Prevalence and risk factors for hepatitis C virus in Beta thalassemic patients attending blood diseases center in Ibn- AL -Baladi Hospital, Baghdad

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ABSTRACT

Background: Thalassemias are a group of heterogeneous genetic disorders, in which the rate of production of hemoglobin is partially or completely suppressed due to reduced rate of synthesis of α or β- chain

Objectives: to estimate the prevalence of Hepatitis C infection among B thalassemia patients attending Ibn-AL-Baladi center of blood diseases in AL-Sader city, in AL-Resafa Quarter of Baghdad and to determine the possible risk factors.

Type of the study: Cross- sectional study.

Methods: A cross sectional study conducted on B Thalassemia patients attending the blood diseases center in Ibn-AL-Baladi hospital during the period from 1st of July till the 31st of December 2015.

Results: All of 400 eligible patients, who were recruited to be included in this study, were accepted to participate in the study giving an overall response rate 100%. The prevalence rate of Hepatitis C according to anti HCV antibody test among the study group was 26%.

Conclusions: we conclude that about quarter of Iraqi thalassemic patients visiting the thalassemia center in Ibn-AL-Baladi hospital have HCV infection

Keywords: Thalassemis, hepatitis, infection.

Thalassemias are a group of heterogeneous genetic disorders(1), in which the rate of production of hemoglobin is partially or completely suppressed due to reduced rate of synthesis of α or β- chain (2.3). In thalassemia, the imbalance of globin chain synthesis leads to red cell damage resulting in destruction of red cells in the marrow (ineffective erythropoiesis) and peripheral circulation (hemolysis) (4,5,6). B thalassemia is an autosomal recessive disease, which can be caused by one of 180 mutations in the gene coding for the β chain of haemoglobin tetramer. The beta globin gene located on chromosome 11 (7,8,9). In Iraq, there have been reports for the incidence of thalassemia minor in different provinces and cities with varying results, ranging between 3.7% to 6.5%(10,11,12). Hepatitis C virus (HCV) is an infectious hepatotropic virus belonging to the Flavi virus family (13,14,15,16). It is a single-stranded, enveloped RNA virus with a genome about 10,000 nucleotides in length(17,18,19). Infected individuals are usually asymptomatic, but some patients ultimately develop cirrhosis or liver cancer (20,21). The virus may be transmitted by any percutaneous blood exposure, most commonly among intravenous drug users (22). Less frequently, it spread through sexual activity, perinatal, or after accidental blood contact. Blood and blood products not screened for HCV have been sources of infection. However, about 10% of people with HCV infection have no recognized risk factor (23).

The aim of this study was to estimate the prevalence of Hepatitis C infection among B thalassemia patients attending Ibn-AL-Baladi center of blood diseases in AL-Sader city, in AL-Resafa Quarter of Baghdad and to determine the possible risk factors.

Method: A-cross sectional study conducted on B Thalassemia patients attending the blood diseases center in Ibn-AL-Baladi hospital during the period from 1st of July till the 31st of December 2015. All blood transfusion dependents B thalassaemic patients attending the center during the study period and meeting the eligibility criteria were informed about the purpose of the study and those who agreed to participate were enrolled in the study after they had given a verbal consent. Patients with thalassemia minor, alpha thalassemia, sickle disease or other hemoglobinopaties, and patients who had not attended the center for ten months period for unknown reasons were excluded from the study. A convenience method of sampling was used in this study. The total number of sample was about 400 patients. The researcher had made regular visits to the center for data collection in a system of4-5 hours a day, 2-3 days per week for 6 months. Each day an 8-12 thalassemic patient were interviewed and examined. An appropriate sample size was calculated according to the following equation: n= (z²pq)/d² (n=sample size, z= is the statistic corresponding to level of confidence, p= is expected prevalence, q= (1-p), d=precision). We took a confidence level of 95% (Z=1.96) and precision of 5% and proportion of HCV prevalence of 0.50 to increase the sample size to the maximum. So we need 384 patients. An extra number of patients was added for patient's non-response to reduce sampling bias, giving a final sample of 400 patients. A questionnaire-form paper had been developed for the process of data collection and it had been piloted on a sample of thirty patients selected randomly to know the time needed to fill the questionnaire, and to figure out any difficult, sensitive or unclear questions. Upon the notes recorded in the pilot project, the questionnaire was changed and adjustments were made for the best acceptable form. Patients who participated in the pilot project were not included in the sample of the study. The questionnaire forms include information on demographic criteria of parent, blood transfusion history of the patient, family history of HCV according to mother statement, questions regarding mode of transmission: Including history of previous injection, history of previous tattoo and desferal

injection. All patients examined carefully for organomegaly and blood aspirated for investigations which include SGPT, SGOT, TSB, anti HCV antibody test and ALP. Statistical package for social science version 18 (SPSS18) was used for both data entry and analysis. Discrete variable presented as frequency and percentage. Chi-square test (or fisher exact test when appropriate) was used to test the significance of association for discrete variable. P-value of < 0.05 was considered significant.

Results: All of 400 eligible patients, who were recruited to be included in this study, were accepted to participate in the study giving an overall response rate 100%. The prevalence rate of Hepatitis C according to anti HCV antibody test among the study group was 26% as shown in figure 1.
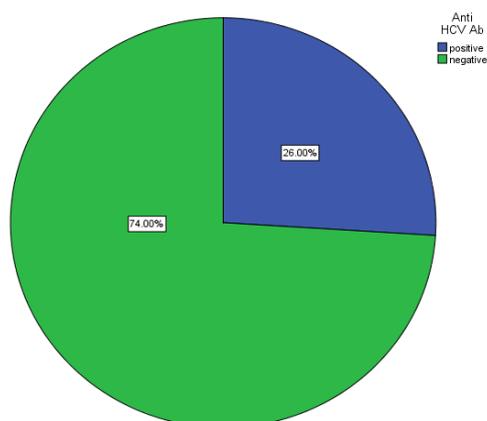
Figure 1: The prevalence rate of Hepatitis among the study group.

Table 1 demonstrate the occurrence of HCV in thalassemia patients according to demographic characteristics. Patients older than 20 years and those whose mother has primary school level of education, had a statistically significant higher anti HCV antibody rate (61.5% and 38.5% respectively). Table 2 demonstrate the prevalence of HCV in thalassemia patients according to thalassemia characteristics. A higher prevalence of anti HCV antibody was found among patients with thalassemia major and patients with age less than 6 months and older than one year at the

time of diagnosis (P value 0.001). Positive family history of thalassemia was not found to have a role in patient acquiring the anti HCV antibody.   Table 3 demonstrate the prevalence of HCV in thalassemia patients according to blood transfusion history. Duration of blood transfusion, frequency of blood transfusion per month and compliance with blood transfusion were found to have a statistically significant association with anti HCV antibody (P value 0.003, 0.001, 0.002 respectively). Table 4 demonstrate the occurrence of HCV in thalassemia patients according to knowledge of parents and patient about blood transfusion associated HCV infection and family history of HCV. No significant association between knowledge of parents and patient about blood transfusion associated HCV infection and positive anti HCV antibody found (p=0.36). From total positive patients, patients without family history of HCV had the highest positive anti HCV antibody (76.9%) with significant p-value (p=0.002). Table 5 demonstrate the occurrence of HCV in thalassemia patients according to possible rout of transmission. None of the studied possible rout of transmission for HCV, namely previous injection, history of previous tattoo, blood transfusion, and desferal injection was found to be significantly associated with acquisition of the infection (P value > 0.05). Table 6 demonstrate the occurrence of HCV in thalassemia patients according to blood group. From total positive patients, patients with blood group O had the highest positive Anti HCV antibody   (49.0%), followed by patients with blood group A (32.7%), while patients of blood group AB had the lowest positive Anti HCV antibody (3.8%). The difference was statistically significant (P value 0.007**).** Table 7 demonstrate the occurrence of HCV in thalassemia patients according to clinical finding. From total positive patients, patients with hepatomegally and or splenomegally had significantly higher rate of positive anti HCV antibody (53.8%, P value 0.012). Although patients with splenectomy had higher positive anti HCV antibody (76.9%) from total positive patients, there were no significant association (P value 0.43). Table 8 demonstrate the occurrence of HCV in thalassemia patients according to liver functional test. Most patients with thalassemia had elevated liver function test but there were no significant association found with the occurrence of HCV(P value > 0.05).

Table 1: Demographic characteristics of HCV positive thalassemic patient.

Variables		Anti HCV antibody				P-value
		Positive		Negative		
		No.	%	No.	%	
Age group	< 10 years	16	15.4	112	37.8	0.001
	10-20 year	24	23.1	152	51.4	
	> 20 years	64	61.5	32	10.8	
Gender	Male	48	46.2	136	45.9	0.97
	Female	56	53.8	160	54.1	
Educational level of mother	Illiterate	16	15.4	24	8.1	0.003
	Primary	40	38.5	56	18.9	
	Secondary	24	23.1	144	48.6	
	Higher education	24	23.1	72	24.3	

Table 2: The Prevalence of anti HCV antibody according to thalassemia characteristics.

Variables		Anti HCV antibody				P-value
		Positive		Negative		
		No.	%	No.	%	
Type of Thalassemia	Major	88	84.6	200	81.1	0.001
	Intermediate	16	15.4	96	18.9	
Age when first diagnosed with thalassemia according to mother statement by questionnaire	< 6 months	40	38.5	72	24.3	0.001
	6 months to 1 year	24	23.1	48	16.2	
	> 1 year	40	38.5	176	59.5	
Family history of thalassemia	Yes	56	53.8	176	59.5	0.31
	no	48	46.2	120	40.5	

Table 3: The prevalence of Anti HCV antibody according to blood transfusion history.

Variables		Anti HCV antibody				P-value
		Positive		Negative		
		No.	%	No.	%	
Duration of blood transfusion	< 10 years	16	15.4	144	48.6	0.003
	10-20 year	32	30.8	136	45.9	
	> 20 year	56	53.8	16	5.4	
Frequency of blood transfusion per month	One time per month	8	7.7	152	51.4	0.001
	Two times per month	80	76.9	128	43.2	
	Three times or more per month	16	15.4	16	5.4	
Compliance with blood transfusion	Yes	104	100	256	86.5	0.002
	No	0	0	40	13.5	

Table 4: The relationship of knowledge of parents and patient about blood transfusion associated HCV infection and family history of HCV with occurrence of HCV.

Variables		Anti HCV antibody				P-value
		Positive		Negative		
		No.	%	No.	%	
Knowledge of parents and patient about blood transfusion associated HCV infection	Yes	56	53.8	144	48	0.36
	No	48	46.2	152	51.4	
Family history of HCV infection	Yes	24	23.1	16	5.4	0.002
	No	80	76.9	280	94.6	

Table 5: The relationship of mode of transmission with occurrence of HCV.

Variables		Anti HCV antibody				p-value
		Positive		Negative		
		No.	%	No.	%	
History of previous injection	Yes	84	80.8	237	80.1	0.87
	No	20	19.2	59	19.9	
History of previous tattoo	Yes	2	1.9	6	2.0	0.94
	No	102	98.1	290	98.0	
History of previous blood transfusion	Yes	1	1.0	8	2.7	0.30
	No	103	99.0	288	97.3	
Desferal injection	Yes	72	69.2	184	62.2	0.196
	No	32	30.8	112	37.8	

Table 6: The relationship of blood group with occurrence of HCV.

Variable	Anti HCV antibody				P-value
	Positive		Negative		
	No.	%	No.	%	
Blood group O	51	49.0	152	51.4	0.007
Blood group A	34	32.7	56	18.9	
Blood group B	15	14.4	80	27.0	
Blood group AB	4	3.8	8	2.7	

Table 7: The relationship of clinical finding with occurrence of HCV.

Variables		Anti HCV antibody				P-value
		Positive		Negative		
		No.	%	No.	%	
Hepatomegaly and or splenomegaly	Yes	56	53.8	200	67.6	0.012
	No	48	46.2	96	32.4	
Splenectomy	Yes	80	76.9	216	73.0	0.43
	No	24	23.1	80	27.0	

Table 8: The relationship of liver functional tests with occurrence of HCV.

Variables			Anti HCV antibody				P-value
			Positive		Negative		
			No.	%	No.	%	
SGPT		Normal	48	46.2	144	48.6	0.66
		Elevated	56	53.8	152	51.4	
SGOT		Normal	40	38.5	96	32.4	0.26
		Elevated	64	61.5	200	67.6	
TSB		Normal	8	7.7	40	13.5	0.11
		Elevated	96	92.3	256	86.5	
ALP		Normal	48	46.2	112	37.8	0.13
		Elevated	56	53.8	184	62.2	

Discussion: The HCV infection is a widespread disease that affects a large number of thalassemia patients worldwide and is considered as a major public health problem in these high risk groups. These patients act as a reservoir of this infection and are one of the main obstacles for HCV infection control in the community. The incidence of transfusion - associated hepatitis has been substantially reduced after the implementation of screening of blood donors for anti HCV antibodies at 1996. Nevertheless, chronic transfusion recipients such as thalassemia patients still suffer a high frequency of liver disease due to transfusion related iron over load and infection with blood borne agents. Hepatitis C virus is responsible for the majority of cases of post-transfusion hepatitis in patients with thalassemia major (24). Infection with HCV may lead to disabling symptoms, cirrhosis and hepatocellular carcinoma (25,26). WHO studies revealed that 170 million of people infected with HCV all over the world (27). The current study showed that the seroprevalence of HCV among the study group was 26%. In comparison of this result with studies from other countries, we found a low prevalence for HCV infection among our thalassemic patients. The prevalence of HCV seropositivity in multi transfused β- thalassemia patients have been observed to vary greatly from 11 to 60% (28). Two factors probably explained this wide variability, the prevalence of HCV in the general population and hence in blood donors and the practice of strict screening for HCV antibody before blood transfusion.The countries with a higher HCV prevalence in general population had a higher prevalence rate among thalassemia patients, too. For Instance, a study in Egypt reported 75% of HCV prevalence among thalassemia patients, considering the fact that the prevalence in their blood donor population was 14.5% (29). In Iraq, the prevalence of anti-HCV among the general population was very low,0.4% (21). In a previous study in the same center, Ibn AL-Baladi

hospital, during 2006, the prevalence of anti HCV antibody was high, 67.3% (24). Difference in the sample and technique use for the diagnosis is probably behind this difference. In a study from Mosul, a seroprevalenc of anti HCV antibody among thalassemic patient were 26.20% (30) a result close to the current study. Other studies from some neighboring Arabic countries reported a HCV infection rate of 33% in Kuwait in 1998 (31), 40% in Bahrain at1995 (32), and 40.5% Jordan at 2001(33). Study of Allavian, et al (2010) provides a comprehensive and reliable tabulation of available data on the epidemiological characteristics and risk factors for HCV infection in thalassemic patients in eastern Mediterranean countries. He concluded that there is enormous heterogeneity in the available study results in this region, and distribution of HCV infection among these patients living in this region is still unknown. The data were available from 50% of countries in this region and most of these data suffered the low sample size and outdatedness. Among the major eastern Mediterranean countries, Iran has the least seroprevalence of HCV infection among thalassemia patients, indicating more advanced blood safety in this country compared with other countries with comparable population in this region (6). Regarding age of the patients, the prevalence of hepatitis C was significantly higher among older age group. This is expected, since older thalassemic patient had longer duration and higher frequency of transfusion. A similar result reported by other studies from different countries (34,35). Regarding gender of patients, this study showed that no statistical difference in HCV infection found between males and females because there is no gender difference of HCV infection and thalassemia syndrome This is similar to the study from Iran by Ghafourian Boroujerdnia M(36). In the current study a significant association between HCV infection and educational level of patient's mothers was found. Mothers with secondary and higher educational level

had low prevalence of HCV infection in their thalassemic children because they are more aware and more cautious when the matter related to blood transfusions or other risky procedures. But patients whose mothers with primary educational level had high prevalence of HCV infection than those with illiterate mothers. This is a probably a bias because large number of registered patients' mothers were of primary educational level. The current study revealed that patients with thalassemia major and younger age when first diagnosed are at more risk for have the infection with HCV. Both these factors making patients receive frequent blood transfusion at an earlier age, similar results reported by other studies (35, 37). On the other hand, no significant association of HCV infection with family history of thalassemia was found. This is because Lack of transmission of hepatitis C in household contacts of children with homozygous beta-thalassemia as concluded by study of Papanastasiou (38).The majority of HCV infection were found among those having more than 20-year duration of blood transfusion. It is clear that as the period of blood transfusion increase, the risk of infection with HCV is greater. Additionally, screening test of blood donor was first implemented in Iraq in 1996 making older patient with long duration at more chance of getting HCV from transfusion before 1996. In Iran, the prevalence of HCV infection dropped significantly from 22.8% to 2.6%, after the implementation of blood donor screening programe in 1995. In addition, patients who received unscreened blood were exposed to HCV infection more than six times as much as those transfused after starting the screening program. He conclude that the meticulous screening of blood donors will eventually eliminate the incidence of HCV infection among thalassemics patients in Iran(39). The current study showed a significant association of infected patients and frequency of blood transfusion per month, the majority of patients with positive anti HCV antibody have two times per month frequency of blood transfusion. Furthermore, all seropositive HCV patient have good compliance with regular visit to the center. Angelucci and Pilo in 2008 postulated that among thalassemic patients transfused before the 1990s, the prevalence of HCV infection was shown to be proportional to the number of units of blood received, and approached 80% in the adult patients (40).

The current study reveals no significant association between knowledge of parents and patient about blood transfusion associated HCV infection and seropositivity for anti HCV antibody. However, patients from families with history of HCV have low prevalence of anti HCV antibody. This is may be because family members are a very important source not only for information, but also of behavioral modeling and these patients are more cautious and more aware when the matter related to blood transfusion or other risky procedures. The current study showed no significant association between possible rout of acquiring the infection (history of previous injection, history of previous tattoo, desferal injection) and positive anti HCV antibody , this is in contrast to the result of study done by Bair RM which indicates that drug injection was linked with majority of HCV infection (41). We though the blood transfusion to be the main rout of acquisition the HCV in our thalassemic patients. Our study also find a significant association between blood group and HCV infection in thalassemic patient. Blood group O patients have high percentage from patients infected with HCV . This result

is in accordance with the findings of Ansari study (42), his results suggest that the highest prevalence of hepatitis C existed among the patients with O blood group (20.6%); he postulated that this blood group is more susceptible to contamination than other blood groups. No other explanations can be given. Therefore, careful screening of this blood group is more important. The presence of hepatomegaly and /or splenomegaly found to have a statistically significant association with seropositivity for HCV. Furthermore, seropositive HCV antibody were higher among splectomized than non splenectomized patients, this was statistically non-significant. Hypersplenism increase the requirement for blood transfusion and hence increase the risk of hepatitis. So, early splenctomy (after 4 years and before 15 years) may decrease the chance of getting HCV infection by decreasing the need for blood transfusion, this is supported by study done by Al-Salem (43). Although no significant association found between liver functional tests and seropositivity for HCV, most of positive HCV infected patients had elevated liver functional test. This abnormal liver function test might be related to HCV infection or is due to deposition of iron in the liver in patients who were noncompliant with desferrioxamine infusion program. This finding supported by study from Iran by Touran S (44) which observed an association between the presence of HCV and abnormal liver tests. Another study from Iran also demonstrate a close association between elevated ALT with iron overload, transfusion index, age, and anti-HCV positivity (45). The main limitation of this study is that it involves one public, tertiary hospital in the Baghdad city. Therefore, the transferability of the findings to other settings will be challenging. From the results of this study we conclude that about quarter of Iraqi thalassemic patients visiting the thalassemia center in Ibn-AL-Baladi hospital have HCV infection. The prevalence of HCV infection was higher among patients with thalassemia major, from older age group, whose mothers had primary educational level, with 20 years of blood transfusion, two times per month frequency of blood transfusion and patient who has good compliant with blood transfusion. Patients without family history of HCV, of blood group O and with hepatomegally and or splenomegaly also have higher prevalence of HCV infection.

We recommend that effective screening program should be apply to all donor of blood for thalassemic patient. Strict measures for the controlling the spread of HCV are needed by introducing advanced techniques for blood donor screening, education of families about the transfusion program, iron chelating therapy, importance of regular visits and other optimum preventive measures like detection of carrier state. Further studies are recommended in other centers in the country to have a better idea on the exact prevalence of HCV in thalassemic patients.

References:

1. Giardina PJ, Forget BG. Thalassemia syndromes. In: Hoffman R, Benz EJ, Shattil SS, et al., eds. *Hematology: Basic Principles and Practice*. 5th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; 2008: chap 41.p.485

2. Noguchi, C.T.; Butterwoth, J.R.; Karawajew, L.; Küppers, R.; Favaloro, E; and Jacobsohn, D. Reducing oxidative damage and cell

dehydration in thalassaemia. Hematological. 2004; 89:1281- 1283.

3. DeBaun MR, Frei-Jones M, Vichinsky E. Hemoglobinopathies. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, eds. Nelson Textbook of Pediatrics. 19th ed. Philadelphia, Pa: Saunders Elsevier; 2011: chap 456.

4. Patel J, Patel A, Patel J, Kaur A, Patel V. Prevalence of haemoglobinopathies in Gujarat, India: A cross-sectional study. Int J Hematol. 2009;5:1.

5. DJ (2010). The thalassaemia: Disorders of globins synthesis. In K Kaushansky et al., eds., Williams Hematology, 8th ed., pp. 675-707.New york:McGraw-Hill

6. Alavian SM, Tabatabaei S, Lankarani K. Epidemiology of HCV infection among thalassaemia patients in eastern Mediterranean countries: A quantitative review of literature. Iran Red Crescent Med J. 2010;12:365-76.

7. Wong, Li Ping, Elizabeth George, and Jin-Ai Mary Anne Tan. ‾Public Perceptions and Attitudes toward Thalassaemia: Influencing Factors in a Multi- Racial Population.‖ *BMC Public Health* . 2011;11: 193.

8. Hanan A. Hamamy, Nasir A. S. Al-Allawi. Epidemiological profile of common haemoglobinopathies in Arab countries. J Community Genet. 2013 Apr; 4(2): 147-167.

9. Laila Zahed. The spectrum of β-thalassemia mutations in the Arab populations. Journal of Biomedicine and Biotechnology. 2001; 1:3 : 129-132.

10. Al-Allawi NAS , Jubrael JMS, Anwar A.,Fariq F.(2007) Service indicators from a regional hemoglobinopathy preventive program in Duhok-Iraq. Proceedings of the2 ndpanaArab human genetics conference, Dubai, CAGS, 20-22 2nd November pp78.

11. Hassan MK, Taha JY, Al-Noama LM, Widad NM, Jasim SN Frequency of Hemoglobinopathies and Glucose-6-phosphate dehydrogenase Deficiency in Basra. East. Mediterr. Health J. 2003; 9(1/2): 1.

12. Yahya HI, Khalel KJ, Al-Allawi NAS,Thalassaemia genes in Baghdad-Iraq. East. Mediterr. Health J . 1996;2(2):315-319.

13. Jalal SD, Al-Allawi NAS, Faraj AH, Ahmed NH, Prevalence of Hemoglobinopathies in sulaimani-IRAQ Dohuk Med. J. 2008; 2: 71-79.

14. National Institutes of Health. National Institutes of Health consensus development conference statement: management of hepatitis C: 2002--

June 10-12, 2002. Hepatology. 2002;36(suppl 1):S3-S20. Abstract

15. Alter MJ. Prevention of spread of hepatitis C. Hepatology. 2002;36:S93-S98. Abstract

16. Farahnaz Fallahian, Atabak Najafi. Epidemiology of Hepatitis C in the Middle East .Saudi J Kidney Dis Transplant 2011;22(1):1-9.

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

18. 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.

19. Alter MJ. Epidemiology of hepatitis C virus infection. World Journal of Gastroenterology: *WJG*. 2007;13(17):2436-2441.

20. Sy TY, Mazen Jamal M. Epidemiology of hepatitis C virus infection. Int J Med Sci. 2006;3:41-46.

21. Ata allah M.Tarky , Wijdan Akram , Ahmed S. Al-Naaimi , Ali Rijab Omer. Epidemiology of viral hepatitis B and C in Iraq: a national survey 2005-2006. ZJMS. 2013; 17 (1) : 370-380.

22. Lavanchy, D. The global burden of hepatitis C. Liver Int. 2009; 29(1):74-81.

23. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558-567.

24. Al-Kubaisy WA. Al-Naib KT &Habib M. Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. Eastern Mediterranean Health J, 2006; 12(1-2): 204-10.

25. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med. 1995; 332:1463-6.

26. di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ. Long-term clinical and histopathological follow-up of chronic post-transfusion hepatitis. Hepatology. 1991; 14:969-74.

27. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat. 1999; 6:35-47.

28. Muhammad Younus, Khalid Hassan, Nadeem Ikram. Hepatitis C Virus Seropositivity in

Repeatedly Transfused Thalassemia Major Patients, International Journal of Pathology; 2004; 2(1):20-23

29. El-Gohary A, Hassan A, Nooman Z. High prevalence of hepatitis C virus among urban and rural population groups in Egypt. Acta Trop 1995;59(2):155-61.

30. Al-Hamadany, B.S. The prevalence of hepatitis B and C in Mosul Province. A thesis submitted to the Iraqi committee for medical specialization in partial fulfillment of the requirement for the degree of fellowship of Iraqi committee for medical specialization in Pediatric, 2008 (not published).

31. Al-Fuzae L, Aboolbacker KC, Al-Saleh Q. Betathalassemia major in Kuwait. *J.* Trop.Pediatr.1998;44:311-312.

32. Al-Mahroos FT, Ebrahim A. Prevalence of hepatitis B, hepatitis C, and human immune deficiency virus markers among patients with hereditary hemolytic anaemias. Ann Trop Paediatr. 1995; 15: 121 – 128.

33. Al-Sheyyab M, Batieha A, El-Khateeb M. The prevalence of hepatitis B, hepatitis C, and human immune deficiency virus markers in multi transfused patients. J Trop Pediatr. 2001; 47: 239 – 242.

34. Ghafourian Boroujerdnia M, Assareh Zadegan MA, Zandian KM, Haghirizadeh Rodan M. Prevalence of Hepatitis C virus (HCV) among Thalassemia Patients in Khuzestan Province, Southwest Iran. Pak J Med Sci 2009;25(1):113-117.

35. Chung-JL; Kao-JH; Kong-MS; Yang-CP; Hung-IJ; Lin-TY. Hepatitis C and G virus infections in polytransfusedchildren.Eur-J-Pediatr. 1997; 156(7): 546-9.

36. Ghafourian Boroujerdnia M, Assareh Zadegan MA, Zandian KM, Haghirizadeh Rodan M.Prevalence of Hepatitis C virus (HCV) among Thalassemia Patients in Khuzestan Province Southwest Iran. Pak J Med Sci 2009;25(1):113-117.

37. Laosombat V, Pornpatkul M, Wongchanchailert M, Worachat K, Wiriyasatienku A. The prevalence of hepatitis C virus antibodies in thalassemic patients in the south of Thailand. Southeast Asian J Trop Med Public Health.1997;28(1):149-53.

38. Papanastasiou, D. A., I. Spiliopoulou, S. Katinakis, A. Karana-Ginopoulou, and M. Repanti. ‖Lack of Transmission of Hepatitis C in Household Contacts of Children with Homozygous Beta-Thalassaemia.‖ Acta Hematologica .1997; 97(3): 168–73.

39. MirmomenS;AlavianSM;Hajarizadeh B; et al. Epidemiology of hepatitis B, hepatitis C, and human immunodeficiency virus infections in patients with betathalassemia in Iran: a multicenter study.Arch Iran Med. 2006;9(4):319-323.

40. Angelucci, E and Pilo, F .Treatment of hepatitis C in patients with thalassemia.haematologica.2008; 93(8):1121 - 1123.

41. Bair RM, Baillargeon PJ, et al: Prevalance and risk factors for hepatitis C virus infection among adolescents in detention. Arch Pediator Adolesc Med 2005;159:1015-1018.

42. Ansari, H. Kamani,H. and ArbabiSarjo,A. Prevalence of Hepatitis C and Related Factors among Beta-Thalassemia Major Patients in Southern Iran in 2005- 2006. . J. Medical Sci. 2007;7:997-1002.

43. Al-Salem, A.H. Is splenectomy for massive splenomegaly safe in children ?.Amm. J. Surg. 1999;178(1)42-5.

44. TouranS, Mansour Shahraki, EsmaielSaneiMoghaddam, MehriNajafi and Ali Bahari. Determination of Hepatitis C Genotypes and the Viral Titer Distribution in Children and Adolescents with Major Thalassemia.Iran J Pediatr. 2010; *20 (1):75- 81*.

45. Ameli M, Besharati S, Nemati K, Zamani F. Relationship between elevated liver enzyme with iron overload and viral hepatitis in thalassemia