Seroprevalence of HDV Infection among HBsAg Positive Blood Donor in Baqubah City, Iraq

Linda Munjed Hadi (BSc)[1], **Areej Atiyah Hussein**(MSc, PhD)[2]**and Ali Musa Ja'afer** (FICMS)[3]

Abstract

Background: Hepatitis B virus (HBV) is the most important causative agent of blood borne hepatitis in humans. Hepatitis D Virus (HDV) infection occurs either as a co-infection or super infection in HBV carriers.

Objective: To determine the seroprevalence of HDV among blood donors (HBsAg positive individuals) in Baqubah city.

Patients and Methods: Serum samples were collected from 89 HBsAg positive blood donation (66 males and 23 females, age range 19-60 years) from blood bank in Baqubah teaching hospital, during the period from 11th August 2016 till 17th January 2017. All of them were tested for HBsAg, HBcIgM, HBcIgG, HDVIgM and HDVIgG using separate ELISA kits and full information were collected from each participant by use questioner sheet which included the sociodemographic and clinical characteristics such as age, gender, educational level, marital status, economic, occupation, family history, blood transfusion, surgical operation, dental history, endoscope, any medical problem, tattoo and hjamaa also were recorded.

Results: In the present study minimum age group was 19 year and maximum was 68 year with mean age 36 year. According to ELISA technique, 5 cases (5.6%) were positive to HDV IgG and they all were males with mean age 49 year, while 84 (94.3%) were negative for HDV infection. Among study group the percentage in males (74.1%) was more than in females (25.8%). Also significant differences was found between the viral hepatitis infection and family history, blood transfusion, surgical operation, dental history, endoscope, tattoo and hjamaa, while there was no significant difference noticed in social state, economic and educational level.

Conclusion: Infection rate of HDV among blood donor is high, early screening of HDV is essential for establishing diagnosis and should be depended in routine work further investigation is needed to validate the findings.

Key words: Hepatitis, HBV, HDV, Blood donor.

Corresponding Author: areej@medicine.uodiyala.edu.iq

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[1,2]Department of Microbiology - College of Medicine - Diyala University -Diyala- Iraq.

[3]Department of Medicine - College of Medicine - Diyala University -Diyala- Iraq.

Introduction

Hepatitis delta is an inflammatory liver disease caused by infection with Hepatitis Delta viruse [1]. Hepatitis D is a disease caused by the hepatitis D virus (HDV), a small spherical RNA enveloped viroid, this is one of five

known hepatitis viruses: A, B, C, D, and E. HDV is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus (HBV) [2]. Hepatitis D virus is a defective RNA virus which requires the help of hepatitis B virus for its replication and assembly of new virions [3]. Both HBV and HDV are transmitted through the same routes including blood-borne, sexual, percutaneous and vertical (perinatal) transmission [4].

Hepatitis delta virus propagates only in an individual who has coexisting HBV, due either to co-infection of the two viruses or super-infection of a chronic HBV carrier [5]. Hepatitis delta virus affects an estimated 15 to 250 million individuals worldwide and the clinical significance of HDV infection is more sever forms of viral hepatitis, acute or chronic, and a higher risk of developing cirrhosis, hepatocellular carcinoma in comparison to HBV mono-infection[6].

The infection is endemic in Southern Europe, Africa, the Middle East and, probably, Latin America. Delta is present in most of the countries of the world where it has been looked for, the one possible exception being China and Japan. Most of the countries of the Far East at the moment appear to have quite a low incidence of Delta with the exception of Taiwan [7].

Many investigations on the prevalence of HDV have been carried out in different parts of the world, and have shown variations in the prevalence of infection. In Iraq, although some investigations have been done about HDV in different places to detect the rate of infection in general population such as Mosul in 2002, Baghdad in 2011 and Duhok in 2015 and they found that 8.3%, 2.5% and 6.6% [8][9][10].

In our Knowledge there is no Iraqi study done in Diyala province, so the preset study designed to determine the infection rate of hepatitis D viruse among hepatits B surface antigen positively population (blood donor). And study the relationship between HDV infection and sociodemographic and clinical characteristic such as age, gender, educational level, marital status, economic level, occupation, family history, blood transfusion, surgical operation, dental history, endoscope, any medical problem, tattoo and hjamp also were recorded.

Materials and Methods

Serum samples were collected from 89 individuals who volunteered for blood donation and found to be HBsAg positive by routine screening. All were from blood bank in Baqubah City during the period from 11th August 2016 till 17th January 2017. Informed consent was obtained from each donor in this study.

All participants completed a questionnaire included the socio-demographic and clinical characteristics such as age, gender, educational level, marital status, economic, occupation, family history, blood transfusion, surgical operation, dental history, endoscope, any medical problem, tattoo and hjamaa also were recorded.

Five ml of blood was collected from each patient by vein puncture using disposable syringes the blood was placed in plastic disposable tube then the serum were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20Co until the time of test.

HBsAg (foresight, USA), anti-HBcIgM (EIA-3895-DRG,Germany), anti-HBcIgG (EIA-3894-DRG, Germany), anti- HDV IgM (EIA-4120-DRG,Germany) and anti- HDV IgG (EIA-4269-DRG,Germany) were determined by using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Fisher's exact test and t-test were used to obtain statistically significant differences, ($p<0.05$) being considered statistically significant.

Result

The results of table (1) show that HBV infection percentage of males 66 (74.10%) was more than females 23(25.80%) out of total number 89. Among patients minimum age was 19 years and maximum was 68 years with mean age 36 year also majority of cases were in age group 19-34 years.

Regarding to residence and marital state highest rate of infection was recorded in urban 58(65.10%) and married groups73(82.00%). According to educational level patients with secondary school show high frequency 31(34.80%) than other, unemployed and intermediate economic level demonstrate high percent of infection 73.00% and 88.70% respectively.

Table (1): Socio-demographic characteristics among studied group.

Parameters	**No. (%)**
Gender type	
Male	66 (74.10%)
Female	23 (25.80%)
Age groups	
19-34 year	52(58.40%)
35-50 year	20(22.40%)
51-66 year	16(17.90%)
≥ 66 years	1(1.10%)
Residence	
Urban	58(65.10%)
Rural	31(34.80%)
Marital status	
Single	11(12.30%)
Married	73(82.00%)
Divorced	1(1.10%)
Widow	4(4.40%)
Education	
Illiterate	13(14.60%)
primary school	28(31.40%)
Secondary school	31(34.80%)
High education	17(19.10%)
Occupation	
Employed	24(26.90%)
Un- employed	65(73.00%)
Economic level	
High	8(8.90%)
Intermediate	79(88.70%)
low	2(2.20)

In table 2 highest rate of infection was noticed among patients with dental history 50(56.17%). And also there were highly infection rate among other risk factors especially patients with family history, surgical, hijamaa, blood transfusion and smoking habit.

Table (2): Distribution of blood donor (HBsAg positive) according to risk factor.

Risk Factor	Yes No. (%)	No No. (%)
Family History	31(34.83%)	58(65.16%)
Surgical history	26(29.21%)	63(70.78%)
Dental history	50(56.17%)	39(34.82%)
Blood transfusion	13(14.6%)	76(85.39%)
Smoking habit	28(31.46%)	61(68.53%)
Arkilaih smoking habit	11(12.35%)	78(87.64%)
Drinking alcohol	1(1.12%)	88(98.87%)
Under to Endoscope	5(5.61%)	84(94.38%)
Tattoo	4(4.49%)	85(95.50%)
Hjamaa	24(26.96%)	65(73.03%)
Have knowledge about disease	21(23.59%)	68(76.40%)

According to table 3 only two cases (2.24%) were positive for anti-HBc IgM while all patients 89 (100%) were positive for IgG. And the statistical analysis revealed significant differences between them.

Table (3): Comparison between anti-HBcIgM and IgG in studied group.

Parameters	ELISA result No. (%)	No No. (%)	Chi-square p-value
IgM	Positive%	2(2.24%)	170.17 0.01*
	Negative%	87(97.75 %)	
	Total	89(99.99%)	
IgG	Positive%	89 (100%)	
	Negative%	0	
	Total	89(100%)	

* Significant at $p < 0.05$

All cases were negative for anti-HDV -IgM while 5(5.61%) were positive for anti HDV IgG. Statistical analysis show significant differences between them as shown in table (4).

Table (4): Comparison between anti-HDV IgM and IgGin studied group.

Parameters	ELISA result No. (%)	No No. (%)	Chi-square p-value
IgM	Positive%	0(0.00%)	5.145 0.02*
	Negative%	89(100%)	
	Total	89(100%)	
IgG	Positive%	5(5.61%)	
	Negative%	84(94.38%)	
	Total	89(100%)	

* Significant at $p < 0.05$

In table 5 all patients with HDV were males rather than females and the statistical analysis didn't revealed significant differences, also most patients were recorded in age group 35-50 year.

The statistical analysis show highly significant differences with the following parameters urban, married, primary school, unemployed and intermediate economic level.

Table (5): Distribution of studied group according to positive and negative anti-HDV IgG.

Parameters	Positive No. (%)	Negative No. (%)	Chi-square p-value
Gender type			1.84 0.17**
Male	5(7.57%)	61(92.42%)	
Female	0(0.00%)	23(100%)	
Age groups			98.50 0.0001
19-34 year	0(0.00%)	52(100%)	
35-50 year	3(15%)	17(85%)	
51-66 year	2(12.5%)	14(87.5%)	
≥ 66 years	0(0.00%)	1(100%)	
Residence			1.65 0.19**
Urban	4(6.89%)	54(93.1%)	
Rural	1(3.22%)	30(96.7%)	
Marital status			89.33 0.0001
Single	0(0.00%)	11(100%)	
Married	5(6.84%)	68(93.15%)	
Divorced	0(0.00%)	1(100%)	
Widow	0(0.00%)	4(100%	
Education			89.0 0.0001
Illiterate	0(0.00%)	13(100%)	
primary school	2(7.14%)	26(92.85%)	
Secondary school	1(3.22%)	30(96.77%)	
High education	2(11.76%)	15(88.23%)	
Occupation			0.45 0.49**
Employed	2(8.33%)	22(91.66%)	
Un- employed	3(4.61%)	62(95.38%)	
Economic level			112.8 0.000
High	0(0.00%)	8(100%)	
Intermediate	5(6.32%)	74(93.67%)	
low	0(0.00%)	2(100%)	

**Non- Significant at $p < .05$

Based on data show in table 6, there was no significant differences noticed with other risk factor except family history.

Table (6): Risk Factor in studied group according to result of IgG to HDV.

Risk Factor	Yes No. (%)	No No. (%)	Chi-square p-value
Family History Positive Negative Total	 0(0.00%) 31(100%) 31(100%)	 5(8.62%) 53(91.37%) 58(99.99%)	2.83 0.09
Surgical history Positive Negative Total	 2(7.69%) 24(92.30%) 26(99.99%)	 3(4.76%) 60(95.23%) 63(99.99%)	0.29 0.58
Dental history Positive Negative Total	 2(4.00%) 48(96.00%) 50(100%)	 3(7.69%) 36(92.30%) 39(99.99%)	0.56 0.45
Blood transfusion Positive Negative Total	 1(7.69%) 12(92.30%) 13(99.99%)	 4(5.26%) 72(94.73%) 76(99.99%)	0.56 0.65
Smoking habit Positive Negative Total	 2(7.14%) 26(92.85%) 28(99.99%)	 3(4.91%) 58(95.08%) 61(99.99%)	0.17 0.64
Arkilaih smoking habit Positive Negative Total	 0(0.00%) 11(100%) 11(100%)	 5(6.41%) 73(93.58%) 78(99.99%)	0.74 0.38
Drinking alcohol Positive Negative Total	 0(0.00%) 1(100%) 1(100%)	 5(5.68%) 83(94.31%) 88(99.99%)	0.6 0.80
Under to Endoscope Positive Negative Total	 0(0.00%) 5(100%) 5(100%)	 5(5.95%) 79(94.04%) 84(99.99%)	0.31 0.57
Tattoo Positive Negative Total	 0(0.00%) 4(100%) 4(100%)	 5(5.88%) 80(94.11%) 85(99.99%)	0.24 0.61
Hjamaa Positive Negative Total	 2(8.33%) 22(91.66%) 24(99.99%)	 3(4.61%) 62(95.38%) 65(99.99%)	0.45 0.49
Have knowledge about disease Positive Negative Total	 1(4.76%) 20(95.23%) 21(99.99%)	 4(5.88%) 64(94.11%) 68(99.99%)	0.45 0.60

Non- Significant at p <0.05

Discussion

The infection rate of HDV among blood donor admitted to Blood bank in Baquba city was 5.61% is comparable to those of Gomaa *et al* (2013) in Egypt and Uzun *et al* (2014) in Turkey which reported 4.7% and 3.4% respectively. However, it is higher than that reported by Meşe *et al* (2009) in Turkey 1.56%, Attaran *et al*.,(2013) in Iran and Hussain *et al* (2015) in Duhok (2.5%) also among chronic. And it was less than study done in Iran by Alizadeh *et al* (2010) who found that 17.3% of patient had positive result for HDV and Khair *et al* (2016) in Sudan (13.2%). Higher rates (14.29%) had been HDV RNA positive reported in other

study Attaran *et al* (2013). Although the prevalence of HDV infection in our country is not exactly known, it has been introduced that it has decreased in the world. The decrease can be related with the general practices to prevent the transmission of HBV as well as hepatitis B vaccination [17][18].

According to gender it has been found that all positive cases were recorded among males, which is in agreements with study done by Değertekin *et al* (2007) who showed no statistically significant difference was detected between the genders, delta positivity was found more frequently in men than in women [19]. And disagreement with study of Khair *et al* (2016) who found that 7(12.2%) were male, and 6(17.6%) were female. In this case, the risk of transmission of hepatitis can be explained by the high-risk behaviors among men or most blood donation were males in our population. The higher rate of chronic HBV infection among males may be due either to a prolonged replicative phase of the virus in boys or to differences in sexual behavior [20]. Dual infections probably depends on aspects such as the endemicity of HDV in the area, the degree of HBV viremia, and the genotypes of HBV and HDV [21].

Hepatitis D virus infection was higher in age group 51-66 years than others age groups as recorded, this result is relatively comparable with that reported by other studies such as Gomaa *et al* in 2013 who showed that high rate of infection was in age group 29-43 and Karagag in 2014 indicate that HDV infection is mostly a disease of elderly in the region (i.e. 45.63 years; 16-74 years); while it is less prevalent in the younger population. Also another study showed comparable results to our study [11][23][24]. This may be related to the rising incidence with age may be explained by the thymus function is known to decline with age. The thymus reaches its maximal size at puberty and then atrophies, with a significant decrease in both cortical and medullary cells and an increase in the total fat content of the organ. Whereas the average weight of the thymus is 30 grams in human infants, its age-dependent involution leaves an organ with an average weight of only 3 grams in the elderly [25]. Moreover, may be related with decline in the number of NK, which play an important role in early natural surveillance against cancer and infectious disease, a progressive age-related shift in the circulating lymphocyte population from conventional T cells to NK cells [26]. In this study, high average age of patients with HDV positivity may indicate that HDV infection is mostly a disease of elderly in the region, while it is less prevalent in the younger population most probably due to the increasingly effective nation-wide vaccination program followed in the last several decades. Not to detect any positivity in children and teenagers is being considered as a favorable situation for our region.

Concerning the residence, the current study showed that higher number of subject where live in urban, seems to be similar with those participated in other studies such as [27][28]. While in other studies the subjects living in rural areas showed a higher prevalence of HDV antibodies than the urban population. This is consistent with the study of Mumtaz *et al.*, 2005 from Pakistan [29]. Earlier studies conducted in Egypt showed different results. In 1988, El-Zayadi *et al.*, reported the prevalence of delta infection among urban Egyptians sera of 44 HBsAg positive chronic liver disease (CLD)[30].

The results of present study demonstrated high frequency of infection occur in married patients; this result in agreement with other study [24] [31]. These results also could be explained due to main rout of infection is sexual intercourse, also were comparable

with result of study done by Hussain *et al* (2015) who recorded infection among 683 married cases [10]. And agreed with other study that done by Ghaffari-fam *et al* in (2016) showed same result[28].

In economic level, the result of current study which revealed that most of the infections with HBV and all infections with HDV were in patients with intermediate economic level, these results disagree with a study done by Al-alwan *et al* in 1986, Hussein in 2008 [32][33].

Regarding to the educational levels in the percent work we divided the patients into four categories but statistical analysis not revealed significant differences between each groups, that mean each patients has the same chance for infection, or may be related to the limited sample size.

Based on occupation the result showed that most of the infections was recorded in non-employed and this result agree with study presented by Ghaffari-fam *et al* in (2016)[27].

According to risk factor the data obtained from our results indicated that most patients had dental history and surgical history, which agrees with similar study that has reported increased among patients with dental history, include Nawfal *et al* in 2015 [10]. And Elah *et al* in 2011[32]. In patients with surgical history also the percentage similar to the patients with dental history and this comparable to result of study done by Hussien *et al* in 2015 [10]. This may be related with contaminated tools used or less efficiently of disinfection.

This study delineates the effect of infection with smoking habit on the blood donor, data obtained from our results indicated that most patients were non-smoker.

Our results revealed that there was no patients that had history of tattoing and this result disagree with a study done by Elah *et al* (2011) [34]. And this may be related with country concepts.

Small number of patients had family history of infection and this agree with study performed by Hussien *et al* (2015) who show small percent of patients with family history[10]. This could be related with limited sample size.

In conclusion, infection rate of HDV in Baqubah city was comparable with that found elsewhere in Iraq. All infections with HDV were occurring in males and in the age group of 35-50 year. Further investigation is needed with large sample size to clarify this issue and great attention should be paid for diagnosis and treatment of blood donor. Study the role of other viruses among this group of population.

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