INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 11, No. 2, p. 1-6, 2020

Open Access RESEARCH PAPER

The prevalence of HBV in the city of Zawia, Libya

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Keywords: HBV, ELISA, Zawia, HBsAg

Publication date: February 12, 2020

Abstract

Hepatitis B virus (HBV) is a major cause of chronic liver disease worldwide. It is development of end-stage liver disease and carcinoma. Studies have shown that patients infected with HBV may respond to different antiviral therapy and information about HBV prevalence is very important in helping physicians to provide better treatments. The aim of the work was to study the prevalence of HBV in the city of Zawia- Libya. 348 samples were analyzed using ELISA test at Zawia Reference Laboratory, the study showed that out of 348 patients, males were 237 and females were 111, their ages ranged from 14 to 83 years, and they were distributed into four age groups. The percentage in first group (4.88%), in the second group (62.64%), in the third group (28.44%), and in the fourth group (4.02%). The non-Libyan patients represent about 28% of the infections and most of the neighboring countries. The vast majority of HBV infections were in the age range of 21 - 60 years (91.08%), which are more subjected to risk factors since they are at the working age. Furthermore, people older than 60 years represent only about 4.02%, suggesting that HBV infections were less common earlier or infected patients did not survive to reach age older than 60 years. The data also showed that both males and females are equally vulnerable to the infection and most of the neighbor countries are represented in these patients.

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Introduction

The prevalence of Hepatitis B infections worldwide is about 6% of the population, whereas it is only about 2.2% of the population in Libya (Madour A, Alkout A, Vanin S 2013). This percentage is considered in the intermediate prevalence; usually more than 8% is considered as a high prevalence and less than 2% is considered a low prevalence (Ott JJ *et al.,* 2012). The Libyan obligatory vaccination program and world health organization vaccination guidelines helped in decreasing the infection rates (Madour A, Alkout A, Vanin S 2013. WHO 2016). Hepatitis B virus (HBV) is a highly contagious virus that can cause liver damage. The virus is easily spread via hepatitis B–positive blood, semen or other body fluids (Trépo C *et al.,* 2014). Pregnant women who have hepatitis B might also transmit the virus to their babies, usually during birth (Vyas AK *et al.,* 2019). People who have not been infected with HBV can be vaccinated against the virus to prevent infection (Cho EJ *et al.,* 2017).

There are two phases of hepatitis B infection: acute and chronic. Acute refers to a new infection that is less than six months old; an HBV infection that lasts more than six months is chronic (Wu J *et al.,* 2020). Acute hepatitis B will resolve on its own without serious complications in the majority of newly infected teens and adults; when this occurs, people are no longer contagious and are immune to further HBV infections (Wu J *et al.,* 2020). However, in people whose infection does not resolve, HBV may be transmitted to others. Chronic hepatitis B can cause fibrosis (mild to moderate liver scarring), cirrhosis (serious liver scarring), liver cancer, liver failure and death (Trépo C *et al.,* 2014, Chen T *et al.,* 2019).The risk that a hepatitis B infection will become chronic varies according to the age at the time of infection where children are more likely to develop chronic disease (Gerlich WH 2013). Many people will have no symptoms during the initial HBV infection; some develop a rapid onset of sickness with vomiting, yellowish skin, tiredness, dark urine and abdominal pain (National Health Service 2019, Davis K 2018). Often these symptoms will last for few weeks and rarely does the initial infection result in death. It may take 30 to 180 days for symptoms to begin (Davis K 2018). About90% of the newborns who are infected at birth will develop chronic hepatitis B while less than 10% of those infected after the age of five do (Gerlich W H 2013). Most of those with chronic HBV disease have no symptoms; however, cirrhosis and liver cancer may eventually develop (Lanini S *et al.,* 2019). These complications result in the death of 15 to 25% of those with chronic disease (Chen T *et al.,* 2019).

Material and methods

Sample collection

The Reference Laboratory in the city of Zawia provided 348 samples that were included in this study. Blood samples were collected by vein puncture and were allowed to clot completely and naturally. Serum and plasma were collected quickly to avoid hemolysis of RBCs. It was ensured that the serum samples were clear and uncontaminated and all visible particles were removed by centrifugation at 3000 RPMs for 10 minutes at room temperature.

ELISA Procedure

All the reagents and samples were allowed to reach room temperature (18 – 30°C) for 15 – 30 minutes before they were used. Solutions were wormed up to 37°C in order to dissolve any possible crystals found in them. Stock wash buffers were diluted 1 to 20 with distilled water. The needed strips were set in the strip-holder alongside the required wells, including three negative controls, two positive controls, and one blank containing the conjugate. The wells were gently rocked for twenty second, and then covered. For better precision, the solutions were mixed well with pipette. The wells were incubated for 60 minutes at 37°C. Once incubation is over, the mixture was removed by emptying into a wash container. Each well was filled with diluted wash buffer and shacked gently for 30 seconds.

The wash solutions were then discarded and the plates were taped on absorbent paper. These steps were repeated four times.

A total of 50µl of Chromogen A and 50µl of Chromogen B solutions were dispensed into each well including the blank, and mix by tapping the plate gently. The plates were incubated at 37°C for 15 minutes in dark place. The enzymatic reactions between the Chromogen solutions and the HRP Conjugate produces blue color in Positive control and HBsAg positive sample wells.

A 50µl Stop Solution were added to each well and mix gently, using a multichannel pipette. Intensive yellow color develops in Positive control and HBsAg positive sample wells. The plate reader with the blank well was calibrate and the absorbance was read at 450nm. the Cut-off value was calculated and the results evaluated.

Results

Blood samples

This study was conducted for a four-year period from 2015 to 2018. All samples collected from patients referred to Reference Laboratory in the city of Zawia, to identify HBV for the purpose of inclusion in antiviral therapy programs. Plasma samples were collected from 348 patients and tested by ELISA.

The year of recording the cases

The data obtained were recorded in the consecutive years 2015, 2016, 2017, and 2018. (as it is presented in Fig. 1). A total of 125 infected cases were recorded in 2015, 84 of them were males and 41 females. In 2016, there were 94 infected cases, 67 of them were males and 27 females. In 2017 there were 66 infected cases, 42 of them were males and 24 females. In 2018 there were 63 infected cases, 46 of them were males and 17 females. The year 2015 had the highest percentage of infected cases (35.92% of the total), whereas the year 2016 had the second highest (27.1% of the total), the year 2017 came third with a percentage of (18.97% of the total) and the year 2018 had the lowest (18.11% of the total). The year 2018 had the highest infected male patients' percentage with 73% males and 27% females. The year 2016 had the second higher percentage of male infections with 71.3% males and 28.7% females. The year2015 came third with 66.4% infected males and 33.6% infected females. The year 2017 had the lowest infected male patients' percentage with 63.6% males and 36.4% females. The average of male to female patients' ratio was about 2:1.

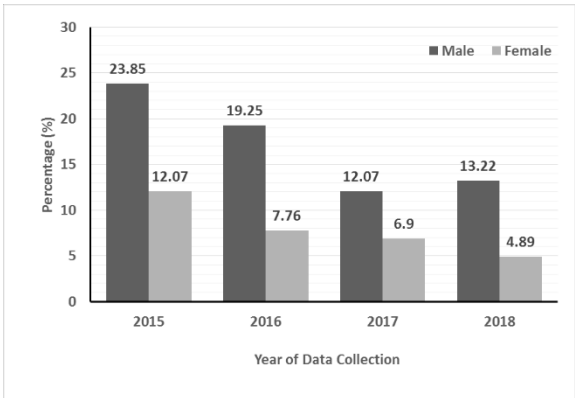

Fig. 1. HBV distribution according to the year of data collection. The data was expressed as the number of patients in each year. The total number of infected patients in the first year (2015) was 125 persons (35.92%), about 2/3 of them are males and 1/3are females. The total number of infected patients in the second year (2016) was 94 persons (27.01%), about 2/3 of them are males and 1/3females. The total number of infected patients in the third year (2017) was 66 persons (18.97%), about 2/3 of them are males and 1/3 females, and the total number of infected patients in the fourth year (2018) were 63 people (18.11%), about 3/4 of them are males and 1/4 females.

The age group distribution

As it was mentioned earlier, the ages of infected cases were distributed from 14 to 83 years with a mean of 43.95 years. As it is shown in fig. 3, the cases were divided according to their ages into four groups. The first group is from 1 to 20 years,

the second group was from 21 to 40 years, the third group was from 41 to 60 years, and the fourth group was older than 60 years. The first group (1-20 years) contains17infected cases (4.88%), 15of them were males (4.31%) and two females (0.57%). The second group (21-40 years) contains218 infected cases (62.64%), 143 of them were males (41.09%) and 75females (21.55%). The third group (41-60 years) contains 99infected cases (28.45%), 69 of them were males (19.83%) and 30 females (8.62%). The fourth group (>60 years) contains14 cases (4.02%), 10 of them were males (2.87%) and 4 females (1.15%). The fourth group (>60 years) showed the lowest number of cases representing only 4.02%. The highest number of infected cases were in the second group (21-40 years) representing about 62.64% of the total.

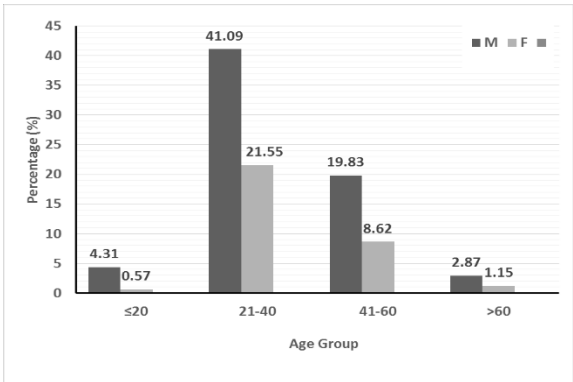

Fig. 2. HBV distribution among different age groups. The data were expressed as the number of patients in each group. The first group is the young people aged (1-20) years contains 17 patients, 15 of them are males and 2 females. The second group is the age 21 to 40 years contains 218 patients, 143 of them are males and 75 females. The third group is the age 41 to 60 years contains 99 patients, 69 of them are males and 30 females. The fourth group is the age older than 60 years contains 14 patients, 10 of them are males and 4 females.

Distribution of HBV infections according to the patient's nationality

The data obtained were also analyzed according to the nationality of infected cases (as it is presented in fig. 3). The infected cases recorded with Libyan nationality were 250 cases (71.8%), 147 of them were males and 103 females. The infected cases recorded with Sudanese nationality were 29 cases (8.3%). The infected cases recorded with Tunisian nationality were 19 cases (5.5%). The infected cases recorded with Chadian nationality were 12 cases (3.4%). The infected cases recorded with Nigerian nationality were 11 cases (3.2%). The infected cases recorded with Egyptian nationality were 9 cases (2.6%). The infected cases recorded from all other countries were18cases (5.2%). The highest percentage of cases were with Libyan nationality (71.8% of the total), whereas the non-Libyan patient were (28.2% of the total).

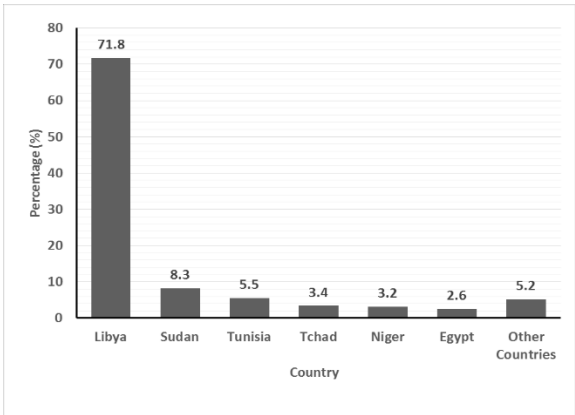

Fig. 3. HBV distribution according to the nationality. The data were expressed as the number of patients. The Libyans were 250 patients (71.8%), the Sudanese were 29 cases (8.3%), the in Tunisians were 19 cases (5.5%), the Chadians were 12 cases (3.4%), the Nigerians were 11 cases (3.2%), the Egyptians were 9 cases (2.6%), and all other nationalities were 18 cases (5.2%).

Discussion

HBV is a member of *Hepadnaviridae* family viruses with significant clinical problems throughout the world in humans and it is responsible for the second most common cause of viral hepatitis (Gerlich WH 2013, Karayiannis P 2017). Data obtained from different parts of the world showed increasing interest in mass

screening of HBV as it is useful in answering epidemiological questions and in development of vaccines against HBV; furthermore, it is beneficial in facilitating therapeutic decisions and strategies and to demonstrate the severity of the disease (Wright CM *et al.,* 2018)

Even though Libyan Health service is following the guidelines of World Health Organization (WHO) including three doses of HBV vaccinations in the first year and a poster vaccination at the age of 6years, some people are not immunized against this virus (WHO 2016 , Ezzikouri S *et al.,* 2013). This could be due to an impaired immune system and / or not taking all or some of the vaccines. In this study, blood samples suspected with HBV infections were tested for patients from the city of Zawia. All the provided 348 serum samples tested by ELISA and all of them were HBV positive. The most commonly affected age group with HBV is 21-40 with a percentage of 62.64%. This result agrees with that obtained by many other reports worldwide where this age group is more affected by HBV infections; The percentage of infections of this age group reached 58.76 in Pakistan and 49.58 in southern China (Khan F *et al.,* 2011, Xie Q *et al.,* 2015). The vaccination against HBV might be an important factor in reducing the HBV infections in people younger than 20 year and none of them under the age of 14 years (Nelson NP *et al.,* 2016).

The results also showed that more men are infected than women in all age groups. This is consistent with results obtained from other countries[19,20]. In fact, in many countries the ratio is less than 2:1 male to female infections; in united states the ratio was 1.6:1 male to female ratio[20]. In our results it reached 7:1 ratio in the age group 1-20 years, but in the overall ratios it is more 2:1 male to female ratio. This could be due to the fact that men are exposed more to infections than women. On the other hand, since the overall percentage of people from other countries is not known, it is not clear whether the non-Libyan infected persons represent the percentage of HBV infections in their countries or not. In fact, 28.2% of infections being non-Libyans suggest that this high percentage should be considered as an alarm for the health authorities in Libya to take actions in controlling the infection of HBV.

Conclusion and recommendations

1-The results suggest that the authorities of public health services need to put more effort in controlling HBV infections, these actions should include providing HBV vaccinations for adults and using all the possible communication ways to increase the public awareness to the risks and effects of HBV on the public health.

2- A mass screening for HBV is needed to have a good estimation of the number of infected people and the percentage of people who are aware that they have been infected with HBV and whether they are using medical treatment.

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