

SEROPREVALENCE OF VIRAL HEPATITIS B IN THE PROVINCE OF TIZNIT, SOUTHERN REGION OF MOROCCO

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ABSTRACT

Introduction: Hepatitis B virus (HBV) infection is a major public health problem worldwide. The World Health Organization (WHO) reveals that an estimated 257 million people worldwide live with chronic hepatitis B virus (HBV) infection.

Objective: Morocco is so far considered, according to the WHO, to have an intermediate prevalence of viral hepatitis B. Currently, few studies report on the epidemiology of HBV in Morocco. The objective of this study is the assessment of the prevalence of HBV infection and risk factors in the southern region of Morocco precisely the province of Tiznit.

Methods: This is a retrospective study conducted over a period of 5 years, from January 2014 to December 2018. Including 10,823 blood donors at the Tiznit Blood Bank. The search for the HBs antigen (HBs Ag) was carried out by ELISA technique "Murex HBs Ag version 3", by the BEP 2000 Advance plc.

Results: The seroprevalence of hepatitis B in the study population is 0.20%, with a male predominance and a sex ratio of 4.5. The study of risk factors for hepatitis B in infected people found that informal dental care (31%), risky sex (23%) are the main risk factors for HBV transmission.

Conclusion: According to this study, the seroprevalence of HBV in the province of Tiznit places Morocco as a country of low prevalence of viral hepatitis B. This figure is consistent with data reported to other regions of the country including Agadir Marrakech and Rabat. WHO should consider Morocco as a country of low endemicity to HBV.

Keywords: Seroprevalence, Hepatitis B virus, Blood donors, Tiznit

INTRODUCTION

Viral hepatitis B (HBV) is a major public health problem worldwide. The World Health Organization (WHO) estimates the global prevalence of HBV infection in the general population at 3.5% and about 257

million people living with chronic HBV infection. It causes nearly 887,000 deaths per year and puts people at risk of serious chronic liver disease [1,2].

HBV is a hundred times more contagious than HIV, and can remain stable at 25°C for

seven days in dried blood. It is transmitted by skin intrusion, by contact of mucous

membranes with contaminated blood or other body fluids or from mother to child in perinatal care. Morocco is a country considered, according to WHO data, to have an intermediate or moderate endemic prevalence of hepatitis B.

Currently, the epidemiology of HBV is not precisely known in Morocco. Thus, the main objective of this study is to assess the seroprevalence of HBV infection in the province of Tiznit in a population of blood donors.

MATERIALS AND METHODS

The study involved 10,823 voluntary blood donors at the Tiznit Blood Bank, they were routinely screened for HBV infection, based on inclusion and exclusion criteria. This research involves:

- The application of the basic rules including the ethical principles of donation and the rules of limits of age of donors, volume and frequency of donations.
- A medical interview and clinical examination in order to select the eligible donor and deem suitable for the donation (inclusion and exclusion list)
- Identification of risk factors for HBV transmission
- The assessment of risk factors and transmission factors for viral hepatitis B in Tiznit is carried out using the risk factor questionnaire usually described for hepatitis B.

1-Sampling

The sample is taken with serum samples or plasma samples taken from an EDTA tube, usually contains an anticoagulant:

- Un tube pour sérologies (VHB, VHC, TPHA, VDRL, HIV).

- A tube for immunological study (grouping + phenotyping).

2-Screening

Surface antigen (HBsAg) is the most commonly used serological marker for HBV screening. In the present study, the search for HBs Ag is carried out by the 3rd generation ELISA (Enzyme Linked Immuno Sorbent Assay) type ELISA test, manually. The ELISA technique is carried out using the "Murex HBs Ag version 3" Kit by the BEP 2000 Advance PLC from SIEMENS DAD BEHRING.

3-Principles of the technique

The ELISA technique used is done by the Kit "Murex HBs Ag version 3". In this test, the sample is pre-incubated in cups coated with a mixture of monoclonal antibodies from mice specific to different epitopes of the HBsAg determinant "a". Goat antibodies purified by affinity chromatography directed against HBsAg conjugated to horseradish peroxidase are then added to the sample contained in the cup. During the two incubation stages, any HBsAg present in the sample forms an antibody-antibody-enzyme complex in the cup.

If there is no HBsAg, the conjugate will not be bound. A solution containing the substrate TMB (3,3', 5,5'-tetramethylbenzidine) and hydrogen peroxide is added to the cups. Cups that contain HBsAg, and therefore the bound conjugate, will develop a purple color that turns orange when the enzymatic reaction is stopped by sulfuric acid.

4-Confirmation technique

In the event that the ELISA 1 test is positive, the blood bag is destroyed, an ELISA 2 test is performed. ELISA 2 "Murex HBs Ag version 3" made for both the donor sample and the corresponding pouch pudding.

If the EISA 2 test is positive for the sample and the pudding; the result is therefore positive, the blood donor is referred to a specialist (Gastroenterologist, Infectiologist).

RESULTS

1-Breakdown by year

Our study consists of 10,823 voluntary blood donors present at the CHP Hassan 1 Tiznit Blood Bank over a period of 5 years; From January 2014 to December 2018. This population is distributed according to the following graph:

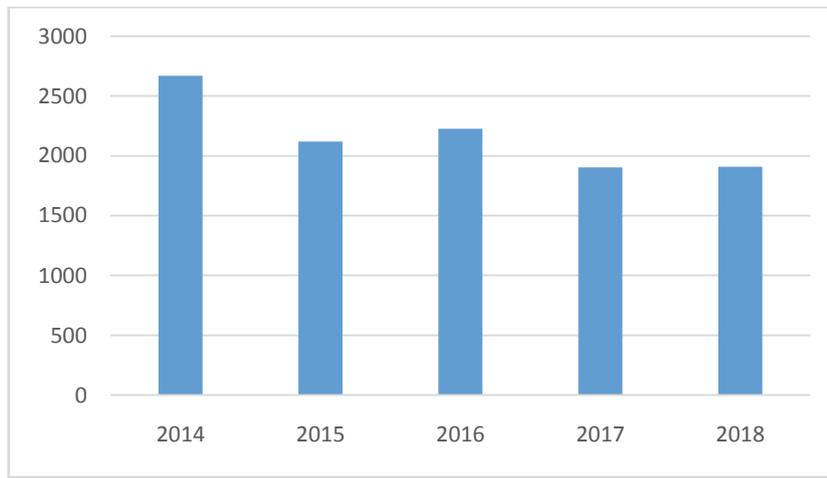


Figure 1: Distribution of Blood Donors

2-Gender distribution

Of the 10,823 donors, n=8225 were male (76%) versus n=2598 female (24%). The sex ratio (M/F) was 3.16 in favour of men.

3-Age Distribution

The average age of donors was 37.8 years with extremes of 18 and 65 years, the age range between 30 and 39 years is the most

represented with 25% of the population studied during the period 2014-2018.

4- Characteristics of the HBV positive population

4.1-General seroprevalence

Of the 10,823 samples analyzed, 22 donations were positive for HBV, for an overall HBV seroprevalence of 0.20%.

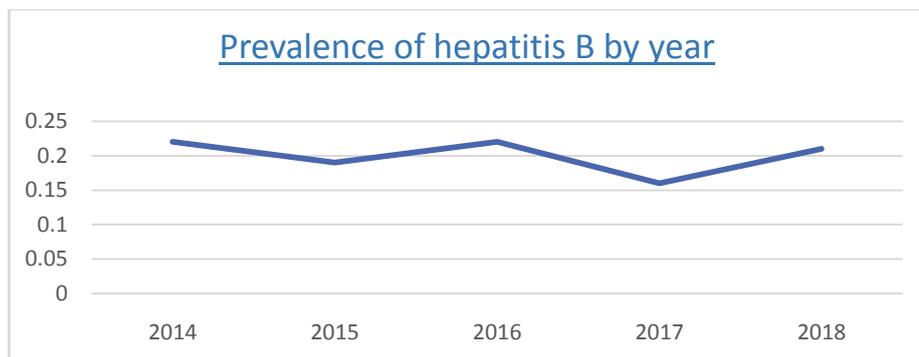
Table I: Number of HBsAg positive cases

Serology	VHB positive	HBV negative	Total
Actual	22	10801	10823
percentage	0,20%	99,80%	100%

The assessment of HBV seroprevalence by year showed the following results.

Table II: Seroprevalence by year of HBV

Year	Number of donors	Number of positive cases	Seroprevalence %
2014	2669	6	0,22%
2015	2118	4	0,19%
2016	2225	5	0,22%
2017	1903	3	0,16%
2018	1908	4	0,21%

**Figure 2: Prevalence of hepatitis B by year****4.2-Seroprevalence by sex**

The prevalence of HBsAg was significantly higher in male $n=18$ (0.22%) than in female $n=4$ (0.15%).

Table III: General Seroprevalence of HBsAg by sex

sex	Don name	Number of positive cases	prevalence
H	8225	18	0,22%
F	2598	4	0,15%
Total	10823	22	0,20%

4.3- Seroprevalence by age

To analyze the seroprevalence of HBsAg as a function of age, the data showed that the predominant age group for both sexes is that of 30 to 39 years. No HBV infection was detected in subjects under 20 years of age.

4.4- Seroprevalence of co-infections (HIV, HCV, SYPHILIS) with HBV

Co-infection was very low $n=1$ (0.009%), only one case was reported, it is an HBV-HIV case.

Table IV: Seroprevalence of co-infections.

Co-infection	Number of cases	percentage
VHB-HIV	1	0.009%
VHB-TPHA/VDRL	0	0%
VHB-VHC	0	0%
VHB-HIV-TPHA/VDRL	0	0%

5. Risk factors for viral hepatitis B

Blood donors with viral hepatitis B serology were contacted by telephone for an interview. Of the 22 people with HBs Ag positive, only 13 people were contacted and questioned. The risk factors for HBV infection assessed in our group are summarized in the following table:

Table V: Distribution of Risk Factors for HBV Infection

Risk factor	Number	Percentage
Risky sexual behaviours	3	23%
Informal dental care	4	31%
Hijama, Piercing, Tattoos, Acupuncture	2	15%
Close contact with HBV carriers within the family or community	1	8%
Multi-purpose glass syringes	0	0%
Healthcare Professional	0	0%
Blood transfusion	0	0%
Hémodialyse	0	0%
Risk factor not identified	3	23%
Perinatal transmission from mothers with HBV	0	0%

DISCUSSION

1-seroprevalence of national hepatitis b

Few studies have been conducted to estimate the seroprevalence of HBV in the general population in Morocco. However, several studies have been carried out among blood donors, particularly in Agadir, Rabat and Casablanca, with prevalences estimated at 0.75%, 1.34% and 0.63% respectively [3, 4, 5].

The socio-demographic characteristics of the population covered by our study, which also concerns a sample of blood donors whose hepatitis B virus screening by HBs antigen was systematically carried out. At the Tiznit Blood Bank, we collected 8225 male (76%) and 2598 female (24%) donors, a sex ratio of 3.16.

Over a period of 5 years we tested 10,823 blood donors, 22 were carriers of the HBs antigen, a seroprevalence of 0.20%. However a higher prevalence of HBsAg was found in men 0.22% versus 0.15% in women with a sex ratio of 4.5.

No HBV infection was detected in subjects less than 20 years of age. This can be explained by the fact that this age group includes in its majority young people who have benefited from systematic vaccination from childhood according to the WHO program (Introduction of the hepatitis B vaccine to the national immunization program in 1999) according to the Ministry of Health of Morocco.

The hepatitis B vaccination coverage rate in Morocco exceeds the global target of 90%, and it is currently 98% [6]. The seroprevalence in this study is 0.20%, which is in line with the figures found in other cities of the kingdom including Rabat (0.4%), Marrakech (0.55%) and Agadir (0.75%) [7, 8.3], while it is 8 times lower

than that found in Ouarzazate [9]. These results place Morocco among the countries with low endemicity.

Internationally, HBV Seroprevalence among blood donors in the Maghreb and sub-Saharan African countries are higher than in our study, with a rate of 2.4% in Libya [10], 1.46% in Tunisia[11], 11.8% in Mauritania [12] 12.6% in Cameroon and 3.9% in Ethiopia [10], stressing the importance of efforts with regard to raising awareness and informing the Moroccan population.

2-Seroprevalence of Hepatitis b Co-infection

In our study, only one case of co-infections was described, it is an HBV-HIV case. These results are consistent with national rates (0.0039%) [7] and are much lower than those obtained in sub-Saharan African countries [13,14]. In Cameroon, the rate of co-infections among blood donors is 1.4% with a rate of 0.6% for HIV – HBV, 0.6% for HBV-HCV and 0.1% for HIV-HBV-HCV [13].

3-seroprevalence of international hepatitis b

The result of our study is in agreement with the data reported in Spain (0.7%), China (0.51%), Algeria (0.25%) and Mexico (0.11%) [15, 16, 17.18], significantly higher than that observed in blood donors in France whose seroprevalence is 0.00066%, and in the United States of America represents 0.0078% [19, 20].

4-Risk factors for viral hepatitis B in Tiznit province

In our study, a questionnaire was developed to assess risk factors and modes of transmission of hepatitis B in Tiznit province. Of the 22 HIV-positive donors,

only 13 people could be contacted. Informal dental care was the most implicated risk factor in the transmission of HBV infection n=4 person (31%) in our study followed by risky sex n=3 (23%). In the absence of sterilization of the instrument holders rotating between each patient during dental care. This is explained by the low level of hygiene and sterilization in dental care settings and also sex education in this region of Morocco.

The prevention of hepatitis B must be the subject of improvement actions requiring a national strategy, a multidisciplinary and multi-professional mobilization to organize the care channels.

CONCLUSION

Morocco is located among the countries with medium endemicity for viral hepatitis B according to the WHO, which estimates the prevalence of HVB between 2 and 2.5% in the general population. However, our study, conducted from 2014 to 2018, shows that HBV seroprevalence in 10,823 of blood donors, at the level of the province of Tiznit is 0.20%. This should classify Morocco as a country with low endemicity to HBV.

This result is of great importance in measuring the effectiveness of prevention strategies. Hence the need to strengthen information, education and communication programmes on HBV and all sexually transmitted infections. The present study also looked at the assessment of risk factors for hepatitis B in people who tested positive for HBsAg. The use of the structured questionnaire indicates that informal dental care and sexual intercourse are the main risk factors for HBV transmission.

The prevention of hepatitis B must be the subject of action plans through a national strategy, a multidisciplinary and multi-professional mobilization to organize the care channels.

Abbreviation

CHP :Provincial Hospital Centre
 HBV:Virus Hepatitis B
 HIV :Human Immunodeficiency Virus
 TPHA :Treponema Pallidum Hemagglutinations Test
 VDRL:Venereal Disease Research Laboratory
 HCV :Hepatitis C Virus
 WHO :The World Health Organization
 ELISA:Enzyme Linked Immuno Sorbent Assay
 EDTA :Ethylenediamine TetraaceticAcid

REFERENCES

- [1] World Health Organization, Geneva, 2017. Access on www.who.int/wer/
- [2] World Health Organization, Geneva, 2017. Global Hepatitis Report 2017. Disponible sur <http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1>.
- [3] Saghir.k et al. The Seroprevalence of viral hepatitis B in the Agadir region 2017.
- [4] B.Adouani et al. Hepatitis B in the blood donor population in Morocco: a comparison of the prevalence of HBsAg among different categories of donors, a CRTS in Rabat, Morocco, 2011.
- [5] Baha W, Foulous A, Dersi N, et al Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco. BMC Public Health. 2013;14:50.
- [6] Ministry of Health. <https://www.sante.gov.ma/Pages/Communi-ques>
- [7] Uwingabiye J; ZAHID H; Unyendje L; Hadeef R. Seroprevalence of viral markers on blood donations at the Blood Transfusion Center, Mohamed V Military Training Hospital in Rabat. Pan Afr Med J. 2016; 25: 185.
- [8] Baadi.F; Zouhair.S.; Moutaj . M and Al. Seroprevalence of viral hepatitis B in the Marrakech region.2016

- [9] Mouflih H and Al. Seroprevalence of viral hepatitis B in the Ouarzazate region 2017.
- [10] Khmmaj Abdulatif, Habas Elmukthar, Azabi Massoud. Frequency of hepatitis BC and HIV viruses among blood donors in Libya. *Libyan J Med.* 2010;5:5333.
- [11] Ben Jemia R, Gouider E. Seroprevalency of transfusion-transmitted infections in first-time volunteer and replacement donors in Tunisia. *Transfusion Clinique et Biologique* (2014).
- [12] Boushab, B.M., Et Al. Estimation of seroprevalence of HIV, hepatitis B and C virus and syphilis among blood donors in the hospital of Aïoun, Mauritania. *Pan African Medical Journal*, 28 (2017).
- [13] F. Ankouane, D. Noah Noah, M.M. et al. Seroprevalence of hepatitis B and C viruses, HIV-1/2 and syphilis at blood donors at the central hospital of Yaoundé, central region, Cameroon. *Clinical and Biological Transfusion* Volume 23, Issue 2, May 2016.
- [14] Hundie, G.B., Raj, V. S., Gebremichael, D., & Haagmans, B. L. Seroepidemiology of hepatitis B and C virus infections among blood donors in Ethiopia. *Journal of Medical Virology*, 89(7), 1300–1303. (2017).
- [15] José Antonio Muñoz-Gámez and Javier Salmerón, Prevalence of hepatitis B and C in Spain: more data needed, Digestive System Unit. San Cecilio University Hospital. CIBERehd. Granada 2013.
- [16] Yang, S., Jiao, D., Liu, C., Lv, M., Li, S., Chen, Seroprevalence of human immunodeficiency virus, hepatitis B and C viruses, and *Treponema pallidum* infections among blood donors at Shiyan, Central China. *BMC Infectious Diseases*, 16(1) 2016.
- [17] Chekroun Wassila Amira. Seroprevalence of hepatitis B virus in blood donors at the Tlemcen Blood Transfusion Centre. 2016. 42-49.
- [18] Lopez- Balderas, N., Bravo, E., Camara, M., & Hernandez- Romano, P. Seroprevalence of hepatitis viruses and risk factors in blood donors of Veracruz, Mexico. *The Journal of Infection in Developing Countries*, 9(03), 274. (2015).
- [19] Public Health France. Epidemiological surveillance of blood donors: HIV, HCV, HBV, HTLV, syphilis. Available from: (<http://invs.santepubliquefrance.fr/Dossiers-thematiques/InfectiousDiseases/HIV-AIDS-STIs/BloodDonors/Epidemiological-Surveillance-of-Blood-Donors-HIV-HCV-HBV-HTLV-Syphilis>). Accessed May 2018.
- [20] Zou S, Stramer SL, Dodd R Y. Donor Testing and Risk: Current Prevalence, Incidence, and Residual Risk of Transfusion- Transmissible Agents in US Allogeneic Donations. *Transfus Med Rev.* 2012; 26(2): 119–28.