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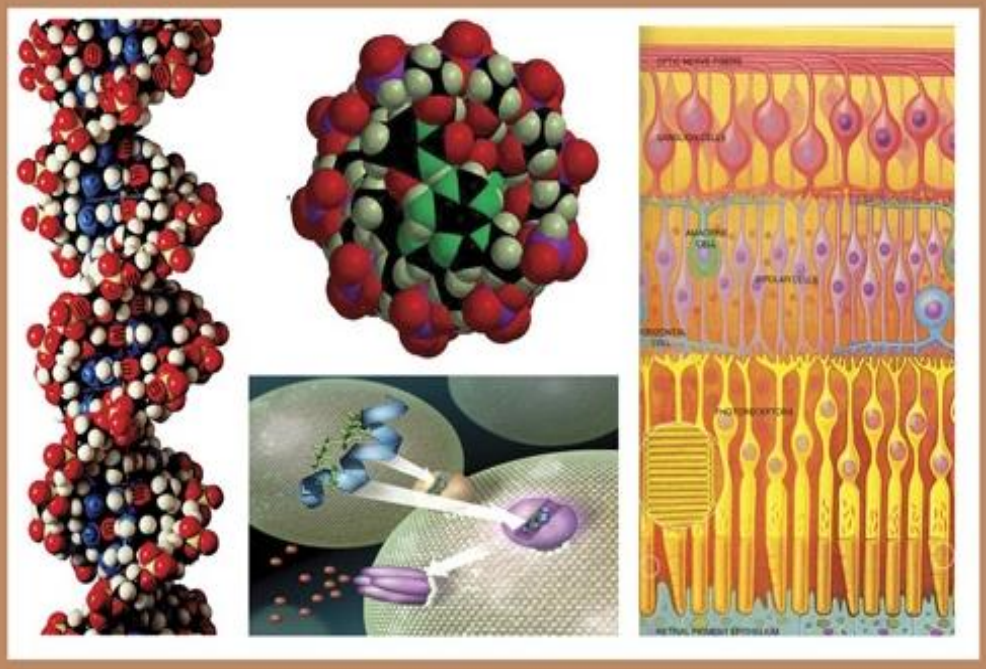

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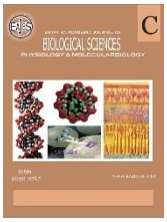

Incidence and Clinical Implications of Isolated Hepatitis B Core Antibody Serologic Profile Pattern Among Egyptian Patients with Chronic Hepatitis C

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ABSTRACT

Background: Hepatitis B viral infection is an oncogenic lethal infection, which is represented by different features. Those features are interpreted according to the coordination of different serological HBV markers. One of the most controversial features is the isolated HBc-Ab serologic profile pattern. In HBV/HCV co-infection, HCV can suppress HBV replication, thus HBV infection may decline. Isolated HBc-Ab serologic profile pattern represents a challenge in patients with chronic HCV, because it may represent a reservoir of latent HBV infection, which may be reactivated later. **Objective:** This study aims to investigate the incidence of the isolated HBc-Ab serologic profile pattern in samples of Egyptian patients chronically infected with HCV, and to demonstrate its clinical implications. **Methods:** The current study proceeded on blood samples of a total of 154 subjects (124 patients with chronic HCV infection and 30 controls). They were evaluated for liver function parameters and AFP as a tumour marker. The serum samples were tested serologically by ELISA for HBs-Ag, HBs-Ab, and HBc-Ab total. Real-time PCR was applied to measure HCV-RNA and HBV-DNA in samples of those patients. Upon interpretations of HBV infection, HCV- patients were divided into 5 groups from G1 to G5. **Results:** A significant increase ($p = <0.001$) in the incidence of HBc-Ab was detected in samples of HCV- patients (68/124, 54%) compared to controls (2/30, 6.66%). Likewise, a significant increase ($p = 0.009$) in the incidence of isolated HBc-Ab serologic profile pattern was detected in samples of HCV-patients (28/124, 22.58 %) compared to controls (0/30, 0%). Based on the comparison study, samples of HCV- patients with isolated HBc-Ab serologic profile pattern (G5) showed a significant decrease ($p = 0.017$) in albumin, and a significant increase ($p = 0.032$) in total bilirubin compared to samples of HCV-patients who resolved HBV infection (G2). Also, the former group of patients (G5) showed a non-significant change in any of the clinical biochemical parameters or AFP compared to the group of HCV- patients who were susceptible to HBV infection (G1)

Conclusion: Isolated HBc-Ab serologic profile is a pattern of HBV infection, which increases significantly in chronic HCV- patients. The increase in hypoalbuminemia and hyperbilirubinemia are the clinical implications associated with the presence of the isolated HBc-Ab serologic profile pattern in HCV-patients who cannot develop HBs-Ab compared to other HCV-patients who can develop HBs-Ab in the presence of HBc-Ab and resolve HBV infection.

INTRODUCTION

Numerous studies have declared that hepatitis B virus (HBV) is a major cause for public health problems, as out of two billion people who were previously infected with HBV, 240 million suffer from chronic infection, which causes several alterations in liver physiology leading to subsequent severe complications (Abdelaziz *et al.,* 2019). In Egypt, HBV is one of the major causes of liver inflammation, fibrosis, cirrhosis, and liver cancer (El-Adley *et al.,* 2020). By progression or resolving of HBV infection, clinical states of such infection are reflected by HBV serological markers. The presence of the major characteristic marker, HBs-Ag is a sign for active acute or chronic infection of HBV (Chang *et al.,* 2018). Inadequate immune response for HBV due to host factors such as age at onset of the exposure, or immunosuppression can increase the risk for HBV chronicity leading to undesired complications (Chang *et al.,* 2018). The mechanisms regarding immune control of HBV infection along with the presence of cccDNA, which refers to covalently-closed circular DNA that persists after HBV exposure, can permit the possibility of HBV reactivation even following recovery and clearance of HBs-Ag. The next characteristic marker is HBc-Ab, which can be seen in either acute or chronic infection. It persists throughout life after the development of immunity (resolved infection). By resolving HBV infection, HBs-Ab can subsequently develop. Furthermore, the presence of HBs-Ab in the absence of both HBs-Ag and HBc-Ab can refer to immunity due to hepatitis B vaccination. The isolated HBc-Ab serologic profile pattern is defined by the presence of HBc-Ab in the absence of both HBs-Ag and HBs-Ab (Bhattacharya *et al.,* 2016; Moretto *et al.,* 2020). It not only represents a challenge for the interpretation of HBV infection but also confuses the entire management for HBV infection (Chang *et al.,* 2018).

In fact, several clinical interpretations of isolated HBc-Ab serologic profile pattern have important considerations regarding the evaluation of HBV serologies. The most important of those clinical interpretations may include; 1. HBV resolved infection with a very low level of HBs-Ab titre, 2. The occult HBV infection, which is characterized by the presence of HBV viremia and the absence of HBs-Ag due to mutation. The occult HBV is a clinical feature of HBV infection, which may develop in HCV-infected patients. Different definitions for occult HBV infection were stated across different studies, some define such cases of infection by the presence of HBV viremia with the absence of HBs-Ag regardless of HBc-Ab status. On the other hand, other studies defined occult HBV infection in the term of HBV viremia only in the actual setting of the isolated HBc-Ab serologic profile pattern (Chang *et al.,* 2018). There is strong evidence for the suggestion that HBV/HCV co-infection can accumulate more impairments regarding liver physiology, and it may accelerate liver disease progression with a subsequent increase in the risk for liver cancer development (Esmat *et al.,* 2015). Previously, interferon-based therapy for HCV can lead to HBV clearance (Yu *et al.,* 2013; Chang *et al.,* 2018). Currently, the management of isolated HBc-Ab serologic profile pattern before treatment for HCV infection is required due to the high-risk regarding HBV reactivation during treatment of HCV by direct-acting antiviral (DAA) drugs (Wu *et al.,* 2017), which may appear in 30 % of dually infected patients. The hypothesis refers to the non-inhibitory effect on HBV by using DAA agents for HCV treatment in a contract to treatment with interferon therapy. So, the ability of HBV to assume viral replication driving the virus to its pathogenicity after HCV clearance may be attributed to HBV reactivation (Chen *et al.,* 2017). In other words, the high risk of HBV reactivation is dependent on both host and viral factors (Loomba and Liang, 2017). The host factors include male gender, older age, and the presence of cirrhosis. On the other hand, the viral factors include baseline HBV-DNA, the occurrence of hepatitis D virus (HDV), and non-A genotype of HBV (Borentain *et al.,* 2010; Tohme *et al.,* 2013). This reactivation is characterized by a

significant elevation of HBV viral load titre and liver enzymes with severe subsequent complications, which may lead to liver failure and fatality (Karvellas *et al.,* 2017; Moretto *et al.,* 2020).

Upon serological markers testing, the absence of all three HBV markers, HBs-Ag, HBs-Ab, and HBc-Ab can refer to individuals without prior exposure to HBV infection and lack of vaccination. Those individuals should be in a need of HBV vaccination, they do not require additional monitoring. The absence of HBs-Ag and HBc-Ab with the presence of HBs-Ab only is an indication for previous vaccination against HBV without the need for any further monitoring. The absence of HBs-Ag and the presence of both HBc-Ab and HBs-Ab can demonstrate a past HBV infection. Furthermore, the absence of HBs-Ag and HBs-Ab in the presence of HBc-Ab usually signifying an isolated HBc-Ab serologic profile pattern. The presence of HBV-DNA in blood requires HBV therapy initiation. The clear understanding of the nature and management of the isolated HBc-Ab serologic profile pattern in the context of HBV infection among HCV - infected patients is very important in deciding appropriate therapy, due to the possibility of HBV reactivation and the increase in the chance of its relapse (Shuttler *et al.,* 2002; Chang *et al.,* 2018).

MATERIALS AND METHODS

Study Population:

The work was done on preserved samples of Egyptian patients. The blood samples of a total of 154 subjects, included 124 patients chronically infected with HCV and 30 subjects as controls were enrolled in this study. The samples of HCV-infected patients were characterized by the presence of both HCV-Ab and HCV-RNA. The samples of controls were characterized by the absence of HCV-Ab, HIV-Ab, HBs-Ag, and HBV-DNA. Those samples were subjected to routine clinical biochemical investigations of liver function parameters including alanine amino-transferase (ALT), aspartate amino-transferase (AST), total bilirubin, direct bilirubin, indirect bilirubin, and albumin. Also, haemoglobin (Hb) and white blood cells (WBCs) were previously measured. The tumour marker, alpha-fetoprotein (AFP) was measured in samples of HCV-infected patients. In all samples, HBV serological markers including HBs-Ag, HBs-Ab, HBc-Ab were determined. Both HCV-RNA quantitative and HBV-DNA quantitative were determined. The statistical data analysis was performed using SPSS program version 20. The study was proceeded according to the World Medical Association's Declaration of Helsinki guidelines.

Detection of HBV Serological Markers:

Detection of Hepatitis B Surface Antigen (HBs-Ag) in serum Samples:

It was determined in all samples using a commercial enzyme-linked immunosorbent assay [ELISA] kit according to instructions of the manufacturer (ACON laboratory, Inc, USA). It depends on double-antibody sandwich ELISA. The wells are coated with monoclonal antibodies with high reactivity to HBsAg. When the samples are incubated with the enzyme conjugate, a sandwich complex of antibodies is formed and bound to the well. The enzyme conjugate is a monoclonal antibody linked to horseradish peroxidase (HRP). Briefly, 100 µl of positive controls, negative control, and specimens were added into corresponding wells in microwell plate, and then 50 µl of conjugate horseradish peroxidase (HRP- conjugate) were also added to each well except the blank well. Then the incubation for microwells at 37°C for 60 minutes represented a crucial step. The washing for each well was proceeded by a diluted wash buffer. Also, 50 µl of chromogen A and 50 µl chromogen B solutions were added to each well including blank followed by incubation at 37 °C for 15 minutes with the avoidance of light. This could allow the enzymatic reaction between HRP- conjugate and chromogen solutions to produce a blue colour that was achieved in wells of positive control and HBs-Ag positive sample. Finally, the addition of 50 µl of stop solution was applied to all wells. The intensive yellow colour appeared in wells of positive control and HBs-Ag positive sample. The reading of

the optical density (OD) was performed at 450 nm using a multi-well plate reader (TECAN; SUNRISE, Austria GmbH). The presence or absence of HBs-Ag could be determined by comparing the absorbance value of the sample to a cut-off value determined from the negative and positive controls.

Detection of Hepatitis B Core Antibody (HBc-Ab) Total in Serum Samples:

It was determined using a commercial ELISA kit according to manufacturer instructions (ACON laboratory, Inc, USA). It depends on the competitive ELISA mechanism. Briefly, 50 µl of cutoff reference, positive control, negative controls, and diluted 1:30 specimen were dispensed into each well, in addition to one blank well. The addition of 50 µl of enzyme conjugate (HRP- conjugate) was added to each well and mixed for 1 min, then incubated at 37 °C for 30 min. The diluted wash buffer was used to wash each well 4 times. Additionally, 50 µl of substrate solution A followed by another 50 µl of substrate solution B were dispensed to each well, and then the plate was incubated at 37 °C for 15 min. Finally, 50 µl of stop solution was dispensed to each well in order to stop the proceeding of colour reaction. The recording of OD values of each well was performed by ELISA reader (TECAN; SUNRISE, Austria GmbH) at 450 nm. The presence or absence of HBc-Ab was determined by comparing the absorbance values of samples to a cut-off value, which was determined from negative and positive controls.

Detection of Hepatitis B Surface Antibody (HBs-Ab) in Serum Samples:

It was determined in all samples using a commercial ELISA kit according to the manufacturer's instructions (DRG International lnc., USA). Briefly, 50 µl of serum samples and controls were added to the microwell plates coated with purified HBs-Ag, then conjugate of HRP labelled with HBs-Ag was added to the serum and incubated at 37 °C for one hour followed by a washing step. 50 µl of stop solution was added. The absorbance was measured at 450 nm using a well plate reader (TECAN; SUNRISE, Austria GmbH). Also, the presence or absence of such serological marker (HBs-Ab) was determined from comparing the absorbance value of the specimens to a cut-off value, which was determined from negative and positive controls.

HCV-RNA Quantification Assay:

HCV-RNA quantification was proceeded by real-time PCR, COBAS AmpliPrep/ COBAS TaqMan HCV automated assay [CAP/CTM HCV v2.0 assay] (Roche Diagnostics, USA). In such a technique, HCV-RNA was determined from 650 µl of the serum specimen (Chevaliez *et al.,* 2013). The nucleic acid was extracted by automated extractor of Cobas AmpliPrep instrument using magnetic silica beads, followed by the nucleic acid amplification with primers specific to 5′ untranslated region (5′ UTR) of the HCV genome. The detection of HCV-RNA was proceeded by using a hydrolysis probe labelled fluorescently through Cobas TaqMan thermal-cycler for the detection of the target HCV-RNA and an internal quantitative standard (QS). The levels of HCV-RNA were expressed in international units per milliliter (IU/mL). The dynamic range of quantification regarding CAP/CTM HCV v2.0 is from 15 to 100,000,000 IU/ml (corresponding to 1.2 to 8.0 log10 IU/ml), with the lower limit detection limit of quantification as 15 IU/ml (Chevaliez *et al.,* 2013; Esmat *et al.,* 2015).

HBV-DNA Quantification Assay:

The quantification of HBV-DNA was performed by using COBAS AmpliPrep /COBAS TaqMan HBV assay [CAP/ CTM v2.0] (Roche Diagnostics, USA). The CAP/CTM v2.0 is an automated real-time PCR method, which applying specific primers. Those primers target specific regions of the HBV genome (the pre-core and core regions). HBV-DNA was determined from 650 µl of plasma samples. The nucleic acid was extracted and eluted in a definite volume of 65 µl, then a specimen of 50 µl were analyzed. Amplification proceeded for two targets, HBV-DNA and the internal quantitation standard. The detection of HBV-DNA was performed by using a fluorescently

labelled hydrolysis probe by the Cobas TaqMan thermal-cycler for the detection of the target HBV-RNA and an internal quantitative standard (QS). HBV-DNA different levels were expressed in IU/mL. The lower detection limit is 20.0 IU/mL with the quantification dynamic range of 20.0 to 1.7×10^8 IU/mL [1.3−8.2 log10 IU/mL] (Caliendo *et al.,* 2011; Han *et al.,* 2017).

Data Statistical Analysis:

Data analysis was performed using SPSS (a statistical program for social science version 20). Such analysis included subject number, percentage, mean and standard deviation (SD) to describe quantitative variables. The qualitative variables were analyzed using the Chi-square test. The t-test was used to compare the quantitative data between the two groups. Furthermore, the analysis of quantitative variables was done by ANOVA test to compare the data among more than two groups. Tukey's test was used as an ANOVA post-test to compare quantitative variables. The significant differences were determined at $p \leq 0.05$ (significant).

RESULTS

Cohort Characteristics of HCV–Patients And Controls:

Demographic parameters (gender and age), clinical biochemical parameters (ALT, AST, AST/ALT ratio, albumin and bilirubin [total, direct and indirect], and haematological parameters (Hb and WBCs) are reported in table 1. A high significant change (p = <0.001) is observed between controls (n=30) and HCV–patients (n=124) for parameters including; age, ALT, AST, albumin, bilirubin total, bilirubin direct, bilirubin indirect, and Hb. Also, a significant change is observed for AST/ALT ratio.

Table 1: Demographic data and parameters of clinical features of HCV-infected patients and controls

Parameter	Subjects of study		Statistical analysis	
	Controls (n=30)	HCV-patients (n=124)	SE	P-value
Gender (M/F)	25/8	105/19	$X^2 = 1.456$	0.228
Age (years)	31.061 ± 10.093	52.137 ± 6.912	T=-14.015	<0.001*
ALT (U/L)	23.520 ± 10.576	44.802 ± 25.020	T=-4.152	<0.001*
AST(U/L)	24.440 ± 9.372	73.752 ± 42.125	T=-5.801	<0.001*
AST/ALT ration	1.134 ± 0.354	1.849 ± 1.262	T=-2.798	0.006*
Albumin (g/dL)	4.489 ± 0.549	2.878 ± 0.552	T=13.669	<0.001*
Bilirubin total (mg/dL)	0.928 ± 0.923	3.439 ± 1.935	T=-5.810	<0.001*
Bilirubin direct (mg/dL)	0.276 ± 0.268	1.995 ± 1.332	T=-6.320	<0.001*
Bilirubin indirect (mg/dL)	0.476 ± 0.210	1.448 ± 1.039	T=-4.582	<0.001*
Hb (g/dL)	14.148 ± 1.827	11.958 ± 1.987	T=4.180	<0.001*
WBCs (cmm)	6.536 ± 1.707	5.518 ± 2.544	T=1.717	0.092

Where; ALT= alanine amino- transferase , AST = aspartate amino- transferase, Hb = haemoglobin, WBCs= white blood cells count, n = number, SE= statistical expression, T= t-test , X^2 = Chi- square test, and * = significant value. Reference normal ranges are as the following; up to 42 U/L for ALT, up to 35 U/L for AST, from 3.5 to 5 g/dL for albumin, up to 1.2 mg/dL for bilirubin total, from 0.1 to 0.3 mg/dL for bilirubin direct, from 0.1 to 0.9 mg/dL for bilirubin indirect, from 13 to 17.5 g/dL for Hb in males and from 12 to 15.5 g/dL for Hb in females, and finally from 4 to 11 thousands/cmm for WBCs.

The incidence of HBV Markers Among the Entire Cohort Of Study:

The markers of HBV infection including (HBs-Ag, HBs-Ab, HBc-Ab, and HBV-DNA) are recorded in table 2**.** A high significant increase (p = <0.001) in incidence is observed for HBc-Ab in HCV- patients, whereas a non-significant change is observed for both HBs-Ag and HBs-Ab among HCV-infected patients versus controls.

Table 2: Incidence of HBV markers among HCV- patients versus controls

HBV markers		Subjects of study				Chi-Square	
		Controls (n=30)		HCV-patients (n=124)			
		N	%	N	%	X^2	P-value
HBs Ag	Negative	30	100	123	99.19	0.598	0.440
	Positive	0	0	1	0.81		
HBs Ab	Negative	22	73.33	74	59.68	1.892	0.169
	Positive	8	26.66	50	40.32		
HBc Ab	Negative	28	93.33	56	45.16	16.502	<0.001*
	Positive	2	6.66	68	54.84		
HBV-DNA	Negative	30	100	123	99.19	0.598	0.440
	Positive	0	0	1	0.81		

Where; HBs Ag = hepatitis B surface antigen, HBs Ab = hepatitis B surface antibody, HBc Ab = hepatitis B core antibody, HBV-DNA= hepatitis B virus DNA, n = number, and * = significant value.

In figure 1, the light columns show the percentage of HCV-infected patients regarding either positivity or negativity areas in the chart for HBV serological markers, whereas the dark columns refer to the percentage of controls in such respect. A high significant change between such columns for HCV- infected patients and controls is accumulated in HBc-Ab area.

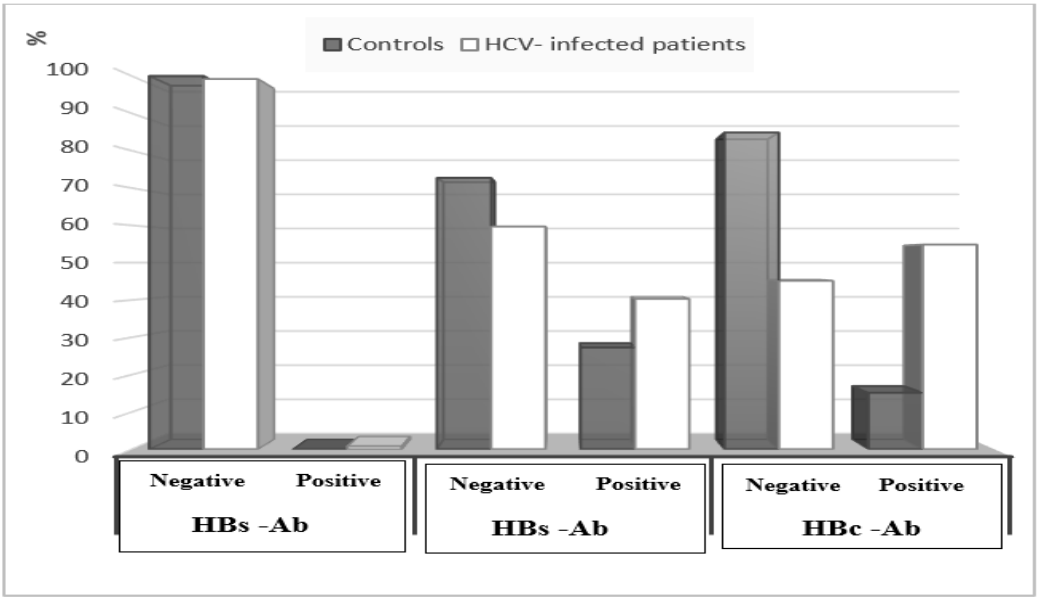

Fig. 1: The percentage of HCV-infected patients and controls regarding the incidence of the different HBV serological markers

Interpretation of HBV Infection and Incidence of Isolated HBc-Ab Serologic Profile Pattern Among HCV-Infected Patients and Controls :

According to the results of the different HBV markers, HCV-infected patients could be classified into five different groups from G1 to G5 based on the interpretation of HBV infection among those patients. In table 3, the comparison between HCV-infected patients and controls for the incidence of different groups regarding HBV infection are recorded.

A significant increase (p = 0.009) of G5 percentage (patients with isolated HBc-Ab serologic profile pattern) is observed for HCV-infected patients. Also, a significant increase (p= 0.012) of G2 percentage (patients with resolved HBV) is observed for such HCV- patients. Contrastly, a significant increase (p= 0.001) of G1 percentage (subjects who are susceptible to HBV infection) is showed for controls.

Table 3: The different groups regarding interpretations of HBV infection among HCV-infected patients and controls

Group number	Group description	Group characterization				Subjects of study				Chi-Square	
						Controls		HCV-patients			
		HBs-Ag	HBs-Ab	HBc-Ab	HBV-DNA	N	%	N	%	X^2	P-value
G1	Susceptible to HBV infection	Neg	Neg	Neg	Neg	22	73.33	45	36.29	12.021	0.001*
G2	Recovered from HBV infection	Neg	pos	Pos	Neg	2	6.67	39	31.45	6.380	0.012*
G3	HBV immunized or vaccinated	Neg	pos	Neg	Neg	6	20.00	11	8.87	2.019	0.155
G4	Active infection of HBV	pos	Neg	Pos Or Neg	pos	0	0.00	1	0.80	0.598	0.440
G5	Isolated HBc-Ab profile pattern	Neg	Neg	Pos	Neg	0	0.00	28	22.58	6.831	0.009*
Total						30	100	124	100		

Where; HBs-Ag = hepatitis B surface antigen, HBs-Ab= hepatitis B surface antibody, HBc-Ab = hepatitis B core antibody, HBV-DNA= hepatitis B virus DNA, G1 = group1, G2 = group2, G3 = group3, G4 = group 4, G5 = group 5, Neg= negative, Pos= positive, n = number, and * = significant value.

In figure 2, the light columns show the percentage of different groups of HCV-infected patients based on the interpretation of HBV infection, whereas dark columns refer to controls in such respect. The chart refers to the significant increase in the percentage of incidence of isolated HBc-Ab serologic profile pattern among HCV-infected patients in G5 area. Where; G1, G2, G3, G4, and G5 refer to group 1, group 2, group 3, group 4, and group 5 respectively.

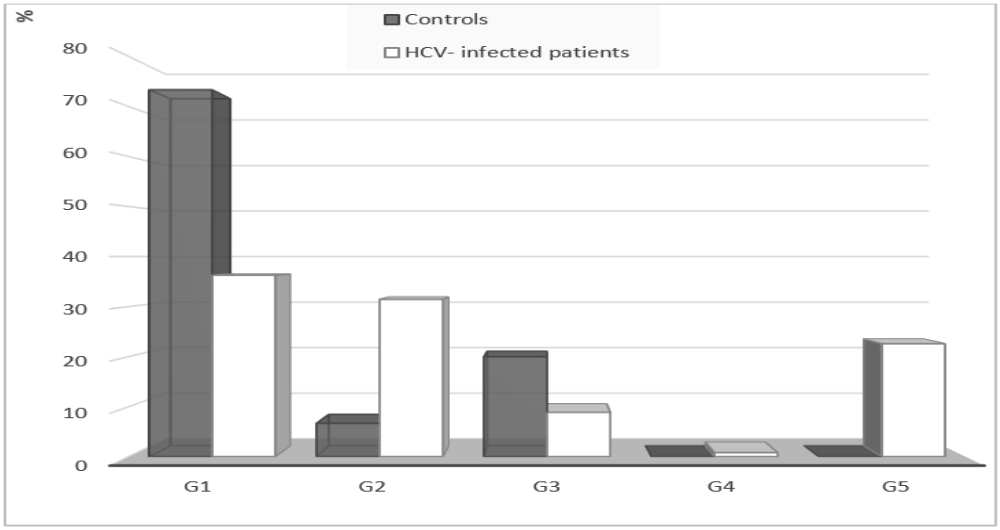

Fig. 2: The percentage of different groups of HCV-infected patients and controls regarding the different interpretations of HBV infection

Clinical Implications of Isolated HBc-Ab Serologic Profile Pattern in HCV-Infected Patients:

HCV-infected patients with positive HBc-Ab are represented by G5 (patients with isolated HBc-Ab serologic profile pattern) and G2 (patients with resolved HBV infection). These groups were selected to study the clinical implications of isolated HBc-Ab serologic profile pattern in HCV-infected patients. Also, G1 (HCV-patients who were susceptible to HBV infection) was included. Contrastly, both G3 (HBV immunized or vaccinated) and G4 (patients with active infection of HBV) were excluded due to the low number of included subjects.

In table (4), The comparison by ANOVA test among G1, G2, and G5 refers to a significant change in parameters including age, albumin, bilirubin total, and bilirubin indirect with p-values 0.011, 0.014, 0.050, and 0.039 respectively. Tukey's test is complementary to the ANOVA test to explore the significant role of the incidence of isolated HBc-Ab serologic profile pattern (G5). The comparison of G1 with G5 refers to a significant increase in the age of patients with isolated HBc-Ab serologic profile pattern. By contrast, the comparison of G2 with G5 refers to a significant decrease [p = 0.017] in albumin (hypoalbuminemia) and a significant increase [p = 0.049] in total bilirubin (hyperbilirubinemia) in HCV-infected patients with isolated HBc-Ab profile (G5). The significant change for AFP could not be detected in any of such comparisons.

Table 4: The comparison of G1, G2, and G5 patients' groups referring to the clinical impact of isolated HBc-Ab serologic profile pattern among HCV- infected patients

Parameter	HCV-patients regarding incidence of different HBV markers			Statistical analysis				
	G1	G2	G5	SE	P-value	TUKEY's test		
						G1 & G2	G1 & G5	G2 & G5
Gender (M/F)	35/10	33/6	26/2	X^2=2.931	0.231	-	-	-
Age (years)	49.822 ± 7.380	53.410 ± 6.269	54.286 ± 6.411	F = 4.724	0.011*	0.045*	0.020*	0.861
HCV-RNA (IU/ml)	332415.676 ± 667301.95	375029.192 ± 687581.945	220578.33 ± 386001.812	F = 0.385	0.682	0.962	0.790	0.669
ALT (U/L)	42.205 ± 21.514	46.424 ± 26.474	44.318 ± 24.732	F = 0.274	0.761	0.740	0.942	0.946
AST(U/L)	75.872 ± 45.650	72.273 ± 38.819	65.091 ± 36.025	F = 0.481	0.619	0.928	0.591	0.802
AST/ALT ratio	1.820 ± 0.685	1.790 ± 0.711	1.518 ± 0.396	F = 1.731	0.183	0.978	0.185	0.275
Albumin (g/dL)	2.808 ± 0.581	3.100 ± 0.501	2.682 ± 0.518	F = 4.485	0.014*	0.067	0.659	0.017*
Bilirubin total (mg/dL)	3.976 ± 1.789	2.524 ± 1.670	5.513 ± 1.125	F = 3.589	0.050*	0.216	0.369	0.049*
Bilirubin direct (mg/dL)	2.451 ± 1.507	1.450 ± 1.063	2.657 ± 0.318	F = 1.581	0.235	0.273	0.967	0.374
Bilirubin indirect (mg/dL)	1.525 ± 0.985	1.089 ± 0.796	2.857 ± 0.931	F = 3.938	0.039*	0.607	0.098	0.032*
Hb (g/dL)	12.445 ± 2.284	12.313 ± 1.671	11.500 ± 1.114	F = 0.357	0.704	0.988	0.687	0.776
WBCs (cmm)	6.764 ± 3.079	4.455 ± 1.617	4.383 ± 1.237	F = 2.655	0.095	0.127	0.237	0.999
AFP (ng/mL)	15.415 ± 14.843	38.370 ± 81.416	16.271 ± 18.005	F = 2.076	0.132	0.158	0.998	0.237

Where; M= male, F= female, ALT= alanine amino- transferase, AST = aspartate amino- transferase, Hb = haemoglobin, WBCs = white blood cells count, AFP= Alpha feto protein (Reference normal value is up to 10.9 ng/ml) , n = number, SE= statistical expression, X^2 = Chi-square test , F = ANOVA test, and * = significant value.

DISCUSSION

Recent findings refer that the isolated anti-HBc pattern is a serologic profile, which mostly indicates past exposure regarding HBV with lacking the development of HBs-Ab immunity (Moretto *et al.,* 2020). Some studies referred to the association of incidence of such serologic profile with those patients who were particularly exposed to HCV infection. Also, some studies demonstrated an association of such serologic profile pattern with severe liver disease complications, while others did not. In fact, meta-analyses studies have referred to an association between isolated HBc serologic profile pattern with severe liver disease and liver cancer in cases of occult HBV infection (Chang *et al.,* 2018). In the current study, the significant increase ($p = <0.001$) of the incidence of HBc-Ab in HCV- infected patients was recorded with a percentage of 54.84 %. These results differ from Esmat *et al.,* (2015), who reported the percentage of HBc-Ab incidence as 27.3 % in their study on a cohort of HCV infected Egyptian patients to detect occult HBV infection. The increased incidence of HBc-Ab could be explained as during the course of HBV infection, the virus establishes itself by a way of dynamic processes involving viral and hosts immune responses. Once the interaction between HBs-Ag and hepatocyte surface proteins takes place, HBV relaxed circular DNA (rcDNA) is transformed into covalently closed circular DNA (cccDNA). The latter can serve as a transcriptional template for the production of viral mRNA and pre-genomic RNA (pgRNA). This can mature into nucleocapsid with viral polymerase, and then it aids for viral propagation outside hepatocytes as virions, or can return to the nucleus as cccDNA (Blondot *et al.,* 2016). The persistence of cccDNA is the chief reason why the cure of HBV remains a very difficult issue (Bowden *et al.,* 2015), as it can provide a transcriptional reservoir for continual replication of the virus (Zoulim, 2005). Also, the presence of cccDNA leads to the production of the core antigen (HBc-Ag) to which the antibody is formed in the form of HBc-Ab. This explains why HBc–Ab is present in all cases of HBV infection involving acute, chronic, and resolved infection (Chang *et al.,* 2018).

The results of the current study refer to a significant increase ($p= 0.009$) of the incidence of isolated HBc-Ab in HCV-infected patients. The percentage of incidence of isolated HBc-Ab serologic profile pattern was detected as 22.58 % in such patients. These results agree with Moretto *et al*., (2020) who declared that the increased prevalence of isolated HBc-Ab is associated with HCV infection (Moretto *et al.,* 2020). Also, these results are slightly agreed with Bhattacharya *et al.,* (2016), who reported the percentage of incidence of isolated HBc-Ab serologic profile pattern as 31 % in HCV-patients co-infected with HIV, they concluded that the higher incidence of isolated HBc-Ab was associated with HCV rather than HIV infection. Our results differ from the results of Hudu *et al.,* (2013), who reported the percentage of incidence of isolated HBc-Ab serologic profile pattern as 5 % among HBV vaccinated cohort free from HCV infection in Malaysia. Also, current results come in accordance with the previous report of Wedemeyer *et al.,* (2004), who reported a significant increase ($p < 0.001$) of incidence of isolated HBc-Ab serologic profile pattern in HCV-Ab positive patients with a percentage of incidence as 22 %, compared to those HCV-Ab negative subjects with a percentage of incidence as 13 %. They concluded that the association of incidence of isolated HBc-Ab serologic profile pattern was achieved by increased HCV replication. The significant increase of isolated HBc-Ab serologic profile pattern among HCV-infected patients may be explained due to mechanisms underlying increased HCV replication in patients with such profile pattern. These mechanisms include immune response cross-reactivity, as the development of HBs-Ab and partial resolution of HBV may account for stronger immune responses to both viruses, which subsequently can allow suppression of HCV. Contrastly, lacking or subsequent loss of HBs-

Ab may allow for the increase in HCV activity and viremia (Wu *et al.,* 2017).

In this study, HBV-DNA was present in the blood of only one patient, form a total of 124 HCV-infected patients. This patient was characterized by positivity for both HBs-Ag and HBc-Ab and this case was interpreted as active HBV infection. So, the incidence of HBV-DNA was 0.66 % among all HCV-infected patients who showed positivity for HBc-Ab, and it was recorded as 0 % for all HCV-infected patients with the presence of isolated HBc-Ab serologic profile patten. These results agree with Esmat *et al.,* (2015), who reported 0 % of HBV-DNA in the entire cohort of HCV- infected patients with positive HBc-Ab. This can be explained by previous studies, which proved that HCV can suppress HBV replication in HBV/ HCV co-infection, as concluded from in vivo study in mouse models showed that core protein of HCV could exert inhibition of HBV replication (Zhu *et al.,* 2012; Chang *et al.,* 2018). Furthermore, other in vitro studies revealed that the core protein of HCV could suppress HBV replication (Schutller *et al.,* 2002; Esmat *et al.,* 2015). Also, HCV could limit HBs-Ag expression resulting in a declining level of HBV viremia and enhancing the clearance of HBs-Ag (Schutller *et al.,* 2002). Additionally, both HBV and HCV may exert varying hegemony during the course of infection regarding each virus (Konstantinou and Deutsch, 2015; Chang *et al.,* 2018).

Clearly, occult HBV infection, which is indicated by the presence of HBV viremia in absence of HBs-Ag among HCV-infected patients with positive HBc-Ab, cannot be detected in our study cohort by using conventional real-time PCR method to detect HBV-DNA from EDTA blood samples. These results agreed with the results obtained by Esmat *et al.,* (2015), from their study to detect occult HBV in chronic HCV infected cohort. They reported 0 % of HBV-DNA by conventional real-time PCR using blood samples and hence 0 % of occult HBV depending on such method. Contrastly, the results in the current study differs from the results obtained from the previously mentioned study by Esmat *et al.,* (2015), when they used Orecin stain test for the detection of HBV particles in liver biopsy in the same cohort of the study. Finally, they reported the percentage of occult HBV infection as 13.48% in HCV- infected patients with positive HBc-Ab and 3.7 % among total studied patients (Esmat *et al.,* 2015).

Regarding the clinical implications of the incidence of isolated HBc-Ab serologic profile pattern among HCV-infected patients, a significant increase ($p = 0.020$) in age was reported, while non-significant changes of the parameters of clinical implications either biochemical or haematological were recorded, depending on the comparison between the HCV- patients with isolated HBc-Ab serologic profile pattern (G5), and other HCV- patients who were susceptible to HBV infection (G1). Also, a non-significant change was reported for tumour marker AFP indicating a non-significant role of incidence of isolated HBc-Ab serologic profile pattern in the elevation of primary HCC marker. Moreover, a significant decrease ($p = 0.032$) of albumin and a significant increase ($p = 0.049$) in total bilirubin was recorded to be associated with the incidence of isolated HBc-Ab serologic profile pattern, when G5 group of patients compared to G2 group of patients who resolved HBV infection with the presence of both HBs-Ab and HBc-Ab serological markers. This can be explained by the increased activity of HCV due to loss in the development of HBs-Ab and the decrease in immune tolerance (Wu *et al.,* 2017).

Conclusion

Incidence of isolated HBc-Ab serologic profile pattern has a significant increase in chronic HCV- patients especially for those with significantly higher ages. It does not contribute to the increase in the risk of the elevation in primary HCC marker in those patients. However, it is associated with the increase in hypoalbuminemia and hyper-bilirubinemia, which reflects liver disease deterioration in those HCV-patients with isolated HBc-Ab serologic profile pattern, who lack HBs-Ab development, compared to other HCV-patients who is characterized by

the presence of HBc-Ab and can develop HBs-Ab in resolving HBV infection.

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