OCCULT HEPATITIS B VIRUS INFECTION IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION AND HEPATOCELLULAR CARCINOMA

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ABSTRACT

Background: Hepatocellular carcinoma is one of the most common cancers worldwide. Primary known risk factors for HCC are Hepatitis C virus (**HCV**), Hepatitis B virus (HBV) and alcoholic liver disease. There is little information about occult HBV and its relation to HCC in chronic . We aimed to evaluate the prevalence and the possible clinical impact of occult HBV infection in chronic HCV patients with hepatocellular carcinoma.

Patient and methods: A total number of 140 patients with hepatocellular carcinoma were examined, 118 patients were included in this study. They had positive HCV-RNA, negative HBsAg and radiological evidence of Hepatocellular carcinoma, previous antiviral therapy or serological evidence of autoimmune hepatitis and were divided into two groups according to their HBc-Ab status: **Group (I)** 93 patients positive for HBc-Ab (**Occult HBV/HCV dual infection**), **Group (II)** 25 patients negative for HBc-Ab (**HCV mono infection**). The rest of HCC patients were coinfected with HCV and HBV, 8 patients had HBV infection **(+ve HBV sAg and –ve HCV Ab**) and 4 patients had negative tests for both HCV and HBV were excluded.

Results: A statistically significant increase in serum levels of AST, ALT, bilirubin, Alpha-fetoprotein with more cirrhosis, ascites, liver decompensation (**according to Child-Turcott-Pugh score**) and more hepatic focal lesions and malignant portal vein thrombosis (**through radiological diagnosis**) in Group (I) as compared to Group (II), while there was no statistically significant difference among Group (I) and Group (II) as regarding PT% or spleen size.

Conclusion: Occult HBV infection is extensively common among HCC chronic hepatitis C infected patients and presence of occult HBV infection is associated with more deterioration of liver status.

Key words: Occult HBV, chronic HCV, HCC.

INTRODUCTION

Hepatitis B and C viruses are the main etiological agents of chronic hepatitis related to the emergence of liver cirrhosis and hepatocellular carcinoma **(1&2).** The prevalence of co-infection of Hepatitis C and manifest Hepatitis B viruses, is variable in different studies ranging from 15%-30%, and 40%-50% when serum and liver tissue are tested, respectively and differs with sensibility of test used **(3).** Routinely the diagnosis of HBV infection is usually based on the detection of hepatitis B surface antigen (HBsAg). Occult hepatitis B is defined by the presence of HBV DNA in serum or liver in the absence of HBsAg **(4&5).** In such condition, HBV infection may occur in HBsAg negative patients with or without serologic markers of previous infection (antibodies to HBsAg [anti-HBs] or to the hepatitis B core antigen [anti-HBc]) **(6).** The subject of occult HBV have been discussed in many studies and articles citing that HBV DNA can be detected in patients with chronic liver disease who are negative for HBsAg but positive for antibodies to hepatitis B core antigen (anti-HBc) **(7&8).** It is well known that the prevalence of latent HBV infection in anti-HBc–positive patients with HCV-related chronic liver disease, including chronic hepatitis, cirrhosis, and HCC **(9).** And, several reports have revealed that the HBV genome is frequently detectable in liver tumors in anti-HBc–positive, HBsAg negative patients with HCV-related liver disease, which suggests that occult HBV infection may contribute to the progression of liver damage and the development of HCC in HCV-positive patients **(10).** Needless to mention, that Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide **(11);** ranging between 3% and 9% annually **(12);** with a geographic correlation between the incidence of HCC and the prevalence of chronic hepatitis B and C, suggesting that these two viral infections are the most important risk factors of HCC worldwide **(13).** Moreover, it has also been indicted as a cause of non response to HCV treatment **(14).** In conditions of immunocomp-etence, the occult infection is inoffensive in itself, but when other important causes of liver damage co-exist ‹the minimal lesions produced by the immune response to the occult virus might contribute to making the course of the liver disease worse over time **(3).** Other studies show that occult infection does not interfere with the natural history of the disorder in this population **(15).** There is little information of occult HBV and its relation to HCC in chronic HCV despite the importance of this infection. **Subjects:** A total number of 140 patients

with hepatocellular carcinoma were examined, 118 patients were included in this study. They had positive HCV-RNA, negative HBsAg and radiological evidence of Hepatocellular carcinoma without any evidence of co-existing liver disease, previous antiviral therapy or serological evidence of autoimmune hepatitis and were divided into two groups according to their HBc-Ab status: Group (I) 93 patients positive for HBc-Ab (Occult HBV/HCV dual infection), Group (II) 25 patients negative for HBc-Ab (HCV mono infection). The rest of HCC patients were coinfected with HCV and HBV, 8 patients had HBV infection (+ve HBV sAg and –ve HCV Ab) and 4 patients had negative tests for both HCV and HBV were excluded.

METHODS

All subjects of study were subjected to full clinical examination, routine laboratory investigations including liver function tests, Alpha-foetoprotein (AFP) , anti HCV antibodies and serological markers of HBV (HBs Ag, antiHBs, HBe Ag, Anti-HBe, anti HBc total (IgG+ IgM), using enzyme linked immunosorbent assay and specific investigations including abdominal Ultra Sound and Triphasic CT scanning.

STATISTICAL ANALYSIS

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data, the following tests were used to test differences for significance; Differences between frequencies (qualitative variables) in groups were compared by Chi-square test. Differences between means (quantitative variables) in two parametric groups were compared by Student's t-test and non parametric group by Mann Whitney Pearson correlation for parametric correlation. P value was set at <0.05 for significant results. Data were collected and submitted to statistical analysis**(16).**

RESULTS

Table (1): comparing both groups regarding Age, Sex and Residence

	Group (**I**)	Group (**II**)		
	Age (years)			
			t	**P**
Mean±SD	56.48±7.751	50.24±6.118	3.724	<0.001 **HS**
	Sex			
	%	**%**	(x^2)	**P**
Male	81.7%	68.0%	2.2	NS
Female	18.3%	32.0%		
	Residence			
	%	**%**	(x^2)	**P**
Rural	75.2	64	0.34	< 0.05 **S**
Urban	24.8	36		

It was reported that the Mean±SD of age was higher in Group (**I**) as compared to Group (**II**) and the majority of cases in Group (**I**) had more patients from rural areas than in Group (**II**) while there was no significant difference between either groups concerning sex.

Table (2): comparing both groups regarding history of diabetes, hypertension, blood transfusion and surgical operations.

	Group (**I**)	Group (**II**)	(x^2)	**P**
	%	**%**		
Diabetes	62.4%	52%	6.33	< 0.05 **S**
Hypertension	41.8%	36%	5.24	< 0.05 **S**
History of blood transfusion	22%	16%	4.34	< 0.05 **S**
operations	47%	40%	3.78	< 0.05 **S**

Group (**I**) had significantly more patients with diabetes, hypertension and history of blood transfusion and surgical operations than Group (**II**)

Table (3): comparing both groups regarding clinical evaluation

		Group 1	Group 2	t	P
	BMI (Mean)	24.23%	22.31%	4.641	< 0.05 **S**
		%	**%**	(x^2)	**P**
Symptoms	Accidental discovery	53.7	48	0.76	NS
	Abdominal pain	70.9	64	6.1	< 0.05 **S**
	Fever	20.4	20	7.2	< 0.05 **S**
	Weight loss	48.4 0	40	5.4	< 0.05 **S**
	Haematemesis	13.9	12	7.1	< 0.05 **S**
	Easy fatigability	69.9	60	6.3	< 0.05 **S**
	Bleeding tendency	49.5	40	5.1	< 0.05 **S**
Signs	Pallor	16.1	16	6.4	< 0.05 **S**
	Cachexia	7.5	8	4.8	< 0.05 **S**
	Encephalopathy	8.6	8	7.7	< 0.05 **S**
	Jaundice	31.2	24	4.9	< 0.05 **S**
	Lower limb edema	52.6	48	6.7	< 0.05 **S**
	Ascites	40.8	36	5.1	< 0.05 **S**

Regarding BMI, symptoms and clinical signs Group (**I**) was significantly more overweight and more clinically deteriorated than Group (**II**)

Table (4): Comparison of Mean±SD of different laboratory parameters in both groups

	Group (**I**) (Mean±SD)	Group (**II**) (Mean±SD)	t	P
Hemoglobin Concentration (g/dl)	9.43±0.98	9.7±0.904	0.56	NS
WBCs count	4.8 ±1.37	6.01±2.004	7.73	NS
Platelet count	70.56±14.82	92.4±10.38	13.911	<0.001 **HS**
AST(u/l)	65.2±8.95	47.15±6.68	9.363	<0.001 **HS**
ALT (u/l)	63±6.98	52.8±7.03	6.471	<0.001 **HS**
Serum albumin (mg/dl)	2.72±0.26	3±0.14	7.315	<0.001 **HS**
Total bilirubin (mg/dl)	2.4±0.53	1.5±0.3	11.186	<0.001 **HS**
PT (%)	64.3±16.6	66.8±6.01	1.181	NS
Urea (ul/l)	33.63±10.36	32.23±10.95	9.363	<0.001 **HS**
Creatinine (mg/dl)	1.24±0.49	1.18±0.47	8.451	<0.001 **HS**
AFP (ng/ml)	4513.57 ± 43336.96	3977.43 ± 31766.73	18.758	<0.001 **HS**

Serum levels of AST, ALT, bilirubin and Alpha-fetoprotein were significantly higher serum level of albumin was significantly lower in Group (**I**) as compared to Group (**II**) while PT showed no significant difference among studied groups. Also Group (**I**) had significantly worse renal functions than Group (**II**). Platelet count and WBCs count were highly significant lower among Group (**I**) than Group (**II**), while there was no significant difference between Group (**I**) and Group (**II**) as regarding hemoglobin level.

Table (5): Comparison between Group (**I**) and Group (**II**) regarding ultrasonographic data

		Group (**I**)		Group (**II**)		(x^2)	P
		No	**%**	**No**	**%**		
Liver size	Shrunken cirrhotics	52	55.9	16	64	4.8	< 0.05 **S**
	Normal sized cirrhotics	41	44.1	9	36		
Degree of ascites	No ascites	23	24.7	3	12	5.6	< 0.05 **S**
	mild	34	36.6	7	28		
	moderate	21	22.6	10	40		
	massive	15	16.1	5	20		
						t	**P**
Spleen Size	Mean±SD	15.2±2.9		15.1±3.2		0.38	NS

Regarding ultrasonographic and CT imaging evaluation, Group (**I**) showed significantly more cirrhosis and ascites than Group (**II**), but no significant difference in spleen size.

Table (6): Comparison between Group (**I**) and Group (**II**) regarding Child classification

Child Classification	Group (**I**)		Group (**II**)		(x^2)	P
	%	**No**	**%**	**No**		
A	13	14	7	28	18.5	<0.001 **HS**
B	41	44.1	7	28		
C	39	41.9	11	44		

Group (**I**) was significantly more liver decompensation than Group (**II**)

Table (7): Comparison between Group (**I**) and Group (**II**) regarding radiological characters of the tumors

		Group (**I**)		Group (**II**)		(x^2)	P
		No	**%**	**No**	**%**		
Site	Right lobe	67	72	15	60	4.8	< 0.05 **S**
	left lobe	19	20.4	7	28		
	Bilobar	7	7.6	3	12		
Number	Single	59	63.4	14	56	5.9	< 0.05 **S**
	Multiple	34	38.6	11	44		
Portal vein thrombosis		13	14	3	12	6.3	< 0.05 **S**

Table (4) shows that there was more hepatic focal lesions and malignant portal vein thrombosis (**through radiological diagnosis**) in Group (**I**) as compared to Group (**II**).

DISCUSSION

Hepatocellular carcinoma is one of the most common cancers worldwide. It is rarely detected early and is usually fatal within a few months of diagnosis**.** Primary known risk factors for HCC are Hepatitis C virus (HCV), Hepatitis B virus (HBV) and alcoholic liver disease. Other risk factors for (HCC) include diabetes mellitus (DM) and obesity **(17).** The rising incidence of HCC over a decade proportion may be explained by the increasing risk factors such as the emergence of HCV over the same period of time, the contribution of HBV infection, improvement of the screening programs and diagnostic tools of HCC, as well as the increased survival rate among patients with cirrhosis to allow time for some of them to develop HCC **(18).** It seems that cirrhosis is the common pathway by which HCV promotes carcinogenesis, but HCV also may play a direct role through involvement of viral gene products in inducing liver cell proliferation **(19).**

HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens. Approximately one third of the world's population has serological evidence of past or present infection with HBV and 350- 400 million people are chronically infected. The spectrum of disease and natural history of chronic HBV infection is diverse and variable, ranging from a low viremic inactive carrier state to progressive chronic hepatitis which may evolve to cirrhosis and hepatocellular carcinoma (HCC) **(20).**

Occult hepatitis B infection is generally defined as the detection of HBV-DNA in the sera

or tissues of subjects who have negative tests for HBsAg, with or without anti-HBc or (anti-HBs), outside the pre-seroconversion window period **(21).** Several studies have reported HBV DNA detection in tumourous liver tissue of HBsAg-negative HCC patients, with prevalence rates ranging from 30-80 %**.**Several mechanisms have been considered for occult infections by HBV such as low HBV DNA and HBsAg levels, mutations in HBV DNA sequence, viral DNA integration in the host genome, infection of peripheral blood mononuclear cells, altered host immune response, and interference of other viruses (mainly HCV) **(22).**

One of the most important clinical implications of OHB is usually observed in the setting of liver transplantation. In particular, livers from donors with OHB carry a risk of HBV transmission with infection occurring in 25–95 % of the liver grafts donated from patients who are HBsAg negative but anti-HBc positive **(23).** Occult HBV may contribute to liver inflammation through episodes of increased viral replication, increased immune activity and subsequent liver injury .In chronic HCV infection, the presence of Occult HBV has been associated with liver enzymes flare, increased severity of liver disease towards advanced fibrosis and cirrhosis, poor response to standard interferon-a in many studies, and increased risk of HCC **(24&25).** The mechanisms of carcinogenesis in HBV infection have been extensively studied, and a major factor is chronic necroinflammation with subsequent fibrosis and hepatocyte proliferation. However, HCC may occur in HBsAg carriers without cirrhosis. Both HBV and host hepatocytes may contribute to the final pathogenic outcomes, either individually or synergistically. Therefore, it is reasonable to consider that apart from host factors, viral factors are likely involved in HBV-related hepatocarcinogenesis **(26).** HBV may encode oncogenic viral proteins that may contribute to hepatocarcinogenesis**.** HBx protein has been shown to complex the tumor suppressor p53 protein and to suppress its function**.** HBV exerts its oncogenic potential through a multi-factorial process, which includes both direct and indirect mechanisms that likely act synergistically **(27).** Liver cirrhosis itself, resulting from sustained inflammatory damage and hepatocyte regeneration, has been considered as a pre-neoplastic condition **(28).** The transactivating potential of several viral oncoproteins, such as HBx and the truncated Pre-S2/S, on the regulatory cellular pathways is a further crucial oncogenic consequence of the integration**.** Due to spontaneous errors in viral reverse transcription, variations along HBV genome occur naturally. These mutations that arise during the course of HBV chronic infection have consequences at both clinical and epidemiological level **(29).**

The strong association between cirrhosis and HCC suggests a hepatocarcino-genic process that is largely mediated by inflammation, leading to repeated cycles of cell death and regeneration that increase hepatocyte proliferation turnover **(30).** The sustained stimulation of liver cell to progress towards the cell cycle can ultimately overcome DNA repair mechanisms in the presence of mutational events. The accumulation of critical variants in the host genome may heavily contribute to transformation of hepatocytes into malignant clones and the cells, designed to the elimination through the apoptosis program or immune response, will become fully transformed **(31).** Concurrently, liver fibrosis disrupts the architecture of hepatic structure. As a consequence, cell-to-cell interactions are modified, and this ultimately leads to loss of control over cell growth. Thus, the persistent inflammatory changes, caused by chronic infection, promote liver cancer development through an integrated multi-step process **(32).**

Therefore, in this study, we tried to explore the prevalence and the possible clinical impact of occult HBV infection in chronic HCV patients with hepatocellular carcinoma, so we studied blood samples and the CT images of chronic HCV infected patients with HCC, who were HBsAg negative.

The present study revealed a significant association between combined occult HBV/HCV infection and the older age of HCC patients. This is supported by Kumar et al. **(33)** who reported that incidence of HCC increases with age. The development of HCC is uncommon before 40 years of age in western world. However, the pattern of HCC incidence by age is sometimes dependent on the geographical pattern or on etiologic factors **(34)**. This also is in agreement with Velazquez et al. **(35)** who found that cirrhotic patients older than 54 years are at 4 times greater risk to develop HCC. But our results come in contrast with Emara et al. **(36)** who reported that average age was the same in occult HBV/HCV dual infection and in HCV monoinfection; yet it should be noted that his population were not HCC patients. As regarding sex, no significant difference between both groups

was detected .In agreement with our study, Emara et al. **(36)** showed no significant difference between both groups regarding sex.

Our study revealed that most cases in both Groups were from rural areas. This agrees with the studies done by Shaker et al. **(37)** and Mohamed et al. **(38)** which revealed that most of HCC cases came from around the Nile banks. Also, another study done by Arthur et al. **(39)** correlates the prevalence of HCV infection in blood donors to residence, found that governorates with highest seroprevalence were those around the Nile River.

In our study, Group (**I**) had significantly more patients with diabetes than Group (**II**)**.** Davila et al. **(40)** explained this finding by the fact that Diabetes, as part of insulin resistance syndrome, has been implicated as a risk factor for non-alcoholic fatty liver disease (NAFLD), including in its most severe form, non-alcoholic steatohepatitis (NASH) and NASH has been identified as a cause of both "cryptogenic cirrhosis" and HCC.

In our study in Group (**I**) was significantly more overweight than Group (**II**).This finding is supported by Ohki et al. **(41)** who stated that visceral fat accumulation is an independent risk factor of HCC recurrence after curative treatment in patients with suspected NASH, and excess weight is involved in the transition from healthy HBV carrier state to HCC and liver-related death among men. Increased BMI is associated with increased risk for early HCC development in HCV-infected patients. Decreasing BMI and reducing alcohol intake could help prevent hepatic carcinogenesis **(42)**.

As regarding clinical evaluation of both groups, we found that the prominent symptom was the newly developed right hypochondrial pain which was present in 70.9% of patients in Group (**I**), and 64% of patients in Group (**II**). This is in agreement with Shaker et al. **(37)** and Mohamed et al. **(38)** who reported right hypochondrial pain in 66.3% of the patients. Fever and loss of weight were present in 20.4% and 48.4% of Group (**I**) patients respectively, and 20% and 40% of Group (**II**) patients respectively likewise. Also, Johnson **(43)** found that the most common mode of presentation of HCC was the triad of right upper quadrant abdominal pain, weight loss and hepatomegaly. Patients who present in this manner usually have tumors larger than 6 cm in diameter. The pain is usually dull ache, sometimes referred to the shoulder. Sudden attacks of more severe pain may be caused by spontaneous bleeding into the tumor.

In our study, ascites, LL edema, jaundice, hematemesis, and encephalopathy were more frequent in Group (**I**) as compared to Group (**II**), exhibiting similar results to Shaker et al. **(37)** and Kumar et al. **(33)**. With our findings, Johnson **(43)** stated that in a patient with previously well-controlled cirrhosis and who develops ascites, recurrent variceal hemorrhage or encephalopathy, HCC must always enter the differential diagnosis. He explained these findings as in cases with HCC the bleed will have been from esophageal varices, this is may be due to portal vein invasion by the tumor.

In our study, we found that AFP level was more than 400 ng/ ml in 37.6% and 36% of the cases of Group (**I**) and Group (**II**) respectively concomitant with that study done by Kumar et al. **(33)** and less than 20 ng/ml in 32.9% and 40% of the cases of Group (**I**) and Group (**II**) respectively as reported by Shaker et al. **(37)**.This means that, in around one third of the cases, AFP level was normal as AFP is not secreted in all cases of HCC and may be normal in many patients with early HCC **(44)**.

In our study, we found that the mean platelets count was significantly lower in Group (**I**) as compared to Group (**II**), which was further supported by Matsouka et al. **(45)** and Branco et al. **(3)** who reported that the mean platelets count was lower in occult HBV/HCV dual infection in comparison to HCV monoinfection.While comparison of other hematological parameters in our work between both groups was statistically non significant. Pierluigi et al. **(46)** explained these results as co-infection causes more progression of liver fibrosis leading to worsening of portal hypertension with consequent increased platelet sequestration and destruction in an enlarging spleen.

The present study revealed significantly higher serum levels of AST, ALT and bilirubin and lower serum level of albumin in Group (**I**) as compared to Group (**II**), while PT% showed no significant difference between both groups. This is in agreement with the study done by Selim et al. **(47)** and Shavakhi et al. **(48)** who reported that the serum levels of AST and ALT were significantly higher among occult HBV/ HCV dual infection than HCV monoinfection. This result was explained by Okuda et al. **(49)** who stated that co-infection leads to more progression of liver fibrosis which may reduce liver enzymes

clearance, leading to increased serum levels, in addition, liver disease may be associated with mitochondrial injury resulting in further liver enzymes release from the hepatocytes. While Branco et al. **(3)** didn't agree with us, as he reported no significant difference between occult HBV/ HCV dual infection and HCV monoinfection regarding serum AST or serum ALT levels.

According to Child-Turcott-Pugh score we found that Group (**I**) was significantly more decompensated than Group (**II**) according to Child-Turcott-Pugh score

While patients with HCC are usually asymptomatic during the early stages of disease, unfortunately, 80% of patients with HCC will be diagnosed with advanced stage disease **(43)**. In our study, 53.7 % of patients in Group (**I**) were accidentally discovered to harbour hepatic focal lesions on ultrasound examination and 48% of patients in Group (**II**) also were accidentally diagnosed with HCC.

In our study, Group (**I**) patients , right lobe was the site of HCC in (72%) of them , followed by left lobe in (20.4%) and in both lobes in (7.6%) meanwhile in Group (**II**), right lobe was involved in (60%) of cases ,right lobe and left lobe (28 %) with bilobar affection in (12%) of them. Similar results were obtained by El-Zayadi et al. **(50)** who found 65% affecting the right lobe, 13.4% the left lobe and 21.6% affecting both lobes. Also, Kumar et al. **(33)** found that HCC most commonly involved the right lobe of liver (48%), followed by bilobar involvement (34%). The left lobe was involved in approximately one fifth of cases.

Our study reported that, single lesion was seen in (63.4%) of patients from Group (**I**) and multiple lesions were found in (38.6%) but in Group (**II**) (56%) of patients had single lesions and (44%) had multiple lesions of studied patients. Similarly, Kumar et al. **(33)** found that single lesion was the most common presentation of HCC observed in two-third cases.

Portal vein thrombosis was present in 14% of our patients of Group (**I**) and 12% of patients in Group (**II**). Similar results were obtained in an Indian study by Kumar et al. **(33)** who found that the incidence of gastrointestinal bleed was high in their study (22%).

So, we can conclude that HCC is a major health problem in Egypt and its incidence is increasing. The high prevalence of Occult HBV makes screening programmes, and the surveillance of HCV patients a very important tool to early detect cases of small HCCs. Further studies focusing on risk factors other than viral infections are warranted

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