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Research Article

Pre-elimination Plan Seroprevalence Epidemiological Study of Hepatitis C Infection in General Population in Tunisia

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Abstract

Background: Viral Hepatitis C (VHC) is a major public health concern. The global prevalence of VHC infection is estimated to more than 170 million people, nearly 3% world's population. In Tunisia, the latest prevalence estimates date back to the nineties with most studies on small populations and specific regions. This study aimed to estimate the national seroprevalence of VHC infection in Tunisia and to identify the associated risk factors in order to guide prevention and management measures. **Methods:** We conducted a cross-sectional survey in the general population from January to December 2015, using a two-stage cluster sampling design based on 2014 national census. The expected sample size was 22 275 participants. Data collection was based on standardized questionnaires administered by trained doctors, blood samples were collected in the nearest healthcare centre and tested for anti-VHC IgG antibodies in the same reference laboratory. Data were entered using EpiData and analysed with SPSS-20. Results were adjusted to the 2014 population. Ethical considerations were respected. **Results:** Anti-VHC antibodies prevalence was 0.88% [0.78% – 0.99%]. Prevalence did not vary with the gender but increased significantly with age (p<10-3). Rates ranged from 0.13% in the centereast region to 2.57% in the northwest region (p<10-3). Univariate analysis revealed that associated risk factors were marital status, profession, blood transfusion before 1992, diabetes, hypertension, hospitalization, invasive procedures, drug use and scarification. Multivariable analysis showed that age (adjusted OR= 1.04 [1.03–1.06]); region (adjusted OR= 3.15 [1.11–8.99] Center vs South and adjusted OR= 12.53 [4.56–34.45] North vs South); rural area (adjusted OR=1.57 CI95% [1.15–2.14]); hospitalization (adjusted OR= 1.51 [1.06–2.15]); drug use (adjusted OR= 11.1 [4.94–24.9]) and having a VHC chronic carrier relative (adjusted OR = 3.99 [2.17-7.36]) were independently associated with VHC infection. **Conclusions:** While overall VHC prevalence can be considered low in Tunisia, some areas are at high risk, probably related to predominance of some risk factors. Prevalence increased with age and higher rates were registered in rural areas and the north region. Our results suggest that besides socio-demographic factors, the main risk factors of VHC transmission in Tunisia were healthcare-related factors, drug use and having VHC infected relatives. Thus the need to strengthen standard precautions of hygiene in healthcare settings, harm reduction programmes as well as screening among those at risk populations.

Keywords: Cross-Sectional Studies; Viral Hepatitis C; Prevalence; Risk Factors; Sero-epidemiologic Studies;

Introduction

Viral Hepatitis C (VHC) is an inflammation of the liver caused by the hepatitis C virus, a bloodborne virus. Although it can lead to short-term illness, most infected people develop a long-term, chronic infection which can result in cirrhosis and liver cancer. In the absence of a vaccine for hepatitis C, prevention is mainly based on reducing the risk of exposure to the virus by avoiding behaviors that can spread the disease, in health care settings and in higher risk populations [1,2].

VHC infection remains a leading cause of morbidity and mortality worldwide. The WHO estimates the global prevalence of chronic VHC infection to more than 70 million people in 2015 [3]. Three to four million new infections and nearly 400 000 attributed deaths occur each year, mostly from cirrhosis and hepatocellular carcinoma [4]. A recent meta-analysis estimated the overall global prevalence of hepatitis C in the general population to 1.8% [5].

The prevalence of VHC infection varies across and within the countries. It is low in north America, moderate in Europe and some African and South American countries, but it's considered to be high in the Middle East and North Africa [6,7]. In fact, the Eastern Mediterranean region seems to be the most affected region with an overall prevalence of 2.3% and at least 23 million infected people [8,9]. However, this prevalence is heterogeneously distributed with rates ranging between 1 and 2% in most countries except Egypt with an estimated prevalence of 14.7% and the highest in the world [8,10].

In Tunisia, reliable VHC epidemiological data are limited to specific regions or populations such as blood donors [11] or dialysis patients [12,13]. The VHC burden in Tunisia has long been thought low. However, prevalence estimates in the general population are lacking. Several studies have attempted to understand the magnitude of VHC prevalence in Tunisia, but most have methodological limitations or focus on specific sub-populations rather than on the general population, with considerable variation in their estimates of prevalence rates. The last population-based study in 1996 showed a prevalence ranging from 1.7% in the North-Western region to 0.2% in the Southern region [14]. A literature review reported an overall prevalence of VHC infection of 0.7% [15].

Regarding VHC transmission, blood transfusion was the main mode of transmission but since the establishment of the National Program of Screening of VHC among Blood Donors in 1994, nosocomial transmission was more incriminated [16]. Recently, VHC treatment has been transformed with the advent of direct-acting antivirals (DAA), which are better tolerated than interferon-based treatment and offer high cure rates [17]. DAA agents has been available in Tunisia since 2016 as a pillar of the national plan for VHC elimination [18].

Our study aimed to estimate the national prevalence of VHC infection and to identify the associated risk factors immediately before the implementation of the national plan for VHC elimination (NPVHCE) in Tunisia. These estimations constitute the baseline to monitor the effectiveness of the NPVHCE in Tunisia.

Materials and Methods

Study design, study population and sampling

A national household cross-sectional population-based survey was conducted from January to December 2015 in Tunisia. A two-stage cluster design with probability proportional to size (PPS) was used with random sampling of districts and households: First, a random selection of districts was conducted in each of the 24 governorates, then, in each of the selected district, 20 households were randomly selected (Fig. 1). All eligible and consent individuals present during the investigators households visits were included.

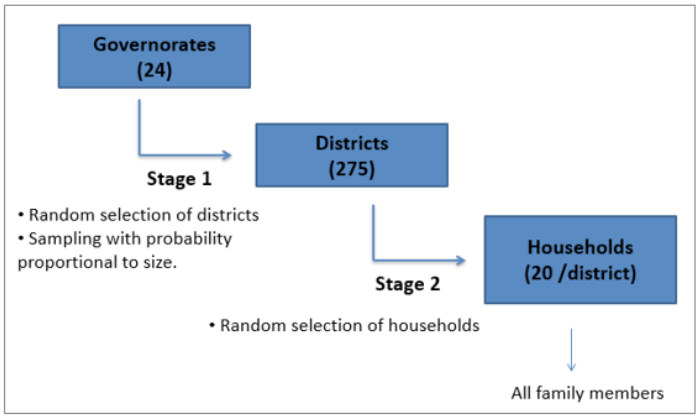

Figure 1: Sampling procedure, VHC national serosurvey, Tunisia-2015.

The sampling and computation of sampling fractions, the sampling frame of districts and households were based on the data provided by the National Institute of Statistics (INS) according to the national census of population of 2014.

Sample sizes were calculated using EpiInfo software based on the given precision (d), the estimations of prevalence (p), the a error risk of 5% (za/2=1.96) and a correction factor DE equal to 1.5 (n= DE (za/2 p(1-p)) / d2) [19]. Table 1 shows the sample size calculation based on an estimated of VHC prevalence by region.

Considering a refusal rate of 15%, the final sample size of 22 275 individuals was necessary for this study.

Region	Prevalence (p)	Precision (d)	Minimal sample size	Expected sample size
North	2,4%	0,4%	8784	10540
Center	0,8%	0,3%	5291	6348
South	0,6%	0,3%	3976	4771
Total			18051	21660

Table 1: Sample size calculation by region.

Data and Blood Sample Collection

A face-to-face standardized, pre-piloted questionnaires were administered to households and individuals by trained 24 teams (1 Medical doctor, 1 nurse). Investigators interviewed all the members of the seleted households through door-to-door visits. Blood samples were taken in the nearest primary healthcare center (Supplementary file).

All family members available during the investigators visits, were included regardless of their age and sex. Those unavailable during the first visit were called for a second appointment and interviewed within the same month.

Were excluded those not permanent resident in the visited district, those having a contraindication to blood sampling and those didn't give their consent to participate

Questionnaires included information on socio-demography and individual history of VHC exposure and risk factors and were divided in two parts. The first part included information on the family (family size, socio-economic level, social security, house type…) and the second part refered to the individual questionnaire for each family member and provided socio-demographic data (date of birth, gender, occupation, education level, insurance coverage…) as well as data on risk factors for VHC transmission (nosocomial risk, sexuality, drug use, etc...). It also contained information of whether the subjects had serology before and if they were known VHC positive, details of medical care and follow-up.

Risk factors for seropositivity identified a priori demographic variables, spatial variables, medical variables (history of blood transfusion and blood donation, history and location/provider type for medical injections, surgery and delivery, dental and gum treatment, type of contraception, miscarriage and abortion) and behavioral variables (tattoos, piercing, IV drug use, pedicures, manicures and frequenting of barbershops).

After being codified to ensure anonymity and confidentiality, all the blood samples were sent to the reference laboratory; the Microbiology-Biochemistry laboratory of Aziza Othmana Hospital in the capital Tunis. All samples were tested for antibodies anti-VHC IgG using (electro) chemiluminescence technique on the «Cobas e411» automate Search for the viral HCV RNA was performed by RT-PCR in real time in subjects diagnosed positive for the screening test for VHC antibodies to distinguish between chronic VHC and healed subjects (Fig. 2).

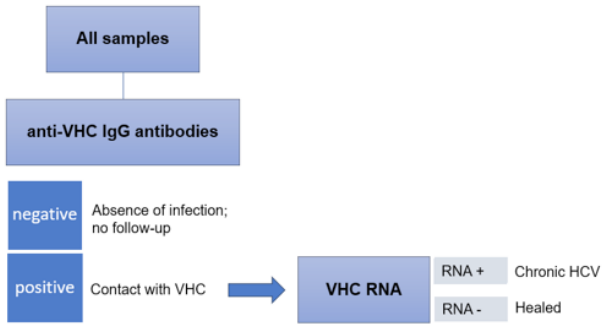

Figure 2: Algorithm for analysis of VHC infection markers

Serological validation of the results was conducted for all samples by a group of biologists and epidemiologists of the steering committee.

Data Entry and Analysis

After initial validation in each region by the regional coordinator, all questionnaires were sent to the National Observatory of New and Emerging Diseases for final validation and data entry using EpiData 1.3. Laboratory results were added in the same database when received.

Statistical analyses were conducted using probabilities and sampling weights calculated for each stage of the sampling (District, household and individual) and analysis considered the finite population correction factor. The prevalence of VHC infection [95%CI] was estimated globally, by region and sociodemographic factors.

Univariate analysis was performed using the Pearson chi-square test and the Student t-test to compare the qualitative and quantitative variables, respectively and a binary logistic regression to estimate crude Odds Ratios [95%CI] associated to different VHC studied risk factors.

We conducted a multivariate analysis, accounting for the sampling design, to identify risk factors for VHC infection among the population. The association between the seroprevalence and the explanatory covariates was quantified by fitting multivariate logistic regression model. The multivariate analysis retained

variables from the univariate analysis with p-value less than 0.2. Estimates of the regression coefficients of the model and odds ratios with their confidence interval were presented. All statistical analysis were conducted using SPSS.20 software.

Accounting for the sampling design, the survey package (Analysis of Complex Survey Samples, Thomas Lumley) version 3.34 estimated parameters, including standard errors (Horvitz-Thompson-type standard errors) were used everywhere in the survey package. Confidence interval calculations usually used the scaled Chi squared distribution for the log likelihood from a binomial distribution.

Community Engagement

Prior to the start of the survey, a national workshop was organized to present the study protocol and data collection tools and needs to all investigators, coordinators and local authorities. Meetings were also organized with local authorities at all levels to introduce the objectives of the survey and to discuss the timeline and request for support. Mobilisers (identified by the chief of each village) visited selected households prior to the data survey to request the household's presence.

Ethical Considerations

Our study had the approval of the Higher Council of Statistics, the National Ethic Committee and the National Instance of Personal Data Protection. Before participating to the study, all individuals had information note on the objectives and modalities of the survey and had to sign informed consent. These two documents were both in Arabic, native language.

All subjects had the right to refuse to participate and were respected in their decision. Confidentiality and anonymity were ensured throughout the study as well as the feedback and support of positive people. The serological results were sent to the concerned subjects through the regional coordinator and positive people were sent to the gastroenterology service for further exploration and proper medical supervision. Patients with VHC positive result were referred to reference VHC elimination program to measure viral load and initiate treatment.

Results

Description of the Study Population

From the expected sample size of 22 275 subjects, a total of 21 720 subjects were surveyed, (participation response rate of 97.5%). Of these, we collected 19 693 blood samples (90.6% response rate).

Male to female sex-ratio was 0.67 with 59.9% females (Fig. 3). Adults aged 30 to 40 years and 40 to 50 years were the most represented (14.9% and 14.7% respectively) (Fig. 4).

Most of the participants were living in the north of the country (48.8%) followed by the center (36.6%) and the south (14.5%) with a predominance of urban areas (67.8%). The highest participation rates were in Tunis (9.7%), Sfax (8.7%) and Nabeul (7.2%).

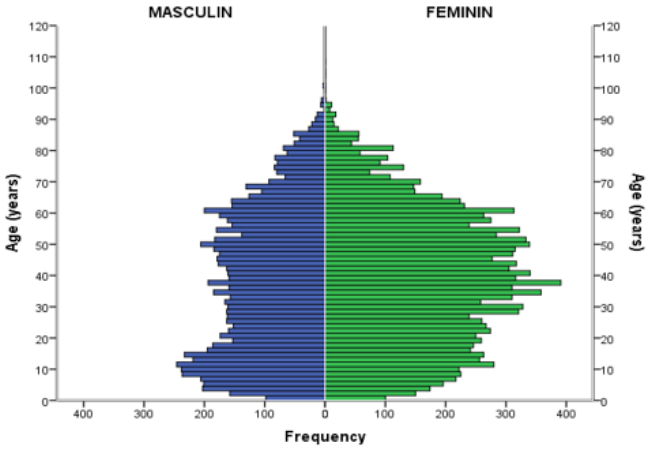

Figure 3: Distribution of study population by gender, VHC national serosurvey, Tunisia-2015.

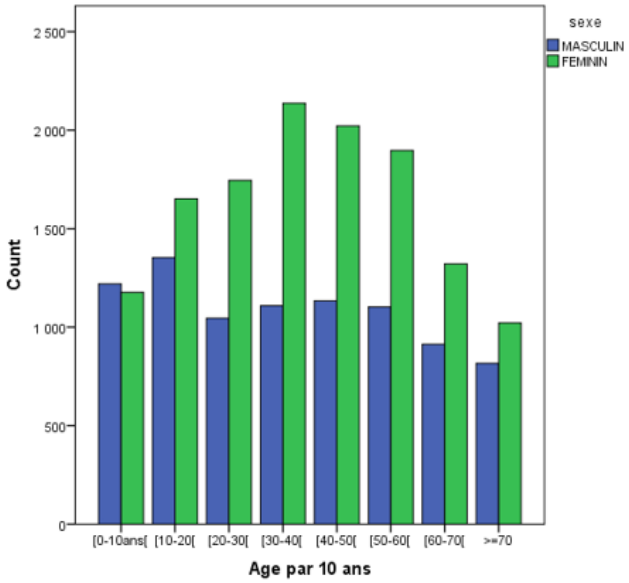

Figure 4: Distribution of study population by age groups, VHC national serosurvey, Tunisia-2015

Prevalence of anti-HCV positive sera

National HCV Prevalence was 0.88% (95%CI [0.78% – 0.99%]) estimating 88130 VHC positive subjects in the general population. There was no significant difference in the prevalence of VHC infection between males and females (0.88% vs 0.89% respectively). However, rates increased significantly with age (Fig. 5) with the highest prevalence among participants aged 70 years

and more (2.82%). The mean age of positive subjected was 21.19 (SD ± 1.31) years. Prevalence ranged from 0.13% in the center-east to 2.57% in the north-west region (p<0.001) (Fig. 6).

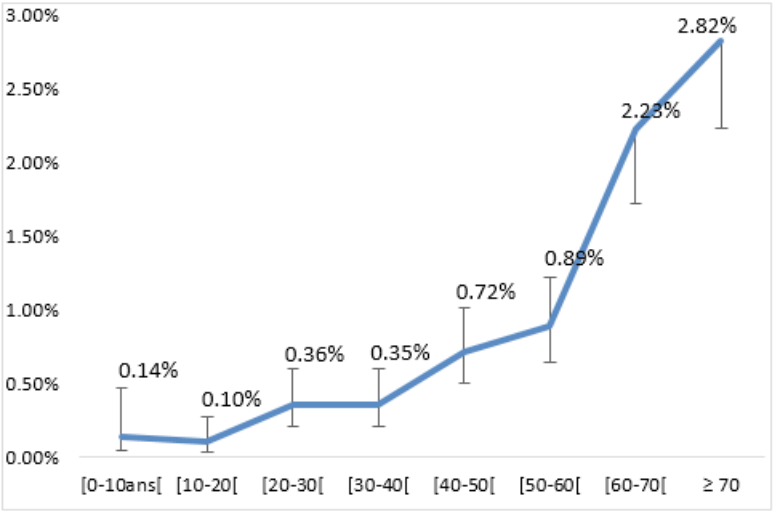

Figure 5: Distribution of anti-HCV prevalence by age groups, VHC national serosurvey, Tunisia-2015

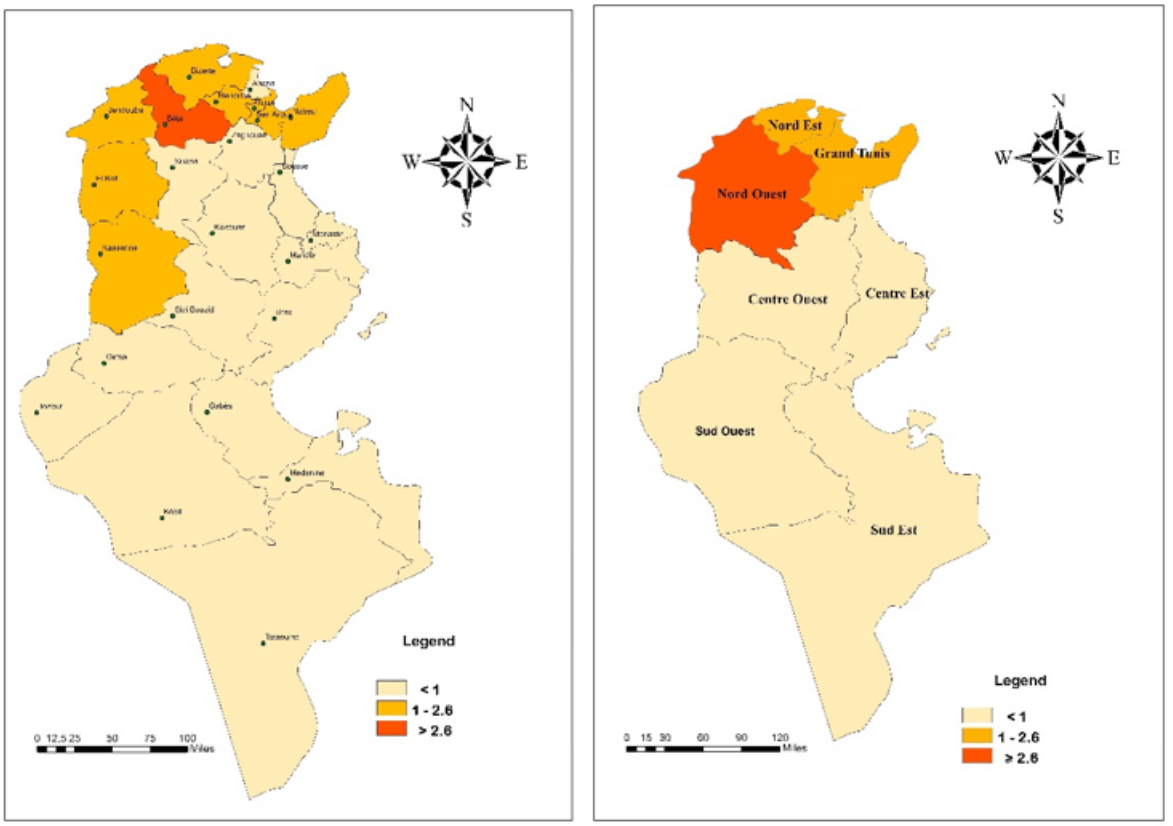

Figure 6: Distribution of anti-HCV prevalence by region/governorate, VHC national serosurvey, Tunisia-2015

VHC infection associated risk factors

Univariate analysis showed that age over 40 years was associated with HCV prevalence with a significant increase of OR; from 5.16 for the age group 40-50 years to 20.72 for those aged =70 years. VHC infection prevalence was higher in the North region (OR=8.06). People living in rudimentary houses were more likely to be positive (p=0.01) but there was no significant difference between rural and urban areas. Besides, VHC infection was higher among married subjects (OR=2.08) and divorced or separated or widowed (OR=5.14) comparing to single participants. Prevalence was significantly higher among those without a professional activity and varied significantly with profession type as well as education level (table 1).

Regarding hygiene, sharing personal items such as towels, toothbrushs or scissors wasn't found to be an associated risk factor for VHC infection as well as sharing razor blades among men. However, the risk of being infected was significantly lower among men shaving at the barber (OR=0.67) and when the barber is using new razor blades each time (OR=0.39) (table 2).

Variable	N	Anti HCV Prevalence [CI 95%]	OR [CI 95%]	p
Sex	**19166**			
Female	11836	0.88 [0.75-1.04]	--	0.97
Male	7330	0.89 [0.72-1.1]	1.01 [0.76 – 1.32]	
Age	**19120**			**< 10^{-3}**
[0-10]	1575	0.14 [0.04-0.48]	--	--
[10-20]	2561	0.10 [0.04-0.28]	0.75 [0.15-3.6]	0.72
[20-30]	2452	0.36 [0.21-0.61]	2.56 [0.67-9.73]	0.16
[30-40]	2973	0.35 [0.21-0.60]	2.52 [0.66-9.61]	0.17
[40-50]	2909	0.72 [0.51-1.02]	**5.16 [1.44-18.45]**	0.012
[50-60]	2794	0.89 [0.65-1.23]	**6.42 [1.81-22.8]**	0.004
[60-70]	2117	2.23 [1.72-2.88]	**16.25 [4.64-56.9]**	< 10^{-3}
≥70	1739	2.82 [2.23-3.57]	**20.72 [5.94-72.27]**	< 10^{-3}
Region	**19166**			**< 10^{-3}**
South	2720	0.18 [0.08-0.39]	--	--
Center	6787	0.38 [0.27-0.53]	2.13 [0.92–4.9]	0.075
North	9659	1.43 [1.24-1.65]	**8.06 [3.7–17.55]**	**< 10^{-3}**
Area	**19166**			
Rural	6363	1 [0.8-1.2]	--	--
Urban	12803	0.8 [0.7-1]	0.87 [0.67–1.14]	0.31
Habitation type	**18583**			
Modern/Appartment	9675	0.7 [0.6-0.9]	--	--
Rudimentary	8908	1 [0.9-1.2]	**1.43 [1.09–1.88]**	**0.01**
Water supply	**18463**			
Running water	16480	0.9 [0.7-1]		
Others	1983	1.1 [0.8-1.6]	1.34 [0.93–1.92]	0.11
Water disposal	**18428**			

Waste water system	11554	0.9 [0.7-1]		
Others	6874	0.9 [0.7-1.1]	1.04 [0.79–1.36]	0.78
Marital Status	**14051**			**< 10^{-3}**
Single	3325	0.5 [0.3-0.7]	Ref	
Married	10388	1 [0.9-1.2]	**2.08 [1.33–3.27]**	0.002
Divorced/separated/widowed	338	2.5 [1.3-4.8]	**5.14 [2.3–11.49]**	< 10^{-3}
Education	**18567**			**< 10^{-3}**
Universitary	1800	0.3 [0.1-0.6]	Ref	
Middle School	2054	0.3 [0.2-0.7]	1.23 [0.44-3.43]	0.69
Primary	6517	0.6 [0.5-0.8]	2.2 [0.98-4.89]	0.054
Secondary	4004	0.6 [0.4-0.9]	**2.32 [1.01-5.34]**	0.047
preschool	547	0.8 [0.3-1.7]	2.74 [0.87-8.55]	0.082
Illiterate	3645	2.3 [2-2.8]	**8.67 [3.99-18.84]**	< 10^{-3}
Professional activity	**14692**			
Yes	4681	0.7 [0.5-0.9]		
No	10011	1.3 [1.1-1.5]	**1.89 [1.36-2.62]**	< 10^{-3}
Profession	**18167**			**< 10^{-3}**
Mid-level employees	1756	0.3 [0.2-0.7]		
Workers	2012	0.7 [0.5-1.1]	2.2 [0.98-4.92]	
No professional activity	12357	0.9 [0.8-1.1]	2.74 [1.35-5.56]	
Agriculture workers	393	1.1 [0.5-2.2]	3.15 [1.12-8.84]	
Retirees	1321	1.7 [1.1-2.4]	5 [2.27-11.03]	
Business leaders, Trade employees	328	1.9 [0.9-3.7]	**5.69 [2.14-15.13]**	

Table 2: Socio-demographic factors associated with HCV infection

Unlike blood transfusion and blood donation, history of surgical intervention, invasive procedures, hospitalization, medical injections were significantly associated with VHC prevalence, as well as dental procedures. The OR associated with diabetes and hypertension was 3.06 and 4.20 respectively. Other risk factors found significantly associated with VHC infection were intravenous and intranasal drug use, traditional scarification and tattoos in addition to multiple sexual partners. Prevalence was also higher among those who had a relative infected with VHC (sister) (table 3 and 4).

Variable	N	Anti HCV Prevalence [CI 95%]	OR [CI 95%]	p
Personal hygiene	**15212**			
No sharing of personal items*	3343	1.1% [0.9-1.5]		
Sharing at least 1 item	11869	1% [0.9-1.2]	0.87 [0.63-1.2]	0.4
Sharing scissors	**14146**			
No	3746	1.3% [1-1.6]		
Yes	10400	1% [0.8-1.1]	0.76 [0.56-1.02]	0.07

Sharing razor blades	**11468**			
No	10921	1.1% [1-1.3]		
Yes	547	0.7% [0.3-1.6]	0.62 [0.27-1.46]	0.27
Shaving at the barber shop	**7044**			
No	2903	1.4% [1.1-1.8]		
Yes	4141	0.9% [0.7-1.2]	**0.67 [0.46-0.97]**	**0.036**
New razor used by barber	**4133**			
No / don't know	358	2.1% [1.1-4.1]		
Yes	3775	0.8% [0.6-1.1]	**0.39 [0.18-0.81]**	**0.01**
Circumcision	**6916**			
Medical professional	2302	0.6% [0.4-0.9]		
Traditional	4614	1% [0.8-1.3]	**1.7 [1-2.8]**	**0.037**

Table 3: Life-related factors associated with HCV infection

Variable	N	Anti HCV Prevalence [CI 95%]	OR [CI 95%]	p
Blood transfusion	**15185**			**0.17**
Never transfused	14267	1% [0.9-1.2]		
Transfused after 1992	673	1.3 [0.7-2.3]	1.3 [0.72-2.35]	0.39
Transfused before 1992	245	2% [0.9-4.2]	**1.95 [0.9-2.25]**	**0.09**
Blood donation	**15398**			
No	11977	1.1% [1-1.3]		
Yes	3421	0.9% [0.6-1.2]	0.76 [0.55-1.07]	0.11
Follow-up for at least one disease	**19075**			
No	14161	0.6% [0.5-0.7]		
Yes	4914	1.8% [1.5-2.1]	**3.1 [2.4-4.1]**	**$<10^{-3}$**
Diabetes	**19063**			
No	17507	0.8% [0.7-0.9]		
Yes	1556	2.3% [1.7-3.1]	**3.06 [2.19 – 4.28]**	**$<10^{-3}$**
Hypertension	**19063**			
No	16949	0.7% [0.6-0.8]		
Yes	2114	2.7% [2.1-3.4]	**4.20 [3.17 – 5.57]**	**$<10^{-3}$**
Relative chronic carrier of HVC	**17431**			
No	17104	0.8 [0.7-0.9]		
Yes	327	3.3 [2-5.5]	**4.15 [2.38 – 7.24]**	**$<10^{-3}$**
Family relationship with the chronic carrier of HVC	**433**			**0.004**
Others	307	1.5 [0.7-3.4]		

Mother	60	3.6 [1.1-11.1]	2.46 [0.45-13.39]	0.29
Partner/ conjoint	44	4.3 [1.3-13]	2.95 [0.43-20.25]	0.27
Sister	22	12.7 [4.1-33.2]	**9.58 [1.35-67.73]**	**0.02**
Surgical procedure	**18716**			
No	92	0.7 % [0.6-0.8]		
Yes	82	1.4% [1.2-1.7]	**2.15 [1.65-2.8]**	**$<10^{-3}$**
Hospitalization	**18788**			
No	9440	0.5% [0.4-0.6]		
Yes	9348	1.3% [1.1-1.5]	**2.75 [2.03-3.72]**	**$<10^{-3}$**
Medical injection	**18878**			
No	2751	0.6% [0.4-0.9]		
Yes	16127	0.9% [0.8-1.1]	**1.5 [1.01 – 2.24]**	**0.047**
Invasive procedures	**19166**			
No	14106	0.8% [0.7.0.9]		
Yes	5060	1.2% [1-1.5]	**1.54 [1.17 – 2.03]**	**0.002**
Dental procedures	**19054**			
No	7583	0.5% [0.4-0.7]		
Yes	11471	1.1% [1-1.3]	**2.06 [1.52-2.79]**	**$<10^{-3}$**

Table 4: Medical history, healthcare-associated factors and HCV infection

By multivariate analysis, factors independently associated with VHC infection (Table 5) were divided in socio-demographic factors, healthcare-related factors and lifestyle habits. Socio-demographic factors included age and area, with older participants and those living in rural areas more at risk of being VHC infected; the corresponding AOR were 1.04 (95%CI [1.03–1.06]) and 1.57 (95%CI[1.15–2.14]) respectively. In addition, the geographic region was also significantly associated with VHC and the most associated category was the north region (AOR was 12.53 (95%CI[4.56 - 34.45]).

Variable	N	Anti HCV Prevalence [CI 95%]	OR [CI 95%]	p
Intranasal drug use	**168**			
No	163	1% [0.9-1.2]		
Yes	5	4.7% [2.3-9.7]	**4.78 [2.19-10.45]**	**$<10^{-3}$**
Intravenous drug use	**??**			
No	161	1% [0.9-1.2]		
Yes	6	26.7% [13.6-45.8]	**34.59 [14.77-81.02]**	**$<10^{-3}$**
Intranasal and/or intravenous drug use	**15223**			
No	15120	1% [0.9-1.2]		
Yes	103	7.6% [4.1-13.7]	**8.01 [4.11-15.6]**	**$<10^{-3}$**
Partner intravenous drug user	**14873**			
No	14826	1% [0.9-1.2]		
Yes	47	11.4% [5.2-23.1]	**12.16 [5.14-28.74]**	**$<10^{-3}$**

Sexual partners	**14846**			**<10^{-3}**
No sexual partner	2519	0.4% [0.3-0.7]		
1 sexual partner	9220	0.9% [0.8-1.1]	**2.15 [1.25-3.67]**	**0.006**
Multiple sexual partners	906	2% [1.4-3]	**4.68 [2.44-8.98]**	**<10^{-3}**
Do not want to answer	2201	2.1% [1.6-2.7]	**4.82 [2.74-8.48]**	**<10^{-3}**
Tattooing	**18697**			
No	154	0.8% [0.7-1]		
Yes	18	2.1% [1.4-3.1]	**2.52 [1.62 – 3.91]**	**<10^{-3}**
Scarification	**18665**			
No	15974	0.8% [0.7-0.9]		
Yes	2691	1.5% [1.2-2]	**1.96 [1.42-2.7]**	**<10^{-3}**
Acupuncture	**18665**			
No	165	0.9% [0.8-1]		
Yes				**<10^{-3}**

Table 5: High-risk behaviors and HCV infection

Variable	p	OR	CI 95%
Sex	0.71	0.94	
Age	**<10^{-3}**	1.04	1.03 – 1.06
Region			
south			
center	**0.032**	3.15	1.11 – 8.99
north	**<10^{-3}**	12.53	4.56 - 34.45
Area			
Urban			
Rural	**0.005**	1.57	1.15 – 2.14
Hospitalization	**0.021**	1.51	1.06 – 2.15
Drug use	**<10^{-3}**	11.1	4.94 – 24.9
Relative chronic carrier	**<10^{-3}**	3.99	2.17 – 7.36

Table 6: Multivariate analysis

Besides, participants who had at least one hospitalization in their life were more likely to have positive VHC antibodies (AOR = 1.51; 95%CI [1.06–2.15]). The risk of being infected was multiplied by 11.1 (95% CI [4.94 – 24.9]) among persons who used drugs compared to those who never did. Lastly, having a relative VHC infected was also found a risk factor (AOR = 3.99; 95% CI[2.17–7.36]).

Discussion

This study provides the first large-scale general adult population prevalence data on VHC infection in Tunisia. It is the first national household-based study determining hepatitis C prevalence and risk factors in Tunisia based on a large sample of 21720 surveyed participants representing the total population regardless of age, sex and region.

Infection status did not differ between males and females and was more likely to occur in old persons and people living in rural areas. Higher prevalence was confirmed in the Northwest region, which prompts us to study the specific risk factors in the region in order to adapt preventive measures. Besides, hospitalization was a significant risk factor of being HCV infected, covering a large group of healthcare exposures and emphasizing the need to strengthen hygiene preventive measures in healthcare settings.

The overall prevalence of anti-VHC antibodies was 0.88%. Some authors have previously estimated this rate to 0.7% by reviewing studies conducted in the general population, all dating back to the late nineties [15,20]. Another systematic review studying the epidemiology of hepatitis C in the Maghreb region estimated a lower prevalence at 0.6% (95% CI: 0.5–0.8) [21]. According to the same study, Algeria and Tunisia had the lowest endemicity for VHC (0.3% and 0.6% respectively) and overall VHC prevalence in the Maghreb countries was about 1%, very different from the adjacent neighbor Egypt (14.7%) [10,22]. Prevalence of anti-VHC in Tunisia was also lower than in European region except some countries such as France (0.75%), Germany (0.4%) and Belgium (0.1%) [23,24].

Positivity did not vary significantly between males and females, which was similar to previous studies [14,25]. VHC positive individuals were significantly older than those negative, and this was consistent to most national and international studies [15,26,27]. However, a higher prevalence among children was observed in some countries and within specific settings such as Egypt and Pakistan, where the incriminated transmission in both cases was unsafe injections [28,29].

Most of the Tunisian studies have reported a heterogeneity in the geographical distribution of VHC infection with the North-Western region the most affected [14,15]. Our study has confirmed this finding with a prevalence increasing from 0.1% in the south-east to 2.6% in the North-West. A previous study was conducted to assess the associated risk factors in this high-endemic area [16]. Besides, regional variability in VHC prevalence in the same country was also observed in other countries like Japan [30] and other north-African countries such as Libya and Egypt [31,32]. This may be explained by differences in risk factors across the countries which need to be further explored.

Regarding hepatitis C transmission, history of hospitalization was an independent associated factor, which we believe covers a group of healthcare exposures such as surgical interventions, medical injections and invasive procedures. This was consistent with many studies reporting nosocomial transmission in VHC epidemiology [33-35].

Although the last Tunisian studies still reported high anti-VHC prevalence rates among hemodialysis patients and blood donors [25,36], these known risk factors were not found associated with VHC infection which may be due to the low number of individuals part of these high-risk populations. High-risk healthcare exposures such as dialysis and blood transfusions were found as major contributors to HCV transmission in the Maghreb region [21].

Prevalence was higher among people with hypertension as well as diabetes. Limited data is available regarding this association but some authors previously showed similar findings [16,37]. A systematic review showed that type 2 diabetes is associated with increased susceptibility to hepatitis C and that overall diabetics were 3.5 more likely to acquire VHC infection than non diabetics [38]. Although diabetes was suspected to be an additional metabolic complication of VHC infection, further studies are needed to explain reasons for this relationship [39].

Drug use, another known risk factor, was also found highly associated with VHC infection. Globally, the prevalence of VHC infection among IDUs is estimated to 61.7% [40]. Although IDU is one of the main modes of transmission in developed countries [34,41], its role is not dominant in the maghrebian countries and data on IDU prevalence and epidemiology is lacking [21]. Nevertheless, some countries of the region are reporting a change in the epidemiology of VHC infection due to increasing drug use such as Iran [42,43]. Association with intranasal drug use was also observed in other series [41,42], but its contribution in VHC transmission remains controversial as it may be a surrogate for other risk behaviors associated with VHC or be explained by sharing blood-contaminated devices [44].

People who had a relative infected with VHC were found more at risk of Hepatitis C. This was discordant with the study of Mejri and al. who reported non significantly higher prevalence rates among household contacts of VHC positives than those in the general population [14]. Contribution of intra-familial transmission was however documented in other studies and household contacts to VHC cases were found exposed to increased risk of HCV infection, although with a minor role [44,45].

Limitations

This study has some limitations. Like all cross-sectional studies, there is no evidence of temporal relationship between the studied risk factors and VHC infection. Under estimation of some risk factors mainly sexual behavior and drug use is possible due to the cultural context and taboos. Confidentiality and anonymity were ensured to minimize the risk of information bias. Recall bias could also have resulted from differences in reporting some life-related factors or medical history. Other limitations may have occurred from the logistic difficulties due to the big sample size, mainly in samples' transport and conservation, as well as from some missing data.

Conclusion and Recommendations:

Our study allowed an adequate estimation of anti-VHC seroprevalence in the general population of Tunisia and classified the level of endemicity as low.

Following this survey, the Tunisian Ministry of Health (MoH) established a VHC elimination plan and initiated Direct Acting Antiviral (DAA)-based treatment. This study establishes a robust estimate of the VHC prevalence and seropositivity risk factors for the general population and is considered as baseline for the evaluation of the VHC elimination plan implemented in Tunisia since 2016. Another sero-prevalence study is considered in the future to assess the changes in prevalence after the availability of DAA, part of this elimination plan.

Our findings strongly support the introduction of screening programs, early diagnosis, treatment and health education of high-risk groups to achieve the goal of elimination of VHC in Tunisia by 2030.

Declarations

The authors declare no competing interest

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Supplementary file: Collection and management of blood samples

Collection of blood samples

All samples were taken from the nearest basic health center. A first sample were taken by a qualified nurse or senior technician investigator after obtaining the informed consent of individuals or their parents (for minors) by following the following steps:

- Complete the questionnaire;
- Put the corresponding code on the sampling sheet;
- Code the tube, paying close attention to the correspondence between this code and that of the questionnaire and the sampling sheet;
- Once this check is completed, take two 10 cc tubes of blood (almost full tube) by needle from the vein.
- Place the clogged tubes in the rack;
- Allow blood to settle for at least two hours at room temperature;
- Send the blood to the designated laboratory or other structure according to the arrangements made according to the regions for centrifugation 10 mn to 3000 rpm;
- Retrieve the serum using a single-use pipette from a sterile, red-capped tube that has been previously labelled and coded with the same code as the original sample tube;

According to the arrangements made by each governorate, the samples were:

- sent within 48 hours after collection to the microbiology-biochemistry laboratory of the Aziza Othmana hospital for the governorates of greater Tunis.
- kept in the freezer at -20°C and sent later for other governorates. Transportation to the reference laboratory was ensured at a temperature of –20°C (carbo ice) and following the regulations in force applicable to potentially infectious diagnosed samples.

Management of blood samples

A specification detailing good practices for carrying out biological analyzes was produced by the microbiology-biochemistry laboratory of the Aziza Othmana hospital. For each sample, a code was matched on the sampling sheet and on the other hand on the questionnaire. This code was kept secret for data management and analysis purposes and allowed to establish the correspondence between the questionnaire and the biological results.

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