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Hepatitis B DNA quantification in the serum of hepatitis B surface antigen-positive patients in Benghazi

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الملخص:

عدوى التهاب الكبد الفيروسي ب هي حالة طبية ذات انتشار عالمي حيث تُستخدم التقنيات المصلية عادةً لتشخيصه. ومع ذلك، فإن القرار بشأن أي مريض يجب علاجه أو عدم علاجه يظل صعبًا بسبب الحساسية الضعيفة للعلامات المصلية كعلامات تنبؤيه أو شديدة. يعد تحديد الحمل الفيروسي (VL) باستخدام تقنيات تفاعل البلمرة المتسلسل تعتبر أداة مفيدة في اتخاذ القرار. الهدف من هذه الدراسة هو تحديد نمط مستويات الحمل الفيروسي HBV لدى الأشخاص من مختلف الأعمار والفئات الجنسية، للتنبؤ بخطر الإصابة بسرطان الخلايا الكبدية وتليف الكبد في المرضى المصابين بفيروس التهاب الكبد B في شرق ليبيا باستخدام نتيجة الإصابة بفيروس التهاب الكبد B (HBV) وتحديد الحمض النووي باستخدام دراسة استعادية ووصفية. تم إجراء اختبار HBV VL واحد فقط على كل مريض قبل أي علاج موجه لفيروس التهاب الكبد B في دراسة على مدى 40 شهرًا (من يناير 2014 إلى أبريل 2018) من لتحليل نتائج التهاب الكبد B DNA VL باستخدام Microsoft Excel 2010 و Minitabالإصدار 17. تم اشتمال الدراسة على 431 مريضاً منهم 51.3٪ (210) ذكور و48.7٪ (221) إناث. من بين 431 مريضاً، 31.3٪ (135) كانوا دون 30 سنة، بينما 68.7٪ (296) كانوا 30 سنة فما فوق. حوالي 31.1٪ (134) لديهم مستويات فحص قابلة للقياس (20 - 2000(Taq) IU / ml) ؛ 21.8 ٪ (94) كان أقل من 20 وحدة دولية / مل و13.2 ٪ (57) أعلى من 2000 وحدة دولية / مل. ما يقرب من 21.1 ٪ (91) لم يكن لديهم HBV DNA قابل للاكتشاف في عيناتهم. حوالي 12.8٪ (55) من المرضى لديهم مستويات أكثر من 2،000،000 وحدة دولية / مل [الجدول 2]. يظهر توزيع إيجابية HBsAg بين المرضى اتجاهًا متزايدًا من الفئة العمرية الأدنى 0-10 إلى 21-30 عند الذكور ومن 31-40 في الإناث، ثم اتجاه تنازلي من 31-40 إلى 81-90 فئة عمرية عند الذكور ومن 41-50 إلى 81-90 الفئة العمرية للإناث. تم فحص إجمالي 431 مريضاً من أجل الحمل الفيروسي لالتهاب الكبد B حيث كانت 90.2٪ سلبية HBeAg و9.8٪ كانت إيجابية .HBeAg كانت أعلى إيجابية HBeAg بين 21-30 عامًا عند الذكور و31-40 عامًا في الإناث. الاستنتاج: سيحسن اختبار الحمض النووي HBV المستخدم وفقًا لإرشادات العلاج الحالية جودة الرعاية لتجنب خزعة الكبد غير الضرورية.

الكلمات المفتاحية: التهاب الكبد B، المستضد السطحي، مقايسة الحمض النووي الفيروسي.

Abstract

Background: Hepatitis B virus (HBV) infection is a global public health problem, with about 240 million people affected worldwide. Serologic techniques are typically used to diagnose it. However, the decision as to which patient to treat or not remains challenging due to the poor sensitivity of serologic markers as prognostic or severity markers. Viral load (VL) determination using polymerase chain reaction techniques is a useful tool in decision-making.

Aim: The aim of this study was to determine the pattern of HBV viral load levels in people of different age and sex groups, to predict the risk of hepatocellular carcinoma and liver cirrhosis in HBsAg positive patients in the east of Libya.

Materials and Methods: This is a retrospective and descriptive study. Only one HBV VL test carried out on patients attending the Benghazi university center (from east of Libya) for specialized medical services prior to any HBV targeted therapy were included in the study over a period of 40 months (January 2014 to April 2018) . Hepatitis B DNA VL determinations were analyzed using Microsoft Excel 2010 and Minitab version 17.

Results: A total of 431 patients, of whom 51.3% (210) were males and 48.7% (221) were females, were included in the study. Of the 431 patients, 31.3% (135) were below 30 years old, while 68.7% (296) were 30 years old or above. Around 31.1% (134) had measurable assay levels (20 - 2000 (Taq) IU/mL); 21.8% (94) had below 20 IU/mL; and 13.2% (57) had above 2000 IU/ml approximately 21.1% (91) had no detectable HBV DNA in their samples. 12.8% (55) of the patients had levels greater than 20000 IU/mL. The distribution of HBsAg positivity among the patients shows an increasing trend from the lower age group 0-10 to 21-30 in males and from 31-40 in females, then a decreasing trend from 31-40 to 81-90 age group in males and from 41-50 to 81-90 age group in females. A total of 431 patients were investigated for hepatitis B viral load, of whom 90.2% were HBeAg negative and 9.8% were HBeAg positive. The highest HBeAg positivity was between 21 and 30 years of age in males and 31- 40 years in females. There is association between the positivity of HBe antigens and high concentration of HBV VL and opposite relationship between the positivity of HBe antibodies and concentration of HBV VL Conclusion: HBV DNA assays used in accordance with existing treatment guidelines will improve the quality of care and help avoid unnecessary liver biopsies.

Keywords: Hepatitis B, Surface antigen, Viral DNA assay.

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1. INTRODUCTION

Hepatocellular carcinoma and liver cirrhosis are two of the most common worldwide health problems and an important cause of cancer-associated death(1). Areas where the Hepatitis B virus is highly prevalent develop hepatocellular carcinoma (2). They reported that chronic HBV and HCV are the major causes of worldwide hepatocellular carcinoma. Over 2 billion people worldwide have some evidence of current or past hepatitis B virus infection, and 350 million of them are chronic carriers (1). West Africa has high endemicity rates, particularly among infants, due to vertical transmission (3). In Nigeria, the infection rate is between 7.3% and 24% (2, 4, 5). Diagnostic investigations for hepatitis B virus infection (HBV) include hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), hepatitis B envelope antigen (HBeAg), antibodies to HBsAg (HBs-ab), antibodies to HBeAg (HBe-ab), and HBV DNA. While the levels of HBV DNA are associated with a higher risk of developing liver complications, HBeAg is measured as well in some strategies (1, 4).

Usually, HBV DNA levels above 2×10^3 IU/mL are linked with an increased risk of hepatocellular carcinoma (HCC) and liver cirrhosis (6, 7). The treatment decision usually depends on the level of viral load (VL) in addition to liver function tests, liver histology, and age at presentation. Still, liver function tests and histological results do not always correlate (8-10). Persistent suppression of hepatitis B DNA is important to avoid the development of hepatitis B virus complications (4).

To study the markers of HBV infection changing aspects and their influence on the progression of the disease risk, a follow-up study with repeated quantitative of these markers is the best way (11). C Chronic HBV infection is variable from patient to patient, depending on the age of the infected patient and the time of infection. Perinatal infection occurs in a state of immune tolerance, where the patient lives with the virus without any problems. The HBV DNA load is high at this age. Later, progressing to an immune clearance phase followed by a tolerance phase where the hepatitis B virus becomes active, the immune system makes efforts to clear infected hepatocytes. During this phase, hepatic inflammation, elevations in liver enzymes, and a reduction in the level of circulating HBV DNA load occur. The frequency and duration of the immune clearance phase vary greatly, but recurrent acute liver inflammation episodes may occur as a result of repeated cycles of injury and regeneration, resulting in an increased risk of developing hepatocellular carcinoma and liver cirrhosis (12).

Using real-time PCR for molecular analysis has made a great impact on HBV viral quantification. Quantification of HBV DNA load plays an important role in defining the infection phase, determining the exact treatment, and monitoring the responses to the therapy (13). As stated in the prevention, care, and treatment guidelines for chronic hepatitis B patients from the WHO and China, quantification of hepatitis B DNA is recommended in the management of chronic hepatitis B infections (13, 14). The real-time PCR for molecular analysis for the diagnosis of viral infections has been stated to have high sensitivity and is considered the standard viral load quantification method (3, 15). There is inadequate information on Hepatitis B viral load detection in developing countries due to its cost. Although the hepatitis B viral load test is important for the management of hepatitis B, due to the cost of the assay and the availability of highly trained staff, HBV DNA technology is not always available. The aim of this study was to determine the pattern of HBV viral load levels in people of different ages and sex groups, to predict the risk of hepatocellular carcinoma and liver cirrhosis in HBsAg positive patients.

2. MATERIALS AND METHODS

A retrospective descriptive study was done among chronic hepatitis B patients attending the Benghazi university center for specialized medical services. All patients were referred for HBV DNA viral load (HBV DNA VL) testing following a positive HBsAg screening test. Age and gender were involved in the analysis, and those without documented age and gender were excluded from the analysis. Only one HB VL test was carried out on a patient prior to any HBV targeted therapy was included in the study. The laboratory records of all 431 patients who met the inclusion criteria and had HB VLs test done from January 2014 to April 2018 were accessed for biodata (age, gender), and VL results. The data were analyzed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and Minitab 17. Patients' undergoing VL testing had 5 ml of venous blood collected into ethylene diamine tetra acetic acid containers, and then the blood samples were shipped to Bioscientia laboratory, Germany to undergo viral load determination. Ethical clearance was obtained from the Benghazi university center for specialized medical services.

3. RESULTS

A total of 431 patients, of whom 51.3% (210) were males and 48.7% (221) were females.. Males had higher VLs than females [Table 1]. Regarding age, 31.3% (135) were below 30 years old, while 68.7% (296/) were 30 years old or older. Around 31.1% (134) had measurable assay levels (20 − 2000 (Taq) IU/mL); 21.8% (94/431) had below 20 IU/mL; and 13.2% (57) had above 2000 IU/mL. Approximately 21.1% (91) had no detectable HBV DNA in their samples. Around 12.8% (55) of the patients had levels greater than 2000000 IU/mL [Table 2]. Distribution of results of presences Hepatitis B Viral Load in different Age Categories was summarized in Table3. Distributions of the presences of HBeAgs (Hepatitis B envelope antigens) as well as the presences of HBe-antibodies among males and females were shown in Tables 4 and Table 5. Meanwhile, the distribution of measurable viral load parameters by age groups (n=90) was summarized in Table 6. The distribution of HBsAg positivity among the patients shows an increasing trend from the lower age group 0-10 to 21-30 in males and from 31-40 in females, then a decreasing trend from 31-40 to 81-90 age group in males and from 41-50 to 81-90 age group in females as shown in Figure 1. A total of 431 patients were investigated for hepatitis B viral load, of whom 90.2% were HBeAg negative and 9.8% were HBeAg positive. The highest HBeAg positivity was between 21 and 30 years old in males and 31-40 years old in females (Figure 2). The distribution of positive HBeAgs and positive HBe Abs results among different age groups shows the highest positivity rate of HBeAgs was in the age group 21-30, while HBe Antibodies at this age group was zero as shown in Figure 3.The high concentration of HBe antigens was associated with high hepatitis B viral load, and high concentration of HBe antibodies were associated with low HB viral load as shown in Figure 4 and Figure 5

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Table 1: Age and sex distribution of hepatitis B surface antigen positive (HBeAgs) patients

Age (Years)	Male. No. (%)	Female No. (%)
0--10	3 (1.43)	2 (0.9)
11--20	11 (5.2)	12 (5.4)
21--30	54 (25.7)	55 (24.9)
31--40	44 (20.95)	57 (25.79)
41--50	47 (22.4)	46 (20.8)
51--60	30 (14.3)	27 (12.22)
61--70	10 (4.76)	19 (8.6)
71--80	11 (5.24)	3 (1.36)
total	**210 (51.3)**	**221 (48.7)**

Table 2: - Association of hepatitis B viral load with gender (431 case)

HBV-DNA (Taq) IU/mL	Number of Male	Frequency	Number of Female	Frequency	OR	P. value
Not detected	54	25.7	37	16.74	1.5359	0.06
< 20	38	18.09	56	25.34	0.7141	0.14
20-2000	63	30	71	32.13	0.9190	0.67
2001-20000	26	12.38	31	14.02	0.8826	0.65
>200000	29	13.80	26	11.76	1.1738	0.57
Total	**210**		**221**			

Table 3: Distribution of hepatitis B viral load results into age categories

	Male		Female			
HBV-DNA (Taq) IU/mL	**Less than 30 years**	**More than 30 years**	**Less than 30 years**	**More than 30 years**	**OR**	**P. value**
Not detected	14	40	13	24	0.6462	0.34
< 20	11	27	14	42	1.2222	0.67
20-2000	20	43	20	51	1.2143	0.60
2001-20000	12	14	11	20	1.5584	0.41
>20000	9	20	11	15	0.6136	0.38
Total	**66**	**144**	**69**	**152**	**1.0097**	**0.97**

Pearson Chi-Square = 8.059, DF = 5, P-Value = 0.153

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Table 4: Distribution of HBeAgs (Hepatitis B envelope antigens) in HBsAg Positive Patients

HBe antigens	Total No.(%)	Male. No.(%)	Female No.(%)
Negative	**81**	**35 (89.7)**	**46 (90.2)**
Positive	**9**	**4 (10.3)**	**5 (9.8)**

Pearson Chi-Square = 0.522, DF = 2, P-Value = 0.770

Table 5: Distribution of HBe-antibodies Results among Males and Females

HBe antibodies	Total. No. (%)	Male. No. (%)	Female No. (%)
Negative	**9**	**4 (10.3)**	**5 (9.8)**
Positive	**75**	**29 (74.4)**	**45 (88.2))**

Pearson Chi-Square = 2.277, DF = 2, P-Value = 0.320

Table 6: Distribution of measurable viral load parameters by age groups (n=90)

Age	Males No. (%)	Females No. (%)
10--20	**4 (10.26)**	**6 (11.8)**
21--30	**10 (25.6)**	**9 (17.65)**
31--40	**7 (17.95)**	**16 (31.37)**
41--50	**7 (17.95)**	**10 (19.6)**
51--60	**9 (23.1)**	**7 (13.7)**
61--70	**2 (5.1)**	**3 (5.9)**
total	**39 (43.3)**	**51 (56.7)**

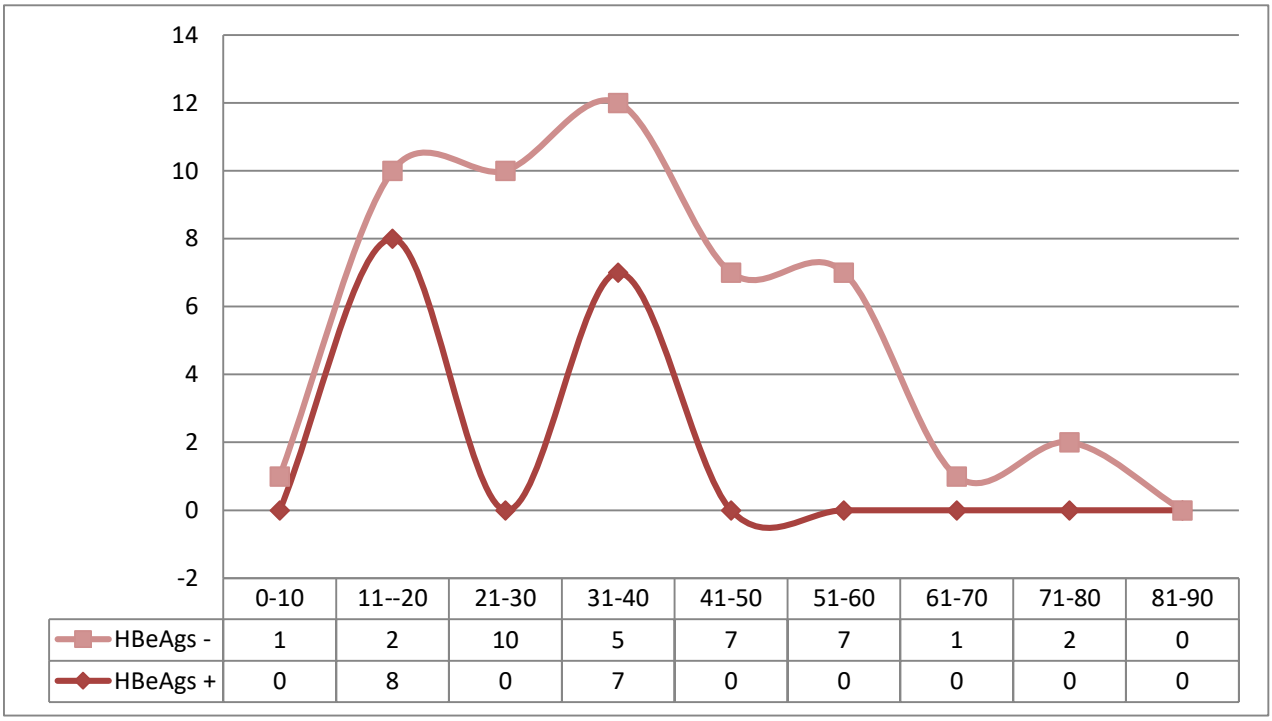

Figure 1:- Association between presence of Hepatitis B Viral Load and Hepatitis B envelope Antigens (HBeAgs) among age groups

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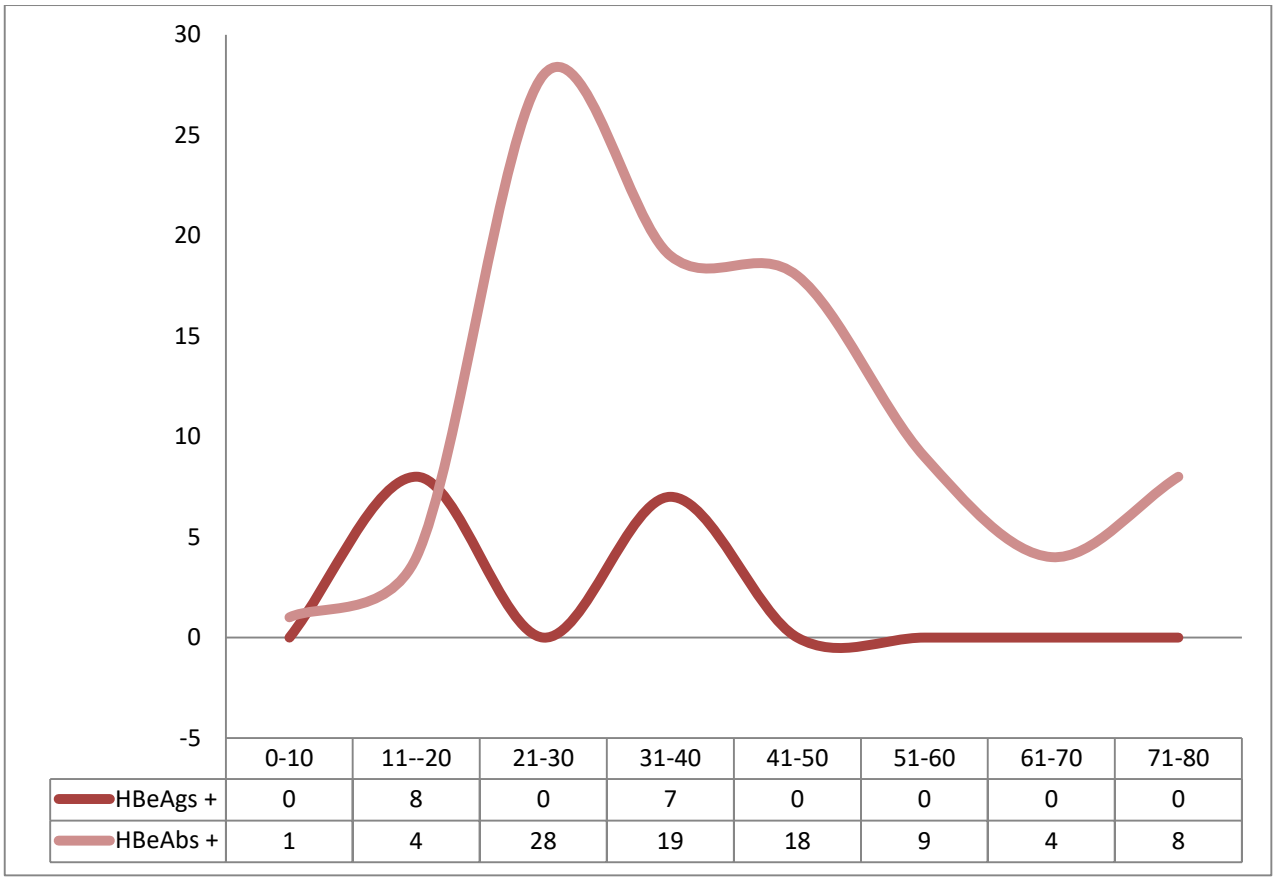

	0-10	11--20	21-30	31-40	41-50	51-60	61-70	71-80
HBeAgs +	0	8	0	7	0	0	0	0
HBeAbs +	1	4	28	19	18	9	4	8

Figure 3: Association between HBeAgs positive and HBe Abs positive results among Different Age Groups

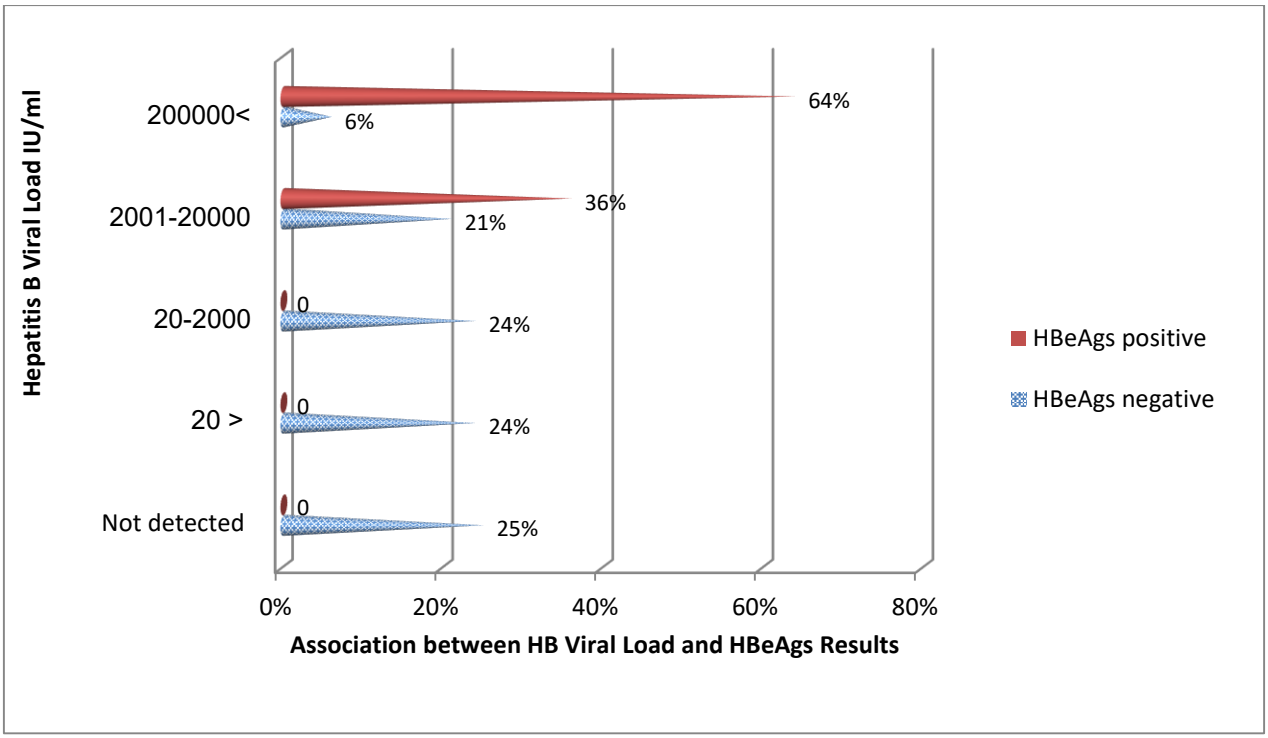

Figure 4: Association between Hepatitis B Viral Load and Hepatitis B envelope antigen (HBeAbs) Results

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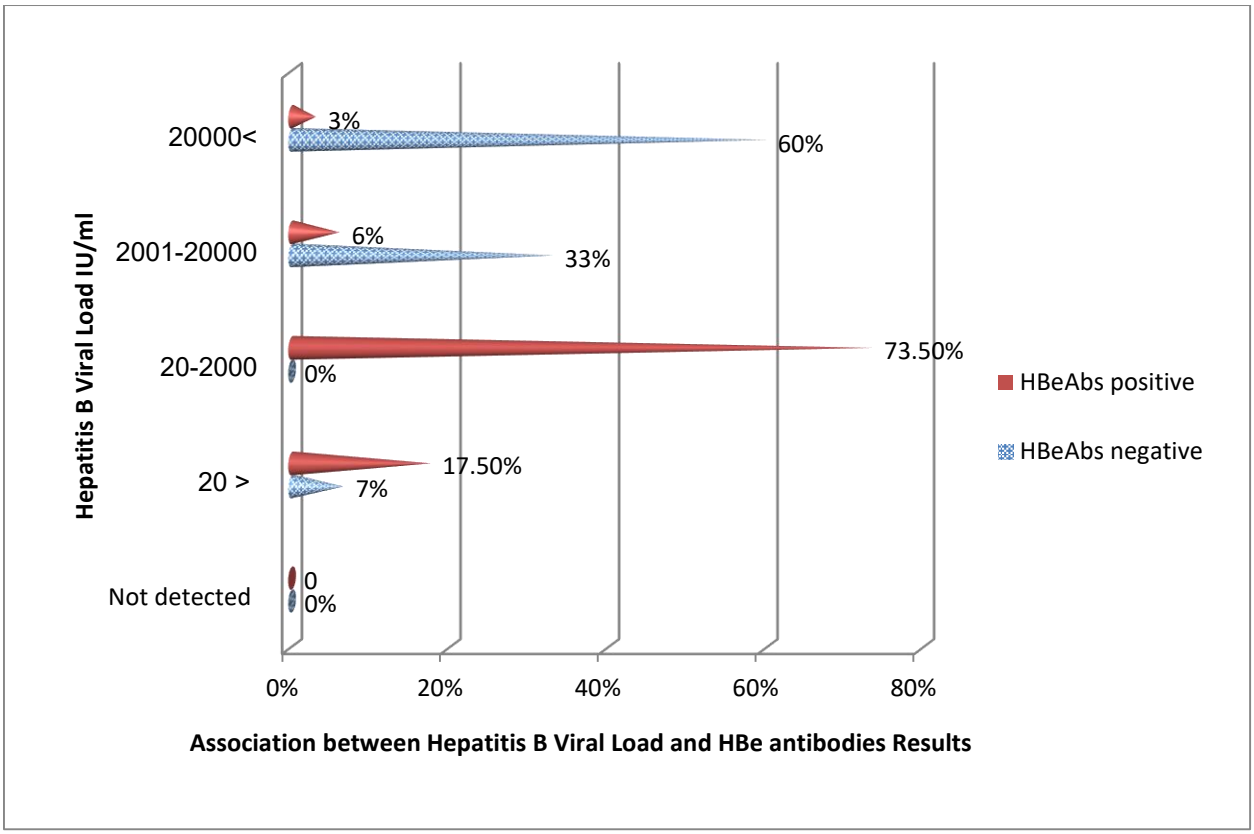

Figure 5: Association between Hepatitis B Viral Load and Hepatitis B envelope Antibodies (HBeAbs) Results

4. DISCUSSION

Hepatitis B virus infection and its outcome are determined by the replication degree of the virus and the status of the immune system. The prevalence of HBV infection is lower in developed countries in comparison to developing countries (15). In this study, the HB-viral load pattern was studied in plasma of patients attending the infectious care Centre, in the East of Libya. Samples were received from different cities in this region. The number of male participants was slightly higher but not significantly different. In contrast to this study, previous studies (16, 17) found that females with detectable Hepatitis B viral load in blood donors were more. Also, it was in contrast with study by Okwuraiwe and his colleagues. (12) , they found that males had a higher detectable hepatitis B viral load and proposed that it might be due to the higher financial incomes available to males to do the tests than females. To determine whether females acquire the infection more than men, a well-designed study may be needed. The distribution of HBsAg positivity among the patients shows increasing trend from the lower age group 0-10 to 21-30 in males and from 31-40 in females, then a decreasing trend from 31-40 to 81-90 age group in males and from 41-50 to 81-90 age group in females. The positivity rate of HBsAg in different age groups shows that the higher and lower age groups are not as exposed to risk factors when compared to the intermediate age groups. This study found similar results for 30-39 year olds in Lagos (12) and 36–50 years in Bangladesh (18). This may be due to the higher prevalence of activities related to hepatitis B virus acquisition or existing HBV infections reactivation in this age group (19, 20). Therefore, the results indicate that the positivity rate is directly related to the exposure. The highest positivity rates were seen in the age group 21-30 (25.7%) in males and in age group 31-40 (25.79%) in females, which related to sexually active age groups. The result of the hepatitis B DNA load is the same as concluded in the previous studies of the other countries. In the contemporary WHO hepatitis B virus standard and consent, one IU/mL is equivalent to five genome copies. Previous studies stated that risk for liver cirrhosis and hepatocellular carcinoma outcomes was statistically significant at more than or equal to 10,000 copies/mL (21-25). A study in Hong Kong for a followed-up 1006 patients for more than 7 years showed similar results, that serum HBV DNA load are associated significantly with later development of hepatocellular carcinoma (26).

In the current study, 9.8% were HBeAg positive. The highest HBeAg positivity was between 21-30 years of age in males and 31-40 years in females. The number of patients with HBeAg positive proportionally to concentration of viral load .The highest number of patients (64%) was found in the range more than 20000 IU/mL, and 36% of them were in range 2001-20000 IU/mL. None of the patients were found to be in the undetectable range or less than 2000 IU/mL. On the other hand, high concentration of HBe antibodies was associated with low hepatitis B viral load, and opposite relationship between HBeAgs and HB viral load with HBeAbs. A current recommendation established by the Society for Gastroenterology and Hepatology considers HBeAg status a major factor (13).

In patients with positive HBeAg, the critical viral load level is 20000 IU/mL. Hepatitis B DNA load exceeding this level with abnormal liver function tests is an warning sign for chemotherapy, while Hepatitis B DNA load less than 2000

IU/mL with normal liver function tests on two repeated times, need abnormal results of liver biopsy prior considering chemotherapy (23).

The Lowest measurable Hepatitis B DNA load range was recorded in the 1–10 years age group, which may due to childhood vaccination against HBV infection. This was in contrast to a study in Nigeria which found that highest viral load was found in 1-10 age groups (22).

5. CONCLUSION

Hepatitis B viral load testing is vital in the management of HBV infection. It may aid in avoiding unnecessary therapies and saving money. The strong correlation between HBeAg levels and viral load suggests that we can rely on HBeAg measurements when following up on patients infected with the hepatitis B virus. More research is needed to further fine-tune the local guidelines. Access to HBV viral load as well as HBeAgs assays needs to improve the quality of health care and research.

6. ACKNOWLEDGMENT

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