

EPIDEMIOLOGY OF HEPATITIS C IN CENTRAL PORTUGAL

Prevalence of anti-HCV in the population of the Coimbra district

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S U M M A R Y

Anti-hepatitis C virus antibody (anti-HCV) screening was performed in a sample of the adult population of the Coimbra District. 657 persons were included (267 male and 390 female, mean age of 42.7+13.1 years), aleatorily chosen from four characteristic regions. Anti-HCV was detected using an ELISA-2 test and all positive sera were also tested with RIBA-2. General prevalence of anti-HCV was 0.46%. All positive patients live in urban areas and presented risk factors for HCV infection. Anti-HCV was found in 33.3% of intravenous drug abusers, in 1.8% of transfused individuals, in 1.3% of alcoholics (higher than 80 g/d alcohol ingestion), in 1% of cases with history of surgical operations, and in 0.65% of persons who lived in risk regions for hepatitis B. We conclude that anti-HCV prevalence is low in our region. We think it is important to perform other studies on larger samples of general population and to study risk groups.

INTRODUCTION

During the last decades, a great volume of knowledge has been acquired on non-A, non-B hepatitis (NANBH), despite not knowing the virus and the absence of a serological test for diagnosis. This information is the result of the study of clinical data, based on the serological exclusion of infection by hepatitis A and B viruses, and on the experiments performed on chimpanzees¹. In 1988, Houghton et al., at the Chiron Corporation, and Bradley, at the Centers for Disease Control, were able to clone a virus² and develop a test to detect antibodies against the proteinic products of these clones³, which proved to be specific and sensitive, when tested on a panel of serum taken from patients with the diagnosis of NANBH⁴. It was therefore possible to have a marker, the anti-hepatitis C virus antibody (anti-HCV), the conditions then existing for a sounder knowledge of this entity, namely in the epidemiological aspect. The development of the *recombinant immunoblot assay* (RIBA) test and, later, of 2nd generation tests, ELISA and RIBA, allowed the gathering of even more precise

data⁴. However, we are still far from having a complete knowledge of hepatitis C as we have, for example, of hepatitis B.

Since then, data on the prevalence of anti-HCV in various groups considered at risk began to emerge from all over. Thus, it was observed that high prevalence existed in patients with post-transfusion (70 to 80%)^{5,6,7} or sporadic (82%)⁸ NANBH, in haemophiliacs (60 to 90%)^{5,9}, in intravenous drug addicts (60 to 75%)^{5,10,11} and in haemodialysis patients (20%)⁵. Screening of blood donors and of selected population groups showed that the prevalence of anti-HCV is relatively constant in the world, varying between 0.3 and 1% in the USA and in Europe¹²⁻¹⁹ and 4.2% in Africa²⁰, having intermediate values in some regions of southern Europe (1.2 to 2.9%)^{21,22}, in Latin America²³ and in Japan²⁴.

In Portugal, the known data regarding the general population result from the extrapolation of data from blood donors, in which the prevalence of anti-HCV, by 2nd generation ELISA, has been estimated at less than 1%²⁵⁻²⁸. However, the data from blood donors may not

be representative of the general population, bearing in mind that they are, previously, subjected to a process of selection.

As our objective is to know the prevalence of anti-HCV in the general population of the central region of the country, with this study we seek to assess, in a first phase, the prevalence in the adult population of the Coimbra District.

METHODS

Population - The universe of the study was the population of the Coimbra District, with ages between 20 and 65 years, estimated at 238 732 individuals²⁹.

In the selection of the sample, the following methodology was used: four regions were chosen, with an overall population of 104002 persons and with characteristics which are empirically representative of the whole district: 1 urban (Coimbra), 1 semi-urban (Montemor-o-Velho), and two rural (Tábua and Góis). For an expected prevalence of 2% and a maximum difference of 1%, with a confidence interval (CI) of 95%³⁰, a sample of 751 individuals was defined. In each region the corresponding sample was selected, by aleatory choice of files from the Health Centres, through the systematic extraction of files; in the case of an individual who did not belong to the age group being studied, the file immediately before was chosen, the one immediately after or thus successively; the period defined was, however, always maintained. To prevent possible failures, over 50% of the number of people required were selected.

The individuals were sent a letter asking them to go to their respective Health Centre, where a medical team, after written consent, would fill out a personal file which included identification and an epidemiological questionnaire (Appendix 1) and a blood sample.

657 individuals (57%), of the 1150 people summoned went to their health centre and were studied, 267 males and 390 females, with an average age of 42.7±13.1 years. The distribution by councils and age groups is described in *Table 1*.

Table 1 – Population studied

Distribution by regions		Age distribution	
County	N° Individuals	Age (years)	N° Individuals
Coimbra	257 (39.1%)	20-29	140 (21.3%)
Mont.-O.-Velho	75 (26.6%)	30-39	149 (22.7%)
Tábua	140 (21.3%)	40-49	138 (21%)
Góis	85 (13%)	50-59	153 (23.3%)
		60-65	77 (11.7%)

Total of individuals: 657

Sex distribution: Male 267 (40.6%) Female 390 (59.4%)

The most significant epidemiological characteristics of the group studied are summarised in *Table 2*.

Table 2 – Epidemiological characteristics of the group studied

Previous	%	Previous	%
Transfusions	8.7	Acute hepatitis	8.1
Hospitalisation	67.4	Other hepatic diseases	6.8
Surgery	44.6	Contact with hep. cases	12.8
Stomatological treatment	87.4	Stay in hep. B risk reg.	23.4
Intravenous Drug Addiction	0.9	Habit. alcohol consump.	55.6
Homosexuality	0	Alcohol consump.>80g/day	11.4
Sexually trans. diseases	6.2	Health professionals	3.6

After centrifuge, the serums were frozen at -20°C and, later, processed together.

The research on anti-HCV was made using a second generation ELISA test (Abbott HCV EIA 2.0). The positive results were repeated for confirmation. The repeatedly positive serums were tested with a supplementary system of 2nd generation RIBA (Ortho Diagnostic Systems).

In determining the prevalence, the confidence interval at 95%³⁰ was used.

RESULTS

Anti-HCV (ELISA) was positive in 3 cases (0.46%; CI, 0.42 to 0.49%) (Table 3). All the ELISA positive serums were RIBA reactive.

All the subjects who were anti-HCV positive were male and lived in the region of Coimbra. They were aged 27, 28 and 36 years, respectively. All of them had had a history of surgical intervention, blood transfusions in 1, drug addiction by intravenous means in 2 and multiple sexual contacts in 1.

Table 3 – Data regarding the cases with anti-HCV +

	Case 1	Case 2	Case 3
Age	28	27	36
Sex	Male	Male	Male
County	Coimbra	Coimbra	Coimbra
Hospitalisations(n°)	Yes(4)	Yes(1)	Yes(2)
Transfusions	-	-	Yes
History of surgery	Yes	Yes	Yes
Stomatological treatment	Yes	Yes	Yes
Intravenous drug addict.	Yes	-	Yes
Multiple sexual contacts	-	Yes	-
Contact with hep. cases	-	Yes	-
Stay in hep.B risk reg.	Yes	-	-
ALT	2xNormal	n/determined	Normal
Hepatitis B markers	HBsAg+	n/determined	Negative

The prevalence in the urban area (n=257) was of 1.16% (CI, 1.02 to 1.31%). Considering only the urban area and the age group of 20 to 39 years (n=117), the prevalence was of 2.56% (CI, 2.1 to 3.03%).

Anti-HCV was negative in all the health professionals (n=21) and in all the subjects with a history of acute

hepatitis (n=53), of other hepatic diseases (n=45) and of sexually transmitted diseases (n=41). Anti-HCV was positive in 33.3% of the subjects with a history of intravenous drug addiction, in 1.8% of those with blood transfusions, in 1% of those submitted to surgical intervention, in 1.33% of alcoholics consuming more than 80 g/day, in 0.7% of those hospitalised, in 0.65% of those who had been in regions of greater hepatitis B risk and in 0.5% of those who had had stomatological treatment (Table 4).

Table 4 – Prevalence of anti-HCV and personal history

Risk factor	Total N°	Prevalence (%)
Drug addiction	2/6	33.3(CI,6.6 to 60.0)
Transfusions	1/57	1.8(CI,1.3 to 2.21)
Alcoholism>80g/day	1/75	1.33(CI,1.03 to 1.64)
Surgical interventions	3/293	1.0(CI,0.91 to 1.14)
Hospitalisation	3/443	0.7(CI,0.61 to 0.74)
Stay in hep.B risk regions	1/154	0.65(CI,0.55 to 0.75)
Stomatological treatment	3/574	0.5(CI,0.48 to 0.57)

DISCUSSION

This study is the first epidemiological study of hepatitis C on the general population to be made in our country.

We can not rule out the fact that as only 57% of the people summoned took part in the study, which is a common occurrence in work of this nature, there may be an error factor in the results. This is due to the high number of people who did not respond and not to the number of subjects included in the sample, since, foreseeing 751 and having summoned 1150, 657 were gathered. An eventual error would be subject to correction by assessing the motivations of the people who did not respond, however the logistic conditions of this work would not allow this study to be made. Thus the results obtained should be interpreted bearing this reality in mind.

We found the prevalence of anti-HCV (0.46%) to be in agreement with the data available in our country regarding blood donors. Therefore, according to recent data, the prevalence of anti-HCV (ELISA-2) in blood donors is about 0.9% in the southern region^{26,28}, 0.56% in the northern region²⁵ and 0.26% in the central region²⁷. We hope to find, in this study, a value of prevalence superior to that verified in blood donors, considering the fact that these are selected populations in which, on the outset, individuals with a presumable greater risk are ruled out.

The rate we found is also comparable to that found in studies made on groups of general population, in the USA(0.3%)¹² and in Europe (0.6 to 0.9%)^{17,18,19}, but is lower than that of studies made in Mediterranean Europe (2.9%)²¹.

We point out the contrast between the prevalence of anti-HCV found in the urban area (1.16%) and in the rural regions, where no case was detected. We found that

the sample we used, obeying an appropriate methodology, showed itself to be, despite this, insufficient for the study, above all in the rural regions. In these regions, where the prevalence is predictably lower, a greater quantity of people would be necessary. The same does not occur in urban areas, where the figure found is adequate to what was expected, although at the lower limit of the prevalence and margin initially estimated.

The results obtained suggest that hepatitis C is, among us, a situation that is not frequent in the general population, acquiring greater significance in urban areas and, above all, in risk groups. In fact, in our country, infection by HCV reaches significant proportions in subjects with politransfusions, in intravenous drug addicts, in chronic haemodialysis patients and chronic haemophiliacs, as well as in patients with other hepatic diseases^{31,32}.

All the cases detected by ELISA method were RIBA reactive. The population being low risk, a situation in which the ELISA test has less specificity⁴, we expected to find a few false positive results, which, withal, did not occur. The small number of positive anti-HCV cases does not allow any conclusion to be drawn from this fact. The ELISA test is a screening method, so the use of RIBA in the positive cases is important, above all when low risk groups are studied⁴.

Although it is not permissible to forecast, from the results of the current study, the prevalence in the country, we admit, however, that infection by HCV may reach, in other regions, greater significance. It is possible that, similar to what was shown for the markers of infection by the hepatitis B virus³³, certain areas of the country, predominantly urban and with greater social and economic development, such as Lisbon, Setúbal and the districts of the North coast, present, by the local conjugation of infection risk factors, higher prevalence of anti-HCV. Thus, it seems important to us to point out the fact that the prevalence in the urban population we studied is greater than that in the rural population, where, in fact, we did not find any case. This fact is, obviously, in contrast with what occurs with hepatitis A, of faecal-oral transmission.

In this study, all the cases of positive anti-HCV occurred in subjects with risk factors for parenteral transmission. This fact is not a rule, also in our personal experience, in which we did not identify any potential source of transmission in 25% of the patients with hepatitis C and only 26% had received transfusions³⁴. In fact, percutaneous transmissions are the most important for HCV¹ infection. However, the frequency of sporadic cases suggests the existence of non parenteral forms of transmission³⁵.

It is not justifiable to apply statistical tests with only three positive cases. However it is important to emphasise the higher prevalence of anti-HCV in drug addicts and patients with blood transfusions and the apparent low risk of stomatological treatment, of hospitalisation and stays in hepatitis B risk regions. We also point out the low prevalence in excessive drinkers, contrary to what has been referred in various foreign

studies^{36,37}, but which we do not confirm, even with patients with alcoholic liver disease³⁸. This fact should be emphasised, justifying more studies aimed at this population group, since the data quoted most in literature, mainly from the Mediterranean region, do not seem to be corroborated among us.

On the other hand, we did not find the anti-HCV in any of the cases in which a history of sexually transmitted diseases had been referred. There is evidence of the possibility of sexual transmission of HCV, but this situation seems to be scarce³⁹; the screening of homosexuals⁵, of sexual partners of drug addicts⁵, of prostitutes^{40,41}, of individuals with sexually transmitted diseases⁴⁰ and of heterosexuals with multiple partners⁴², showed prevalence of anti-HCV only moderately higher than that of the general population. In the same way, the risk of infection with HCV is low in health care workers^{43,44}, as we also verified in this current study.

In conclusion, we may state that, in the entire population of our region, the prevalence of markers of HCV infection is very low, not being at the base of the high prevalence in risk groups. Drug addiction and transfusions are, among us, the most important risk factors. However, we can not forget another important group, not represented in this work: the patients on haemodialysis^{5,45}. It is important to do other epidemiological studies, if possible with greater samples, which was indicated by the results we obtained, following the estimated prevalence of an initial 2% and final of 0.46%. Finally, these data indicate the need to focus attention on risk groups.

REFERENCES

- ALTER HJ: The hepatitis C virus and its relationship to the clinical spectrum of NANB hepatitis. *J Gastroenterol Hepatol* 1990; 5 (Suppl. 1): 78-94
- CHOO QL, KUO G, WEINER AJ, OVERBY LR, BRADLEY DW, HOUGHTON M: Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; 244: 359-361
- KUO G, CHOO QL, ALTER HJ, et al: An assay of circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*; 1989; 244: 362-364
- ALTER HJ: Descartes before the horse: I alone, therefore I am: the hepatitis C virus in current perspective. *Ann Intern Med* 1991; 115: 644-649
- ESTEBAN JI, ESTEBAN R, VILADOMIU L et al: Hepatitis C virus antibodies among risk groups in Spain. *Lancet*; 1989; 2: 294-297
- VIOLA L, PIMENTEL E, MORGANTE P, FERNANDEZ JL: Are hepatitis C virus antibodies involved in chronic liver diseases other than non-A, non-B hepatitis? *Hepatology*; 1991; 14: 1303
- ALTER HG, PURCELL RH, SHIH JW, MELPOLDER JC, HOUGHTON M, CHOO QL et al: Detection of antibodies to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989; 1494-1500.
- ALTER MJ, MARGOLIS HS, KRAWCZYNSKI K et al: The natural history of community acquired hepatitis C in the United States 1992; *N Eng J Med* 327: 1899-1905
- BRETHER DB, ALTER HJ, DIENSTAG JL, FORSBERG AD, LEVINE PH: Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. 1990; *Blood* 76: 254-256
- GUADAGNINO V, ZIMATORE G, ROCCA A et al: Anti hepatitis C antibody prevalence among intravenous drug addicts in the Catanzaro area. *Arch Virol Suppl* 1992- 4: 335-336.
- VAN DEN HOEK JA, VAN HAASRECHT HJ, GOUDSMIT J, DE WOLFF, COUTINHO RA: Prevalence, incidence, and risk factors of hepatitis C virus infection among drug users in Amsterdam. *J Infect Dis* 1990; 162: 823-826
- HYAMS KC, STRUEVING JP, GRAY GC: Seroprevalence of hepatitis A, B and C in a United States military recruit population. *Mil Med* 1992; 157: 579-582
- AGUELLES O, JANOT C: Epidemiology of anti-HCV antibodies in France. *Viral Hepatitis Study Group of the French Blood Transfusion Society. Arch Virol Suppl* 1992; 4: 249-252.
- ARCHER GT, DURING ML, CLARK B et al: Prevalence of hepatitis C virus antibodies in Sydney blood donors. *Med J Aust* 1992; 157: 225-227
- JANOT C, COUROUCE AM, MANIEZ M: Antibodies to hepatitis C virus in French blood donors [letter]. *Lancet*. 1989; 2: 796-797
- STEVENS CE, TAYLOR PE, PINDYCK J et al: Epidemiology of hepatitis C virus. A preliminary study in volunteer blood donors 1990, *JAMA* 263: 49-53
- DAL RE R, AGUILAR L, CORONEL P: Current prevalence of hepatitis B, A and C in a healthy Spanish population. A seroepidemiological study. *Infection*. 1991; 19: 409-413.
- CAMPELLO C, MAIORI S, POLI A, PACINI P, NICO-LARDI L, PINI F: Prevalence of HCV antibodies in health care workers from northern Italy. *Infection* 1992; 20: 224-226.
- RAPICETTA M, ATTILI AF, MELE A et al - Prevalence of hepatitis C virus antibodies and hepatitis C virus RNA in an urban population. *J Med Virol* 1992; 37: 87-92
- COURSAGET P, BOURDIL C, KASTALLY R et al: Prevalence of hepatitis C virus infection in Africa: anti HCV antibodies in the general population and in patients suffering from cirrhosis or primary liver cancer. *Res Virol* 1990; 141: 449-454
- ALBANO A, PIANETTI A, BITTI MR, BRUSCOLINI F, DAFFONE W, ALBANO V: Prevalence of anti-HCV in subjects of various age groups. *Eur J Epidemiol* 1992; 8: 309-311
- ESTEBAN JI, LOPEZ-TALAVERA JC, GENESCA J et al: High rate of infectivity and liver disease in blood donors with antibodies to hepatitis C virus. *Ann Int Med* 1991; 115:443-449
- MULLER G, ZABALETA M, CALDERA LH, BIANCO N, MACHADO IV: Hepatitis C in Venezuela. Preliminary report (abstract). *G EN* 1990; 44: 336-342
- KASHIWAGI S: Epidemiological study of hepatitis B and C virus in Okinawa and Kyushu, Japan [abstract]. *Rinsho Byori*. 1992; 40: 910-924
- PINTO T, JUSTIC, A B: O valor do RIBA na confirmação serológica do anticorpo anti-HCV. I Congresso Nacional de Hepatite C, Lisboa, 1993
- NASCIMENTO F: Prevalência do anti-HCV nas dádivas de sangue da Região Sul. I Congresso Nacional de Hepatite C, Lisboa, 1993
- OLIVEIRA Z, TOMAZ J, SIMÕES I, PAIS L: HCV em dádivas de sangue (poster). I Congresso Nacional de Hepatite C, Lisboa, 1993
- GUERREIRO H, ESTEVENS J, ESMAEL MJ, CHARNECA MM, CARVALHEIRA C: Hepatite C: problemas de diagnóstico e epidemiologia no Algarve. I Congresso Nacional de Hepatite C, Lisboa, 1993
- INE: XII Recenseamento Geral da População (1981). Imprensa Nacional - Casa da Moeda. Lisboa, 1984
- BERNARD P-M, LAPOINTE C: Mesures statistiques en epidemiologie. Presses de l'Université du Québec. Québec, 1987
- RAMALHO F, MARINHO R, VELOSA J, SEREJO F, MOURA MC: Epidemiologia da infecção pelo vírus da hepatite C. *Revista do Interno* 1993; 1 (Supl. A): A29-A32
- RIBEIRO AT: Epidemiologia da infecção pelo vírus da hepatite C. *Arq Hepato-Gastr Port*. 1992; 1: 80-87
- LECOUR H: Hepatite virica: epidemiologia e diagnóstico (tese de doutoramento). Porto, 1983
- CARVALHO A, BENTO D, SANTOS A, PERDIGOTO R, SANTOS R, CRESPO J, VELEZ J, ALMIRO E, SEVERO F, PORTO A: Hepatite C: análise de 82 doentes com anti-VHC positivo. XI Jornadas de Medicina Interna de Coimbra, Coimbra, 1993
- ALTER HJ: Clinical, virological and epidemiological basis for the treatment of chronic non A, non B hepatitis. *J Hepatol* 1990; 11(Suppl 1): S19-S25
- HALIMI C, DENY P, GOTHEIL C, TRINCHET JC, MAL F, SCAVIZZI M, BEAUGRAND M: Pathogenesis of liver cirrhosis in alcoholic patients: histological evidence for hepatitis C virus responsibility. *Liver* 1991; 111: 329-333
- PARES A, BARRERA JM, CABALLERIA J, ERCILIA G, BRUGUERA M, CABALLERIA L, CASTILLO R, RODES J: Hepatitis C virus antibodies in chronic alcoholic patients: association with severity of liver injury. *Hepatology*. 1990;12: 1295-1299
- CARVALHO A, SANTOS A, PINTO A, AMARO P, LOPES RP,

- ALMIROE, SEVERO F, PORTO A: Low prevalence of anti-HCV in alcoholics with liver disease in the central region of Portugal (abstract). *J Hepatol* 1992; 16 (Suppl. 1): S83-S84
39. FRIED MW, SHINDO M, FONG TL, FOX PC, HOOFNAGLE JH, DI BISCEGLIE AM: Absence of hepatitis C viral RNA from saliva and semen of patients with chronic hepatitis C. *Gastroenterology* 1992; 102: 1306-1308
40. NAKASHIMA K, KASHIWAGI S, HAYASHI I et al: Sexual transmission of hepatitis C virus among female prostitutes and patients with sexually transmitted diseases in Fukuoka Kyushu, Japan. *Am J Epidemiol* 1992; 136: 1132-1137
41. ORDUNA A, BRATOS MA, GUTIERREZ P et al: Infection by hepatitis B and C virus in non intravenous drug using female prostitutes in Spain. *Eur J Epidemiol* 1992; 8: 656-659
42. VAN DOORNUM GJ, HOOYKAAS C, CUYPERS MT, VAN DER LINDEN MM, COUTINHO RA: Prevalence of hepatitis C virus infection among heterosexuals with multiple partners. *J Med Virol* 1991; 35: 22-27
43. PEREZ TRALLERO E, CILLA G, ALCORTA M, CLOSEGUI ME, SAENZ DOMINGUEZ JR: Bajo riesgo de adquisición del virus de la hepatitis C para el personal sanitario. *Med Clin Barc* 1992; 99: 609-611
44. SCHIFF ER: Hepatitis C among health care providers: risk factors and possible prophylaxis. *Hepatology* 1992; 16: 1300-1301
45. ALVES H, BARROS H, TEIXEIRA V, LAMAS F, MARTINS P, DIAS M, MENDES A: Infecção pelo vírus da hepatite C em hemodialisados. I Congresso Nacional de Hepatite C, Lisboa, 1993

Appendix 1

Epidemiology of Hepatitis C in Central Portugal
Anti-HCV antibody screening in the District of Coimbra

Health Centre _____ File Nº _____

Identification

Name: _____ Nº _____
Sex: M F Age _____ years Marital Status: _____
Profession: _____

History

Acute hepatitis: N Y, _____ ago; Type: A B C NANB _____
Hepatic disease: N Y _____
Stay in risk regions: N Y _____
Hospitalisations: N Y _____ Surgery: Y N _____
Parenteral treat.: N Y Stomatological treat.: N Y Transfusions: Y N
Alcohol consumption: N Y <80 - >80 g/day, for _____ years.
Medication: N Y _____
Drug addiction: N Y _____
Sexual hist.: homo - multiple partners - suspicious relationships - STD
Contact with patients with hepatitis: N Y

Pathology

1. _____ 3. _____
2. _____ 4. _____

Anti-HCV

ELISA 2: _____ RIBA 2: _____

Authorization

I hereby agree to participate in the study of the prevalence of hepatitis C in the District of Coimbra, with the guarantee that medical confidentiality will be maintained in all the fases of the study.
Signature: _____