role in protection against hepatic carcinogenesis.

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العوامل المؤدية الى انتشار سرطان الكبد فى مصر الإصابة بفيروس سى او بى

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يعتبر سرطان الكبد من اكثر الأورام السرطانية شيوعا فى البلاد النامية كما تعتبر العوامل المؤدية الى سرطان الكبد عوامل محددة ومتعارف عليها لكن مازالت ميكانيكية تسرطن خلايا الكبد غير مكتشفة حتى الان ويبدو أنها تختلف بأختلاف المسبب .

ومن العوامل المؤدية الى سرطان الكبد الآصابة بفيروس سى أو بى أو الاصابة بالآثنين معا وذلك عن طريق الدور الذى تلعبة السيتوكينات والعوامل الؤكسدة فى كل من خلايا الكبد المصابة أو حتى الطبيعية .

وتحاول هذه الدراسة المقدمة القاء الضوء على بعض هذه السيتوكينات ممثلة فى عامل النخر الورمى ألفا أنترلوكين واحد بتيا وانترلوكين 6 بقياسهم فى دم مرضى المصابين بسرطان الكبد شاملة بعض المرضى المصابين بفيروس سى أو بى أو كلاهما معا فى نفس الوقت أو غير المصابين بأى فيروس على الإطلاق كما تحاول هذة الدراسة معرفة دور كل من دلائل الأكسدة الفوق الدهنية وكذلك أكسيد النتروجين كعوامل حماية ووقاية أو كعلاج مستقبلى فى هذه الحالات :-

وقد أجريت هذة الدراسة على 98 مريضا يعانون من الاصابة بسرطان الكبد مقسمون الى مجموعات :-

المجوعة الآولى:- وتشمل 28 مريضا يعانون من سرطان الكبد مع الاصابة بفيروس سى

الجموع الثانية :- وتشمل 11 مريضا يعانون من سرطان الكبد مقرونة بالاصابة بفيروس بى.

المجموعة الثالثة :- وتشمل 10 مرضى مصابون بسرطان الكبد مع الاصابة بفيروس سى وفيروس بى معا فى نفس الوقت

المجموعة الرابعة :- وتشمل 9 مرضى مصابون فقط بسرطان الكبد دون أن يصاحبها الاصابة بأى من فيروس سى أو بى

المجموعة الخامسة :- وهى المجموعة الضابطة وتشمل 40 شخص ذو صحة سليمة مماثلين للمجموعات السابقة من حيث أعمارهم ومستواهم الاجتماعي .

وقد تم قياس كل من المواد التالية فى دم هؤلاء المرضى عامل النخر الورقى ألفا أنترلوكين بينا , أنترلوكين 6 أكسيد النتروجين – دلائل الآكسدة فوق الدهنية الى جانب قياس البروتين الجنينى ألفا ووظائف الكبد قد اجريت ايضا دراسة هستوباثولوجية لعينات كبدية أخذت من المرضى المصابين بسرطان الكبد المصاحبة بالإصابة بفيروس سى نظرا لشيوع هذة الحالات .

ولقد دلت نتائج هذه الدراسة على زيادة كل من عامل النخر الورمى ألفا , أنترلوكين واحد بيتا ,انترلوكين 6 , أكسيد النيتروجين , دلائل الأكسدة فوق الدهنية فى مختلف

مجموعات المرضى مقارنة بالمجموعة الضابطة . بينما كانت هذه الزيادة ذات قيمة إحصائية فى المرضى المصابين بسرطان الكبد المصاحب بالإصابة بفيروس سى , بى أو الإصابة بالاثنين معا مقارنة بالمرضى المصابين بسرطان الكبد الغير مصاحب بالإصابة بأى من الفيروسين . وقد سجلت أعلى مستويات الزيادة فى المرضى المصابين بسرطان الكبد المصاحب بالاصابة بالفيروس سى , بى معا .

ولقد أوضحت النتائج أيضا وجود علاقات إحصائية ايجابية بين كل من عامل النخر الورمى وأنترلوكين واحد بيتا , انتروكين 6 , أكسيد النتروجين وكل من البروتين الجينى ألفا والبيليروبين وأنزيم الفوسفاتيز القلوى وايضا درجة الورم السرطانى . أيضا علاقة إيجابية بين أكسيد النتروجين ودرجات الورم

بينما أظهرت النتائج علاقات ذات دلالة سلبية بين كل من :-

- انترلوكين واحدبيتا وتركيز البروثروبين .
- البروتين الجينيتى ألفا وكل من تركيز البروثروبين وتركيز البروتين الكلى بالدم .
- تركيز البروثروبين وكلا من البيليروبين الفوسفاتيز الكلوى وأكسيد النيتروجين ودرجات الورم .
- أكسيد النتروجين وكل من الالبومين والبروتين الكلى .
- أكسيد النيروجين وكل من الالبومين والبروتين الكلى .

وقد بينت النتائج الهستوبوثولوجية للعينات المأخوذة من المرضى الذين كانوا يعانون من سرطان الكبد مجتمعة مع الاصابة بفيروس سى أن هناك 15 مريضا يعانون من ورم كبدى من الدرجة الثانية بينما كان 13 مريضا منهم يعانون من ورم من الدرجة الثالثة .

ومن جهة اخرى أضهرت التجارب وجود زيادة فى مستويات كل من البروتين الجنينى ألفا والآنزيم الناقل للمجموعة الأمنية . زأنزيم الفوسفاتير القلوى والبليروبين فى جميع المرضى مقارنة بأشخاص المجموعة الضابطة ولقد كانت هذة الزيادة ملموسة اكثر فى كل المصابين بسرطان الكبد مع أصابتهم بكل من فيروس سى , بى معا يليهم المرضى المصابين بفيروس سى فقط ولقد أظهرت النتائج التهاب الواضح فى زيادات الدلائل المدروسة فى مرضى سرطان الكبد والمصاحب بفيروس سى والاخرين المصابين بفيروس بى وهذا يعزى الى قدرة كل من الفيروس على تخليق هذة الجزيئات بنفس القدرة

وقد تفسر زيادة قياسات الدلالات بآلاتي :-

-إفراز الخلية السرطانية للستيوكنيات .

-تنبية للجينات المسئولة عن تكوين هذة الستيلوكنيات كدفاع من الخلية ضد هذا المرض

-التخليق الجديد للأوعية الدموية يمكن أن يؤدى الى زيادة عامل النخر الورقى بصفة خاصة .

-أيضا نتيجة لدور الموت المبرمج للخلية

-تحطيم الخلية السرطانية عن طريق التأثير على الجينات والمادة الوراثية فى الخلية عن طريق تراكم كل من شقوق الاكسجين والاكاسيد فوق الدهنية وأكسيد النتروجين والتى تحدث فى خلايا هؤلاء المرضى .

وفى النهاية توحى هذه الدراسة :-

-بأن بروتين وهى 130 المنشط لانترلوكين 6 الذى بدورة يساعد على نمو خلايا الكبد وقد يكون أملا لمرضى الكبد .

-أمكانية استخدام عامل النخر الورقى الفا كعلاج للخلايا السرطانية بالكبد .

-أستخدام وسائل لتشخيص المرضى ميكرافيل دراسة نشاط أنزيم تيلوميرير

-مجايهة شقوق الاكسجين النشطة فى الخلايا الكبدية سواء المصابة بالسرطان أو غيرها باستخدام المواد المانعة للاكسدة مثل فيتامين سى وجلوتاثيون وفيتامين ه مع وجوب تعميمها .

-جعل التطعيم ضد فيروس بى اجباريا .

-تكثيف الجهود لآكتشاف مصل ضد فيروس سى

-الادوية التى لها علاقة بالموت المبرمج للخلية والتكوين الجديد للآوعية الدموية قد يكون لها دورا فعالة فى تلك الحالات .