Review

Epidemiological and clinical characteristics of HCV infection in transfusion-dependent thalassemia

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SUMMARY

Hepatitis C virus (HCV) is considered the principal etiologic agent of post-transfusion hepatitis and is the main cause of chronic liver disease in multi-transfused subjects. Transfusion-associated HCV infection has become rare since blood donor screening was initiated in 1990.

There is an impressively wide range of anti-HCV(+) prevalence in thalassemics worldwide. This range varies from 2.7% to 97%. Studies in the early 90's report higher anti-HCV rates compared to studies after 1993 when anti-HCV screening was largely available in transfusion centres. However large differences in anti-HCV(+) between centers in the same country seem to need further explanation.

HCV genotype distribution also varies significantly between countries and between thalassemia centres within the same country. In studies from Asian countries, it seems that HCV infection genotypes 3 and 6 predominate. In studies from Europe there is a genotype 1 predominance, while in Greece it seems that genotype 3 predominates.

Patients with β-thalassemia in Northwest Greece have an HCV seroprevalence of 22.7% which is the lowest described in Greece, while serum HCV-RNA was not detectable in the majority (74%) of HCV(+) patients. No HCV(+) patient was co-infected with HIV, HBV, HSV1, HSV2 and CMV. HCV-RNA was detectable in 4 (26%) patients and transaminasemia was frequent (73%).

Alpha-interferon (a-IFN) is the first-line treatment for thalassemic patients diagnosed with HCV infection. There is, therefore, particular clinical importance to determine the incidence of HCV infection in thalassemic patients in order to facilitate monitoring and treatment policy.

Key words: HCV infection, thalassemia, prevalence, HCV-RNA, anti-HCV(+).

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1. INTRODUCTION

Patients with thalassemia are at high risk of acquiring a number of viral infections during multiple blood transfusions. Of these infections, hepatitis B and C and human immunodeficiency virus infections are extremely important. Preventing these infections is mandatory for improving survival and quality of life of thalassemic patients. Additionally, iron overload is a major cause of chronic liver disease in patients with β-thalassemia major (βMT). Although iron overload is an independent cause of liver dysfunction in thalassemics, the relationship between liver disease and the iron status in anti-HCV(+) patients has been poorly investigated. Hepatitis C virus (HCV) is considered the principal etiologic agent of post-transfusion hepatitis and is the main cause of chronic liver disease in multi-transfused subjects such

Abbreviations used in the text

HCV= *hepatitis C virus*
HCV-RNA= *Hepatitis C virus ribonucleic acid*
PCR=*polymerase chain reaction*
ALT= *alanine aminotransferase*
βTM= β*-thalassemia major*

as patients with β-thalassemia major (βTM). For this reason, HCV infections are found in many βTM patients worldwide. HCV isolates also display high levels of sequence heterogeneity, allowing classification into at least 11 types and 90 subtypes. Thalassemic patients may acquire hepatitis C through the administration of HCV-infected blood collected during the donor window period. Moreover, they have frequent hospitalizations, which is an additional risk factor for HCV infection.

Transfusion-associated HCV infection has become rare since blood donor screening was initiated in 1990. β-Thalassemia is an extremely rare genetic disorder among Northern European and North American populations. However, it still remains a challenge for many health care systems due to continuous immigration of people, especially from countries around the Mediterranean Sea, which are endemic for β-thalassemia.[1-2]

2. WORLDWIDE PREVALENCE OF ANTI-HCV(+) IN THALASSEMICS.

The prevalence of antibodies to HCV infection (anti-HCV) varies widely among multi-transfused patients including patients with thalassemia. In fact, according to a large number of epidemiological studies, it seems that hepatitis C virus infects a great proportion of transfusion-dependent β-thalassemic patients although transfusion-associated HCV infection has become rare since blood donor screening started in the early 1990s.[3-4]

There is an impressively wide range of anti-HCV prevalence in thalassemics worldwide[5-63] [Table 1]. This range varies from 97% in Italy[19] to 2.7% in Gouadeloupe (France).[41] However it must be mentioned that studies in the early 90's report higher anti-HCV rates compared to studies after 1993 when, anti-HCV screening became largely available in transfusion centres [Table 2].

In Asia the anti-HCV prevalence ranges from 5.8% (Malaysia)[36] to 63.8% (Iran)[27] according to papers published from 1991 to 2002. Differences between centres in the same country also exist, suggesting local HCV endemicity irrespective of transfusion-acquired HCV infection [Table 1]. Generally, it seems that one third to one fourth of Asian thalassemics is anti-HCV positive.

In South America (Brasil) the anti-HCV positivity reached 46.8% of the total thalassemic population in a study published in 1993.[48]

There are many studies in thalassemic populations around the Mediterranean basin, showing large discrepancies in HCV prevalence numbers.[5-15] This phenomenon may represent either real difference across Mediterranean Sea countries or a lack of homogeneity as far as the thalassemic population origin and hospital care is concerned.[1]

In Middle East anti-HCV (+) prevalence presents a remarkably stable range from 40% to 70% according to studies published from 1993 to 2001 [Table 1]. The highest prevalence has been reported in Egypt (75.6%)[11] and the lowest (40%) in Bahrain (Saudi Arabia).[46]

In southern European countries the prevalence of anti-HCV (+) in the thalassemic population varies among countries and among centres in the same country. In detail anti-HCV (+) prevalence in Italy has ranged from 14.8%[2] to 97%[19] according to studies in the 1992-2001 period [Table 2]. In studies during the period 1993-1996 in Romania the range was 33.8% to 91.8% and in studies from Greece during the 1995-2001 period the range was 57%[22] to 91%.[6]

In European studies there is a remarkable difference of anti-HCV (+) prevalence between northern and southern Europe [Table 1]. However it must be emphasized that the vast majority of northern European patients are second or third generation immigrants of from South Europe and the Middle East. Thus the anti-HCV (+) prevalence in Northern Europe ranges from 2.7%[41] to 33%.[43] One fifth to one third of northern Europian thalassemics are anti-HCV (+) according to studies from UK, Germany and France.

It is generally accepted that anti-HCV(+) prevalence seems to have decrease in more recent studies [Table 2]. However, large differences in anti-HCV(+) between centres of the same country seem to need further explanations.

3. WORLDWIDE PREVALENCE OF HCV GENOTYPES IN THALASSEMICS.

Genotype distribution varies significantly between countries and between thalassemia centres within the same country [Table 3]. In studies from Asian countries, which have been published recently, it seems that HCV infection genotypes 3 and 6 predominate in Pakistan and Hong Kong respectively. In detail, the Hong Kong study[54] showed a 50% prevalence of HCV genotype 6, although the number of HCV-RNA(+) patients was restricted (twelve). Among 70 HCV-RNA(+) patients from Pakistan, 89% were diagnosed with 3a and 3b genotypes.[50] In the same year another thalassemia centre from Pakistan with 19 HCV-RNA(+) patients reported a

Table 1. Distribution of prevalence of anti-HCV (%) in thalassemia in several geographical areas.

Geographic area	Period of publications	Prevalence of anti-HCV (%)
North Europe	***1990-2002***	***2.7-33***
UK	1990-92	11.1-30
Germany	2000	22
France	1992	33
Gouadeloupe .(France)	2002	2.7
South Europe	***1992-2001***	***14.8-97***
Greece	1995-2001	57-91
Italy	1992-2001	14.8-97
Romania	1993-96	33.8-91.8
Middle East	***1993-1999***	***40-75.6***
Libanon	1999	47.1
Egypt	1995	44-75.6
Saudi Arabia/ Bahrain	1993-96	40-70
Jordan	2001	40.5
Asia	***1991-2002***	***5.8-63.8***
India	1991-2002	14.3-35.9
Iran	2001-2002	15.7-63.8
Pakistan	1995-2002	14-60
Burma	2000	50
Malaysia	1993-98	5.8-22.4
Thailand	1997	23.8
Taiwan	1996	42.6
Hong Kong	1993	34.3
South America	***1993***	***46.8***
Brasil	1993	46.8

different genotype distribution:[51] 1a(21%)/1b(16%)/2b(5%)/3a(21%)/4(37%). In five HCV-RNA(+) thalassemics from Taiwan, genotype 1 was diagnosed in 4 of them.[18]

In studies from Europe there is also a noteworthy genotype variety. In Romania[53] and Italy[52] there is a genotype 1 predominance (in over 50% of HCV-RNA(+) thalassemics) while in Greece it seems that genotype 3 predominates.[49] It is noteworthy that in these studies and according to the authors, in some patients genotyping was technically impossible (including mixed genotypes).

4. CLINICO-EPIDEMIOLOGIC CHARACTERISTICS OF HCV INFECTION IN NORTHWEST GREECE *B*-THALASSEMICS.

The results of an HCV clinico-epidemiological study of a cohort of β-thalassemic patients followed over a mean period of eleven years by the only referral centre for thalassemia in Epirus, a well-defined geographical area in Northwest Greece, are briefly presented here.

The subjects included 66 thalassemic transfusion dependent patients (41 with major β-thalassemia, 25 with intermediate thalassemia, (mean age of 29.6 years, range 18-55 years) followed by the referral centre of thalassemia in Epirus, Northwest Greece [Figure]. None of these patients had a previous history of anti-viral treatment, intravenous drug abuse, thrombosis, recurrent abortion or thrombocytopenia.

All samples were screened for HCV, hepatitis B, herpes simplex virus, cytomegalovirus and human immunodeficiency virus (HIV) infection.

Anti-HCV antibodies were carried out by third-gen-

Table 2. Prevalence of HCV-RNA positivity in thalassemic patients.

Author	Country	Publication year	No of patients	% of patients anti- HCV (+)
Irshad et al[29].	India	2002	50	30%
Jaiswal et al[30].	India	2001	104	21%
Juneja et al[33].	India	1998	64	21%
Choudhury et al[32].	India	1998	39	35.9%
Jaiswal et al[34]	India	1996	*	24.45%
Agarwal et al[37].	India	1993	72	16.7%
Amarapurhar et al[60].	India	1992	40	17.5%
Bhattacharya et al[39].	India	1991	70	14.3%
Ansar et al[27].	Iran	2002	105	63.8%
Karimi et al[28].	Iran	2001	466	15.7%
Ramia et al[51].	Pakistan	2002	395	14%
Bhatti et al[35].	Pakistan	1995	35	60%
Le Turdu-Chicot et al[41].	Gouadeloupe	2002	331	2.7%
Spiliopoulou et al[6].	Greece	1995	24	91%
Lambropoulou et al[21].	Greece	1999	108	59.2%
Spiliopoulou et al[20].	Greece	1999	122	61%
Sougleri et al[22].	Greece	2001	28	57%
Al-Sheyyab et al[3].	Jordan	2001	143	40.5%
Perniola et al[55].	Italy	1999	88	60%
De Luca et al[61].	Italy	1999	48	81.2%
Prati et al[2].	Italy	1998	1481	14.8%
Sampietro et al[19].	Italy	2001	68	97%
Bagnulo et al[24].	Italy	1998	19	21%
Mangiali et al[9].	Italy	1997	75	56%
Conjia et al[56].	Italy	1996	420	74.7%
Livrea et al[62].	Italy	1996	42	73.8%
Erer et al[12].	Italy	1994	98	51%
Angelucci et al[5].	Italy	1994	256	60%
Lai et al[42].	Italy	1993	135	55.5%
Locasciulli et al[13].	Italy	1993	25	76%
Resti el al[14].	Italy	1992	78	50%
Cacopardo et al[15].	Italy	1992	152	47%
Nigro et al[7].	Italy	1992	36	33.3%
Giordano et al[63].	Italy	1998	68	73.5%
Okada et al[31].	Burma	2000	*	50%
Jamal et al[25].	Malaysia	1998	85	22.4%
Isahak et al[36].	Malaysia	1993	52	5.8%
Taher et al[59].	Libanon	1999	17	47.1%
Antipa et al[8].	Romania	1993	61	91.8%
Antpa et al[53].	Romania	1996	18	33.8%
Laosombat et al[26].	Thailand	1997	101	23.8%
Al-Fawaz et al[45].	S. Arabia	1996	28	57.1%
Al-Fawaz et al[47].	S. Arabia	1993	20	70%
Ni et al[67].	Taiwan	1996	61	42.6%
Al-Mahroos et al[46].	Bahrain	1995	59	40%
El-Nanawy et al[10].	Egypt	1995	18	44%
El-Cohary et al[11].	Egypt	1995	*	75.6%
Covas et al[48].	Brasil	1993	32	46.8%
Lau et al[38].	Hong Kong	1993	99	34.3%
Williams et al[4].	UK	1992	54	11.1%
Donohue et al[40].	UK	1990	219	18.8-30%
Wonke et al[44].	UK	1990	73	23.3%
De Montalembert et al[43].	France	1992	305	33%
Cario et al[1].	Germany	2000	152	22%

**not available, data from indirect information.*

Table 3. Prevalence of HCV genotypes in thalassemic patients.

Author	Country	Year	No of patients HCV-RNA (+)	Genotype distribution* 1 / 2 / 3 / 4
Akhtar et al[50].	Pakistan	2002	70	3a & 3b (89%)
Ramia et al[51].	Pakistan	2002	19	1a(21%)/1b(16%)/ 2b(5%)/3a(21%)/4(37%)
Wong et al[54].	Hong Kong	1998	12	Genotype 6 (50%)
Diamantis et al[49].	Greece	1998	16	3a (50%)
Antipa et al[53].	Romania	1996	18	1 (51.1%)/2(13.3%)
Chung et al[18].	Taiwan	1997	5	4 / 1 / 0 / 0
Christophidou et al[16].	Greece	2000	13	2 / 0 /4 / 7
Di Marco et al[52].	Italy	1997	63	1b(65.1%)/ [non-1b type in 20.6%]

**In some patients genotyping was technically impossible (including mixed genotypes) according to authors*

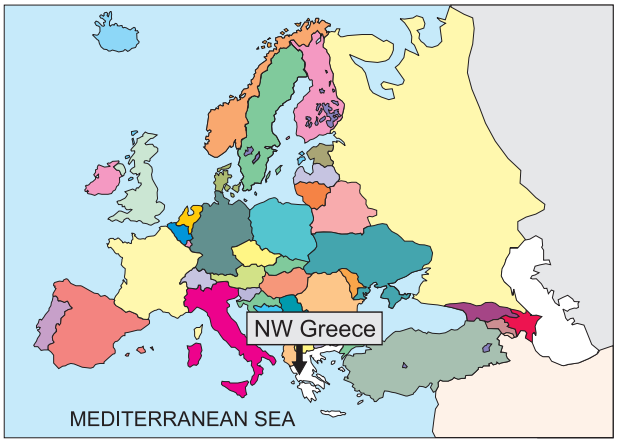

Fig. The area of Northwest Greece

eration ELISA (ELISA test system, Abbot, Germany). Anti-HCV(+) serum samples underwent confirmatory immunoassay (Innogenetics, Belgium). Serum HCV-RNA measurement was performed by PCR (Combas Amplicor, Roche, cut off values of 615 UI/ml)) while HCV genotyping was carried out using a line probe assay (Innogenetics, Belgium).

Hepatitis C prevalence in healthy blood donors in Greece is approximately 0.5%[16,17]. None of the thalassemic patients examined was positive for infection with HIV, HBV (all patients were vaccinated against hepatitis B virus), HSV1, HSV2 (herpes simplex virus type 1 & 2) and CMV (cytomegalovirus). The clinical data of all patients is summarized in Table 4.

Fifteen out of 66 (22.7%) patients were HCV(+) [double tested].Of these 15 HCV(+) patients (10 males, 5 females, mean age 28.3 years, range from 18 to 42 years), 13 were diagnosed with major β-thalassemia and 2 were diagnosed with intermediate β-thalassemia.

HCV-RNA was detected in sera of 4 HCV(+)patients [26% of all HCV(+) patients, range of 10^3 to 10^6 UI/ml]. Of these four HCV-RNA(+) patients [mean age 31 years) two patients were diagnosed with genotype 1a, one with genotype 3 and one with genotype 4 [Table 5]. No HCV-RNA seroconversion was noticed during follow up of these HCV(+)/HCV-RNA(-) patients.

Serum ferritin was increased in all β-thalassemia patients (range of 213 to 7105 ng/ml), while total bilirubin was within expected levels (range of 1.0 to 3.5 mg/dl). Platelet count, INR and aPTT were within normal limits. No patient had any evidence of other coagulation abnormalities or autoimmune disorders. None of all patients examined had any clinical evidence of liver cirrhosis or decompensated liver disease or of other kind of focal liver abnormality on abdominal ultrasound or magnetic resonance imaging. However, organ dysfunction secondary to hemochromatosis was present in 33 patients; 12 patients with hypogonadism, 8 patients with severe osteoporosis, 4 patients with heart failure, 3 patients with diabetes mellitus, 3 patients with hypoparathyroidism and 3 patients with hypothyroidism.

Alanine aminotransferase (ALT) levels were increased in 11 out of 15 HCV(+) patients (73%). Alanine aminotransferase elevation was minimal to mild (0.5 to 4-fold increase) in the great majority of HCV(+) patients and this protype remained stable during a three

Table 4. Clinical and laboratory data on Northwest Greece β-thalassemics

No of patients with β-thallassemia	66
-with major thalassemia	41
-with indermediate thalassemia	25
Hepatis B positive	0 (all patients vaccinated)
HIV positive	0
CMV positive (serum IgM antibodies /PCR)	0
Herpes simplex viruses (HSV1 & HSV2)	0
Platelet number range	150,000-450,000 mm^3
Serum ferritin	213-7105 ng/ml
Serum total bilirubin	1.0-3.5 mg/dl
ALT range [11 of 15 HCV(+) patients]	73 -158 UI/ml

Table 5. HCV-RNA data in the HCV(+)Northwest Greece β-thalassemics.

Anti-HCV status	Patients	HCV-RNA positive	HCV-RNA negative
Anti-HCV positive	15	4*	11
Anti-HCV negative	51	0	51

** Two patients diagnosed with genotype 1a, one patient with genotype 3 and one patient with genotype 4.*

year follow up. Two HCV(+) patients had simultaneously HCV-RNA(+) and mildly elevated ALT levels.

Five out of fifteen HCV(+) patients accepted to undergo percutaneous liver biopsy, three of whom were finally introduced to interferon-a therapy (3MU/three times a week subcutaneously). The two patients with genotypes 3 and 4 respectively showed sustained virological and biochemical response at the end of six months treatment while the third patient (with genotype 1a) did not respond to twelve month treatment.

5. COMPARATIVE EPIDEMIOLOGY IN ANTI-HCV(+) THALASSEMICS USING THE NW GREECE DATA MODEL

The prevalence of HCV(+) in β-thalassemia patients in Northwest Greece according to this study is 22.7%. As there is no previous study in the region of Northwest Greece to compare with, it is interesting to mention that the prevalence of HCV(+) β-thalassemics in Northwest Greece is at least half of the prevalence of HCV(+) β-thalassemics in South Greece according to direct or indirect information from studies published from the area of Patras.[6,20-22] This discrepancy may reflect differences in patient clinical characteristics including disease severity, transfusion requirements or total number of blood units transfused, disease duration in terms of life expectancy and high-risk behavior. Differences in quality of hospital care and blood bank facilities or differences between doctor and nurse education and training could attribute to this difference in HCV prevalence, as this may also happen among countries and continents; however, this is an argument of only theoretical value. This difference in HCV prevalence in these two different regions of Greece may also reflect the difference in risk of acquiring HCV in household contacts.[23] Genetic differences determining host immune reaction to HCV cannot be underestimated; however no such convincing data is available in the current literature.

The prevalence of HCV(+) in β-thalassemics in Northwest Greece is similar or comparable to this reported in Bari (South Italy),[24] in Germany,[1] in Malaysia[25] and Thailand[26] [Table 2]. In addition, this prevalence is within the range-towards the lower limit-of the HCV(+) prevalence reported in β-thalassemics in studies across Asia[27-39] and Europe.[40-44] By contrast, prevalence of HCV(+) in β-thalassemics in northwest Greece is at least half of that reported in the Middle East[45-47] and South America[48] [Table 1]. It can be supported that areas such as the Middle East and South America with high HCV prevalence in β-thalassemics may have also a higher prevalence of HCV infection in the general population. However, the last may not be the only cause for these chaotic differences in HCV prevalence in the transfusion-dependent thalassemic population worldwide.

The prevalence of positive HCV-RNA in β-thalas-

semia in Northwest Greece is 26% in HCV(+) patients which is comparable to the results already published worldwide [data not shown]. It seems that thalassemics with no detectable HCV-RNA are the majority of HCV(+) thalassemics worldwide. However, as HCV virus flares may happen in any such patient during follow up, all HCV(+)/HCV-RNA(-) thalassemics need regular testing for viral load.

The HCV genotype distribution analysis in only four β-thalassemia patients showed the existence of genotypes 1a, 3 and 4. Thus, only speculations can be made about the distribution of prevalence of HCV genotypes in the Northwest Greece thalassemic population. Two studies in South Greek thalassemics showed HCV genotype 3a and 4 predominance and also showed the existance of genotype 1a.[16,49] Studies from Pakistan[50-51] present the same HCV profile as Greece, but studies from Italy[52] and Romania[53] showed genotype 1a predominance [Table 3]. The most common HCV major genotypes in HCV-RNA(+) thalassemics seem to follow the analogy of HCV genotype distribution of their homeland population, but this speculation needs confirmatory national multicentre studies.[54]

CONCLUSIONS

Although the incidence of post-transfusion hepatitis has been greatly reduced by the introduction of blood donor screening for antibodies to hepatitis C virus, multi-transfused patients are still at risk of developing liver dysfunction. In fact, cases of post-transfusion hepatitis have been described that are not related to known viruses and which cannot be prevented by current techniques for donor selection.[18-19]

In addition, despite the fact that the incidence of post-transfusion hepatitis has been reduced by the introduction of hepatitis B vaccination, it seems that research on vaccination against HCV infection still remains far from being applied in routine practice.[2] Consequently, hepatitis C will remain an important clinical issue in multi-transfused patients, including patients with thalassemia.

At present, there is no prophylaxis available against HCV infection. Alpha-interferon (a-IFN) is the first-line treatment for thalassemic patients diagnosed with HCV infection.[64-66] It is, therefore, particularly clinically importance to determine the incidence of HCV infection in thalassemic patients, in order to facilitate monitoring and treatment policy.

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