

The Prevalence of Hepatitis B Virus in High Risk Groups in Nineveh Governorate / Iraq

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Abstract:

Hepatitis B is an inflammation of the liver that caused by Hepatitis B virus (HBV) which is DNA virus that infects the human and some kinds of animals such as chimpanzees and birds. This disease considered as the major disease of mankind and a serious global public health problem. HBsAg, HBeAg, HBcAb, HBeAb and HBsAb are markers used to detect the presence and the stage of infection. The current study included (181) individuals from both sexes, (137) males and (44) females. By ratio 3.11: 1. The mean age of patients 2.4033 ± 0.83519 (range 18-73) years as follows < 20 (11.6%), 21–40 (47.5%), 41–60 (29.8%) and > 60 (11.0%) . These patients are 73 (40.4%) Blood donors from Central Blood Bank, 88 (48.6%) Chronic kidney failure at Ibn – Sina Teaching Hospital and 20 (11.0%) Thalassemic patients at Ibn – Alatheer Teaching Hospital, Nineveh Governorate / Iraq. For the period from July 2011 till May 2012. The results indicated that the number of serum patients infected with HBV was 90 (49.7%) using Enzyme Linked Immuno Sorbent Assay technique. These patients had many markers named HBsAg, HBeAg, HBcAb, HBeAb and HBsAb with percentages 90 (49.7%), 47 (26.0%), 89 (49.2%), 53 (29.3%) and 5 (2.8%) respectively. Ninety three patients infected with HBV ninety of them gave positive results using ELISA and rt-PCR technique, and three gave positive results using rt-PCR only inspite of their negative results in ELISA. We concluded that HBV infection remains a serious issue because it's prevalence is still significant among patients, all viral markers are very important for the diagnosis of infection, rt-PCR is a very sensitive scientific technique gave the exactly number of copies/ml in a closed system.

Key words: Hepatitis B, Liver function tests, Hepatitis B Virus markers, real time – PCR

Introduction:

Hepatitis B is a viral disease caused by Hepatitis B Virus (HBV). This disease considered as the major of disease and a serious global public health problem. HBV is endemic throughout the world and shed in blood and all body fluids by individuals with acute or chronic infections [1]. It's infection may lead to cirrhosis, hepatocellular carcinoma or death. HBV is DNA virus that infects the human and some kinds of animals such as chimpanzees and birds. More

important, HBV infection is a global health problem. Two billion people have been infected worldwide; 360 million are chronically infected, over 520,000 die each year (50,000 of acute hepatitis B and 470,000 of cirrhosis or develop to hepatocellular carcinoma [2]. There are serological markers Hepatitis B surfaces antigen (HBsAg), Hepatitis B e antigen (HBeAg), Hepatitis B patients also contain circulating antibodies against Hepatitis B core antigen, Hepatitis B e antigen

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and Hepatitis B surfaces antigen called Hepatitis B core antibody (HBcAb), Hepatitis B e antibody (HBeAb) and Hepatitis B surfaces antibody (HBsAb). Hepatitis B core antigen (HBcAg) was found only in hepatocytes and not found in blood stream. There are sequential appearance and disappearance of these five serological markers during atypical course of infection [3,4,5]. The first stage of infection is characterized by the presence of HBsAg, HBeAg and IgM class of HBcAb and may last for decades in the intermediate stage. Patients lose HBeAg and develop to HBeAb and often enter in a clinical remission. Finally, the patient lose HBsAg and rises HBsAb which refer to the recovery stage from infection [6]. Polymerase chain reaction (PCR) is a scientific technique in molecular biology used to amplify a single or few copies of DNA across several orders of magnitude for generating thousands to millions copies of a particular DNA sequences [5], but Real Time – PCR is a technique used for the qualitative and quantitative detection of DNA in samples by amplifying and simultaneously quantifying a targeted DNA molecule for one or more specific sequences in DNA sample [7]. We concluded that HBV infection remains a serious issue because its prevalence is still significant among patients, all viral markers are very important for the diagnosis of infection rt-PCR is a very sensitive scientific technique gave the exactly number of copies/ml in a closed system.

Materials and Methods:

A total number of patients was (181) from both sexes, comprising (73) Blood donors from Central Blood Bank, (88) Chronic Kidney failure at Ibn –Sina Teaching Hospital, (20) Thalassemic patients at Ibn-Al atheer Teaching Hospital in Nineveh

Governorate, for the period from July 2011 till May 2012, their ages ranged from (18-73) years with mean of (2.40 ± 0.83) years. Sera were separated by centrifuge (3000 rpm) and stored at -20°C . Sera were tested to detect the presence of HBV using Micro ELISA Screening from (Plasmatec company ,UK). The Absorbance was measured at 450nm by the reader, cut-off value equals 0.100. The positive sample absorbance was greater than or equal to \geq cutoff value but the negative samples absorbance was less than $<$ cutoff value. Sera were also tested to determine the HBV markers HBsAg, HBeAg, HBsAb, HBcAb and HBeAb by One step multi HBV test device consists of 5 chromatographic strips. Each strip detects a certain HBV marker in Immuno chromatography assay using specific kit from (Plasmatec company, UK). Sixty micro liter of serum samples or plasma was added to each well and simultaneously timing starts. The results were recorded after 15 minutes. Finally, sera were tested to determine the Quantitative detection of HBV by rt – PCR technique using specific kit from (Analytik Jena, Germany) for DNA Extraction step and another specific kit from (Primer Design , USA) for amplification step .

Results and Discussion:

Recently, HBV infection has truly become a problem which we still face. In Nineveh city, the number of patients has become noticeably medium. Worth noting is that all cases were detected accidentally during blood transfusion or other undirected tests, noticeably at Oncology unit, Hemodialysis unit, Central blood bank and Public health laboratory in Nineveh governorate. The number of blood donors were 7998. 72(0.90%) of them were infected with HBV and five infected with HCV. A total number of

patients in chronic kidney failure was 130, 17 (13.0%) of them were infected with HBV and 25 (19.2%) infected with HCV. All of them were negative for HIV. Table -1- Showed the number of serum samples which investigated in our study, the results indicated that 90 (49.7%) gave positive and 91(50.3%) gave negative result for HBV using ELISA technique.

Table -1- Percentage of serum samples infected and non infected with HBV using ELISA technique

Serum samples	Infected with HBV(No.%)	Noninfected with HBV (No.%)
Blood donors	72 (39.77)	1 (0.55)
Hemodialysis	17 (9.39)	71 (93.22)
Thalassemia	1 (0.55)	19 (10.42)
Total	90 (49.7)	91(50.3)

This study agree with other studies carried out worldwide such as, McMahan [9] who showed that the prevalence of chronic HBV infection worldwide could be categorized as high, medium and low endemicity. HBV is moderately endemic in parts of eastern and southern Europe, middle east and south America. Between (10-60%) of the population have evidence of infection, and (2-7%) as chronic carriers [8].

Alter [10] who showed that HBV infection prevalence varies markedly in different geographic areas of the world as well as indifferent population subgroups. It ranged over 10% in some Asian and Western Pacific countries to under 0.5% in the United States and Northern European countries. The patients who gave positive results for HBV must be tested to detect the presence of HBV markers to identify and know the stage of HBV infection, these markers are HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. The result of all these markers as illustrated in Table -2 -

Table -2- Percentages of the prevalence of HBV markers using Immuno chromatography assay.

Markers	Positive No. (%)	Negative No. (%)	Non infection No. (%)	Mean± SD
HBsAg	90 (49.7)	5 (2.8)	86 (47.5)	2.87±2.03
HBeAg	47 (26.0)	48 (26.5)	86 (47.5)	2.64±2.28
HBcAb	89 (49.2)	6 (3.3)	86 (47.5)	2.87±2.04
HBeAb	53 (29.3)	42(23.2)	86 (47.5)	2.61±2.31
HBsAb	5(2.8)	90(49.7)	86 (47.5)	2.38±2.50
Total				181 (100.0)

The numbers of patients who gave positive and negative results for HBsAg marker are 90 (49.7%) and 5 (2.8%) respectively, non infection were 86 (47.5%) with the mean 2.87 ± 2.03 who don't have the infection with HBV. The result of this study is similar to other studies conducted in Basrah, Iraq such as Al- Hmudi [11] who showed that 44.52 % had HBsAg marker. Weber *et al.*[12] who showed that HBsAg may turn negative in the early stage of the disease course but in chronic HBV infections patients, this marker can be persistently positive. HBeAg is considered as a better marker of viral blood infection that comes from a core of HBV. When HBV core degrades in the serum, this antigen is created and can be detected, because HBcAg will be totally degraded in the serum. When HBeAg marker becomes positive, it is equivalent to positive HBcAg marker and shows that HBV is replicating actively in hepatocyte and the patient is infectious [13,14].HBeAb is the body's immune reaction to HBeAg and combines with it. It usually appeared after HBeAg turns to negative; which also means that HBV replication activities have decreased and the patient becomes non infectious at all. The development of host Antibodies to HBeAg indicated the assessment of immunity and reduction viral replication in infected individuals [15].The current study indicated that the numbers of patients who gave positive results for HBeAg were 47

(26.0%), negative 48 (26.5%) and non infection 86 (47.5%) with the mean 2.64 ± 2.28 , while for HBeAb were 53(29.3%), negative 42 (23.2%) and non infection 86 (47.5%) with the mean 2.61 ± 2.31 . On comparing this study with other studies done in Iraq , Al Jaaf [16] who showed that the prevalence of HBeAg and HBeAb among chronic group were (36%, 58%) respectively but among the carrier group were (2%, 94%) respectively. Youssif [17] who found that 54.2% had HBeAg marker and 44.58% had HBeAb marker. Other studies done in Iraq showed that a higher prevalence of HBeAg among carrier group, namely Omar and Al – Douri [18] who found that 25% of HBsAg positive female nursery staff were HBeAg positive and 43% positive for HBeAb. HBcAg is not commercially available because it is not found in the serum. Therefore, we cannot find it. But it is available in hepatocyte only. HBcAb is available in serum only and its presence may refer to the replication of HBV in hepatocyte [19]. The current study indicate that the number of patients who gave positive results for HBcAb were 89 (49.2%), negative 6 (3.3%) and non-infection 86 (47.5%) with the mean 2.87 ± 2.04 , and the number of patients who gave positive results for HBsAb were 5 (2.8%), negative 90 (23.2%), non-infection 86 (47.5%) with the mean 2.38 ± 2.50 . This study agree with other studies done in the world such as [20] who showed that HBsAb is an immunoglobulin secreted by Plasma cells that can combine with HBsAg to neutralize it. A long with other immune reactions, HBsAb can eradicate the invading HBV from the body. Either HBV vaccinations can cause HBsAb marker to be positive. This antibody can exit in the blood for a long time and gradually decreases with age. The patients who gave positive results for HBsAb and

negative for HBsAg must be tested in molecular ways such as real – time PCR to know if the patients enter in recovery phase and HBsAg level declined or disappeared from the serum. The results indicated that three of them HBsAg level were very low (less than cutoff + 0.100) therefore, not detected using ELISA technique but detected using rt- PCR and two are not detected using both of them. Therefore, we have to use rt- PCR technique to give the exactly number of particles/ml of blood and confirm the infection with HBV especially in some patients suspected by the infection. So, the results indicated that ninety three patients infected with HBV ninety of them gave positive results using ELISA and rt- PCR and three gave positive results using rt- PCR only inspite of their negative results in ELISA technique. Table -3- Explain the results clearly.

Table -3- Percentage of serum samples who gave positive and negative results using ELISA and rt-PCR techniques.

Serum samples	ELISA technique No. (%)	Real time-PCR No. (%)
HBV positive	90 (49.7)	93 (51.4)
HBV negative	91 (50.3)	88 (48.6)
Total	181 (100.0)	

Enzyme Linked Immuno Sorbent Assay technique depend on the presence of HBsAg in the serum samples, if HBsAg level was high at that time gave positive results but, if HBsAg level was very low at that time gave negative results therefore, we used rt-PCR which is very sensitive technique and can detect few numbers of particles /ml.

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انتشار التهاب الكبد الفيروسي نمط B لدى المجاميع عالية الخطورة في محافظة نينوى/العراق

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الخلاصة:

يعد التهاب الكبد الفيروسي نمط B هو واحد من الامراض الخطيرة الناتج عن الاصابة بفيروس HBV وهو من الفيروسات ذات الحامض النووي المنقوص الاوكسجين. هذا الفيروس يصيب الانسان وبعض انواع الحيوانات كقردة الشمبانزي والطيور. يعتبر المستضد السطحي، المستضد الذائب، الجسم المضاد للمستضد اللبي، الجسم المضاد الذائب، الجسم المضاد السطحي مؤشرا لحدوث الاصابة الكبدية حيث يعتبر ظهورهم ضروري للتأكد من حدوث الاصابة وبيان المرحلة التي وصل اليها المصاب. إذ تضمنت الدراسة (181) شخصاً من كلا الجنسين منهم (137) ذكراً و(44) انثى حيث كانت نسبة الذكور الى الاناث 3.11:1 وكان معدل عمر المرضى 204033 ± 0.83519 الذين تراوحت اعمارهم ما بين (18-73) سنة وكالاتي: (11.6%) >20, (47.5%) 21 - 40, (29.8%) 41 - 60, (11.0%) < 60 على التوالي. شملت الدراسة مرضى من مصرف نقل الدم الرئيسي بنسبة (40.4%) و 73 مرضى الفشل الكلوي المزمن الراقدين في مستشفى ابن سينا التعليمي بنسبة (48.6%) و 88 اخرين من مرضى التلاسيميا الراقدين في مستشفى ابن الاثير التعليمي بنسبة (11.0%) في محافظة نينوى/العراق للفترة ما بين تموز 2011 ولغاية ايار 2012. أشارت النتائج الى ان عددا المصابين بالتهاب الكبد الفيروسي نمط B بلغ (90) (49.7%) بزيادة معنوية قدرها $P < 0.01$ باستخدام تقنية المعايرة الامتصاصية المرتبطة بالانزيم وقد اظهرت النتائج ان المرضى المصابين بالتهاب الكبد الفيروسي نمط B يمتلكون عدد من المؤشرات التي يعد وجودها ضروري لتحديد درجة الاصابة وهذه المؤشرات هي المستضد السطحي، المستضد الذائب، الجسم المضاد اللبي، الجسم المضاد الذائب واخيراً الجسم المضاد السطحي حيث كانت نسب الاصابة بهم كالاتي: 90 (49.7%) و 47 (26.0%) و 89 (49.2%) و 53 (29.3%) و 5 (2.8%) على التوالي. وقد اظهرت النتائج بما لا يقبل الشك أن ثلاثة وتسعين مريضاً مصاباً بالنمط B من بينهم تسعين مريضاً مصاباً بالنمط B باستخدام تقنية الاليزا وتقنية rt-PCR اما الثلاثة الباقية تم تأكيد اصابتهم بالنمط B باستخدام تقنية rt-PCR على الرغم من انهم اعطوا نتائج سلبية باستخدام تقنية الاليزا. نستنتج من ذلك ان الاصابة بالتهاب الكبد نمط B مشكلة مهمة لكون انتشارها لازال معنوياً وواضحاً بين المرضى وقد اشارت الدراسات الى ضرورة اعتماد كل مؤشرات الاصابة بهدف الوصول الى التشخيص الدقيق للاصابة، تعد rt-PCR تقنية حساسة تعطي العدد الحقيقي لجزيئات الفيروس /المليتر الواحد من الدم ضمن نظام مغلق.