COMPARISON OF HEPATITIS A SEROPREVALENCE IN BLOOD DONORS IN SOUTH TUNISIA BETWEEN 2000 AND 2007

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RESUME

L'objectif de cette étude a été de déterminer la séroprévalence du virus de l'hépatite A (VHA) chez les donneurs de sang du sud tunisien sur deux périodes 2000 et 2007. Pour cela, 376 donneurs prélevés en 2000 et 2007 respectivement, ont été testés pour les IgG anti-VHA. Ces donneurs sont âgés de 18 à 30 ans et proviennent de différentes régions du sud tunisien. La séroprévalence globale du VHA a été de 85.9 % en 2007 contre 94.9 % en 2000. La séroprévalence chez les donneurs âgés de 18 à 20 ans a été de 91.9 % en 2000 versus 80.6 % en 2007 et atteint, chez les donneurs de plus de 26 ans, 99% et 92% respectivement en 2000 et 2007. Tenant compte de l'origine géographique, la séroprévalence dans Sfax ville a diminué passant de 88.9 % en 2000 à 62.7 % en 2007 ($p<0.001$). Par contre, elle reste inchangée dans les alentours de Sfax (98.4 % et 96 %) et les gouvernorats du sud tunisien (97.6 % et 99.2 %). En conclusion, le nombre d'adultes non immunisés contre le VHA dans la ville de Sfax a augmenté avec pour conséquence le risque d'une augmentation du nombre de cas d'hépatite A aigue symptomatique voire grave chez les adolescents et adultes jeunes.

Mots clés: *Virus de l'hépatite A, séroprévalence, donneurs de sang, sud Tunisien.*

SUMMARY

The aim of the study was to assess hepatitis A virus (HAV) seroprevalence in blood donors from South Tunisia in two periods 2000 and 2007. Serum samples collected from 376 blood donors in each period aged 18 to 30 years from different regions of South Tunisia were analysed for anti-HAV IgG. The global seroprevalence of HAV infection was 85.9% in 2007 as compared with 94.9% in 2000. The seroprevalence in the 18-20 years age group was 91.9% in 2000 vs 80.6% in 2007, and increased to 99% in 2000 and 92% in 2007 in the subjects over 26. Taking account of geographic area, the HAV seroprevalence in Sfax city decreased from 88.9% in 2000 to 62.7% in 2007 ($p<0.001$), but it is still approximatively the same in rural areas (98.4% and 96%) and in the governorates of South Tunisia (97.6% and 99.2%). In conclusion, the number of adults in the city of Sfax witch are not immunized against HAV is increasing. Thus, adolescents and young adults are at risk to develop symptomatic and potentially severe hepatitis A.

Key words: *Hepatitis A virus, seroepidemiology, blood donors, south Tunisia.*

INTRODUCTION

Hepatitis A virus (HAV) is the leading cause of viral hepatitis worldwide [1]. Infection by this virus is often asymptomatic or accompanied by mild and non specific illness in young children [2]. In adults, this disease can be severe and in some cases, it may cause fulminant and fatal hepatitis [3]. The prevalence of hepatitis A worldwide is classified as high, intermediate and low [4]. The level of endemicity is correlated with the hygienic and sanitary conditions in each area [5, 6]. In most industrialized countries, endemicity is low and rates of HAV infection prevalence in children are generally low. In developing countries, HAV infection occurs early during life and more than 90 of the population have acquired natural immunity before 10 years of age. However, in many countries, the pattern of HAV endemicity has dropped from high to intermediate due to improvement in sanitary conditions in the recent decades [7, 8]. As a result, many children acquired infection during early childhood. But, viral circulation remains high and infection often occurs in adolescents and young adults.

In Tunisia, epidemiological data on HAV are fragmentary, and are limited to certain regions of the country. For instance, studies conducted in different regions of Tunisia and in the middle of Tunisia (Sousse) showed an important decrease in HAV infection [9, 10]. The aim of this study was to determine the seroprevalence of HAV infection in blood donors from different regions of South Tunisia in two periods of time: 2000 and 2007, and to evaluate the change in the epidemiology of this infection.

MATERIALS AND METHODS

This study was conducted as part of the collaboration between the laboratory of Microbiology of the University Hospital Habib Bourguiba of Sfax and the blood transfusion regional center of Sfax (CRTS).

It is a descriptive, cross sectional study involving 752 blood donors of both sexes and varying ages in the governorate of Sfax and many governorates of South Tunisia in the years 2000 and 2007. In 2000, the population of Sfax was 846.500 inhabitants, of whom 248.871 (29.4%) were 15-29 years old. In 2007, it was 894.200 inhabitants, of whom 244.116 (27.3%) were 15-29 years old [11]. The targeted population included blood donors aged between 18 to 30 years. A two-stage cluster sampling was used for random selection of regions that was proportional to the number of blood donors in each region. In the first stage, we sampled according to age; in the second stage, we sampled regions from each age group. The same stage process was used in 2000 and 2007. The sample size was calculated using an alpha error of 5% and a precision of $\pm$ 0.025, with an expected precision rate prevalence of antibodies of 50%, corresponding to a theoretical sample size of 376 blood donors each year. Blood donors were divided into three age groups (Table I): 18-20 years, 21-25 years and 26-30 years.

As for the urbanisation level, the distribution of the regions was taken into account during sampling. Regions of blood donors were allocated to three groups (Table I):

- Group 1: blood donors from the city of Sfax (urban area)
- Group 2: blood donors from different delegations of Sfax (rural areas)
- Group 3: blood donors from different governorates of South Tunisia: Gabès, Gafsa, Sidi Bouzid, Mednine, Gbelli, Tataouine, Tozeur (urban and rural areas).

All serum samples collected in 2000 and 2007 were frozen at -40°C. Total HAV antibodies were determined by a commercial third generation competitive immunometric assay (ETI-AB-HAVK PLUS, DiaSorin, Italia) according to the manufacturer's instructions.

Table I: Description of studied population by age and origin in 2000 and 2007.

	Number of blood donors						
	2000			2007			
Age groups (years) / **Origin**	18-20	21-25	26-30	18-20	21-25	26-30	
Group 1	42	42	42	42	42	42	
Group 2	42	42	42	42	42	42	
Group 3	40	42	42	40	42	42	
Total	**124**	**126**	**126**	**124**	**126**	**126**	**752**

Group 1: *city of Sfax;* ***Group 2:*** *different delegations of Sfax;* ***Group 3:*** *different governorates of South Tunisia.*

Statistical analyses were performed using SPSS11. HAV seroprevalence was studied in subjects taking into account age and regions in 2000 and 2007. Differences in the prevalence of anti-HAV antibodies among different age groups or regions of blood donors were analysed using the Chi-square. A significant level of 0.05 was used for all statistical tests in the analysis. A multivariate analysis with logistic regression that included age and origin was used to determine the influence of these factors in HAV seroprevalence. The odds ratio (OR) and 95%confidence interval (95% CI) were calculated for these variables.

RESULTS

The global seroprevalence of anti-HAV in 2007 was 85.9% which is lower than the prevalence of 94.94% obtained in 2000; the difference was statistically significant ($p<0.001$). The prevalence of hepatitis A antibodies according to age groups increased significantly with age in the two periods of time ($p=0.023$ in 2000, $p=0.032$ in 2007; p calculated with khi-deux of Pearson). In 2007, the 18-20 years, 21-25 years and 26-30 years age groups had prevalences of 80.6%, 84.9% and 92.1% respectively, all inferior to the prevalences of corresponding age groups in 2000 (91.9%, 93.7% and 99.2% respectively); the differences were statistically significant ($p<0.01$, $p=0.02$ and $p<0.01$ respectively). The Details of these results in each age group obtained in 2000 and 2007 are shown in table II. Beside age, the prevalence of HAV antibodies was correlated with the origin of blood donors in both years 2000 and 2007 as shown in table III ($p=0.001$ and $p=0$ respectively). HAV seroprevalence showed significant decline between 2000 and 2007 in the city of Sfax (88.9% in 2000 vs 62.7% in 2007) ($p< 0.001$). However, it remained high in rural areas (98.4% and 96%) and in the governorates of South Tunisia (97.6% and 99.2%).

The results of the HAV seroprevalence by age and region among blood donors in 2000 and 2007 obtained after this univariate analysis were maintained in the multivariate analysis (Table IV). However, no significant differences were detected for the subjects aged 18 to 20 years and those aged 21 to 25 years in 2000 and 2007 and anti-HAV seropositivity, with adjusted OR of 1.5 (0.7-3.2) and 1.4 (0.8 -2.6) respectively.

Table II: Prevalence of anti-hepatitis A virus (HAV) antibodies in blood donors by age group in 2000 and 2007.

	Anti-HAV					
	2000			2007		
Age groups (years)	Number tested	Number Positive (%)	95% CI*	Number tested	Number Positive (%)	95% CI*
18-20	124	114 (91.9)[1]	0.87- 0.96	124	100 (80.6)[1]	0.87- 0.73
21-25	126	118 (93.7)[2]	0.89- 0.98	126	107 (84.9)[2]	0.78- 0.91
26-30	126	125 (99.2)[3]	0.97- 1	126	116 (92.1)[3]	0.87- 0.96
Total	**376**	**357 (94.94)[4]**		**376**	**323 (85.9)[4]**	

[1]: p < 0. 01; [2]: p =0. 02; [3]: p <0. 01; [4]: p<0.001; CI: confidence interval.

Table III: Prevalence of anti-hepatitis A virus (HAV) antibodies in blood donors by origin in 2000 and 2007.

	Anti-HAV					
	2000			2007		
Origin	Number tested	Number Positive (%)	95% CI*	Number tested	Number Positive (%)	95% CI*
Group 1	126	112 (88.9)[1]	0.83 -0.94	126	79 (62.7) [1]	0.54-0.71
Group 2	126	124 (98.4)	0.96 -1	126	121 (96)	0.92-0.99
Group 3	124	121 (97.6)	0.94-0.97	124	123 (99.2)	0,97-1
Total	**376**	**357(94.9)**		**376**	**323 (85.9)**	

[1]: p <0.001; CI: confidence interval; Group 1: city of Sfax; Group 2: different rural delegations of Sfax; Group 3: different governorates of South Tunisia.

Table IV: Odds ratios for the association between age or origin and anti-HAV seropositivity.

	Anti-HAV			
	2000		2007	
Variables	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Age (years)				
18-20	1		1	
21-25	1.5 (0.7-3.2)	0.32	1.4 (0.8-2.6)	0.27
26-30	3.6 (1.5-8.7)	0.004	4.4 (2-9.3)	<0.001
Age (years)				
Group 1	1		1	
Group 2	15.6 (5.8-41.4)	<0.001	13 (5.7-29.7)	<0.001
Group 3	79 (10.6-587.1)	<0.001	22.7 (8-64.5)	<0.001

[1]: referent; CI: confidence interval; Group 1: city of Sfax; Group 2: different rural delegations of Sfax; Group 3: different governorates of South Tunisia.

DISCUSSION

Hepatitis A occurs worldwide and is endemic in several regions. The prevalence of the disease varies widely, as a result of basic sanitary conditions [12]. In Tunisia, although hepatitis A infection is still frequent, mainly in children, few studies of seroprevalence were conducted.

The present study conducted in the Tunisian south have shown that the overall prevalence of antibodies to HAV decreased significantly from 94.3% to 85.6% during the years 2000 and 2007. These results are similar to those of other recent studies carried out in Tunisia; in Sousse (Tunisian center), HAV seroprevalence rate in 2002 was 83% in school children aged over 15 years [9]. In a study published in 2008, the seroprevalence was 91.7% in young adults aged between 16 to 25 years selected from different regions of Tunisia [10].

However, HAV seroprevalence rates in Tunisia are still higher than those reported in European countries (Spain, Italy, United Kingdom) where they do not exceed 25% in adolescents [13, 14, 15].

Moreover, the shift in epidemiological pattern in the current study was observed only in Sfax city, where HAV seroprevalence declined from 88.9% to 62.7% between 2000 and 2007 while it was approximatively the same in the rural delegations of Sfax (98.4% and 96% respectively) and the governorates of South Tunisia (97.6% and 99.2% respectively). The same findings were previously reported by Letaief et al in 2005 who found, regardless of age, only 39.7% of HAV seroprevalence rate in the city of Sousse as compared to 89.9% in the rural areas of Sousse [9]. Similarly, in 2008, Rezig et al have shown a lower anti HAV prevalence in coastal regions (87%) as compared to the inside regions of Tunisia (98.9%) [10]. Those results indicate a changing pattern of HAV epidemiology in Tunisia probably due to socioeconomic and hygienic improvements, although hepatitis A is still considered as an endemic disease. Indeed, the prevalence of HAV infection is very closely related to the socioeconomic status of the population and may vary within the same country according to changes in hygienic conditions [16, 17]. Sfax is an industrialised town and counts as the second most important town of Tunisia. Thus, factors such as source of drinking water, waste water sewage, number of people living in the same housing, type of housing, educational level of parents and crowding [18, 19], vary widely between Sfax city and the rest of South Tunisia. Studies conducted in Chile [20] or in France [6], confirmed the influence of these factors in HAV seroprevalence. Due to this gradual shift in Sfax city, there is a decrease in HAV circulation, and consequently the immunity to HAV infection falls rapidly, resulting in a growing pool of susceptible people. As a consequence, a rapid spread of hepatitis A among susceptibles may happen. The transition from high to intermediate or even low endemicity in HAV prevalence as in China has previously led to explosive outbreaks due to viral cross-contamination from endemic to non-immune sectors of the population via food or water. An epidemic of hepatitis A in 1988 in Shanghai linked to the ingestion of raw clams had an overall attack rate of 4083/100.000 inhabitants (292.301 cases) [16].

Finally, regardless of the origin of blood donors, this study shows an increase of seroprevalence with age

particularly in Sfax city in 2000 and 2007. In 2000, approximatively 15% of young people of urban areas and higher in 2007 (53%), reach adolescence and adulthood without natural immunity to HAV; thus, primary infection with HAV in some regions of Tunisia is progressively shifting to older ages. In relation to this low prevalence of anti-HAV among young adults, one may fear an increase of overt hepatitis cases which are known to be more frequent in adults than in infants. Moreover, any break in hygiene may expose the population to an epidemic. Only immunization with the anti-HAV vaccine could efficiently prevent these individual and community-related risks. The use of effective hepatitis A vaccination as a public health tool has become more and more essential as increasing numbers of individuals reaching adolescence and adulthood without natural immunity to the disease and are therefore at risk of hepatitis A morbidity and mortality [21, 22]. Vaccines against hepatitis A have been shown to provide long-term protection against the disease [23, 24]. The WHO position paper on hepatitis A vaccines states that in countries of intermediate endemicity, large-scale childhood vaccination should be considered as a supplement to health education and improved sanitation [22]. Such immunization programs against hepatitis A have already been implemented in several countries including Spain and Italy, the effectiveness and cost-benefit analysis of hepatitis A vaccination programs proved to be both medically and economically justifiable in these countries [25, 26, 27, 28].

The present work contributes to a better knowledge of epidemiology of HAV infection in Tunisia and especially in South Tunisia. Our results showed that the seroprevalence of hepatitis A decreased globally from 2000 to 2007 and a shift in the epidemiological pattern was observed only in Sfax city, the other areas of South Tunisia still of high endemicity. Improvement of hygiene and socioeconomic conditions has undoubtedly contributed to this epidemiologic shift. Thus, the number of subjects which are not immunized against HAV is increasing, notably adult people, prone to develop symptomatic and potentially severe hepatitis A. This situation can also lead to potentially massive outbreaks involving adults. Currently, Tunisia does not have a national immunization policy against hepatitis A. The shift in epidemiologic pattern observed in some regions of Tunisia and the availability of safe and effective hepatitis A vaccines stress the need to consider appropriate vaccination strategies to reduce the risk of hepatitis A morbidity and mortality in susceptible children and adolescents.

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