Detection of Hepatitis C Virus Infection and Genotypes among Seropositive Blood Donors by Polymerase Chain Reaction in Babylon Governorate\ Iraq

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Abstract

Objectives: To detect the hepatitis C virus infection and genotypes among blood donors by Polymerase Chain Reaction and biochemical measurement of Alanine Transaminase, Aspartate Transaminase & Alkaline phosphatase enzymes' levels and measurement of viral load among the studied sample.

Methods: A descriptive Cross sectional study done on 45 hepatitis C virus sero-positive blood donors(20-53 years old). A non probability (convenient) sample of blood donors at their first donation of blood, who accepted to participate in the study were included, interviewed and blood samples were taken.

Results: The seroprevalence of anti-Hepatitis C virus antibody was 0.29%, whereas prevalence of Hepatitis C Virus-Ribo-Nucleic Acid-positivity after confirmation by Polymerase Chain Reaction was 0.2%. 46.7% of the blood donors were infected with genotype 4 followed by genotype 1b in 15.6% and genotype 1a in 6.6. The presence of Hepatitis C virus infection significantly associated with biochemical parameters in the three groups of genotypes, except in the levels of alkaline phosphatase. Statistically significant association was found between age infections.

Conclusions: The prevalence of anti-Hepatitis C Virus- antibodies was relatively low in blood bank of Babylon province in Iraq compared to other with province in Iraq and other neighboring countries more among males than female and rural than urban area. The most common genotype was genotype 4, followed by 1b then 1a. The main factor associated with transmission of infection was blood transfusion in association with surgical procedures.

Key words: Hepatitis C Virus, Blood donors, Genotypes, Liver enzymes.

التحري عن مرض التهاب الكبد الفيروسي والطراز العرقي له لدى الاشخاص المصابين المتبرعين بالدم بتقنية التضاعف الجيني لسلاسل الدنا في محافظة بابل \العراق

الخلاصة

هدف البحث :الكشف عن الاصابة بالتهاب الكبد الفيروسي نمط سي بتقنية التضاعف الجيني لسلاسل الدنا، وقياس مستوى انزيمات الكبد وشحنة الفيروس لدى عينة البحث

منهجية البحث: اجريت دراسة وصفية على عينة من المتبرعين بالدم مشخصين بالاصابة بالتهاب الكبد الفيروسي نمط سي (عمر ٢٠-٥٣ سنة). عينة البحث عينة غير عشوائية تتبرع بالدم للمرة الاولى ووافقت على الاشتراك بالبحث واجريت معها المقابلة الشخصية وسحب عينة من الدم.

النتائج: ان مدى الانتشار المصلي للاجسام المضادة لالتهاب الكبد الفيروسي نمط سي هو ٠.٢٦% بينما كان انتشار الاصابة بتقنية التضاعف الجيني لسلاسل الدنا هو٠.٢ %. ان ٤٦.٧% من الاصابة هو بالطراز العرقي نوع ٤ يليه النوع ١ب لدى ١٥.٦% ثم

النوع ١أ لدى ٦.٦%. وهناك علاقة معنوية بين الاصابة والمحاور الكيميائية الحياتية في الانواع الثلاثة للطراز العرقي ماعدا مستوى الفوسفات القاعدي.

وهناك علاقة معنوية بين الاصابة وعمر المصاب

الاستنتاجات : ان مدى الانتشار المصلي لالتهاب الكبد الفيروسي نمط سي هو اقل نسبيا من باقي محافظات العراق او من الاقطار المجاورة. الطراز العرقي نوع ٤ هو الاكثر شيوعا يليه ١ب ثم ١أ . العامل الرئيسي المصاحب لحدوث الاصابة كان نقل الدم خلال اجراء العمليات الجراحية.

Introduction

Hepatitis C virus (HCV) infection is a single-stranded, positive sense RNA molecule of approximately 9.6 kb in length. There is a remarkable genetic heterogeneity and divergence among sequences which has lead to the categorization of HCV into "genotypes". Hepatitis C virus genotypes are related to regional distribution, clinical manifestation, response to treatment, and prognosis of HCV infection [1- 3].

Hepatitis C virus (HCV) infection is a major global health problem. Worldwide, 180 million of people are estimated to be infected with (HCV). At least 130 million infected individuals are chronic carriers of HCV and are at significant risk of developing liver cirrhosis and hepatocellular carcinoma [4-8]. More than three million new cases of infection are reported annually, and epidemiological studies indicate a wide variation in its prevalence patterns in different contents and countries [9].

Hepatitis C virus is transmitted most efficiently by percutaneous exposure to infectious blood. Since donated blood is tested for these viruses, high proportion of the new infections are associated with injecting drug use or other risk behaviors, such as tattooing, unprotected sexual contact (especially men having sex with men), piercing, and malpractice in the healthcare system [5, 10-13].

For HCV the number of infected persons, who are considered as chronic carriers is about 2.7 - 3.5 million cases world widely. Chronicity occurs in 80-85% of the infected patients [14-18].

For hepatitis C, 150 000 Americans are yearly infected and it is considered to be the most prevalent transfusion related disease [19].

Every blood transfusion carries a potential risk for transmissible diseases. It is estimated that approximately that 17 million persons in the region have chronic HCV infection. The cost of treating patients with chronic HBV or HCV infection far outweighs the cost of implementing prevention programs [20- 22]. An unsafe blood supply represents a major contributor to the total HCV disease burden in many countries. WHO estimates that blood donations up to 13 million units of the global blood supply are not screened for all relevant transfusion-transmittable infections. Screening of blood for HCV in blood banks in Iraq started in 1996 [23, 24, and 25]. In resources-challenged countries, the expense of currently available assays for blood screening results in a lack of or inconsistent testing of blood donations. In addition, transfusion services and laboratories are hampered by the generally poor specificity of anti-HCV screening assays; these constraints underscore the need to identify diagnostic test kits that are sensitive, specific, and also affordable [23].

In Iraq in 1998, Omer and Mohammed reported, that, the prevalence of anti-HCV among Iraqi normal population and blood donors was 0.5 % and 0.4 %, respectively.

Higher anti-HCV results detected among risky groups; in thalassemics, 62%; in hemophilics, 59%; in renal dialyzed patients, 49%; and only 2.2% among leukemic patients. It is also worthy to know that, Fayadh and Jureidini in 2001 had reported, that there was a low prevalence of HCV carriage among the general population as can be seen from data of blood bank which ranges from (0.2%) to (0.5%) [26].

Methods

A descriptive Cross sectional study. was conducted at two major Public Hospitals (The Main blood banks in central Hilla city and Al-Musaib blood bank), in Babylon governorate for the period from 15th November 2011, till the 15th April 2012. A non probability (convenient) sample of blood donors at their first donation of blood, who accepted to participate in the study.

The target population was blood donors attending the Voluntary and campaign donation of blood. The studied population includes 45 Patients who have anti-HCV antibody positive out of 15605 blood donors examined during the study period (5 months). These Patients belong to different parts of the governorate. Out of the 15605 blood donors, 45 were included in the study, and 15560 were excluded because they were negative to anti-HCV antibodies but have basic information about age, gender, residence, marital status and educational level.

Participants were asked to provide a blood sample and answer a questionnaire by direct interview which contains sociodemographic questions, including age, gender, marital status, education levels, residence and as well as questions related to route of exposure to the virus including history of (dental procedure, blood receiving, surgical procedure, scarification and tattooed and history of travel.

The sample size was calculated as 45 on a seropositive HCV and 31 out of 45 (68.9%) cases confirmed by PCR was positive then work HCV genotypes and 14 out of 45 (31.1%) confirmed by PCR was Negative. The researcher received training in on PCR technique in the Directorate of Health of Babylon Government for one weeks. This study was approved by the Iraqi MOH and the Babil health department the purpose and procedures of the study were Detection of HCV infections and genotypes.

Ten ml of blood sample was obtained from each participant by vein puncture using disposable syringes with needle, transported to two tube EDTA tube and other tube without any anticoagulants, plasma & sera were separated by centrifugation at 2000 RPM for 10 minutes, and then stored at -20°C until used.

Data obtained were entered into a computer database. Statistical package for social science (SPSS version 17) software was used for statistical analysis. Data were recorded as number and percentages. Percentages were compared using the chi-squared test and ANOVA; $P \leq 0.05$ was considered significant. Data were then presented in tables.

Results

To the best of the available knowledge this is the first work carried out in Iraq on blood donors in Babylon province who were assessed using Real time PCR and genotyping by conventional PCR.

The total number of blood donors tested by ELIZA were 15605 the HCV antibodies detected in 45 (0.29%) of cases. Hepatitis C-RNA was positive in 31 (68.9 %) out of 45 ELIZA positive cases while it was negative in

14(31.1%). The overall prevalence of seropositive HCV infection obtained in the present work was 0.29%, while the prevalence of HCV infection after confirmation by PCR was (0.2%) Figure 1. Also the prevalence of anti-HCV Ab seropositive in males was 0.3% while in female was 0.2%. The seroprevalence of anti-HCV Ab positive in rural area was 0.34% while in urban area was 0.27%.

Hepatitis C virus genotypes and subtypes were analyzed in all 31 HCV-RNA positive blood donors and 14 cases had undetected genotypes by this technique. Ten out of 45 (22.2%) cases had HCV genotype 1. Three of them (6.6%) had subtypes 1a, while 7(15.6%) had subtype 1b. Twenty one (46.7%) of blood donors had genotype 4. There was a significant statistical association between HCV-RNA positive and different genotypes (P = 0.000) (Figure 2).

The whole number of the blood donors who have Anti-HCV Ab positive was 45, they aged from 20-53 years (mean & std. deviation: 36.62±8.08). Thirty one HCV-RNA positive recovered blood donors aged from 26-53 was (38.35±7.25) while 14 HCV-RNA negative blood donors aged from (20-45) with mean of (32.79±8.78). The highest percentage of active hepatitis C virus 3 (100%) were more than 50 years old compared with other age groups. Thirteen (76.5%) of blood donors are between 30-39 years of age, 12(70.6%) are between 40-49 years old, and less, 8(17.8%) were between the age of 20-29 years. There is significant association between different age groups and HCV infection Table 1.

Table (2) shows that (57.8%) of the blood donors who had ALT levels were with abnormal liver enzyme, 80.8% of them were positive HCV-RNA in comparison with (52.6%) with normal serum ALT, 42.2% showed positive HCV-RNA, with significant association between ALT level and HCV infection (P=0.044).

About 31 out of 45 (68.9%) of the total number of blood donors have abnormal AST level, 26 (83.9%) of them were HCV-RNA-positive, while 14 out of 45(31.1%), of blood donors have normal AST level, 5 (35.7%) were positive for HCV-RNA and normal AST level . There is highly significant association in between (P = 0.001). The results also showed that 39 out of 45 (86.7%) of the total population of blood.

donors have normal ALP level ,31(79.5%) of them have positive HCV-RNA, while 6 out of 45(13.3%) of the total number of blood donors have abnormal ALP level, no one with abnormal ALP level and HCV-RNA-positive, with very highly significant association in between (P = 0.0001).

Table (3) shows the relation of HCV genotypes with biochemical parameters. Three blood donors out of 31 had genotypes 1a, 7 had genotype 1b and 21 had genotypes 4.

The LFT according to genotypes showed as the following: ALT level of mean (16.67±9.07 IU/I) in genotype 1a, (18.00±7.97 IU/I) in genotype 1b and in genotype 4 was (19.33±8.80 IU/I), with significant association in between (P= 0.012). Also AST levels of mean of (23.00±12.77 IU/I) in genotype 1a, (25.71±4.23 IU/I) in genotype 1b and in genotype 4 was (23.19± 10.54) with significant association in between this test highly significant (P= 0.001). While ALP levels of mean of (48.33±17.47 IU/I) in genotype 1a, (48.29±19.69 IU/I) in genotype 1b, and level of mean in genotype 4 was (55±16.0 IU/I) with non-significant association in between (P= 0.065).

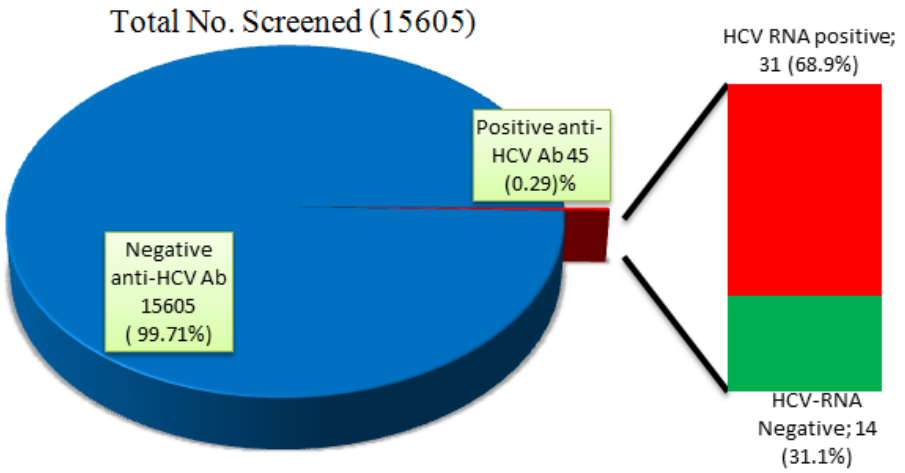

Figure 1 The prevalence of HCV infection among blood donors

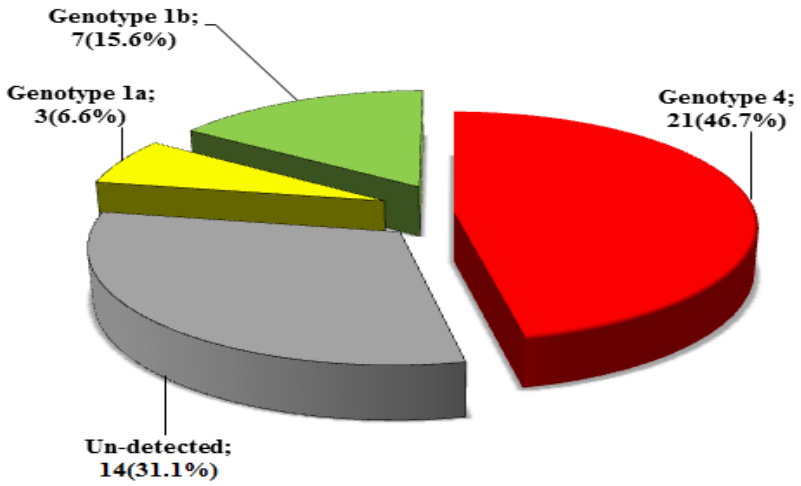

Figure 2 Distribution of HCV Genotypes/subtypes in Babylon province in Iraq

Table 1 The frequency of study sample according to age groups

	HCV RNA		
	Positive	Negative	P value
Age/years	38.35±7.25 (26-53)	32.79±8.78 (20-45)	0.031*

***Significant using Chi-squared test at 0.05 level of significance**

Table 2 The relation of HCV infection according to biochemical parameters of liver enzymes.

Liver Enzymes		HCV RNA						C.S*
		Positive N(31)		Negative N(14)		Total N(45)		
		No	%	No	%	No.	%	
S.GPT	< 12 (Normal)	10	52.6%	9	47.4%	19	42.2 %	0.044* df=1
	> 12 (Abnormal)	21	80.8%	5	19.2%	26	57.8 %	X^2=4.055
S.GOT	< 12 (Normal)	5	35.7%	9	64.3%	14	31.1 %	0.001* df=1
	> 12 (Abnormal)	26	83.9%	5	16.1%	31	68.9 %	X^2=10.43
S.Alk. Phosph.	21-92 (Normal)	31	79.5%	8	20.5%	39	86.7 %	0.0001* df=1
	> 92(Abnormal)	**0**	**0%**	**6**	**100%**	**6**	**13.3 %**	X^2=15.33

***Significant using Chi-squared test at 0.05 level of significance**

Table 3 Mean and SD of biochemical parameters in relation to HCV infection genotypes.

Biochemical parameters(IU/l)	Genotype			
	Genotype 1a n (3) (mean±SD)	Genotype 1b n (7) (mean±SD)	Genotype 4 n (21) (mean±SD)	P value (ANOVA)
ALT(Up to 12)	16.67±9.07	18.00±4.97	19.33±8.80	0.012*
AST(Up to 12)	23.00±12.77	25.71±4.23	23.19±10.54	0.001*
ALP(21-92)	48.33±17.47	48.29±19.69	55.0±16.0	0.065

Discussion

In this study, it was found that among (15605) blood donors selected in two Blood bank in Babylon governorate examined by using ELISA technique, 45 (0.29%) of them showed seropositive result, and prevalence of HCV infection after confirmed by PCR is (0.2%). The infection rate of HCV-RNA positive was (68.9%) for HCV active hepatitis and 14 (31.1%) HCV-RNA negative but positive ELISA is considered resolved infection of HCV.(Figure 1).

The HCV-RNA detection is one of the criteria to start therapy that depends also on genotype, viral load, and the degree of liver damage. The presence of specific antibodies against HCV and absence of HCV-RNA is a common finding and may be related with one of the following causes: a) the

patient has resolved the infection eliminating the virus, b) the infection is so recent and there is no sufficient viral load to detect the virus and the patient should be continuously monitored, or c) there is a cross reaction with antibodies different from anti-HCV [27, 28, 29, 30, 31].

Close to be similar results were mentioned by Hanan et al., (2011) in Baghdad who recorded (0.3%) [32].

The seropositive rate in this study was lower than that recorded by Al-Juboury et al., (2010), who recorded a seropositivity rate of (0.5%) blood donors are infected with hepatitis C virus in Babylon governorate [33], Nineveh governorate by using PCR technique, the infection rate of (1%) blood donors [34], Diyala governorate by Hassan., (2008) by using ELISA technique found ($9.9/10^5$) population, another study in Diyala governorate by Noaman, (2012) who recorded infection rate(1.1%) [35], also study in Kirkuk by Abdul-Aziz et al., (2001) founded the seropositivity of HCV was (0.93%) [36]. While the present study I higher than in Musol by Amin, (2011) who recorded (0.07%) of HCV prevalence among blood donors [37].

In comparison with some Iraqi neighbor countries, it was lower than in Kuwait (0.8%) [38], in Iran (0.13% per 100,000 Iranian blood donations) [39], in Lebanon (0.6%) [40], in Pakistan (7.5%) [41], in UAE (0.6%) [42] and in Egypt (14.5%) in urban blood donors [43]

This different in prevalence of HCV infection in different countries attributed to different epidemiological distribution and risk factors of HCV infection between these countries.

Genotyping is important because it provides information as to strain variation and potential association with disease severity. In addition, it is of epidemiologic value because it sheds light on whether prevalent HCV strains are similar to that endemic in a certain region, such as herein in the Middle East [44].

In the current study, hepatitis C virus genotypes and subtypes were analyzed in all 31 HCV-RNA positive blood donors and 14 cases had undetected genotypes by this technique. Hepatitis C virus genotype 1 was (22.2%) cases. (6.6%) cases of them had subtypes 1a, while (15.6%) cases had subtype 1b. The majority (46.7%) cases of blood donors had genotype 4. There is very highly significant association between HCV infection and genotypes (P= 0.000) (Figure 2). This study is the first study in Iraq among blood donors according to genotypes.

In comparison with studies made in Iraq's neighbor countries, it can be understood that the most common genotype of Yemen, Kuwait, Syria and Saudi Arabia is type 4 [45, 46]. Although genotype 4 is found almost exclusively in Middle East and western countries, the most prevalent genotype of Lebanon and Sudan is HCV genotype 4[47,4,49,50]. The correlation between HCV genotype and the presence of HCV-RNA in blood donors warrants further study.

In the current study, there was a significant association between HCV infection and age groups (*P= 0.013)*. Table 1.

The higher percentage of active HCV infection was seen in ≥50 years old, followed by age group 30-39 years and 40-49 years old and less active HCV infection was seen in between age group 20-29 years.

The present study is consistent with study in Mongolia by Tsatsralt-Od et al, (2005) [51] which was reported that active HCV infection (HCV-RNA-positive) was higher percentage in age ≥ 50 years old (50.0%) comparative with other age groups. Also study in Tanzani that using ELISA test [52]

which was reported that the infection with HCV was higher percentage in age group 50 years and above (36.0%), while contrast with study in India by Thakral et al, (2006) [53] which was reported that higher percentage of HCV infection in age group (18-30) years.

This is may be attributed to the immunological state, that age effect with infection because the HCV infection can be distributed more in persons with immune deficiency and co-infection with other virus that lead to decreasing immunity and also may be younger age group have resolved HCV infection due to having own high immunity.

AST is present in high concentrations in cells of cardiac and skeletal muscle comparative with ALT is present in high concentrations in liver while ALP is present in most tissues. Damage to any of these tissues may increase levels.

In the present study founded significant (P< 0.05) change was observed in ALT, AST, & ALP level of blood donors who had HCV-RNA-positive compared with donors had HCV-RNA-negative.

Elevated ALT and HCV infection, Thus indicating chronic hepatitis in majority of the blood donors biochemically (P= 0.044), Findings regarding AST elevated level (P= 0.001) in blood donors who have HCV-RNA positive is strongly supported by the assay [54] distinguishing Chronic active hepatitis from acute hepatitis. It's agree with study work by the Lutfullah et al, (2009) [55].

This result was in agreement with that recorded by Al-Azzawi et al., (2009) [56] in Baghdad and Ramarokoto et al., (2008) in Madagascar [57] who founded significant association between seropositivity and hepatic enzyme (ALT & AST) activities. Also consistent this study with reported by Jurado et al., (2010) in Mexico [58] and Thakral et al, (2006) in Indian [59] who documented that significant association of HCV-RNA positive donors with ALT levels in blood donors.

ALP levels in blood donors was only normal in all HCV-RNA-positive, with significant association in between (*P= 0.0001*), which is similarity with study by AL- Mola et al, (2006) [60]

The ALT is highly specific for liver, whereas AST was less specific for liver injury, The ALP level is normal in blood donors HCV-RNA-positive this is meaning of the donors not had prognosis of liver disease or no cirrhosis [61.].

Table (3) showed that the ALT level of mean (16.67±9.07 IU/I) in genotype 1a, (18.00±7.97 IU/I) in genotype 1b and in genotype 4 was (19.33±8.80 IU/I), with significant association in between (P= 0.012).

This means that the HCV genotype 4 associated with elevated of ALT level than other genotype (1a and 1b) and genotype 1b higher level of ALT than genotype1a. This is significantly in between (P = 0.044).

Study in Iran by Kabir et al, (2006) [62] who documented elevated of ALT level in genotype 4 as comparative with genotype 1a and 1b, also founded genotype 1b had ALT level more than in genotype 1a but this study was not statistically difference in cases with different genotypes.

Also AST levels of mean of (23.00±12.77 IU/I) in genotype 1a, (25.71±4.23 IU/I) in genotype 1b and in genotype 4 was (23.19± 10.54) with significant association in between this test highly significant (P= 0.001).

The present study revealed that SGPT and SGOT levels varied significantly among the three groups of genotypes. It is, therefore, not a highly

specific indicator of liver injury. ALT is most concentrated in liver and released into the bloodstream as the result of liver injury. It, therefore, serves as a fairly specific indicator of liver status [63]

While ALP levels of mean of (48.33±17.47 IU/I) in genotype 1a, (48.29±19.69 IU/I) in genotype 1b, and level of mean in genotype 4 was (55±16.0 IU/I) with non-significant association in between (P= 0.065).

when the alkaline phosphatase level is normal in an HCV-infected person, the likelihood of significant liver disease is very low [64]. The study concludes that:

1- The prevalence of anti-HCV antibodies positive blood donors and after confirmed HCV by PCR was relatively low in blood bank of Babylon province in Iraq compared to other with province in Iraq and other neighboring countries.

2- The seroprevalence of HCV positive blood donors in male more than female and in rural more than urban.

3- Distribution and prevalent of HCV genotype in blood donors in Babylon province was genotype 4, followed by genotype 1b then 1a.

4- Majority of the infected blood donors are old age ≥ 50 years more than other ages.

5- The liver enzyme (ALT, AST & ALP) is significant with HCV infections and genotypes.

6- The first mode of transmission of HCV in blood donors was during blood received or blood transfusion followed by surgical procedure.

7- Genotype 4 more severe than genotype 1b and 1a, also genotype 1b more severe than genotype 1a.

8- Until a vaccine against HCV becomes available, preventive measures for blood donors and other related factors screening using advanced techniques for detecting HCV infection before transfusion and strict infection control measures are crucial for control of spread of HCV among these high risk blood donors.

Based on the above results, it seems that viral hepatitis is an important public hepatitis problem in Babylon governorate. Thus the following recommendations are made:

1- The PCR screening program should be applied to most the blood donors and blood products; and it should have more than one screening test available to increase its sensitivity as much as possible.

2- Effective educational programs are needed to all the population especially to those at risk of hepatitis virus transmission.

3- All the hepatitis positive patients should be regularly followed up by well-trained health care workers to decrease morbidity and mortality.

4- Provision of drugs and diagnostic equipment for early diagnosis of the cases are needed in order to treat the patients effectively.

5- Screening program for the blood units before transfusion aiding to decrease the chance of getting infection with hepatitis should be done.

6- Further studies are recommended to carry on in this province and other parts of the country to detect seropositive cases of different types of hepatitis in order that restrict measurement should be taken place to prevent the dissemination of infection.

7- Recommended with make periodic studies to determine the prevalence of HCV genotypes in blood donors should performed to monitor the emergence of new genotypes such as genotype 2 (2a& 2b) and genotype 3a and mix genotypes.

8- Proper and reliable HCV screening should include latest ELISA procedure.

9- Better laboratory facilities and investigations should be provided for detection of HCV-RNA in high risk

groups positive & negative HCV antibodies.

10- Establish public health strategies, well-programmed, population-based and certain HCV infection at risk surveys are needed in the Babylon province.

11- Health education for people about the risk of HCV infection from contaminated instruments and certain traditional habits.

12- National strategy should be implemented and specific prevention guide lines have to be followed to reduce risks.

13- Effort programs and projects which mean the activities with risk group population insisting of them to use condoms and another protection measures during sexual activities and professional care.

14- Early diagnosis, prevention programs and therapeutic interventions are necessary inorder to minimize risks involved in the epidemic spread of HCV inside blood banks.

15- Establish strong intersectoral collaboration between the health sector, legislative branch in Babylon province directorate and key ministry of health in order to pass legislation ensuring safety of all risk group especially worker health, universal vaccination of workers with occupational exposure to blood and introduction of strategies for harm reduction.

16- Further studies are recommended make studies to compare HCV genotypes according to the stages of liver disease, to characterize pathogenicity according to HCV genotypes.

References

1. Choo Q, Kuo G, Weiner A, Overby L, Bradley D, Houghton M. Isolation of a cDNA clone derived from a blood borne non-A, non-B viral hepatitis genome. Science 1989;244:359-62.

2. Liselotte V, Verhaest l, Lamzira S. Spread of Hepatitis C virus among European injection drug users infected with HIV:A phylogenetic analysis . J infect Dis 2004;189:292-302.

3. Senevirathna D, Amuduwage S, Weerasingam S, Jayasinghe S, Fernandopulle N. Hepatitis C virus in healthy blood donors in Sri Lanka. 2011;5:23-25.

4. Chuang WL, Yu ML, Dai CY, Chang WY. Treatment of chronic hepatitis C in southern Taiwan. Intervirology 2006; 49:99–106.

5. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.

6. Yang JF, Lin CI, Huang JF, et al. Viral hepatitis infections in southern Taiwan: a multicenter community-based study. Kaohsiung J Med Sci 2010;26:461–469..

7. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009; 49 (4): 1335–1374.

8. Susser S, Welsch C, Wang Y, et al. Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. Hepatology 2009;50:1709–1718.

9. WHO. Hepatitis C. WHO fact sheet N0 164, October 2000b. Available from:URL:http://www.who.int/mediac entre/factsheets/fs 164en/print.htm1. [Cited on 2007 May 14].

10. MacDonald M, Crofts N, Kaldor J. Transmission of hepatitis C virus: rates, routes, and cofactors. Epidemiol Rev. 1996;18:137-148.

11.Holmberg SD. Molecular epidemiology of health care-associated transmission of hepatitis B and C viruses. Clin Liver Dis. 2010;14:37-48.

12. Rauch A, Rickenbach M, Weber R, et al. Unsafe sex and increased incidence of hepatitis C virus infection among HIV-infected men who have sex with men: the Swiss HIV Cohort

Study. Clin Infect Dis. 2005;41:395-402.
13. Dencs A, Farkas A, Gyugos M, et al. Phylogenetic analysis of a nosocomial transmission of hepatitis B virus at a paediatric haematology ward. Acta Microbiol Immunol Hung. 2011;58:23-29.
14. Jones MS, Kapoor A, Lukashov VV, et al. New DNA viruses identified in patients with acute viral infection syndrome.; J Virol. 2005;79(13):8230-6.
15. Benenson AS. Viral Hepatitis. Control of Communicable Disease Manual. 16th edition. Washington. American public health association. 2000;217-233.
16. Chu D. Clinical Update of Viral Hepatitis A-G. Common Medical Disease, Viral Hepatitis Update. 2004;20:20-24.
17. Haslett C, Chilvers E R, Hunter J A et al. Davidson principle & practice of medicine.20th edition. Edinburgh. London. New York. Philadelphia. Sydney. Toronto. Churchill Livingstone.2006: 962-970.
18. Akiba J, Umemura T, Alter HJ, et al; Epidemiology and characteristics of a transfusion-transmitted virus. 2005;45(7):1084-1088.
19. Bernuau S, Jacques R. Acute Viral Hepatitis. European Journal of Gastroenterology&Hepatology.2008;20(3):161-163.
20. Bhawani Y, Rao PR, Sudhakar V: Seroprevalence of transfusion transmissible infections among blood donors in a tertiary care hospital of Andhra Pradesh. Biol Med 2010, 2(4):45–48.
21. Khan S, Attaullah S, Ayaz S, Khan SN, Shams S, Ali I, Bilal M, Siraj S: Molecular epidemiology of HCV among the health care workers of Khyber Pakhtunkhwa. Virol J. 2011, 8(1):105.
22. Hauri AM, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. International Journal of STD and AIDS, 2004, 15:7–16.
23. Diaz S.liu P and El-Nageh M.M.,Development of the International Consortium for Blood Safety(ICBS) HCV panels. Eastern Mediterranean Health J, 2008; 14(2):427-36.
24. Shamsi Al-Dinn A. A clinic-epidemiology study of thalassemia in Baghdad. MSc thesis in community Medicine, Al-Mustansirya University, 2005,:15-30.
25. Al-Kubaisy W.A. , Al-Naib K.T. and Habib M. Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassemic. Eastern Mediterranean Health J.,2006 ; 12(1-2):204-10.
26. Omer A.R., Mohammed D.A.M, Hepatitis C in IRAQ, Reference Laboratory CDC center, Baghdad. 1998.
27. Ren FR, Lv QS, Zhuang H, Li JJ, Gong XY, Gao GJ, Liu CL, Wang JX, Yao FZ, Zheng YR, , Significance of the signal-to-cutoff ratios of anti-hepatitis C virus enzyme immunoassays in screening of Chinese blood donors. Transfusion 2005, 45:1816-1822.
28. MacKenzie WR, Davis JP, Peterson DE, Hibbard AJ, Becker G, Zarvan BS: Multiple false-positive serologic tests for HIV, HTLV-1, and hepatitis C following influenza vaccination, 1991. JAMA 1992, 268:1015-1017.
29. Contreras AM, Tornero-Romo CM, Toribio JG, Celis A, Orozco-Hernandez A, Rivera PK, Mendez C, Hernandez-Lugo MI, Olivares L, Alvarado MA: Very low hepatitis C antibody levels predict false-positive results and avoid supplemental testing. Transfusion 2008, 48:2540-2548.
30. Hyland C, Seed CR, Kiely P, Parker S, Cowley N, Bolton W:

Follow-up of six blood donors highlights the complementary role and limitations of hepatitis C virus antibody and nucleic acid amplification tests. Vox Sang 2003, 85:1-8.

31. Baba SS, Fagbami AH, Olaleye OD: Antigenic relatedness of selected flaviviruses: study with homologous and heterologous immune mouse ascitic fluids. Rev Inst Med Trop Sao Paulo 1998, 40:343-349.

32. Hanan K. A., Ataallah T.M., Maysoun K.S., Sadoon A.A., Prevalnec of hepatitis B and C among blood donors attending the National Blood Transfusion Center in Baghdad, Iraq from 2006-2009, Saudi Med J. 2011 Oct ;32 (10):1046-50 22008925.

33. Al-Jubory A.W.F, Salih H.A.L.M., AL-Assaadi M.K., Ali A.M., Seroprevalence of hepatitis B and C among blood donors in Babylon governorate-Iraq, Medical Journal of Babylon; 7(1-2),2010.

34. Abdullah B. A., Khaled M. D., Maaroof M. N., Detection of Hepatits C Virus (HCV) by ELISA, RIBA and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Technique among Kidney dialysis patients in Nineveh Governorate/Iraq., Thi-Qar science Journal, vol.3(2), 2012.

35. Noaman N.G., prevalence of hepatitis C virus infection among blood donors and certain risky groups in Diyala province, Diyala Journal of Medicine, vol.2, issue 1, April 2012.

36. Abdul-Aziz M., Abdul-Karem K., Shamse-El-den S., Al-Moula G., prevalence of hepatitis B & C among people attending Kirkuk public health laboratory,(2001). Available from Iraq Academic Scientific Journal www. iasj.net.

37. Amin R. M., prevalence of HBV and HCV in blood donors in Mousul city, Technical Institute / Mosul (2011). Available from Iraq Academic Scientific Journal www. iasj.net.

38. Ameen R, Sanad N, Al-Shemmari S, et al. Prevalence of viral markers among first-time Arab blood donors in Kuwait. Transfusion 2005; 45 (12): 1973-80.

39. Kafi-abad SA, Rezvan H, Abolghasemi H, Talebian A. Prevalence and trends of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus among blood donors in Iran, 2004 through 2007. Transfusion 2009; 49(10): 2214-20.

40. Irani-Hakime N, Tamim H, Samaha H, Almawi WY. Prevalence of antibodies against hepatitis C virus among blood donors in Lebanon, 1997-2000. Clin Lab Haematol 2001;23(5):317-23.

41. Mujeed SA, Aamir K, Mehmood K, seroprevalence of HBV, HCV & HIV infections among college going first time vouluntary blood donors J Med Assoc. 2006, 56:524-525.

42. Gohar HH, Al-Amiri AH, Al-Marzouqi, Prevalence of transfusion transmissible viral infection in first time blood donors in UAE, Sharjah blood transfusion and research center, MOH, UAE.

43. Mohamed MK, Hussein MH, Massoud AA, et al. Study of the risk factors for viral hepatitis C infection among Egyptians applying for work abroad. J Egypt Public Health Assoc 1996;71 (1-2):113-47.

44. Kabir A., Alavian S.M., Keyvani H., Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters: a preliminary study, Comparative Hepatology 2006, 5:4. available from: http:// www. comparativehepatology.com/content/5/ 1/4.

45. Kabir A., Alavian S.M., Keyvani H., Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation

with clinical and virological parameters: a preliminary study, Comparative Hepatology 2006, 5:4 doi:10.1186/1476-5926-5-4. Available from: http://www.comparative-hepatology.com/content/5/1/4.

46. Antaki N, Haddad M, Kebbewar K, et al. The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. Epidemiol Infect 2009;137(1):79-84.

47. Sharara AI, Ramia S, Ramlawi F, Fares JE, Klayme S, Naman R. Genotypes of hepatitis C virus (HCV) among positive Lebanese patients: Comparison of data with that from other Middle Eastern countries. Epidemiol Infect 2007;135(3):427-32.

48. Abdel-Wahab MF, Zakaria S, Kamel M, et al. High seroprevalence of hepatitis C infection among risk groups in Egypt. Am J Trop Med Hyg 1994;51(5):563-7.

49. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. Int J Infect Dis 2006;10(4):272-7.

50. Watson JP, Al-Mardini H, Awadh S, Ukabam S, Record CO. Hepatitis C virus genotypes in a cohort of Middle Eastern patients. Ann Saudi Med 1999;19(5):410-2.

51. Tsatsralt-Od B., Takahashi M., Nishizawa T., Inoue J., Ulaankhuu D., Okamoto H., High prevalence of hepatitis B, C and delta virus infections among blood donors in Mongolia, Arch Virol (2005) 150: 2513–2528.

52. Nagu T.J., Bakari M., Matee M., Hepatitis A, B and C viral co-infections among HIV-infected adults presenting for care and treatment at Muhimbili National Hospital in Dar es Salaam, Tanzania, BMC Public Health 2008, 8:416. Available from http ://www.biomedcentral.com/147l-2458/8/416.

53. Thakral B., Marwaha N., Chawla Y.K., Saluja K., Sharma A., Sharma R.R., Minz R.W., Agnihotri S.K., Prevalence & significance of hepatitis C virus (HCV) seropositivity in blood donors, Indian J Med Res 124, October 2006, pp 431-438.

54. N. assay and G.Y. Minuk, Am. J. Gastroenterol, 95, 1545 (2000).

55. Lutfullah G., Akhtar T., Rahim A., Nazil R., Arif S., Khan T.M., Comparison of liver function test in symptomatic and asymptomatic HCV positive patients, J.Chem.Soc.Pak., 31(1),2009.

56. Al-Azzawi R.H., Mohammed G.S., Al-khalidi N.M., The Necroinflammatory activity of HCV in liver biopsies of Iraqi patients that detected by In Situ hybridization technique, Um-Salama Science Journal, Vol.6(1)2009.

57. Ramarokoto C.E., Rakotomanana F., Ratsitorahina M., Raharimanga V., Razafindratsimandresy R., Randremanana R., Andrianarivelo M.R., Rousset D., Andrianaja V., Richard V., Soares J.L., Rabarijaona L.P., Seroprevalence of hepatitis C and associated risk factors in urban areas of Antananarivo, Madagascar, BMC Infectious Diseases 2008, 8:25. available from: http://www.biomedcentral.com/1471-2334/8/25.

58. Jurado F.S., López G.S., Flores B.G., Conde J.I.R., Mena D.M., Maldonado M.T.V., Laguna Y.M., Mioni L.C., Ruiz V.V., Leyva J.R., Hepatitis C virus infection in blood donors from the state of Puebla, Mexico, Sosa- Jurado et al. Virology Journal 2010, 7:18.)) available from http://www.virologyj.com/content/7/1/18.

59. Thakral B., Marwaha N., Chawla Y.K., Saluja K., Sharma A., Sharma R.R., Minz R.W., Agnihotri S.K., Prevalence & significance of hepatitis C virus (HCV) seropositivity in blood donors, Indian J Med Res 124, October 2006: 431-438.

60. AL- Mola G.A., Zaman N.A., Frequency of Circulating Antibodies to Hepatitis C Virus (HCV): A Follow up Study, Raf. Jour. Sci., Vol.17, No.2, pp.1- 7, 2006.
61. Richard, D. and Remington, M., 1985. Statistics with applications to the biological and health sciences., pp.241-313.
62. Kabir A., Alavian S.M., Keyvani H., Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters: a preliminary study, Comparative Hepatology 2006, 5:4. This article is available from: http://www.comparative-hepatology.com/content/5/1/4.
63. Lee YS, Yoon SK, Chung ES, Bae SH, Choi JY, Han JY, et al. The Relationship of Histologic Activity to Serum ALT, HCV genotype and HCV RNA titers in Chronic Hepatitis C. J Korean Med Sci. 2001;16:585–91.
64. Bacon BR. Treatment of patients with hepatitis C and normal aminotransferase levels. Hepatology 2002; 36 (Suppl 1):S179–S184.