European Journal of Internal Medicine xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original article

Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete

I. Drositis [a,*], A. Bertsias [b], C. Lionis [b], E. Kouroumalis [c]

[a] Arkalochori Health Centre-Venizeleio General Hospital of Heraklion, Crete, Greece
[b] Clinic of Social and Family Medicine, School of Medicine, University of Crete, Voutes, 71110 Heraklion, Crete, Greece
[c] Department of Gastroenterology and Liver Research Laboratory, University Hospital and Medical School of Crete, Voutes, 71110 Heraklion, Crete, Greece

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 10 June 2013
Accepted 3 August 2013
Available online xxxx

Keywords:
Crete
Greece
Viral-hepatitis
Markers
Prevalence

ABSTRACT

Aim: An observational seroepidemiological study was carried out in a well-defined primary-care district on the island of Crete in order to determine the recent endemicity of viral hepatitis in Cretan-population.
Setting and participants: The setting consisted of a semi-urban group and a remote & rural group. Serum samples were collected from 876 subjects (437 males, 439 females) aged 15 years or above. Subjects were randomly selected from the permanent population of the area that consisted of 5705 individuals. The aim was to measure the prevalence of selected viral-hepatitis markers.
Results: Hepatitis B surface-antigen (HBsAg) was found positive in twenty-nine individuals, (3.3%). Antibodies to hepatitis B virus core-antigen (HBcAb) were detected in 287 subjects (32.8%) and antibodies to hepatitis C virus (anti-HCV) were detected in nineteen subjects (2.2%). Seropositivities for the semi-urban group were: 3.4%, 19.1%, 2.1% and 3.2%, 48.8%, 2.2% in remote & rural group respectively. Virtually, all subjects >45 years old were seropositive for antibodies to hepatitis A, whereas approximately 80% of those in the 15–44 age-group were found to be seropositive.
Conclusion: A threefold increase in the HBV exposure and carrier proportion was found in Cretan native-population and in rural-areas compared to older studies carried out in other rural-populations of the island. It is still unknown whether the recent economic crisis or the demographic changes in Cretan-population contributed to these findings. HCV endemicity remains relatively constant, however an alteration of hepatitis C genotypes was observed. Exposure to HAV was found to be higher in remote and rural areas compared to semi-urban areas.

1. Introduction

Worldwide, about 350 million persons have chronic hepatitis B virus (HBV) infection, and about 125 million have been infected with hepatitis C virus (HCV), placing viral-hepatitis among the world's greatest infectious diseases [1–4]. Viral hepatitis can lead to liver cirrhosis and subsequently to liver failure and hepatocellular carcinoma. Such complications could be prevented in cases of timely diagnosis and adequate treatment provision. Therefore public health measures aiming at prevention, early diagnosis and treatment are considered to be necessary.

In Greece a high prevalence of hepatitis A and C infection was reported certain years ago [1–4]. The prevalence of HBsAg chronic-carriers was found to be relatively high in mainland Greece compared to western European countries, while a seroepidemiological study reported Greece having the highest prevalence of hepatitis A (82%). Crete is a Greek administrative region where a relatively lower prevalence of both HBsAg chronic-carriers and hepatitis A antibodies was reported probably due to an overall improvement of sanitarian conditions [1–3,11,12]. Contrary to these favorable findings, a high prevalence of hepatitis C antibodies in remote and rural populations of Crete has been published a couple of years ago [2]. An increase in the number of hospital discharges of patients suffering from liver diseases was reported in Crete over the last decades [14,15]. A study on mortality proportion caused by malignant neoplasms reported liver cancer being the fifth most common type of cancer in Crete, being significantly higher from the national proportion [16,17] and this finding still needs an answer. Cretan population became more heterogeneous over the last two decades mainly due to economic migration. Observations based on clinical medical records from primary-care and hospital services of the island have revealed a

Abbreviations: HAV, hepatitis A virus; anti-HAV, antibodies to hepatitis A virus antigen; HBsAg, hepatitis B surface antigen; HBcAb, antibodies to hepatitis B core-Ag; Anti-HBs, antibodies to hepatitis B surface antigen; Anti-HBe, antibodies to hepatitis B e-core antigen; HCV, hepatitis C virus; anti-HCV, antibodies to hepatitis C virus antigen; AHC, Arkalochori Health Centre.

* Corresponding author at: Department of Medical Oncology, Venizeleio General Hospital, Knossos A., 71409 Heraklion-Crete, Greece. Tel.: +30 2813408158; fax: +30 2813408053.
E-mail addresses: i_drossitis@hotmail.com (I. Drositis), antonisbertsias@yahoo.gr (A. Bertsias), lionis@galinos.med.uoc.gr (C. Lionis), kouroum@med.uoc.gr (E. Kouroumalis).

0953-6205/$ – see front matter © 2013 European Federation of Internal Medicine. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejim.2013.08.003

higher incidence of hepatitis B infection. This observation introduced certain questions to what extend the seroepidemiology of viral hepatitis has changed in the population of this island. Despite the wide implementation of hepatitis B vaccination in Greece our working hypothesis inclined towards a steady increase of HBV and a parallel decrease of HCV carrier states. That trend would be more evident in the remote and rural population, in individuals with a low level of awareness of the HBV risk, those living under poor hygiene conditions and in the elderly population.

Thus the aim of the study was to measure the prevalence of selected hepatitis serum markers in the general population of one geographically defined area of Crete and to identify the potential risk factors could raise recommendations for both health care practitioners and policy makers.

2. Ethics

All residents who consented to participate in the study signed a written consent form. In addition a written approval was obtained from the Scientific Committee of the University Hospital of Crete before the start of the study in order to be conducted in accordance with international ethical standards.

3. Methods

3.1. Setting

The study was conducted in a geographically defined area with the target population being the permanent inhabitants of the Arkalochori province in central-southern Crete. The majority of inhabitants were farmers, while the total number of the permanent inhabitants of this area was 5705.

3.2. Participants

The reference populations consisted of the 2988 residents of the Arkalohori semi-urban region aged at least 15 years (semi-urban group) and 2717 residents of 6 remote and rural areas within the same prefecture aged at least 15 years (remote and rural group). The distance of the remote and rural areas from the Arkalohori semi-urban area was at least 15 km in each case. The sampling frame was drawn from the electoral roll for subjects of age 18 years old or above and the municipal registry office for subjects of age between 15 and 18 years. Permission for access to both records was granted upon request. Subsequently, the sample was proportionately stratified by age and gender. Four age strata were used (namely 15–24 years old, 25–44 years old, 45–64 years old and ≥65 years old). Upon stratification, a random sample which comprised one sixth of the population of reference (951 subjects, 498 subjects from semi-urban group and 453 from remote and rural group) was chosen for enrollment. The response rate was 90% for semi-urban group (448 participants) and 84% for remote and rural group (380 participants). Forty-eight additional subjects participated in the study (24 in semi-urban group and 24 in remote and rural). All additional subjects were proportionately distributed in the sample according to their age group in order to replace nonresponders and increase the power of the study. Eventually a total of 876 subjects were enrolled in the study, 437 males and 439 females. Based on measurements of antibodies to hepatitis C virus prevalence from a previous study conducted in the island of Crete [2], a sample size of 266 subjects from a population of 5705 could measure a prevalence of 3% assuming a maximum allowable error of 2.5% (Fig. 1).

3.3. Data collection and measurements

Blood samples were collected from all participants. Information regarding socio-demographic profile, living conditions and medical history (such as blood transfusion, hospital admissions, surgical or dental procedures, intravenous drug abuse and history of miscarriage or abortion in women) was collected locally. Serum samples were stored at −80 °C and analyzed at the Virology and Liver Research Laboratory of the University Hospital of Heraklion Crete. Sample collection and analyses took place between January 2006 and January 2010. Genotyping for hepatitis B and C virus was completed in 2012 (Fig. 2).

3.4. Laboratory measurements

Each serum sample was tested for hepatitis B and C markers by the microparticle capture enzyme immunoassay (MEIA) according to the manufacturer's instructions using the AxSym-Analyzer from Abbott Laboratories (ELIZA-method) for hepatitis B surface antigen (HBsAg), total antibodies to hepatitis B core antigen (HBcAb), antibodies to hepatitis B surface antigen (AntiHBs), and for antibodies to hepatitis C virus (anti-HCV). HBV positive participants were further tested for genotype identification, using a Real-time PCR. For HCV positive participants the HCV-RNA was further assessed using the AMPLICOR-TM quantitative method. HCV genotype identification was performed using the INNO-Lipa method. Sera from the first 220 subjects (~25%) were also tested for anti-HAV total antibody, using ELIZA-method (110 from semi-urban group and 110 from remote and rural group, 55—males and 55—females from each study group).

3.5. Statistical analysis

Descriptive statistics were used in order to estimate the prevalence of HBsAg, HBcAb, anti-HBs and anti-HCV. Comparisons in the prevalence of the serum markers between the two study groups, between gender and among age groups were performed using χ^2 tests.

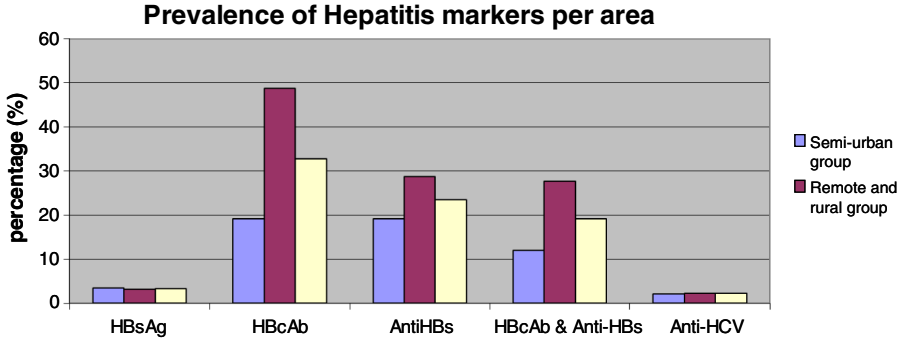

Fig. 1. Viral hepatitis' markers prevalence per area.

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003

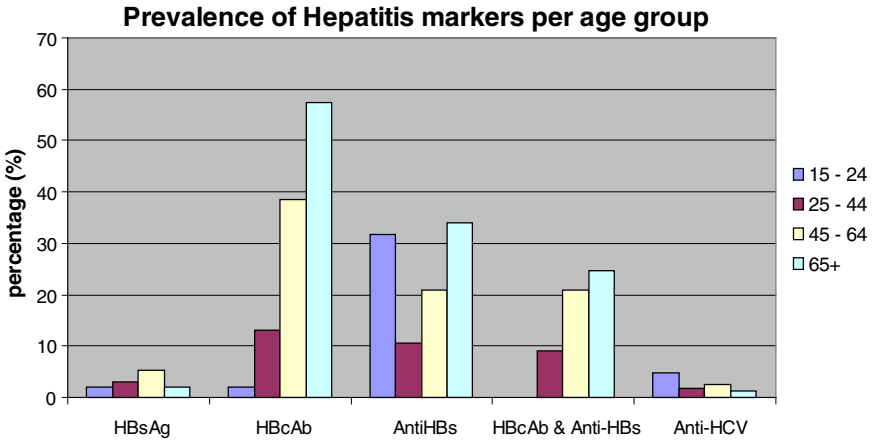

Fig. 2. Viral hepatitis' markers prevalence per age.

Comparison of median age between the two study groups was performed using the non-parametric Mann–Whitney statistical test. Multiple unconditional logistic regression with three independent variables namely age (in years), residence (remote and rural, semi-urban) and gender (male, female) was used to assess possible associations with HBsAg, HBcAb, anti-HBs, HBcAb & Anti-HBs (combined), and anti-HCV seropositivity. Logistic regression results are distributed in tables as odds ratios and confidence intervals. The level of statistical significance was $\alpha = 0.05$. The statistical software package used for sample size estimation was CDC EPI Info™ 7 and for the analyses was IBM SPSS 19.

4. Results

4.1. Basic socio-demographic characteristics of participants

A total of 437 males (49.9%) and 439 (50.1%) females participated in the study. From the initially selected sample, 2.7% denied participation and 10.3% could not be reached due to migration, change of home address or death.

Semi-urban group consisted of 472 individuals, 244 males (51.7%) and 228 females (48.3%). Remote and rural group consisted of 404 individuals, 193 (47.8%) males and 211 (52.2%) females. Median age was 52 years for the entire population and varied significantly between the two study groups (median age, 40 years in group-A and 64 years in group-B; P-value < 0.0001). In semi-urban group, 36.4% of participants were aged between 25 and 44 years while 32.2% were aged between 45 and 64 years. On the contrary, in remote and rural, most participants (47.3%) were aged 65 years or above, while 31.9% were aged between 45 and 64 years. Further details are shown in Table 1.

4.2. Prevalence of hepatitis serologic markers

4.2.1. Anti-HAV prevalence

From the 220 individuals tested in the total population, 191 (86.8%) were found to be positive for anti-HAV. Antibodies were found in 99 (89.9%) males and 92 (83.7%) females. In semi-urban group, 89 out of the 110 tested individuals (80.9%) were found to be positive for anti-HAV, [45 males (81.4%)/44 females (80.4%)]. Parallel to this, in remote and rural group 102 out of 110 tested individuals (92.8%) were also found to be positive for anti-HAV [54 males (93.7%)/48 females (91.9%)]. With regards to the association with age, 82 out of 102 individuals (80.3%) aged between 15 and 44 years were found to be positive, compared to 109 out of 118 (92.4%) subjects aged 45 years or older. There was no significant difference in anti-HAV frequency between males and females.

4.2.2. Hepatitis B markers

The prevalence of HBsAg was 3.3% in the total population, 3.4% in the semi-urban group and 3.2% in the remote and rural group. There were no significant variations in HBsAg prevalence between males and females.

HBcAb was present in 32.8% of all individuals, 19.1% for the semi-urban group and 48.8% for the remote and rural group (p-value < 0.0001). There were no significant variations in HBcAb prevalence between males and females.

Table 1
Basic socio-demographic characteristics of the study participants.

	Semi-urban group	Remote and rural group	Total	P-value
	Tested n = 472	Tested n = 404	Tested n = 876	
Gender				N.S.
Male (n tested, %)	244 (51.7)	193 (47.8)	437 (49.9)	
Female (n tested, %)	228 (48.3)	211 (52.2)	439 (50.1)	
Age (Median years)	40	64	52	<0.0001*
(min, max; IQR)	(14, 86; 28)	(14, 88; 24)	(14, 88; 33)	
Age group (years)				<0.0001**
15–24 (n tested, %)	85 (18.0)	22 (5.4)	107 (12.2)	
25–44 (n tested, %)	172 (36.4)	62 (15.3)	234 (26.7)	
45–64 (n tested, %)	152 (32.2)	129 (31.9)	281 (32.1)	
65+ (n tested, %)	63 (13.3)	191 (47.3)	254 (29.0)	

N.S. = Not significant.
* Median age was compared between the two study groups using the non-parametric Mann–Whitney test (Mann–Whitney U = 48,649.500).
** Comparison of the number of participants in each age group between the two study groups was performed using Chi-Square test (Pearson's Chi-Square = 150.820, in 3 d.f.).

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003

Table 2
Hepatitis markers prevalence per group (area).

Groups	Hepatitis markers				
Semi-urban group Tested n = 472	HBsAg	HBcAb*	AntiHBs†	HBcAb & Anti-HBs‡	Anti-HCV
Male (n positive, %)	8 (3.3)	48 (19.7)	51 (20.9)	33 (13.5)	8 (3.3)
Female (n positive, %)	8 (3.5)	42 (18.4)	39 (17.1)	23 (10.1)	2 (0.9)
Prevalence (n positive, %)	16 (3.4)	90 (19.1)	90 (19.1)	56 (11.9)	10 (2.1)
95% CI	1.9–5.5	15.6–22.9	15.6–22.9	9.1–15.1	1.0–3.8
Remote and rural group					
Tested n = 404					
Male (n positive, %)	8 (4.1)	101 (52.3)	57 (29.5)	54 (28.0)	5 (2.6)
Female (n positive, %)	5 (2.4)	96 (45.5)	59 (28.0)	58 (27.5)	5 (1.9)
Prevalence (n positive, %)	13 (3.2)	197 (48.8)	116 (28.7)	112 (27.7)	9 (2.2)
95% CI	1.7–5.4	43.8–53.8	24.3–33.4	23.4–32.4	1.0–4.2
Both groups					
Tested n = 876					
Male (n positive, %)	16 (3.7)	149 (34.1)	108 (24.7)	87 (19.9)	13 (3.0)
Female (n positive, %)	13 (3.0)	138 (31.4)	98 (22.3)	81 (18.5)	6 (1.4)
Prevalence (n positive, %)	29 (3.3)	287 (32.8)	206 (23.5)	168 (19.2)	19 (2.2)
95% CI	2.2–4.7	29.7–36.0	20.7–26.5	16.6–21.9	1.3–3.3

Comparisons in the prevalence within gender yielded no statistical significant results in all categories.
*, †, ‡ Comparison in the prevalence of HBcAb, AntiHBS and combined HBcAb & Anti-HBs markers between semi-urban group and remote & rural group yielded statistically significant results (P-value < 0.00001, Pearson's Chi-Square = 87.133, 11.259 and 35.318 respectively).

Anti-HBs was found in 23.5% of the total population, 19.1% in the semi-urban group and 28.7% in the remote and rural group (p-value = 0.001) and there was no significant difference according to gender.

Combining HBcAb and Anti-HBs to define past/resolved HBV infection, it was found that the total prevalence (double positive) was 19.2% in the total population, 11.9% for the semi-urban group and 27.7% for the remote and rural population (p-value < 0.0001). Table 2 provides detailed information regarding serologic markers, namely HBsAg, HBcAb, AntiHBs, HBcAb & Anti-HBs (combined), and Anti-HCV.

4.2.3. Hepatitis C markers

Finally, the prevalence of anti-HCV was 2.2% in the total population, 2.1% in semi-urban group and 2.2% in remote and rural group (Tables 2, 3).

Based on the above results, statistically significant differences between semi-urban and remote and rural groups were observed for the HBcAb, AntiHbs and the combined HBcAb & Anti-HBs serologic markers. In all cases, the prevalence was higher in the remote and rural group compared to semi-urban group. As previously mentioned, median age varied significantly between the two study groups. In Table 3 the prevalence of selected serologic markers is presented in different age-groups. A significant variation in prevalence of HBcAb, AntiHbs and in the HBcAb & Anti-HBs among age groups was observed (P-value < 0.0001 for each).

Multiple logistic regression analyses indicated that residents of remote and rural areas and older subjects had higher odds of being positive to HBcAb, Anti-HBs and HBcAb & Anti-HBs compared to residents of semi-urban areas and younger subjects respectively. In particular, residents of remote and rural areas had 2.2 times the odds of being positive for HBcAb, 1.5 times higher odds of being positive for Anti-HBs and 1.6 times higher odds of being positive for combined HBcAb & Anti-HBs compared to residents of semi-urban area (Odds Ratio [OR] 2.17; 95% Confidence Interval [CI] from 1.56 to 3.05 for HBcAb seropositivity, OR 1.45; 95% CI from 1.03 to 2.06 for Anti-HBs serepositivity, OR 1.57; 95% CI from 1.06 to 2.33 for the combined HBcAb & Anti-Hbs). Age was a statistically significant predictor of the odds for being positive for HBcAb, Anti-HBs and combined HBcAb & Anti-HBs. The odds of being positive for HBcAb increased 1.05 times for each passing year of age (OR 1.05; 95% CI from 1.04 to 1.07), the odds of being positive for Anti-HBs increased 1.01 times for each passing year of age (OR 1.01; 95% CI from 1.00 to 1.02) and finally the odds of being positive for combined HBcAb & Anti-Hbs increased 1.05 times

Table 3
Hepatitis markers prevalence within age group (both groups/total no. tested = 876).

Age groups (years)	Hepatitis markers prevalence per area.				
	HBsAg	HBcAb*	AntiHBs†	HBcAb & Anti-HBs‡	Anti-HCV
15–24 (n = 107)					
(n positive, %)	2 (1.9)	2 (1.9)	34 (31.8)	0 (0.0)	5 (4.7)
95% CI	0.2–6.6	0.2–6.6	23.1–41.5	–	1.5–10.6
25–44 (n = 234)					
(n positive, %)	7 (3.0)	31 (13.2)	25 (10.7)	21 (9.0)	4 (1.7)
95% CI	1.2–6.1	9.2–18.2	7.0–15.4	5.6–13.4	0.4–4.3
45–64 (n = 281)					
(n positive, %)	15 (5.3)	108 (38.4)	59 (21.0)	59 (21.0)	7 (2.5)
95% CI	3.0–8.7	32.7–44.4	16.4–26.2	16.4–26.2	1.0–5.1
65+ (n = 254)					
(n positive,%)	5 (2.0)	146 (57.5)	88 (34.6)	88 (34.6)	3 (1.2)
95% CI	0.6–4.5	51.1–63.6	28.8–40.8	28.8–40.8	0.2–3.4
P-value	N.S.	<0.0001[a]	<0.0001[b]	<0.0001[c]	N.S.

N.S. = Not significant.
*, †, ‡ Comparison in the prevalence of HBcAb, AntiHBs and combined HBcAb & Anti-HBs between age groups yielded statistically significant results (P-value < 0.00001, Pearson's Chi-Square = 161.361, 43.967, 80.913 respectively).

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003

for each passing year (OR 1.05, 95% CI from 1.03 to 1.06). The above findings are presented in Table 4. Logistic regression failed to produce significant results for HBsAg and Anti-HCV.

4.3. Distribution of hepatitis genotypes

4.3.1. Distribution of HBV genotypes

Real-time PCR revealed that Genotype D accounted for the vast majority of the tested specimens (27 out of 29, 93.1%). One sample was identified being Genotype A (3.4%) and one remained unidentified.

4.3.2. Distribution of HCV genotypes

The distribution of HCV genotypes was as follows: 1a: 15.7%, 1b: 31.5%, 1c: 5.2% (total genotype 1: 52.4%), 2a: 5.2%, 3a: 42.1%.

5. Discussion

5.1. Key findings

In recent reports, Greece is classified as having intermediate HBV and HCV carriage proportion [29]. However, in most studies to date the assessment of hepatitis prevalence was typically derived from population resources such as blood donors, hospital patients, pregnant women, army recruits or refugees thus a potential selection bias may have been introduced. Combination of previous results seems to provide adequate prevalence estimates, although it is unclear to what extend potential pockets of high prevalence could exist within regions of the country. The present study estimated hepatitis B prevalence (HBsAg) of the general population to be 3.3% and hepatitis C prevalence (Anti-HCV) to be 2.2%. Both key findings of this study and particularly the first one indicate a continuous change in hepatitis seroepidemiology in Crete and call for certain actions. It should also be stressed that findings of this study seem to be in agreement with estimates from the European Centre for Disease Prevention and Control [29]. On the other hand, the endemicity of hepatitis A was found to be high in certain remote rural areas and in older individuals. It was also found lower but still considerably high in semi-urban areas and in younger individuals.

5.2. Comments on study findings regarding hepatitis seroprevalence

A study that was conducted in rural locations of Crete in the early 90's reported variations in the HBcAb prevalence among villages, with an overall estimate of 9.8% [2]. In the present study similar patterns were observed with a significant variability in the HBcAb prevalence between semi-urban and remote-rural areas. Paralleling with other studies, the HBcAb prevalence tended to increase with increasing age. Of interest was the fact that the overall prevalence of HBcAb was found to be 32.8%, higher from that previously reported for the island of Crete [2] and the general population in South-Western Greece as well [30].

Studies have indicated that the epidemiology of viral hepatitis B is susceptible to changes over the years [6,7,29,30]. It is unclear to what extend the recent population heterogeneity or the current financial crisis contributed to these findings. The prevalence of HBcAb and Anti-HBs combined was still found to be zero in the younger age group (15–24 years), whereas the prevalence of Anti-HBs was found to be high (31.8%) in the same age group. This finding is probably due to improved living conditions and the adequate implementation of vaccination in the young, at least until the recent years.

Anti-HCV prevalence was found to be somewhat lower, yet consistent with previous findings from Crete, [2,3] yet higher upon comparison with parts of Central and South-Western Greece as well [30]. Anti-HCV prevalence was found to be higher from that reported for Northern European countries but consistent to that reported for Mediterranean countries. Findings of this study indicated a higher prevalence of anti-HCV among younger individuals which could be attributed to lifestyle related habits such as drug or alcohol abuse, tattoos or piercing [2,3,5,9,10,19,20]. The high anti-HCV prevalence suggests possible existence of pocket areas with higher prevalence as previous studies have shown [1–3].

This epidemiological study was almost in accordance with a former Cretan study regarding Hepatitis A prevalence in the island [2]. The prevalence of hepatitis A Antibodies (Anti-HAV) in remote and rural areas as well as in older age groups was still high, compared to semi-urban areas and younger age groups. The hepatitis A prevalence was below 10% in participants aged less than 20 years old, residents of semi-urban areas. This finding could be attributed to the improved hygienic conditions and implementation of vaccination programs in the young.

5.3. Comments on study findings regarding observed genotypes

In literature, eight HBV genotypes ranging from A through H have been reported [26,27]. Genotype D appears to be predominant in Southern Europe, the Mediterranean basin, North Africa, and the Middle East, while Genotype A is the most prevalent in Northern and Central Europe [22,24,25]. Previous Greek and Mediterranean studies reported Genotype D to be predominant with a proportion up to 98% [7,8,22,24], but no data existed for Crete. In this study this finding was verified as Genotype D accounted for 93% of positive samples. Genotype D is associated with more severe liver histology and liver disease [24–27]. Genotype D infected-patients may be at increased risk for the development of cirrhosis and hepatocellular carcinoma, and HBV recurrence following liver transplantation [14–16,22–27].

In Greece Genotype 3 accounts for almost 30% of HCV infected patients. Genotype 1 accounts for almost 50%, being relatively constant over the years. Genotype 4 comprises almost 14% of the cases, [18,19,28]. Subtype-1b may represent a more aggressive strain and generally associates with more advanced liver diseases such as the development of liver-cirrhosis and hepatocellular-carcinoma [13,17].

Regarding HCV positive samples, a minor increase of Genotype 1 was observed in a proportion of 52.4% (1b: 31.5%). A parallel increase of Genotype 3 (3a: 42.1%), was also observed especially among younger male individuals. Subtype 3a was particularly prevalent in intravenous drug-abusers in Europe and the United States [5,10,13,21], and possibly these results reflected the extensive drug dissemination in rural Crete during recent years. The absence of Genotype 4 and of other genotypes

Table 4
Logistic regression predicting the odds of being positively tested for HBcAb, Anti-HBs and combined HBcAb & Anti-HBs (n = 876).

	HBcAb positive*		Anti-HBs positive†		HBcAb & Anti-HBs positive‡	
Predictor	O.R.	95% CI	O.R.	95% CI	O.R.	95% CI
Remote and rural group	2.17	1.56 to 3.05	1.45	1.03 to 2.06	1.57	1.06 to 2.33
Age (years)	1.05	1.04 to 1.07	1.01	1.00 to 1.02	1.05	1.03 to 1.06
Male	1.28	0.93 to 1.76	1.179	0.86 to 1.62	1.17	0.82 to 1.68

* Overall likelihood ratio X^2(on 3 df) = 208.129, p = <0.0001; pseudo R^2 = 0.211.
† Overall likelihood ratio X^2(on 3 df) = 17.563, p = 0.001; pseudo R^2 = 0.020.
‡ Overall likelihood ratio X^2(on 3 df) = 98.060, p < 0.0001; pseudo R^2 = 0.106.

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003

should also be noted, with the exception of Genotype 2a, which was found to be 5.2%.

The findings for HBV and HCV prevalence along with the parallel existence of more aggressive-genotypes, could potentially explain the reported increase of hospital discharges for liver diseases in Crete [13–18].

5.4. Limitations of the study

The present cross-sectional study reports the burden of viral hepatitis in a population of one geographically defined area of Crete and potential associations with basic socio-demographic characteristics such as gender, age and area. It certainly does not report much about other hepatitis' determinants or causation as all the observation studies. Besides these, the study was focused on one district of the island and while the participation rate was considered high (~87%) its external validity is under discussion. The ongoing heterogeneity of the population potentially has an effect in the interpretation of the study results and drawing definite conclusions about trends of the whole island or the Greek population is not recommended

5.5. Impact of the study

Cretan population became more heterogeneous over the last two decades due to economic immigrants and residents of mainland Greece who moved in this area to live and work. The current study revealed a burden of hepatitis B mainly in rural population that calls to certain actions with the public health sector. Urgent actions regarding closer monitoring of hepatitis B vaccination coverage seems to be imperative particularly in a period where austerity may have a negative impact on vaccination implementation. In addition, health education programs and measures in controlling poverty are obviously needed.

Learning points

- A threefold increase in the HBV exposure and carriage was found in the Cretan native-population studied.
- HCV endemicity remained virtually constant, presenting just a slight decrease.
- HAV endemicity was found to be still high in remote and rural areas and in older age groups, compared to semi-urban areas and younger age groups.
- Increase of more aggressive genotypes, such as Genotype D for HBV infection and 1b and 3a for HCV infection was observed.
- Viral hepatitis in general is revisiting the island of Crete.
- The above findings could explain the observation of continuous increase of hospital discharges with liver diseases in Crete.

In conclusion, this cross-sectional study indicated changes in HBV seroepidemiology in Crete. Low endemicity of hepatitis B seems to have changed, with some remote and rural areas manifesting increased burden, while the HCV endemicity remained virtually unchanged, presenting just a slight decrease in this well-defined area. Although further analyses need to be conducted, our findings have identified the changing prevalence of viral hepatitis and call for a change in preventive policies in Greece.

Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contributions

DI: participated in the design of the study, collected the demographic data, blood samples and medical history of the participants, analyzed the results, was the main author of the article and drafted the manuscript. BA: performed the statistical analysis, was a co-author of the article and reviewed the manuscript. LC: participated in the design and supervision of the study, contributed to the initial draft of the manuscript and to all its subsequent versions and he was a co-author and reviewed the manuscript. KE: conceived and supervised the study and the laboratory work performed, was a co-author of the article and reviewed the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

This study was funded in full by a scholarship provided by the Pythagoras research programs, in part by the Greek Ministry of Education and mainly by the European Community (project no: 2080, 2005).

The help of the staff of the Arkalochori Health Centre and of the Virology Laboratory of the University Hospital of Crete is greatly appreciated. We would also like to thank Dr Moschandreas J. (Assistant Professor of Statistics, Clinic of Social and Family Medicine, University of Crete), for her important contribution to the completion of the project, the former Mayor and the staff of Arkalochori community, the local administrators of the villages and all the people who participated in the study.

References

[1] Lionis C, Frangoulis E, Koulentakis M, Biziagos E, Kouroumalis E. Prevalence of hepatitis A, B, and C markers in school children of a rural area of Crete, Greece. Eur J Epidemiol 1997;13:417–20.
[2] Lionis C, Koulentaki M, Biziaros E, Kouroumalis E. Current prevalence of hepatitis A, B and C markers in a well-defined area of rural Crete-Greece. J Viral Hepat 1997;4:55–61.
[3] Lionis C, Vlachonikolis I, Skliros S, Symeonidis A, Merkouris BP, Kouroumalis E. Do undefined sources of hepatitis C transmission exist? The Greek study in general practice. J Viral Hepat 2000;7:218–24.
[4] Alter MJ. Epidemiology of hepatitis B in Europe and worldwide. J Hepatol 2003;39:S64–9.
[5] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–67.
[6] Tassopoulos NC, Papatheodoridis GV. Epidemiology of hepatitis B virus infection. Hellenic J Gastroenterol 1993;6:67–73.
[7] Hadziyannis SJ. Natural history of chronic hepatitis B in Euro-Mediterranean and African countries. J Hepatol 2011;55:183–91.
[8] Raptopoulou M, Papatheodoridis G, Antoniou A, Ketikoglou J, Tzourmakliotis D, Vasiliadis T, et al. Epidemiology, course and disease burden of chronic hepatitis B virus infection. HEPNET study for chronic hepatitis B: a multicentre Greek study. J Viral Hepat 2009;16:195–202.
[9] Gatselis NK, Rigopoulou E, Stefos A, Kardasi M, Dalekos GN. Risk factors associated with HCV infection in semi-rural areas of central Greece. Eur J Intern Med 2007;18:48–55.
[10] Hatzakis A, Wait S, Bruix J, Buti M, Carballo M, Cavaleri M, et al. The state of hepatitis B and C in Europe: report from the hepatitis B and C summit conference. J Viral Hepat 2011:1–16.
[11] Kyrka A, Tragiannidis A, Cassimos D, Pantelaki K, Tzoufi M, Mavrokosta M, et al. Seroepidemiology of hepatitis A among Greek children indicates that the virus is still prevalent: implications for universal vaccination. J Med Virol 2009;81:582–7.
[12] Papaevagelou G. Epidemiology of hepatitis A in Mediterranean countries. Vaccine 1992;10:S63–5.
[13] Hino K, Sainokami S, Shimoda K, Iino S, Wang Y, Okamoto H, et al. Genotypes and titers of hepatitis C virus for predicting response to interferon in patients with chronic hepatitis C. J Med Virol 1994;42:299–305.
[14] Vlachonikolis I, Philalithis A, Brittan Y, Georgoulias V. Mortality from malignant neoplasms in Crete. J Epidemiol Community Health 1998;52:126–7.
[15] Vlachonikolis I, Mavromanolakis S, Georgoulias V. Descriptive epidemiology of cancer in Crete, Greece. Gastrointestinal cancers. J Buon 1998;4:73–81.
[16] Hadziyannis SJ, Giannoulis G, Hadziyannis E, Kaklamani E, Alexopoulou A, Dourakis S, et al. Hepatitis C virus infection in Greece and its role in chronic liver disease and hepatocellular carcinoma. J Hepatol 1993;17:572–7.
[17] Dalagiorgou G, Vassilaki N, Foka P, Boumlic A, Kakkanas A, Kochlios E, et al. High levels of HCV core + 1 antibodies in HCV patients with hepatocellular carcinoma. J Gen Virol 2011;92:1343–51.
[18] Karatapanis S, Tsoplou P, Papastergiou V, Vasiageorgi A, Stampori M, Saitis I, et al. Hepatitis C virus genotyping in Greece: unexpected high prevalence of genotype 5a in a Greek island. J Med Virol 2012;84:223–8.
[19] Stamouli M, Panagiotou I, Kairis D, Michopoulou A, Skliris A, Totos G. Genotype distribution in chronic hepatitis C patients in Greece. Clin Lab 2012;58:173–6.
[20] Ackerman Z, Ackerman E, Paltiel O. Intrafamilial transmission of hepatitis C virus: a systematic review. J Viral Hepat 2000;7:93–103.
[21] Cornberg M, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C, et al. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. Liver Int 2011;31:30–60.

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003

[22] Katsoulidou A, Paraskevis D, Magiorkinis E, Moschidis Z, Haida C, Hatzitheodorou E, et al. Molecular characterization of occult hepatitis B cases in Greek blood donors. J Med Virol 2009;81:815–25.
[23] Zhang ZH, Zhao XP, Xia JB, Li X, Lu MJ, Yang DL. Characterization of HBsAbs in occult hepatitis B virus infection. Zhonghua Gan Zang Bing Za Zhi 2011;19:506–10.
[24] Zehender G, Ebranati E, Gabanelli E, Shkjezi R, Lai A, Sorrentino C, et al. Spatial and temporal dynamics of hepatitis B virus D genotype in Europe and the Mediterranean Basin. PLoS One 2012;7:e37198 [Epub.2012 May 25].
[25] Deterding K, Constantinescu I, Nedelcu FD, Gervain J, Nemecek V, Srtunecky O, et al. Prevalence of HBV genotypes in Central and Eastern Europe. J Med Virol 2008;80: 1707–11.
[26] Schaefer S. Hepatitis B, virus genotypes in Europe. Hepatol Res 2007;37:S20–6.
[27] Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. Intervirology 2003;46:329–38.
[28] Katsoulidou A, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, Ketikoglou I, et al. Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. J Viral Hepat 2006;13:19–27.
[29] European Centre for Disease Prevention and Control. Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies. Stockholm: ECDC; 2010 .
[30] Gogos CA, Fouka KP, Nikiforidis G, Avgeridis K, Sakellaropoulos G, Bassaris H, et al. Prevalence of hepatitis B and C virus infection in the general population and selected groups in South-Western Greece. Eur J Epidemiol0393-2990 2003;18:551–7.

Please cite this article as: Drositis I, et al, Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete, Eur J Intern Med (2013), http://dx.doi.org/10.1016/j.ejim.2013.08.003