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Original Research Article

Seroepidemiology of Hepatitis B and Hepatitis C virus infections in Diyala province: A population based survey

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A B S T R A C T

Acute and chronic hepatitis is highly prevalent and create a substantial burden to health care systems globally. The World Health Organization (WHO) estimates that over 350 and 250 million people worldwide are chronic carriers of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection respectively. This study was arranged to determine the seroprevalence of HBV and HCV viral infection among Diyala general population, and to figure out their relevance with socio-demographic factors. The present cross-sectional population- based survey was conducted in Diyala province for the period from 1st.Jun 2013 to 30th. March 2014. The overall sample size was 2400 individuals covering the all 6 administrative districts in Diyala governorate. The sample size in each administrative district was based on population proportion of all population in the governorate. Venus blood samples were collected from each participant. Sera were separated and submitted to HBV and HCV markers including HBsAg, anti-HBc total and IgM antibodies as well as anti-HCV antibodies using the third generation of enzyme immunoassays. All data were statistically analyzed using the Epi-info program and P value less than 0.05 were considered significant. The results revealed that the HBsAg positivity rate among Diyala general population was 0.65% with 2:1 male: female ratio, and significantly higher prevalence among those ≥ 45years old. The positivity rate of anti-HBc total and IgM antibodies was 9.56% and 3.67% respectively, with a significantly higher rate among the age group 5-14 years old. All HB viral markers were significantly higher in Baquba district. One of the participants (0.043%) was positive to anti-HCV antibodies. The prevalence of HBsAg and anti-HCV antibodies among Diyala general population is low, but the prevalence of anti-HBc total and IgM antibodies is high particularly among younger age groups.

Keywords

HBV infection, HCV infection, Diyala

Introduction

Chronic viral hepatitis is highly prevalent and creates a substantial burden to health care system globally. The WHO estimates that over 350 and 250 million people

worldwide are chronic carrier of HBV and HCV infection respectively (Ott *et al*., 2012; Te and Jensen, 2010). Both HBV and HCV infections represent a worldwide epidemic causes significant morbidity and mortality accounting for approximately 1 million deaths each year due to their sequelae of acute and chronic liver diseases including primary hepatocellular carcinoma (Lavanchy, 2008; Nebbia *et al*., 2012; Huang *et al*., 2011). In Eastern Mediterranean region, viral hepatitis has emerged as a leading public health concern and continues to be major disease burden. According to WHO estimates, approximately 4.3 million persons are infected with HBV each year (Aljarbou, 2013; Fallahian and Najafi, 2011).

Currently, 10 HBV genotypes have been identified (A-J), and shown to have a distinct geographic distribution with genotypes B and C prevailed in Asia. High prevalence of HBV mutants with various forms, such as the pre-S mutant, basal core promoter mutant, YMDD motif mutant and vaccine escape mutant were identified in Asia and these were found to be related to the severity of liver disease and sensitivity to therapy. HCV, on the other hand, has six genotypes and several subtypes showing important epidemiological and clinical implications (Irshad *et al*., 2010; Araujo *et al*., 2011; Liu and Koa, 2013).

In Iraq, the prevalence of HBV infection was ranged between 0.6–6.5%, while the prevalence of HCV infection among the general population was ranged between 0.5–0.9 and among blood donors between 0.07–0.7 as reported from different Iraqi governorates (Ataallah *et al.*, 2011; Kadir *et al*., 2001; Mahmood *et al*., 2001; Mohammed, 1997). In Diyala province, the prevalence of HBsAg among blood donors for the period from 1989–2000 was 1.5% (Hasan *et al*., 2006). In another study conducted for the period from 2003-2008, the prevalence of HBsAg and anti-HCV antibody was 3.9/1000 population and 1.6/1000 population respectively (Al-Jobouri *et al*., 2010). The prevalence of anti-HCV antibody among blood donors was 0.15 (Hasan, 2008). In 2012, the prevalence of HBsAg, anti-HBc IgM and HBV DNA, as detected by polymerase Chain Reaction (PCR) were 4.3%, 3.2% and 8.1% respectively (Hasan, 2013).

Materials and Methods

Study population

The current cross-sectional population-based survey was conducted in Diyala governorate for the period from 1st. Jun 2013 to 30th. March 2014. The study plan in details was approved by the Iraqi Ministry of Health in advance. The overall sample size was determined by Diyala Directorate of Statistics to be 2400 individuals based on the governorate census in 2012 which was (1,477,648) population. The administrative division of the governorate into 6 districts was adopted. These districts include; Baquba, Mukdadiah, Khalis, Baladruz, Khanaqin, and Munsuriah. The sample size in each administrative district was based on population proportion. The sample size in each district was set ahead of the survey and recruitment continued until the sampling teams fulfilled the required sample size. Those people enrolled from each district were selected randomly. Teams of medical doctors and nurses established the sampling and collected the information required in the demographic and epidemiological data questionnaire form which was previously constructed for this purpose. The questionnaire sought information about age, sex, residential address, tattooing, history of major invasive interventions, blood

transfusion, renal dialysis, presence of chronic diseases or previous complain of jaundice, household contact of HBV or HCV positive cases, and visiting of unqualified nursing private clinics. Those participants of younger age were helped by their parents in the presence of medical doctor and social assistants.

Laboratory tests

A whole blood sample of 4–5 milliliters was aseptically aspirated through vein puncture. Blood samples were collected in plan disposable tubes without anticoagulant, and transported as soon as possible to the local laboratory within the same region. Sera were separated by centrifugation and then stored at -20^{0}C (deep freezer) until delivered by cool boxes to the Baquba Public Health Laboratory for testing. 127 samples were excluded from the study due to uncompleted data form. All other samples were tested for HBsAg (Acon-USA), anti-HBc total (Biokit-Spain), anti-HBc IgM (CTK-USA), and anti-HCV antibody (Dialab-Austria) by using a third generation enzyme linked immunosorbant assays. Samples that were repeatedly positive for any viral markers were sent to the Central Public Health Laboratory (Reference Laboratory) in Baghdad for confirmatory tests.

Statistical analyses

Collected data and the results of the laboratory tests were translated into computerized data base. Statistically analyses were done using the Epi-info program, and P values < 0.05 were considered significant.

Ethical considerations

The study plan was approved in advance by the Iraqi Ministry of Health and directly supervised by the Diyala Directorate of Health, Department of Public Health and Primary Health care. All participants signed an informed consent form witnessed by the local health office before collection of data and blood samples.

Results and Discussion

The results revealed that out of 3373 individuals enrolled as study population, 15 (0.65%) were HBsAg positive, while the remaining 2258 (99.34%) were HBsAg negative. Table 1 and 2 showed the HBsAg positivity rate in relation to the gender. The rate was higher in males as compared to females (66.7% vs. 33.3%). However, the difference was failed to reach the level of statistical significance (χ^2 =0.224, *P*= 0.636). Furthermore, of the 15 HBsAg positive people, 10(66.7%) were married and 5 (33.3%) were single.

The distribution of HBsAg positive cases according to Diyala governorate districts, it was revealed that Baquba district harbor the highest infection rate compared to other districts, Table 3.

The results also revealed that the risk factors of the 15 HBsAg positive people were as follows: 4 (27%) underwent cupping, 3 (20%) had tattoo, 2 (13%) had major surgical intervention, 2 (13%) got blood transfusion, 2 (13%) were household contact of positive cases, 2 (13%) had concomitant chronic disease, 1(7%) underwent renal dialysis, 3 (20%) had previous history of jaundice, 9 (60%) attend unqualified nurses, and 5 (33%) had dental invasive intervention.

The 2258 serum samples which were negative for HBsAg were further submitted to anti-HBc total and IgM tests. The results showed that 216 (9.56%) and 83 (3.67%)

were positive for anti-HBc total and anti-HBc IgM respectively. Table 4 showed the distribution of anti-HBc total positivity rate according to age groups, which was found to be significantly higher in the 5-14 years old group (χ^2 = 26.79, *P*< 0.001).

The distribution of anti-HBc total antibody positivity rate according to the administrative districts revealed that Baquba district has significantly higher rate compared to other governorate districts (χ^2 = 6.196, *P*< 0.05) (Table 5).

On the other hand, the distribution of anti-HBc IgM antibody according to district showed insignificant differences (Table 6).

Table 7 revealed the impact of risky practices on the distribution of anti-HBc total antibody positivity rate. It appears that the positivity rate was significantly higher among those people who had dental invasive interventions (χ^2 = 7.715, *P*= 0.005), and those who attend unqualified nurses (χ^2 = 8.389, *P*< 0.004).

The impact of risk factors on the distribution of anti-HBc IgM was revealed in Table 8. These results revealed that anti-HBcIgM positivity rate was significantly higher among those people with major surgical intervention (χ^2 = 17.15, *P*= 0.00003), dental invasive intervention (χ^2 = 12.33, *P*= 0.005), attendance of unqualified nurse private clinic (χ^2 = 13.79, *P*= 0.0002), and those perform cupping (χ^2 = 4.63, *P*= 0.031), Furthermore, it is higher in people with tattoo and those people with previous history of jaundice, but the differences were failed to reach the levels of statistical significance.

Concerning the anti-HCV, the results showed that only one person was positive, recording a prevalence of 0.043% among the study population.

The importance of the present study may emerge from the fact that both HBV and HCV infections represent a worldwide epidemic. Iraq was ranked in the intermediate zone of endemicity like other East Mediterranean countries (Ott *et al*., 2012). The implementation of universal HB vaccination during early infancy should decline the prevalence of HBV infection, but unfortunately no country-based survey to determine the effectiveness of vaccine was conducted. A comprehensive public health prevention program should include the conducting population-based surveillance and monitoring the effectiveness of prevention activities and setup a research agenda. For the best of knowledge, this is definitely the first population study in Diyala province to determine the prevalence of HBV and HCV infection, since all previous studies were conducted on blood donors or other risky groups, and usually on limited sample size (Hasan, 2006; Al-Jobouri *et al*., 2010; Hasan, 2008).

The results revealed that the overall prevalence of HBsAg in Diyala general population was 0.65%. Comparing with studies conducted on blood donors, the present result is nearly similar to that reported by Al-Jobouri *et al*. (2010) in Diyala and Attaallah *et al*. (2011) in Baghdad. However, it is lower than that reported before the universal HB vaccine era (Kadir *et al*., 2001; Mahmood *et al*., 2001; Mohammed, 1997; Hasan *et al*., 2006). This remarkable decline in the prevalence of HBsAg in Diyala population is undoubtedly related to the introduction of compulsory HB vaccine in the Iraqi Expanded Program of Immunization since 1994, beside the general improvement in community education and awareness of the disease. Similar results were reported from other East Mediterranean, as well as worldwide countries (Te and Jensen, 2010; Lavanchy,

2008; Aljarbou, 2013; Baha *et al*., 2013; Romero *et al*., 2011; Janahi, 2014; El-Raziky *et al*., 2012; Rantala and van de Laar, 2008).

The significantly higher prevalence of HBsAg among males obtained in this study is consistent with most of previous studies in Iraq and other countries (Ataallah *et al*., 2011; Hasan *et al*., 2006; Al-Jobouri *et al*., 2010; Baha *et al*., 2013; Li *et al*., 2012; Khan *et al*., 2011). On the other hand, the significantly higher prevalence of HBsAg among those people with ≥ 45 years old is inconsistent with previous studies conducted in Diyala province before the vaccination era, during which a higher prevalence was reported among 16-29 years old.

However, it is in agreement with most studies carried out after that which documented a decline in HBsAg among lower ages, but increased in older ages (Baha *et al*.,2013; Janahi,2014; Gogos *et al*.,2003; Gheorghe *et al*.,2013). The most acceptable explanation is that as the vaccination program is strictly targeting infants in the first week of life, therefore the prevalence of HBsAg was declined during the childhood and young adulthood, but it is sustained in the older ages. In this regard, it has been reported that vaccination in infancy provides protection for > 20 years (Lavanchy, 2008; Huang *et al*., 2011; Romero *et al*., 2011; Ioannou, 2011).

What are the fascinating results obtained in the current study? are the high prevalence of anti-HBc total and anti-HBc IgM antibodies among people who tested negative for HBsAg, particularly among those 5-14 years old in whom the prevalence was significantly higher compared to other age groups. The question is they had occult hepatitis B infection? In 2008, the European Association for the Study of the Liver (EASL) defined occult hepatitis B virus infection (OBI) as the presence of hepatitis B virus (HBV) DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals testing HBsAg negative (Ocana *et al*., 2011). It has been documented that the prevalence of OBI is quite variable depending on the level of endemic disease in different parts of the world, the different assays utilized, and the different populations studied (Romero *et al*., 2011; Assar *et al*., 2012; Gutierrez-Garcia *et al*., 2011).

Multiple worldwide studies had documented the presence of OBI among general population as well as the blood donors and other risky groups (Emara, 2012; Muselmani *et al*., 2013; Asim *et al*., 2010; Bhatti *et al*., 2007). In Diyala province, we previously reported that 8.1% of HBsAg negative blood donors were positive for HBV DNA by polymerase chain reaction (Hasan, 2012). Taken together and according to the definition of OBI, this study assumes that the prevalence of OBI in Diyala general population is high. Upon reviewing the literature, several studies had reported similar results, affirming that the reason behind this occult or silent HBV infection was the emergence and/or selection of immune escape HBV mutants that enable viral persistence in spite of adequate antibody titers, and suggesting that new preventative strategies are needed to prevent these strains from becoming dominant during the next decennia (El-Raziky *et al*., 2012; Cooreman *et al*., 2001; Tabor, 2006: Lai *et al*., 2012; Gerlich *et al*., 2010).

In blood transfusion services, therefore an understanding of the prevalence of OBI among blood donors is a critical strategy.

Table.1 HBsAg positivity rate of the study population by gender

Gender	No. tested (%)	HBsAg positive No. (%)	χ^2	*P* value
Male	1380 (60.7)	10 (66.7)	0.224	0.636 [NS]
Female	893 (39.3)	5 (33.3)		
Total	2273 (100)	15 (100)		

Table.2 HBsAg positivity rate of the study population by gender

Gender	No. tested (%)	HBsAg positive No. (%)	χ^2	*P* value
< 5	9 (0.4)	0 (0)	4.7	0.030 [S]
5-14	265 (11.7)	1 (0.4)		
15-44	1473 (64.8)	7 (0.5)		
≥ 45	526 (23.1)	7 (1.3)		
Total	2273 (100)	15 (0.65)		

Table.3 HBsAg positivity rate of the study population by district

District	No. tested (%)	No. (positivity rate of district)	No. (positivity rate of total cases)
Baquba	720 (31.6)	7 (0.97)	7 (46.7)
Khalis	380 (16.7)	3 (0.78)	3 (20.0)
Mukdadiah	377 (16.6)	2 (0.53)	2 (13.3)
Khanaqien	488 (21.5)	3 (0.61)	3 (20.0)
Baladruz	214 (9.4)	0 (0)	0 (0)
Mansuriah	94 (4.1)	0 (0)	0 (0)
Total	2273 (100)	15 (0.65)	15 (100)

Table.4 Anti-HBC total positivity rate of study population by age groups

Age (Ys)	No. tested	Anti-HBc total positive (%)	No. (positivity rate of total)	No. (positivity rate of + cases)	χ^2 (*p* value)
< 5	9	0 (0)	0 (0)	0 (0)	**
5-14	264	2 (0.8)	2 (0.088)	2 (0.92)	26.7 (<0.001)
15-44	1466	147 (10.0)	147 (6.51)	147 (68.05)	1.02(0.312)
≥ 45	519	67 (13.0)	67 (2.96)	67 (31.01)	3.19(0.073)
Total	2258	216 (9.56)	216 (9.56)	216 (100)	

Table.5 Anti-HBC total positivity rate of the study population by district

District	No tested	No. positive (% of district)	No. positive (% of total)	No. positive (% 0f + cases)	χ^2 (*P* value)
Baquba	713	71 (9.95)	71 (3.14)	71 (31.87)	6.19 (0.021)
Khalis	377	32 (8.48)	32 (1.41)	32 (14.81)	0.60 (0.435)
Mukdadiah	375	44 (11.73)	44 (1.94)	44 (20.37)	2.44 (0.118)
Khanaqien	485	47 (9.69)	47 (2.08)	47 (21.75)	0.011 (0.916)
Baladruz	214	15 (7.0)	15 (0.66)	15 (6.94)	1.78 (0.181)
Mansuriah	94	7 (7.44)	7 (0.31)	7 (3.24)	0.50 (0.475)
Total	2258	216 (9.56)	216 (6.56)	216 (100)	

Table.6 Anti-HBC IgM positivity rate of the study population by district

District	No tested	No. positive (% of district)	No. positive (% of total)	No. positive (% 0f + cases)	χ^2 (*P* value)
Baquba	713	26 (3.64)	26 (1.15)	26 (31.32)	7.33 (0.483)
Khalis	377	12 (3.18)	12 (0.53)	12 (14.45)	0.013 (0.908)
Mukdadiah	375	18 (4.8)	18 (0.79)	18 (21.68)	0.144 (0.703)
Khanaqien	485	21 (4.32)	21 (0.93)	21 (25.30)	0.991 (0.318)
Baladruz	214	6 92.80)	6 (0.26)	6 (7.22)	0.017 (0.895)
Mansuriah	94	0 (0)	0 (0)	0(0)	0 (0)
Total	2258	83(3.67)	83 (3.67)	83 (100)	

Table.7 Distribution of anti-HBc total antibody according to risk factors

Risk factor	No. tested	No. HBc total + (%)	No. HBc total + (% of total)	No, HBc total + (% of + cases)	χ^2 (*P* value)
Surgical intervention	386	33 (8.54)	33 (1.46)	33 (15.27)	0.5 (0.45)
Renal dialysis	6	1 (0.04)	1 (0.044)	1 (0.46)	0.3 (0.55)
Blood transfusion	97	7 (0.31)	7 (0.310)	7 (3.24)	0.6 (0.42)
Dental intervention	594	84 (3.72)	84 (3.72)	84 (38.88)	19.4 (0.0001)*
Unqualified nurses	1200	135 (11.25)	135 (11.25)	135 (62.5)	8.38 (0.004)*
Tattooing	174	27 (15.51)	27 (1.19)	27 (12.5)	7.71 (0.005)*
Cupping	308	37 (12.01)	37 (1.63)	37 (17.12)	2.46 (0.11)
Household contact	17	3 (17.64)	3 (0.132)	3 (1.38)	1.29 (0.25)
History of jaundice	115	14 (12.17)	14 (0.620)	1 (0.46)	0.95 (0.32)
Coexistane chronic disease	36	3 (8.33)	3 (0.132)	3 (1.38)	0.06(0.80)

*Significant

Table.8 Distribution of anti-HBc IgM antibody according to risk factors

Risk factor	No. tested	No. HBc IgM + (%)	No. HBc IgM + (% of total)	No, HBc IgM + (% of + cases)	χ^2 (*P* value)
Surgical intervention	386	2 (0.51)	2(0.08)	2 (2.40)	17.15(0.0003)*
Renal dialysis	6	1 (16.6)	1(0.04)	1 (1.20)	516 (4725)
Blood transfusion	97	1 (1.03)	1(0.04)	1 (1.20)	1.76 (0.18)
Dental intervention	594	20 (3.36)	20 (0.88)	20 (24..09)	12.3 (0.005)*
Unqualified nurses	1200	39 (3.25)	39 (1.72)	39 (46.98)	13.7 (0.0002)*
Tattooing	174	18 (10.34)	18 (0.79)	18 (21.68)	3.81 (0.050)
Cupping	308	16 (5.19)	16 (0.70)	16 (19.27)	4.63 (0.031) *
Household contact	17	1 (5.88)	1(0.04)	1 (1.20)	0.032 (0.85)
History of jaundice	115	2 (1.73)	2(0.08)	2 (2.40)	3.66 (0.055)

*Significant

In this regard , multiple studies had affirm the high potential risk for transmission of OBI through blood transfusion inducing post transfusion hepatitis, liver cirrhosis and hepatocellular carcinoma (Romero *et al*., 2011; Assar *et al*., 2012; Raimondo *et al*., 2013). Therefore, HBV DNA amplification and/or HBV anti core antibodies screening should be implemented to eliminate risk of unsafe blood donation (Asim *et al*., 2010; Bhatti *et al*., 2007; Cooreman *et al*., 2001; Said *et al*., 2013). Nevertheless, further molecular studies are recommended to addresses this dilemma in our country.

Regarding the risk factors, the results found that the anti-HBc total and anti-HBC IgM antibodies were significantly associated with surgical and dental invasive interventions. These results are in agreement with similar results obtained in other studies, especially in developing countries (Janahi *et al*., 2014; Li *et al*., 2012; Gheorghe *et al*., 2013; Al-Waleedi and Khader, 2012; Mahboobi *et al* .,2013; Radcliffe *et al*., 2013). The source of infection most probably comes from either contaminated surgical utensils or infected healthcare workers (Spijkerman *et al* ., 2002; Enfield *et al*., 2013). It is worth to draw the attention that high prevalence of HBV infection was documented among healthcare workers in Diyala province (Hasan, 2003). Therefore, continuous vaccination of healthcare workers in Diyala healthcare settings and precaution guidelines are recommended (Askarian *et al*., 2011).

Cupping and tattoo are also appeared as risk factors. These social customs as popular remedies were reappeared and flourished during the last years, and usually practiced by unqualified persons away from the supervision of medical authorities. Similar results were reported by other studies (Gheorghe *et al*., 2013; Al-Waleedi and Khader, 2012). Another unsafe practice that may hasten blood transmitted viruses in our society is the visiting private clinics of unqualified non-authorized paramedical staff such as nurses for minor surgical interventions, midwives for home delivery, and circumcisioners for home circumcision. In this regard, our results are in agreement with other studies (Askarian *et al*., 2011; Ashraf *et al*., 2010). It is important to mention that certain rural areas in Diyala

province had witnessed the occurrence of an outbreak of HBV infection due to these primitive unsafe practices (Hasan, 2004).

A part from health care setting, the intrafamilial transmission of HBV is another risk factor that may contribute to a wide spread of the virus among household contacts, especially in extended families, a feature that is previously documented in Diyala society (Hasan, 2005). Several worldwide studies had reported similar results (Bawazir *et al*., 2011; Demirturk and Demirdal, 2014; Urganci *et al*., 2013).

The current study concluded that although the prevalence of HBV and HCV viral infections are low among the general population in Diyala province, the prevalence of HBV anti-core antibodies are high. Renewing of current preventive strategy, particularly in blood transfusion services is recommended.

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