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Serum HBe Ag and HBV DNA Markers among Different Stages of Chronic Hepatitis B Patients in Sana'a City, Yemen

Al-Ofairi, B. A*[1] , Humaid, A. A[2], Al-Huthaifi, A.M[3]
[1, 2, 3] Biology department - Microbiology , Faculty of Science , Sana'a University, Yemen
*Corresponding Author: Bashir Ahmed Al-Ofairi, Biology department- Microbiology, Faculty of Science, Sana'a University, Yemen

Abstract:- The infection with chronic hepatitis B (CHB) continues one of the major problem worldwide , which lead to the progressive liver sequelae and the risk of liver failure (LF). So, our study were performed to detect the serum HBe Ag and HBV DNA markers among different stages of CHB patient and their association with biochemical tests levels and predisposing risk factors. A total of one hundred –eight (108) serum samples were collected from CHB patients whom do not treated with any antiviral drugs and were analyzed: Biochemical tests (ALT & AST) , Virological markers (HBs Ag , HBe Ag, anti-HBc IgG) and HBV DNA viral load . An average of CHB patients ages was [(mean ± SD: 38.21±14.16)] years , there were 82 (75.9%) males and 26 (24.1%) were females and all CHB patients were positive for HBs Ag and anti-HBc IgG . Our results showed there were 17(15.7%) of patients were HBe Ag positive and 91(84.3%) were HBeAg negative, HBeAg positive patients with abnormal ALT were 10 (9.3%) , while HBeAg positive patients with abnormal AST were 8 (7.4%). Also, our findings showed there an association were (P < 0.05) between ALT and AST with HBV DNA levels : Patient with high HBV DNA levels with abnormal ALT were 18 (16.7%) , while patients with abnormal AST were 14 (13 %), but, the patients with low HBV DNA levels have abnormal ALT and AST [56 (51.8%) ,8 (7.4 %), respectively]. Interestingly, our data showed that CHB patients with high HBV DNA serum levels and HBeAg positive were 17 (15.7%),while, patients with high HBV DNA levels and HBeAg negative were 19 (17.6%) , but, patients with low HBV DNA serum levels and HBeAg negative 72(66.7%). Finally, our findings revealed that the clinical different stages of CHB patients included : the inactive CHB carrier were 72 (66.7%), the active CHB patients were 19 (17.6%) , the immune tolerant CHB were 11(10.2%), the immune reactive CHB patients were 6 (5.5%) and occult CHB patients were 0 (0%).

Keywords:- *Chronic hepatitis B, HBe Ag , HBV DNA , CHB stages, Yemen.*

I. INTRODUCTION

According to the medical reports from world health organization (WHO) , which were indicated that 2 billion individuals with hepatitis B virus (HBV) infection , 350-400 million were hepatitis surface antigen (HBsAg) positive more than six month and one million deaths annually from chronic hepatitis B (CHB), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) **(Han *et al.*, 2011; Tan , 2011; Liu *et al.*, 2012 ; Bertoletti and Bert , 2018).**

The HBV infection were classified as : acute , fulminant hepatitis and different stages of CHB infection : the inactive carrier state, chronic active hepatitis, LC and HCC, also, the diagnosis of HBV is not only imperative but also complex, because different viral antigen which bring out with varying serological profiles in different clinical stages of CHB infections , therefore, serological markers: Hepatitis B e antigen (HBe Ag) and HBV DNA serum levels are a prognostic indicators for assessing the severity of HBV infection (**Rodring *et al.*,2001; MacMahon, 2004; Gunal *et al.*, 2014 ; Kirisci *et al.*, 2016**).

Clinically, HBsAg and HBeAg are very important to determine CHB infection stages, HBsAg is a hall mark for CHB infection when positive for at least 6 months, HBeAg usually serves as a marker of active viral replication, in addition, biochemical tests : ALT , AST levels are checked repeatedly to determine the liver cell necrosis and viral activity ,but, HBV DNA levels were performed for understanding the pathogenesis and natural history of CHB infection (**Badur and Akgun, 2001**; **Kao *et al.*, 2010** ; **Nguyen *et al.*, 2010; Tseng and Kao , 2013;Wong *et al.*, 2016).**

CHB infection is one of the universal health problem, 75% of patients were restricted in Asia . Different stages of CHB infections have different clinical features and disease complications, the interpretation of active CHB infection according to the present of HBeAg and their correlated with HBV DNA serum levels for detection the severity of HBV infection **(Hoofnagle *et al.*, 1981; Lavanchy , 2004 ; Liaw and Chu ; 2009 , Keshvari *et al.*, 2015).**

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Basically, in most laboratory the detection of HBV DNA serum levels is limited , due to its high cost, but, HBeAg and ALT is a simple and available , therefore, when polymerase chain reaction (PCR) is not available for HBV DNA, HBeAg and ALT serum levels were determined HBV infection stages ,but , the detection of serum HBV DNA for confirmed the natural history of CHB infectivity **(Funk *et al.*,2002 ; Nguyen *et al.*, 2010 ; Gunal *et al.*, 2014 ; Wong *et al.*, 2016).**

HBeAg is used for detection the active phase of CHB infection, spontaneous seroconversion of HBe Ag to HBe antibody is an important for immune clearance, the most of patients become inactive chronic HBs Ag carriers after HBe Ag seroconversion with disappearance of HBV DNA and normal ALT levels in serum , but, some patients with HBe Ag negative have high HBV DNA serum levels , it may be due to immune escape mutations and can be detected by HBV genotype testing **(Realdi *et al.*, 1980 ; Andres *et al.*, 1981 ; Funk *et al.*, 2002 ; Nguyen *et al.*, 2010 ; Terrault *et al.*, 2016).**

The relationship between ALT and AST serum levels with histological stages were used to predict the clinical consequence of CHB infection , patients with HBeAg positive and normalized of ALT should be checked once every 3–6 months and HBeAg every 6–12 months, whereas , patients with HBeAg negative and persistently normal ALT levels, HBV DNA serum levels can be checked once every 6–12 months, liver biopsy is recommended in HBV DNA serum levels (≥20 000 IU/mL) and HBe Ag positive , also in patients with HBV DNA serum levels(≥2000 IU/mL) and HBeAg-negative, whom ages over 40 years **(Aygun *et al.*, 2010 ; Kirisci *et al.*, 2016).**

Therefore , serum ALT, HBsAg , HBeAg and HBV DNA are the most tests used in CHB patients ,which correlates with hepatic necro-inflammation and allowed us for understanding the natural history and disease progression during different clinical stages of CHB infection . Importantly , more previous studies depend on the HBeAg status were classified the clinical stages of CHB infection from two to three phases in the mid1980s according to detect chronic immune tolerance patients and to four stages in the early 1990s with the diagnosis of "HBeAg negative patients (**Tsai *et al.*, 1992 ; Huo *et al.*, 1998 ; Hsu *et al.*, 2002 ; Chu *et al.*, 2004 ; Nguyen *et al.*, 2010 ; EASL, 2012 ; Park *et al.*, 2016 ; Wong *et al.*, 2016 , Li et al., 2019).**

Clinically , in CHB patients the first stage is immune tolerance which diagnosis serology by the presence of HBeAg and high levels of HBV DNA with normalized of serum ALT and liver biopsy, the second stage is active CHB immune clearance which confirmed by the presence of HBeAg , high or fluctuating HBV DNA levels and persistent or intermittent increase of serum ALT and these patients have active inflammation depend on liver biopsy examination , the third phase is inactive CHB carriers which have HBeAg or HBe antibody with normalized of ALT levels , low or absent of serum HBV DNA and mild inflammation according to liver biopsy and the fourth stage is immune reactive CHB patients identified by absent of HBeAg , presence HBe antibody and HBV DNA with , elevation of ALT levels and the liver biopsy showed continued inflammation . Recently ,the fifth stage is occult CHB patients detected by absence of serum HBsAg at 0.5% to 1% of patients per year with absence of serum HBV DNA, normalized of serum ALT and a worse inflammation according to liver biopsy **(Lok *et al.*, 1987 ; Hadziyannis and Vassilopoulos , 2001 , Chu *et al.*, 2002, Chen *et al.*, 2002 , Manno *et al.*, 2004 ;Yim and Lok ; 2005 , Liaw *et al.*, 2012 ; Terrault *et al.*, 2016 ; Li *et al.*, 2017 ; Jia *et al.*, 2019).**

II. AIMS OF THE STUDY

The current study were designed to : (1) Detect the HBe Ag and HBV DNA markers levels of CHB patients in Sana'a city -Yemen , (2) Determine the clinical significance association between HBe Ag and HBV DNA levels with biochemical parameters (ALT , AST) levels and (3) Explore the relationship between predisposing risk factors (age, sex, duration of infection and family history) with different stages of CHB infection.

III. MATERIALS AND METHODS

The present cross-sectional study had been performed by collection of one hundred–eight (108) blood samples from patients with CHB infections : 82 (75.9%) were males and 26 (24.1%) were females, which attending to the gastroenterology and hepatology clinics at University of Science and Technology Hospital in Sana'a city-Yemen between September 2017 to December 2018. All CHB patients were confirmed by a positive of HBs Ag and Anti-HBc IgG in theirs serum for more than six months and we excluded patients with : Anti-viral treatment, co-infected with hepatitis C virus (HCV) or co-infected with hepatitis D virus (HDV) or co-infected with human immunodeficiency virus (HIV) , with LC , with HCC and with autoimmune hepatitis (AIH). While, the serum ALT and AST levels , HBs Ag , HBe Ag and Anti-HBc IgG of CHB patients were performed according commercial kits and under manufacturer's instructions.

Finally, the quantitative assessment of HBV DNA levels were detected by real-time polymerase chain reaction (rtPCR) , the levels were appeared as : high ≥ 2000 IU/ml and low < 2000 IU/ml (1IU/ml≈5.82 copies/ml). Finally, the data were analyzed using a statistical package of social science program (SPSS) version 21 and the results were presented as percentages, mean, stander division (SD) , Chi-square ,which used for the detection the different between variables, *P-value* < 0.05 were considered statistically significant.

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IV. RESULTS

One hundred-eight (108) of CHB patients were enrolled in the present study ,which were summarized in **Table. 1**, as : The patients ages , the gender and other demographic, clinical characteristics of the CHB patients .

Characteristics	Number (%)
Age groups (Years)	
<30	35 (32.4)
30-39	29 (26.9)
40-49	16 (14.8)
≥50	28 (25.9)
Mean +SD : (38.21± 14.16)	
Gender	
Male	82 (75.9)*****
Female	26 (24.1)
Family history	
Yes	6 (5.6)
No	56 (51.9) *****
Unknown	46 (42.5)
Duration of CHB Infections	
<2	78(72.2) *****
≥2	30(27.8)
Biochemical markers levels	
ALT	
Normal	74 (68.5)*
Abnormal	34 (31.5)
Mean ± SD (41.78 ± 46.77) IU/L	
AST	
Normal	86 (79.6)*
Abnormal	22 (20.4)
Mean ± SD (35 ± 43.51) IU/L	
HBe Ag Status	
Positive	17 (15.7)
Negative	91 (84.3)*
HBV DNA Levels	
High ≥ 2000	36 (33.3)
Low < 2000	72 (66.7)*
Mean ± SD (13037710.44 ± 39003202.39) IU/ml	

P<0.05

Table 1:- The baseline demographic and clinical characteristics of CHB patients.

Our results showed that all CHB patients were HBsAg and Anti-HBc IgG positive , there were 17(15.7%) of patients were HBeAg positive and 91(84.3%) were HBeAg negative, there were no significant difference in all ages groups and gender ($p > 0.05$), there were a high statistically significant association between ALT and AST with HBeAg ($p < 0.05$). HBeAg positive patients with abnormal ALT were 10 (9.3%) , while abnormal AST patients were 8 (7.4%) , the number of HBe Ag negative patients with abnormal ALT were 24 (22.2%) and the number of these patients with abnormal AST were 14 (13%), as showing in **Table.2.**

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Parameters	HBe Ag Positive	HBe Ag Negative
Age (Mean ± SD)	30.53 ± 15.24	39.56 ± 13.57*
Gender N (%)		
Male	14 (13)	68 (63)*
Female	3 (2.8)	23 (21.3)*
Biochemical markers levels N(%)		
ALT		
Normal	7 (6.5)	67 (62)*
Abnormal	10 (9.3)	24 (22.2)*
Mean + SD	57.52 ± 36.89*	38.84 ± 47.99
AST		
Normal	9 (8.3)	77 (71.3)*
Abnormal	8 (7.4)	14 (13)*
Mean ± SD	53.94 ± 58.65*	31.5 ± 39.5

P<0.05

Table 2:- Correlation of HBe Ag serum levels with demographic and clinical parameters.

Our findings showed that there were no a statistical relationship between HBV DNA levels and ages groups or gender (P > 0.05), there were a high statistically significant (P < 0.05) association between ALT, AST and HBV DNA levels . High HBV DNA levels patients with abnormal ALT were 18 (16.7%) , while high HBV DNA levels patients with abnormal AST were 14 (13%), the number of low HBV DNA levels patients with abnormal ALT were 56 (51.8%), whereas the patients with abnormal AST were 8 (7.4%), as showing in **Table.3.**

Parameters	High HBV DNA Levels	Low HBV DNA Levels
Age		
Mean + SD	37.35 ± 16 .8*	38.36 ±12.76
Gender N (%)		
Male	30 (27.8)	52 (48.1)*
Female	6 (5.6)	20 (18.5)*
Biochemical markers levels N(%)		
ALT		
Normal	18 (16.7)	16 (14.8)
Abnormal	18 (16.7)	56 (51.8)*
Mean ± SD	59.06 ± 68.72*	33.15 ± 27.24
AST		
Normal	22 (20.4)	77 (71.3)
Abnormal	14 (13)	8 (7.4)
Mean ± SD	54.19 ± 70.12*	25.53 ±12.34

P < 0.05

Table 3:- Correlation of serum HBV DNA levels with demographic and clinical parameters.

Our findings showed that there were a high statistically significant (P < 0.05) relationship between HBeAg and HBV DNA levels. CHB patients with high serum HBV DNA levels and HBe Ag positive were 17 (15.7%) ,while high HBV DNA levels and HBeAg negative patients were 19 (17.6%), there were no HBeAg positive patients with low HBV DNA levels, while the number of HBe Ag negative patients with low HBV DNA levels were 72 (66.7%), as showing in **Table.4.**

HBe Ag status	High HBV DNA levels	Low HBV DNA levels
Positive N (%)	17 (15.7%)*	0 (0%)
Negative N (%)	19 (17.6%)	72 (66.7%)*
Mean ± SD	65063637.7 ± 59327525* (IU/ml)	33185812.17 ± 24001805.41(IU/ml)

P<0.05

Table 4:- The correlation between serum HBV DNA viral load and HBe Ag status in CHB patients.

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Importantly , our data of the different stages of CHB patients were: The inactive CHB carrier were 72 (66.7%), the active CHB were 19 (17.6%) , immune tolerance CHB were 11 (10.2%) , immune reactive CHB were 6 (5.5%) and occult CHB were 0 (0%), as showing in **Figure.1**

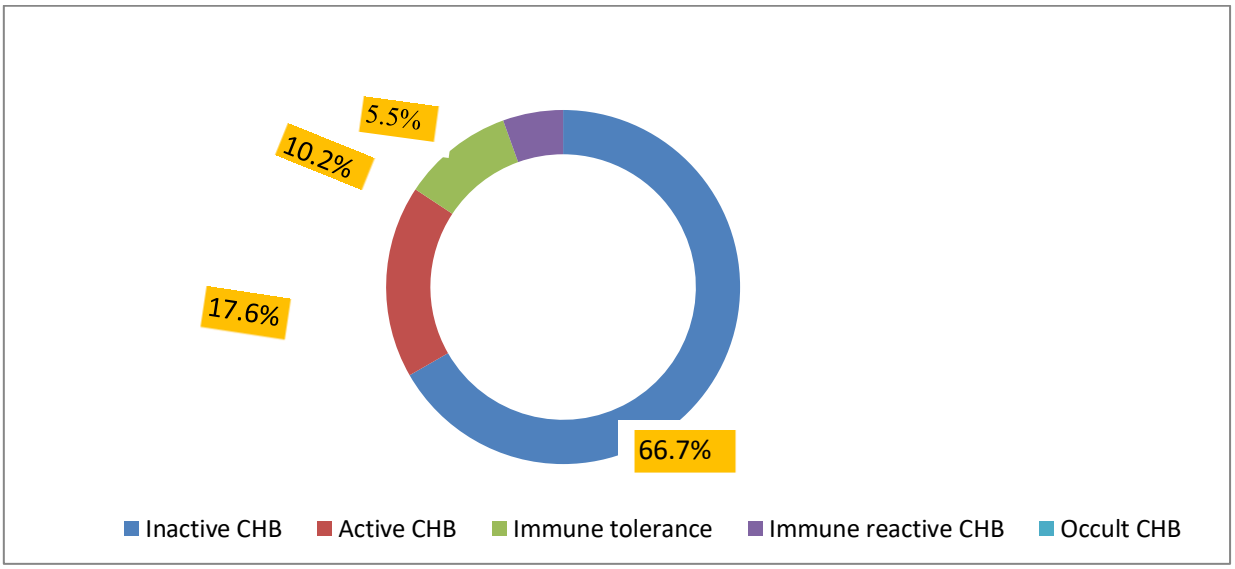

Fig 1:- Distribution of the different stages in CHB patients.

Finally, our findings showed that the most of CHB patients were active CHB stage , the most active CHB patients were males , the ALT and AST serum levels were significance ($P < 0.05$) increased in active CHB patients ,the HBe Ag were positive in immune tolerance and immune reactive CHB patients and the high levels of HBV DNA were found in active CHB and immune tolerance ,while low levels of HBV DNA found in inactive CHB and immune reactive CHB patients, as showing in **Table .5.**

CHB stages / Demographic and Clinical characters	**Inactive CHB**	**Active CHB**	**Immune tolerance CHB**	**Immune reactive CHB**	**Occult CHB**
Age Mean ± SD	35 ± 13.4	39 ± 15.7 *	29 ±12.9	24 ± 14.5	-
Gender N (%) Male Female	52 (48.15) * 20 (18.52)	16 (14.82) 3 (2.77)	9 (8.33) 2 (1.85)	5 (4.63) 1 (0.93)	-
ALT IU/L Mean ± SD	35 ± 17.4	152 ± 45.3*	38 ± 12.8	89 ± 32.6*	-
AST IU/L Mean ± SD	23 ± 10.8	186 ± 17.4*	42 ± 13.9	179± 11.8*	-
HBe Ag	Negative	Negative	Positive	Positive	-
HBV DNA* log10 IU/ml Mean ± SD	2.04 ± 0.66 HBV DNA<2000	6.87 ± 3.31 HBV DNA ≥2000	8.34 ± 7.23* HBV DNA >10^7	4.4± 3.39 HBV DNA ≥2000	-

P<0.05

Table 5:- The correlation between CHB patients stages with demographic and clinical characters.

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V. DISCUSSION

The clinical stages of CHB infection varying from an asymptomatic chronic carrier state with normal liver function tests to symptomatic chronic progressive state with high liver function tests and there were more epidemiological studies support a close relationship between chronic persistent HBV infection with chronic active hepatitis, LC and HCC. Also, Male patients become chronic carriers of HBV infection than women **(Blumberg *et al.*, 1972 ; Tsai *et al.*, 1990 ; Chan *et al.*,1999 ; Tsai *et al.*, 2000 ; Spradling *et al.*, 2016).**

Our results showed there were no a significant differences between ages groups of CHB patients , there were a significant differences between gender (males have CHB infection more than females) and there were no family history between these patients as showing in **Table. 1.** This results agreement with others whom found that the most of CHB patients were in the middle of their lives (adults) and agreement with others which illustrated that males may be exposed to many risk factors than females **(Yalcin *et al.*, 2003 ; Kaya *et al.*, 2006 ; Tsai *et al.*, 2008 ; Hong *et al.*, 2011 ; Demir *et al.*, 2014 ; Tulin *et al.*, 2014).**

Also, our result showed that the levels of ALT were abnormal and AST normal between more of CHB patients , which agreement with other previous reports whom indicated that the levels of ALT were consistently higher than AST with CHB infections (**Bonacini *et al.*, 1997** ; **Myers *et al.*, 2003)** and these result were disagreement with others who found that less of CHB patient with abnormal ALT and AST levels (**Olut *et al.*, 2007** ; **Kirisci *et al.*, 2016**).

In addition, our finding also showed that the most CHB patients in the duration were <2 years , the most of these patients were HBe Ag negative and low levels of HBV DNA , which agreements with many previous studies whom showed that CHB patients may present or absent HBe Ag and its correlated with HBV infectivity and HBeAg positive patients were considered at risk for cirrhosis and HCC **(Yang *et al.*, 2002 ; Liaw and Chu , 2009 ; EASL , 2012 ; Li *et al.*, 2019).**

Also, our data about HBV DNA levels were agreement with others researchers who reported that the reduction of HBV DNA levels in most HBV carriers confirms the importance of natural immunological responses against HBV infections ,which induced the reduction of HBV DNA levels that consider a very good indicator for suppression of HBV infectivity **(Lapinski *et al.*, 2006 ; Issa *et al.*, 2012 ; Li *et al.*, 2019).**

Our results showed that the most of CHB patients were HBeAg negative, there were a significant difference according to ages groups and genders ($p < 0.05$), the ALT levels were a significant higher in HBeAg positive patients ,while AST levels were higher in HBe Ag negative patients as showing in **Table.2.** This results agreement with many previous studies , which suggested that HBV DNA and ALT levels with HBe Ag are very important to determine HBV infection setting and prognosis of viral replication **(Hasan *et al.*, 2002 ; Rabbi *et al.*, 2008 ; Jia *et al.*, 2019) .** Also, other studies showed that the most of HBe Ag positive patients with increased levels of ALT had high serum HBV DNA levels , whereas , few of HBe Ag positive patients with normal ALT had low serum levels of HBV DNA , which, indicated that HBeAg , HBV DNA and ALT levels were explored CHB infection activity and the host defense reaction reactivity **(Chen *et al.*, 2013 ; Bertoletti and Bert , 2018) .**

Our findings showed that there were a statistical relationship between patients with high HBV DNA levels and ages groups ,whereas the most of our patients (males & females) with low HBV DNA levels and the levels of ALT and AST were significantly increased in patients with High HBV DNA levels as showing in **Table.3**. This findings were agreement with others authors who indicated that high ALT levels are thought to be associated with CHB and there were useful with HBe Ag for discrimination of active from inactive CHB patients **,** also, others reported that the high ALT levels in these patients with high HBV DNA levels can classify CHB patient into the inactive and active carrier states **(Dufour *et al.*, 2000 ; Green and Flamm , 2002 ; Prati *et al.*, 2002 ; Keefee *et al.*, 2008 ; Beniashvili *et al.*, 2009 ; Ijaz *et al.*, 2011; Jia *et al.*, 2019).**

Our findings showed that there were a statistically significant ($P < 0.05$) relationship between HBeAg and HBV DNA levels , HBeAg positive patients with high HBV DNA viral load ,while HBeAg negative patients with low HBV DNA levels as showing in **Table.4.** This results were agreement with other researchers who illustrated that the detection of HBV DNA levels in the majority of CHB patients were confirmed the pathogenesis , natural history and monitoring the responsiveness to treatment in these patients , also, previous studies found that serum HBV DNA levels were increased among patients with HBeAg positive and decreased among patients with HBe-antibody and elevated of ALT **(Loeb *et al.*, 2000 ; Li *et al.*, 2017).**

Our data showed that the most of CHB patients were the inactive CHB carrier , followed by the active CHB, immune tolerance CHB and immune reactive CHB .But, there were no occult CHB patients as showing in **Figure.1.**This findings were agreement with others who illustrated that CHB infection have four different stages : Inactive CHB carriers , the immune tolerant patients , the active CHB patients and immune reactivation CHB patients , Also, many previous had found that both HBsAg and HBV DNA levels were increased during the immunotolerance stage and lowest in the inactive carriers Also, others illustrated that serum HBeAg , HBV DNA ALT and AST, levels are the main serologic markers, which changed during different stages of the CHB patient **(Niitsuma *et al.*,1997 ; Gerken *et al.*, 1998 ; Fujiwara *et al.*, 1998 ; Lindh *et al.*, 2000 ; Noborg *et al.*, 2000 ; Kesseler *et al.*, 2000 ; Lok and McMahon, 2001 ; Sablon and Shapiro, 2005 ; EASL , 2009 ; Li *et al.*, 2017) . Chu**

and Liaw, 2004 ; Sablon and Shapiro, 2005 ; Mustafa *et al.*, 2014 ; **Keshvari *et al.*, 2015).**

Interestingly , our findings showed that the most active CHB patients were males , the levels of ALT and AST were significance ($P < 0.05$) increased in active CHB patients ,the HBe Ag were positive in immune tolerance and immune reactive CHB patients and the high viral load of HBV DNA were found in active CHB and immune tolerance ,while low viral load of HBV DNA in inactive CHB and immune reactive CHB patients as showing in **Table .5.** These results agreement with others that were divided CHB different stages of CHB patients into: Immune tolerance patients , active CHB, inactive carrier state in about 20-30% of inactive carriers cover to the immune reactivation CHB patients , also , many authors indicated in the natural history of HBsAg negative patients "occult infection", the HBV DNA viral levels is very important , because it is responsible for genome organization, replication and effects to the host immune response in these patients **(Chen , 1993 ; Green and Flamm , 2002 ; Prati *et al.*, 2002; Conjeevaram and Lok, 2003 ; Chu and Liaw, 2004 ; Yim and Lok, 2006 ; Geller and Petrovic, 2009 ; Liaw and Chu , 2009 ; Liaw et al., 2010 ; Chu and Liaw , 2010 ; Chen *et al.*, 2010 ; Chan *et al.*, 2010 ; Jaroszewicz *et al.*, 2010; Nguyen *et al.*, 2010 ; Brunetto *et al.*, 2010 ; Martinot *et al.*, 2010 ; Bihla *et al.*, 2010 ; Bushra *et al.*, 2011, Tseng *et al.*, 2012 ;Squadrito *et al.*, 2014 ; Koyuncuer, 2014 ; Li *et al.*, 2017 ; Jia *et al.*, 2019).**

VI. CONCLUSION

From our pervious comprehensive study we concluded that the most of CHB patients were inactive CHB carrier , which indicated that the low viral infectivity in these patients , the most of HBeAg positive patients with high HBV DNA levels ,while the most of HBeAg negative patients with low HBV DNA levels and there were a logic correlations between serum HBV DNA levels, HBeAg status with ALT and AST, those main markers were determined the natural history and pathogenesis of CHB infections. Finally, our study indicated that the serum HBV DNA levels is an important markers to confirm the clinical stages of CHB patients.

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➢ *Conflict of Interests*

There is no conflict of interests regarding the publication of this paper.

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