Seroprevalence of Hepatitis B and C among Thalassemic, Haemophilic patients in Babylon Governorate-Iraq

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Abstract

A randomly selected of 60 haemphilic, and 56 thalassemic patients from Babylon Governorate – Iraq were studied during the period from November (2011) to June (2012).A total of (116) blood samples were collected from (71 were males) and (45 were females) with a mean age of (24.5±7.1 years) and tested for anti- HBsAg and anti-HCV IgG antibody by ELISA test .The anti-HBsAg reactivity was confirmed by enzyme –linked fluorescent immunoassay (ELFA) that is performed in the automated VIDAS system while the anti-HCV reactivity was confirmed by Cobas apparatus and the viral load was measured by RT-PCR .The study showed that there are no anti-HBs Ag seropositive cases in both haemophilia and thalassemia groups while the ratio of seropositivty cases of anti – HCV Ab in hemophilia patients were (6.67%) in which (10.52%)were infected males while (4.5%) were infected females. In thalassemia the anti-HCV prevalence were (25%) in which (27.27%) were males while (26.0%) were females. The study showed that there is a higher rate of HCV infections in rural residence than in urban areas. Also HCV had more incidences in people with low economic status, low educational level, and it was more in smokers than in non smokers. The results of RT-PCR and ELISA technique were not compatible for (anti-HCV IgG).

الخلاصه

تم إجراء دراسة عشوائيه شملت من ٦٠ مريض من مرضى نزف الدم الوراثي و ٥٦ مريض من مرضى فقر الدم الابيض المتوسط في محافظة بابل للفترة من اول تشرين الثاني٢٠١١ إلى اخر حزيران ٢٠١٢ حيث تم اختيار (١١٦ عينه دم) وتم اخذ عينه دم عينه دم واحده من كل مريض، (٧١) من الذكور(٦١,٢١%) و(٤٥) من الاناث (٣٨,٧٩ %) . تم فحص جميع المتبرعين للتأكد من وجود إصابات التهاب الكبد الفايروسي نوع (B) و(C) بواسطه فحص الاليزا. تفاعليه anti–HBsAg اكدت بواسطه نظام VIDASالالي بينما اكدت تفاعليه anti–HCV بواسطه جهاز ال Cobas بينما تم قياس الحمل الفايروسي بوسطه RT–PCR

بينت الدراسه انه لا يوجد حالات تفاعل مصلي موجب ل anti–HBsAg في كلا من مجموعتي من مرضى نزف الدم الوراثي من مرضى فقر الدم الابيض بينما نسبه التفاعل المصلي الموجب ل,anti–HCV Ab (٦,٦٧%) وفيها نسبه (١٠.٥٢%) ذكور بينما(٤.٥%) كانت الاناث في مجموعه مرضى نزف الدم الوراثي .اما في مجموعه فقر دم البحر الابيض المتوسط فان نسبه التفاعل المصلي الموجب لanti–HCV Ab (٢٥%) وفيها نسبه (٢٧,٢٧%) ذكور بينما(٢٦%) كانت الاناث.كما بينت الدراسه ان معدل انتشار التهاب الكبد الفايروسي نوع (C) لكلاالمجموعتين كان اعلىعند الاشخاص ذوي الحاله المعيشيه والمستوى التعليمي المتدنين وان الاصابه باالمناطق الريفيه اكثر انتشارا من المدن..واخيرا ان عيار(anti–HCV IgG) المقاس بواسطه الاليزا لم يكن باالضروره متوافقا مع الحمل الفايروسي للنوع ج والمقاس بواسطه فحص (RT–PCR)

Introduction

An increasing number of hepatotropic viruses have been identified and may be acquired either from faecal contamination (hepatitis A and E) or parenterally via body fluids (hepatitis B, C, D and G)[1-3].Among human hepatitis viruses, hepatitis B (HBV) and C (HCV) are able to persist in the host

for years [4]. Chronic infections caused by HBV and/or (HCV) are the main possible inducing factors for the development of hepatocellular carcinoma (HCC) in humans and both viruses cause a wide spectrum of clinical manifestations ranging from healthy carrier state to acute and chronic hepatitis, liver cirrhosis, and HCC. Hepatitis B and C belong to different viral families (Hepadnoviridae and Flaviviridae, respectively); they are characterized by different genetic structures and the clinical manifestations were the results of these viral infections to host hepatocytes (i.e. between viral and cell genomes). Proteins encoded by both viruses play an important role in processes responsible for immortalization and transformation of these cells. Chronic inflammation determined by host immune response to the viral infection, hepatocyte death and their compensatory proliferation, as well as modulation of expression of some regulatory proteins of the cell (growth factors, cytokines, etc.) are the processes that play the major role in liver cancer induced by HBV and HCV [5]

Patients presenting clinical signs and symptoms of hepatitis are typically tested with an acute panel that detects hepatitis B surface antigen (HBsAg), antibody to hepatitis B core-immunoglobulin M (HBc IgM) antibody to HCV (Anti-HCV) and hepatitis A IgM (HAV IgM) to rule out or rule in acute viral hepatitis. Molecular tests to determine viral load can be used to monitor infectiousness and treatment efficacy for HBV and HCV. Also determination of genotype for HBV or HCV can be instrumental in deciding the appropriate type and duration of antiviral therapy [6]. Hemophilia is the most common inherited congenital bleeding disorder and it is estimated to affect between 1 to 5 individuals per 50,000 male population [7]. All multipltransfused patients who received clotting-factor concentrate before 1985 and/or blood transfusions before 1992 are infected with hepatitis C and almost all of them have tested positive for the HCV antibody [8].The infection rate was 36.03% in Pakistan and it was estimated at 55.70% for Tunisia. While the infection rate in Saudi Arabia is reported to be 78.6% [9]. Repeated blood transfusion in thalassemia patients is necessary for their survival; however, such transfusions increase their exposure only to not HCV but also to other blood-borne viruses like hepatitis B, hepatitis G, human immunodeficiency virus, transfusion transmitted virus [10]. Polytransfused thalassemics are still at great risk of infection with HCV virus. This picture accordance with result of [11] in Iraq.

The Aims of this Study

1. Assess the status of viral hepatitis B and C in thalassemic,haemophlic patients in Babylon province through the following technique

a.ELISA technique
b.Minividas technique
c.Cobas technique
d. Real Time-PCR technique

2. Assess prevalence of HBV&HCV in:

a. patient with Different age groups range from ≤10 to ≥40 years
b. Human population for both sexes.
c.Different risk groups include : haemophilia and thalassemia associated with hepatitis.

Patient and Methods

A total of (56) thalasemics patients (age range <10- >30, mean age

19.11 years)were admitted into Babylon Hospital /laboratory department/ were tested. There were (38) males and (22) females. Also a total of (60) haemophilic patients (age range10->40, mean age 20.68 years) were admitted into from Babylon hospital /laboratory department/ were tested; there were (33) males and (23) female. The blood samples were collected for research investigation on random basis .Three to five ml of venous blood were collected from each patients .Of which, one ml was mixed with anticoagulant (EDTA) for plasma collection. The rest was used for sera collection which was separated by centrifuge at 1000 rpm for 10 minute and then the collected serum(supernatants) were frozen at -20 until used for the required test. For determine anti-HBsAg, anti-HCV IgG the third-generation of enzyme immunoassay kits (EIA-3),Acon–USA).The anti-HBsAg reactivity was confirmed by enzyme–linked fluorescent immunoassay (ELFA) that was performed in the automated VIDAS system (Minividas Kit/HBsAg, Biomerieux-france) while the anti-HCV reactivity was confirmed by (Cobas Kit/Anti –HCV, Roche-USA).The viral load was measured by RT-PCR using Exiprep^{TM} viral DNA/RNA kit (Bioneer-Koria) for extraction of the DNA of the two viruses and RT-PCR Amplification kit (Sacace-Italia) was used to amplification.

Results

The Anti-HCV prevalence among haemophilic patients was 6.67% (4:60) and the age group (31-40) had the higher ratio of infection. From this data it appears that haemophilics are of great risk of infection with HCV .These results are in accordance with other study (12) in Iraq .According to the sex ; male was significantly had higher rate of infection than female(x^2 $^{=}$2.11, FD=1, P>0.05) .Table(1) indicated that:

Table 1 The prevalence of anti-HCV IgG in hemophilia patients

Age groups of patients		Male		Female		Total	
	No. of patients	Anti-HCV Abs+ No (%)		No. of patients	Anti- HCV Abs+ No. (%)	No. test of patients	Anti-HCV Abs+ No (%)
10_20	13	1	(5.00)	7	0 (0 .0)	20	1 (5.00)
21_30	11	1	(7.14)	3	0 (0.0)	14	1 (7.14)
31_40	7	2	(11.76)	10	1 (10)	17	2 (11.76)
>40	7	0	(0.0)	2	0 (0.0)	9	0 (0.0)
Tolal No.	38	4	(10.52)	22	1 (4.5)	60	4 (6.67)

Table 2 The test results of anti-HCV IgG in haemophilia patients

Age groups of patients	No. of patients	Anti-HCV Abs+	ELISA			Cobas Value	RT-PCR Viral load/ML
			Mean of O.D of Negative control	Mean of O.D of Positive control	Mean of O.D of Patient titer		
10_20	20	1	0.03	0.006	1.02	9.91	$5.34*10^5$
21_30	14	1	0.05	0.004	1.70	10.52	$7.23*10^5$
31_40	17	2	0.10	0.03	1.96	12.01	$2.11*10^6$
>40	9	0	0	0	0	0	0

Distribution of anti-HCV in thalassemics group

The Anti-HCV IgG prevalence among thalassemic patients was (25%) (16:56). No difference was observed in the prevalence of HCV infection according to sex (x^2 =0.02 DF=1 , P>0.05).The age of patients rang from >10 to< 30 and there are no difference was observed in the prevalence of HCV infection according to age group as indicated in table (3);

Table 3 The anti-HCV IgG in thalassemics group according to age and sex

Age groups of patients	Male		Female		Total	
	No.of patients	HCV Abs+ No. %	No. of patients	HCV Abs+ No. (%)	No. of patients	HCV Abs+ No (%)
<10	9	2 22.22	4	1 (25.00)	13	3 (23.07)
20_11	7	1 14.28	7	1 (14.28)	14	2 (14.29)
30_21	11	3 27.27	6	2 (33.33)	17	5 (29.41)
>30	6	2 33.33	6	2 (33.33)	12	4 (33.33)
Total	33	8 24.24	23	6 (26.00)	56	14 (25.00)

Table 4 Result tests of anti-HCV IgG in the thalassemics group

Age groups of patients	Anti-HCV Abs+	ELISA			Cobas value	PCR viral load /ML
		Mean of O.D of Negative control	Mean of O.D of Positive control	Mean of O.D of Patients samples		
≤10	3	0.007	1.072	1.410	10.1	$1.9*10^5$
11-20	2	0.050	1.264	1.960	10.8	$2.01*10^5$
21-30	5	0.004	0.997	2.360	12.07	$26.12*10^4$
>30	2	0.031	1.322	2.697	16.67	$3.9*10^5$

Table 5 Distribution of hepatitis C infections according to residence

Residency	Hepatitis C virus No. (%)
Rural	6 (33.4)
Urban	12 (66.7)
Total	18 (100)

Table 6 Distribution hepatitis C infection according to economic status:

Economic status	No. of cases (%)
Good	2 (11.11)
medium	4 (22.22)
low	12 (66.67)
Total	18 (100)

Table 7 Distribution of hepatitis C infection according to educational levels:

Educational level	No. of cases (%)
high	3 (16.7)
low	15 (83.3)
Total	18 (100)

Important risk factors contributing to HCV spreading includes barber risk*;* blood transfusion; history of injection with disposable syringes; reuse of syringes, dental risk ,surgical procedure ; history of contact with acute cases of acute hepatitis and smoking as relived in table- 8

Table 8 Major risk factors for transmission of HCV infection in risk patients

Risk Factors	Anti-HCV IgG+ve No. (%)
Barber risk	3 (12)
Blood transfusion	8 (19)
History of injection with disposable syringes	12 (20)
Reuse of syringes	0 (0.0)
Dental risk	3 (7.5)
Surgical procedure	2 (5.0)
History of contact with acute cases of acute hepatitis	7 (17.5)
Smoking risk	18 (45.0)

All above tested blood samples blood samples were negative for anti-HBsAg

Discussion

Hepatitis C virus infection and age: Table-1 indicates that the prevalence of anti-HCV-IgG in haemophila groups was (6.67%) and the highest frequency of infection was found in the age (31-40) with positivity percentage (11.76%) followed by (7.14%) in the age (21-30) while the positivity percentage in the group (10-20) was (5.00%).The rate of infection was increase gradually with age. This difference was statistically not significant (x^2=4.07, DF=1, p>0.05).The result are compatible with other studies like [13].Table-2 indicated that the higher mean of optical density of patient samples of anti-HCV IgG infection by ELISA test was in the age group in (31-40) then (21-30) and finally the age group (10-20).The statistical analysis indicated that there are significant difference between O.D of patients sample compared to the mean of O.D of negative control ($LSD_{0.05}$=0.86) and that referred to actual infection with the virus as revealed in figure-1

Figure 1 The status of anti- HCV in different age groups of hemophilia by ELISA test

Table-3 indicates that the prevalence of anti-HCV-IgG in thalassemic patients was (25.00%) and the highest frequency of infection was found in the age (>30) with positivity percentage (33.33%) followed by (29.41%)in the age (21-30),(23.07 %) in the age group (<10) and finally (14.29 %) in the age group (11-20).This difference was statistically not significant(x^2=0.02 DF=1,P> 0.05).

Table-4 indicated that the higher mean of O.D of patient samples of anti-HCV IgG infection by ELISA test was in the age group in (>30) then (21-30) and finally the age group (10-20)**.**The statistical analysis indicated that there are significant difference between mean of O.D of patients sample compared to the mean of O.D of negative control ($LSD_{0.05}$=098) as indicated in figure-2.

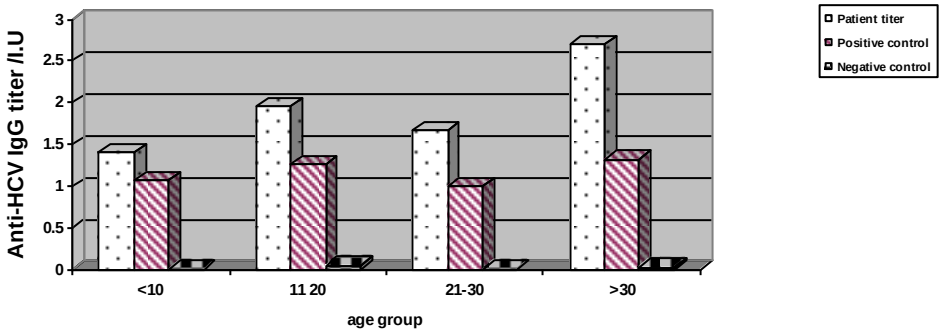

Figure 2 The status of anti- HCV in different age groups of thalassemic by ELISA test.

Hepatitis C virus infection and sex: In the current study male had more rate of infection compare to female but no significant difference between them(x^2=0.02 DF=1, P > 0.05).these result confirm the previous studies in this field in Iraq [12].The rate of chronicity in HCV infection appears to be lower in women particularly younger women. Evidence for this comes mostly from retrospective analyses of two large outbreaks of hepatitis C that occurred among women who received Rh immune globulin that had been contaminated with HCV. In a 17-year follow-up study of 704 Irish women with anti-HCV after receipt of

contaminated immune globulin. The chronicity rate was (55%). The same HCV chronicity percentage(55%) was found in a 20-year follow-up study of 917 German women who had received a similarly HCV contaminated Rh immune globulin [15].The most likely explanation for the higher prevalence among males than females is explains due to that males make more frequent visits to barber shops than females and may share shaving equipment and they may be more likely to get wounded and have other risk factors than females [16].The risk effect of some factors could not be analyzed because the exposure was universal among studied cases such as hair-cutting by community barbers and insect bites or because the exposure was nil such as cauterization ,tattooing and sharing toothbrushes[17].

Hepatitis C virus infection and residency: Distribution of hepatitis C infection according to residency revealed in table -5 which refers that the study shows that there is a relationship between viral hepatitis anti-HCV-IgM infections and residence (rural and urban) ($P>0.05$). This finding is may be due to the health education in the urban is better than in rural areas which leads to early discovery of this disease.

Hepatitis C virus infection and the economic status: Table-6 indicated the relation between susceptibility to infection and the economic status in that more rate of infection in low economic status. The ratio of good, medium and low economic status was about (1:2:3) respectively. These differences was statistically significant ($x^2=2.79$, $DF=2$, $P>0.05$).Some results reported by (Alter,1993) and (Murphy *et al*.,1994) were confirm these results.

Hepatitis C virus infection and the educational levels: Table -7 indicated that the subjects with low educational level are more affected than those with higher educational level at percentage of (4:1) respectively. The present study agrees with other study that revealed the people who have lower educational level were 10 more affected than the higher educational level [18].The results agree with the results obtained by other study was done in Egypt by [19] who they found that the prevalence was significantly higher among non educated people than among university educated (27.8% versus 4.0%) and non educated had about nine times the risk of being positive anti HCV compared to those who had higher education.

Table-8 indicated that important risk factors contributing to HCV spreading includes barber risk with percentage(12%).The reason might include lack of poor sterilization techniques and reuse of contaminated dental and surgical equipment, blood transfusion (19%); history of injection with disposable syringes (20%); reuse of syringes (0%), dental risk (7.50%),surgical procedure (5.00 %) ;history of contact with acute cases of acute hepatitis (17.50%) and smoking appear as major risk factors for the transmission with the percentage (45.00%). This may be be back to the fact that smoking alters the immune system [14].

The absence of anti-HBsAg positive cases this can be back to Immunization which remains the most effective way to control hepatitis B disease. The HBV vaccine has been available since 1982 and is given simultaneously with other universally administrated vaccines at birth and after that and remains highly

immunogenic. Globally, the prevalence of the anti-HBsAg carrier state has changed rapidly since the availability of HBV vaccine and implementation of mass immunization in infant [20].Also this may back to successful routine of checking for the clearing blood from possible blood born antigen including HBV and this lead to earlier detection of infection and prevent it from transmission to another person by the contaminated blood or blood product.

References

1. Mehwish, R., Muhamad, I., Hifza, K. and Firoz, K., (2011).An overview of Triple infection with Hepatitis B, C and D viruses.Virology Journal ;8:368:1-5
2. Golla, K., Epstein, J.B. and Cabay, J.B., (2004).Liver disease: Current perspectives on medical and dental management Oral Surg Oral Med Oral Pathol Oral Radiol Endodntology.98:516-21.
3. Grover, V.and Keays, .R,(2010). Viral hepatitis and transmissible spongiform encephalopathies. Anaesthesia & Intensive Care Medicine .11 (2) :42–46
4. Guido, M. and Borlolotti, F., (2008).Chronic Viral Hepatitis in children:any role for the pathogen .Gut J.57:7-873.
5. Gurtsevitch, V.E., (2008). Human oncogenic viruses: hepatitis B and hepatitis C viruses and their role in hepatocarcinogenesis. Biochemistry (Mosc); 73(5):504-13.
6. CDC,(2008).Hepatitis B FAQs for healthcare professionals
7. Tonbler, L.H., Stramer, S.L., Lee, S.R., Baggett, D., Wright, D., Hirschkorn,. D, (2005). Performance of ORTHO HCV core antigen and trak-C assays for detection of viraemia in pre-seroconversion plasma and whole blood donors. Vox Sang.89 (4): 201–207
8. Ohishi W., Fujiwara, S., Cologne, J.B., Suzuki, G., Akahoshi, M., Nishi, N., (2011). Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma. Hepatology; 53(4): 1237 - 45.
9. Alavian ,S.,M. and Aalaei-Andabili ,S.,H.,(2012). Lack of Knowledge About Hepatitis C Infection Rates Among Patients With Inherited Coagulation Disorders in Countries Under the Eastern Mediterranean Region Office of WHO (EMRO): A Meta-Analysis. Hepat Mon; 12(4): 244–252
10. Al-Kubaisy ,W.A., Al-Naib, K.T. and Habib,M.,(2006). Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. Eastern Mediterranean Health Journal;12, (1,2) .205-210).
11. Ghadir, M.R., Belbasi, M., Heidari, A., Jandagh, M., Ahmadi, I., Habibinejad, H., Kabiri, A., Ghanooni, A.H., Iranikhah, A. and Alavian, S.M.,(2012).Distribution and Risk Factors of Hepatitis B Virus Infection in the General Population of Central Iran. Hepat Mon.12 (2):112-117.
12. Hussin, A.G.,(1997), Seroepidem-iological survey of Hepatitis B surface antigen and antibodies of Hepatitis Cin Babylon province. MSc .thesis. Babylon University. collag of medicine.
13. Gogos, C.A.,Fouka, K.P. ,Niliforidis, G. ,Avgiridis, K . Sakel laropoulos G., Bassaris, H ., Maniatis ,A. and Skoutelis, A. (2003).Prevalence of hepatitis B and C virus infection in the general population and selected groups in South-Western Greece. Europ. J. Epidem.; 18: 551–557.
14. Dennis, L. K., Eugene, B., Anthony, S. F., Stephen, L. H., Dnal L. and Jameson, J. L. , (2005). Harrison

'sprinciples of internal medicine. 16th edition. McGraw-Hill. USA. 1830

15. Chen, S.L. and Morgan T.R., (2006). The Natural History of Hepatitis C Virus (HCV) Infection. Int J Med Sci. 3(2): 47–52

16. Wasfi ، O.A.S. and Sadek,N.A.,(2011). Prevalence of hepatitis B surface antigen and hepatitis C virus antibodies among blood donors in Alexandria, Egypt. Eastern Mediterranean Health Journal.. 17;(3) 238-242

17.Kalil,, K..A.، Farghally, H.S. Hassanein, , K.M. Abd-Elsayed A.A. and. Hassanein F.E، ., (2010) . Hepatitis C virus infection among paediatric patients attendingUniversity of Assiut Hospital, Egypt. Eastern Mediterranean Health Journal. (EMHJ).16 (4) .356-362

18.Murphy,E.,Bryzman,S.,Matijas,L.,Williams,A.,andNema,G.,(1994).Demographicdeterminants of HCV seroprevalence in U.S.blood donors .Am.J. Epidemiology, 139: 31.

19. Eassa,S., Eissa,M., Sharaf,S.M., Ibrahim,M.H., Hassanein,A.,(2007) Prevalence of Hepatitis C Virus Infection and Evaluation of a Health Education Program in El- Ghar Village in Zagazig, Egypt. J Egypt Public Health Assoc; 82(5).380-404

20. Dikici, B.,Uzun,H.,Gozu,A.and Fidan,M.,(2009).Prevalenc of Hepatitis B Infection among Schoolchildren in Southeast Turkey. Turk J Med Sci. 39 (2): 289-293