Scholars Journal of Applied Medical Sciences

Abbreviated Key Title: Sch J App Med Sci ISSN 2347-954X (Print) | ISSN 2320-6691 (Online) Journal homepage: <u>https://saspublishers.com</u>

Biology & Anatomopathology

Serological Analysis of HBsAg and Anti-HCV Antibody in Patients Received at the Mali Hospital Laboratory

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DOI: 10.36347/sjams.2023.v11i04.006

| Received: 26.02.2023 | Accepted: 30.03.2023 | Published: 09.04.2023

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Abstract

Original Research Article

Introduction: Viral hepatitis is a public health threat that has long been underestimated. This infectious disease is the seventh cause of death in the world and even ahead of HIV in terms of epidemiological prevalence. Objective: highlight the seroprevalence of hepatitis B and C viral infections in patients seen in the medical analysis and pathology laboratory department of the Mali hospital. *Method*: This is a prospective and retrospective study that took place from July 2021 to August 2022. This study included 1540 samples from eligible patients with HBsAg, Anti HBe IgG Antibody, Anti HBc Antibody, HBe Ag, Antibody Anti HBs and/or anti-HCV antibodies. Results: These different methodologies allowed us to have the results of 1540 samples concerning this study. The female sex was more represented with 62.92% compared to the male sex or 37.08%. The HBSAg was mainly found positive in the male sex with 5.45%. The average age was 38 ± 17 years with extremes ranging from 2 to 92 years. The age group 21-39 years was the most represented with 47.47% and the most affected by the positivity of the HBSAg with 5.65%. 64.03% of our patients were married and also with increasing positivity for HBS Ag and anti-HCV Ac with 5.91% and 0.91% respectively. The seroprevalence of HBV, HCV and HVB/HCV co-infection were 10.06, 0.9% and 0.84% respectively. Conclusion: Hepatitis B remains largely unknown to the general public but also to many medico-social actors. This lack of knowledge represents a major obstacle to prevention and screening. It also has as a corollary the stigmatization of people who are affected, in romantic encounters or at work in particular. A high seroprevalence of HBsAg obtained indicates the spread of hepatitis B in the Malian population.

Keywords: Viral hepatitis, epidemiological prevalence, pathology, HBSAg, seroprevalence.

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INTRODUCTION

Hepatitis B and C are liver infections caused by the hepatitis B and hepatitis C viruses [1, 2]. More than 80% of cases of primary liver cancer in the world are caused by a viral infection, the hepatitis B virus in two thirds of cases, and the hepatitis C virus in the remaining third.

The bibliographic data showed a carriage rate of 4.6% and 8.2% respectively for the prevalence of HbSAg and anti-HCV antibody [3]. With vaccination efforts, the prevalence in children under 5 years of age decreased from 4.7% in 2000 to 1.3% in 2017 [4]. The

incidence of the hepatitis C virus is 23.7 per 100,000 residents worldwide, or 1.75 million, and 31 per 100,000 in Africa, or 1% prevalence [4, 5].

The hepatitis B virus is transmitted between people through contact with an infected person's blood or other body fluids, while the hepatitis virus is transmitted through direct contact with infected blood. Very rarely, it can also be transmitted through other bodily fluids. Many people infected with hepatitis B and/or C rarely show symptoms, although they can still transmit the virus to others [6].

704

Citation: Yaya Goïta *et al.* Serological Analysis of HBsAg and Anti-HCV Antibody in Patients Received at the Mali Hospital Laboratory. Sch J App Med Sci, 2023 Apr 11(4): 704-709.

Diagnosis is essentially immunoenzymatic looking for serum markers of infection. Thus, depending on the profile obtained, one can make the diagnosis of acute, chronic or cured hepatitis [7].

In Mali, 2.6 million people, or 18% of the global population are carriers of the hepatitis B virus, particularly one in two young people between 18 and 25 years old [8, 9]. HCV prevalence rates in Mali have not been widely documented since the early 2000, but screening sessions have revealed rates of between 4-5% [10]. Some authors have reported that the seroprevalence of HCV was 10% and 19.7%, corresponding respectively to the results of the studies by Diakité Y in 2013 [11] and Coulibaly A in 2021 [12].

These data confirm the seriousness of the infection and the need to undertake screening and treatment programs for infected subjects. This is the reason why we decided to undertake this work in order to highlight the seroprevalence of viral infections of hepatitis B and C of patients received at the laboratory service of medical analysis and anatomopathology of the hospital of Mali.

MATERIALS AND METHODS

Ethical aspects

The study was conducted in accordance with the ethical standards set out in the Declaration of Helsinki (1983). Inclusion in the study was carried out according to the rules of the legislation in force at the hospital in Mali and the strict respect of the confidentiality of the identity of the patients and their analysis results.

Type of Study and Participant

This was a prospective and retrospective study that took place in the laborotory Department of Mali Hospital. This study included 1540 patient samples in the laborotory Department of Mali Hospital from Jully 2021 to August 2022. Eligible patients were selected according to the inclusion criteria as follows: all patients with HBSAg, Ac Anti HBe IgG, Ac Anti HBc, Ag Anti HBs antibody and/or anti-HCV antibody admitted to the Medical Laboratory Laboratory Analysis during the study period.

Collection of Samples

All patients were collected from the venous blood collection room of the said laboratory after observing a fast of 8 to 10 hours. Blood is collected in the dry tube and heparin in order to collect plasma after centrifugation. All plasmas obtained were identified and stored between +2 and $+8^{\circ}$ C prior to immunological analysis. All hemolyzed plasmas were rejected according to the analytical precautions of the various immunological markers. We processed and analyzed blood samples on board the MAGLUMI 800 system, which uses the flash chemiluminescence method.

HbsAg and Anti-HCV Analysis Method

AgHbs is an immunoassay by the chemiluminescence method (CLIA) sandwich. The sample labelled with an anti-AgHbs monoclonal antibody, a buffer and magnetic microbeads coated with another anti-AgHbs monoclonal antibody are thoroughly mixed and incubated at 37 ° C to form a sandwich. The HCV test is а sandwich chemiluminescence immunoassay. The sample (or calibrator/control. if applicable), FITC-labelled recombinant HCV antigen and biotinylated HCV recombinant antigen react to form a sandwich complex. After addition of an ABEI- labelled anti-FITC polyclonal antibody and streptavidin magnetic microbeads, the complex binds to the solid phase by interaction of biotin and streptavidin. After precipitation in a magnetic field, the supernatant is settled, 11 (eleven) and then a washing cycle is performed. The light signal is measured by a photomultiplier within 3 seconds as relative light units, which is proportional to the concentration of AgHbs and anti-HCV present in the sample. The same principle applies to the control and calibrator samples of the MAGLUMI system.

The reagents used for the quantification of HbsAg for hepatitis B and Anti-HCV for hepatitis C were HBS Ag (CLIA) Ref: 130210001M and Anti-HCV (CLIA) Ref: 130210006M. The controls used were the internal quality control kits whose references were respectively 130210001M and 130210006M for AgHbs and Anti-HCV. Reagents and Internal Quality Control Kit were all stored at (2-8°C). If the results of the internal quality control did not correspond to the expected standards or to the values established by the laboratory, corrective measures were taken.

Variables Explored in the Study

Socio-Demographic Data (Age, Sex and Marital Status)

The biological parameters measured were: The reference for HBsAg: Not active: <1; Active: >=1; The reference for Ac HCV: Negative: <20; Positive: >=20; The reference for Ac Anti HBe IGG: - Negative: <100; Positive: >=100; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <1; Negative: >1; The reference for Ac Anti HBc: Positive: <100, Positive: >=100.

Univariate Statistical Analysis

The Mann-Whitney-Wilcoxon non-parametric test was used to compare the means of the quantitative variables while the $\chi 2$ tests or Fisher's exact test, depending on the numbers, were used for the qualitative variables. Differences were considered significant if the probability (*P*) of the observed difference under the null hypothesis was $P \le 0.05$.

RESULTS

These different methodologies allowed us to have the results of 1540 samples concerning this study. The results are broken down into the following tables:

	Négative (%)		Total (%)
		71 (4.61)	969* (62.92*)
Male	487 (31.62)	84 (5.45)	571 (37.08)
Total	1385 (89.94)	155 (10.06)	1540 (100.00) 1.69 ***

The female sex was in the majority (62.92%) with a sex ratio of 1.69 in favor of women. HBSAg was mainly found in the male sex with 5.45%.

able II: Distribution of patients according to age group and HBSAg					
Age Range/HBSAg	Négative (%)	Positive (%)	Total (%)		
2 - 20	181 (11.75)	4 (0.26)	185 (12.01)		
21 – 39	644 (41.82)	87 (5.65)	731 (47.47)		
40 - 58	319 (20.71)	50 (3.25)	369 (23.96)		
59 – 77	205 (13.31)	14 (0.91)	219 (14.22)		
78 – 92	36 (2.34)	0.00	36 (2.34)		
Total	1385 (89.94)	155 (10.06)	1540 (100)		

I	able II: Distribution o	f patients accor	ding to age	group	and HBSAg	

The average age was 38 ± 17 years with extremes ranging from 2 to 92 years. The 21-39 age group was the most represented with 47.47% and the

most affected by the positivity of Ag HBS, either 5.65% of cases.

Table III: Distribution of	patients according to	o marital status of HBS A	Ag and anti-HCV antibody

	HVC-	HVB-	HVC+	HVB+	
Marital status	(anti HVC Ac)	(HBSAg)	(anti HVC Ac)	(HBSAg)	Total (%)
	Effective (%)	Effective (%)	Effective (%)	Effective (%)	
Singles	101 (6.56)	403 (26.17)	6 (0.39)	44 (2.86)	554 (35.97)
Marrieds	347 (22.53)	534 (34.68)	14 (0.91)	91 (5.91)	986 (64.03)
Total	448 (29.09)	937 (60.84)	20 (1.30)	135 (8.77)	1 540 (100.00)

The analysis of the data recorded in this table showed that the majority of our patients were married, with 64.03% and also with a growing positivity of Ag HBS and anti-HCV Ac with 5.91% and 0.91%.

Table IV: Distribution of	patients according	g to serological monitoring	g markers and	positive HBS Ag (HVB+)
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Ag HBS/Marqueurs de suivis sérologique	Négatif (%)	Positif (%)	AgHBS + (%)
Ac Anti HBe IGG	11 (0,71)	7 (0,45)	18 (1,17)
Ac Anti HBc	35 (2,27)	8 (0,52)	43 (2,79)
Ac Anti HBs	3 (0,19)	2 (0,13)	5 (0,32)
Ag Hbe	4 (0,26)	2 (0,13)	6 (0,39)
Non appliqués	-	-	83 (5,39)
Total	53 (3,44)	19 (1,23)	155 (10,06)

The most represented antibody was Ac Anti HBc with 2.79%, a positivity of 0.52% and a negativity of 2.27%.

The positivity of these serological monitoring markers were, Anti HBe Antibody IGG (0.71%), Anti HBs Antibody (0.13%), Hbe Ag (0.13%). and the negative were, Anti HBe Antibody IGG (0.45%), Anti HBs Antibody (0.19%), Hbe Ag (0.26%).

NB: (155/1540): A prevalence of HBsAg obtained was 10.06% or 155 positive cases out of all 1540 patients included.

Sanan navalan aa mankana	нvв	HVC -	HVC +
Seroprevalence markers	Effective (%)	Effective (%)	Effective (%)
HBSAg-	1385 (89.94)	336 (21.82)	7 (0.45)
HBSAg +	155 (10.06)	37 (2.40)	13 (0.84)
anti HVC Antibody	468 (30.39)	448 (29.09)	20 (1.30)

Table V: Distribution of patients according to seroprevalence

- The seroprevalence of HBS Ag carriage was 10.06% in patients.
- The seroprevalence of anti-HCV Antibody carriage was 1.30% in patients.
- The seroprevalence of HBV and HCV coinfections was 0.84% in all patients.

DISCUSSION

Our study took place over a period of 12 months from July 2021 to June 2022. We listed 1540 patients for serology examinations of HBSAg for viral hepatitis B, anti-HVC Antibody for hepatitis C and HBSAg/ Anti HVC Antibody for hepatitis B and C coinfection. During this study, among the 1540 patients who dosed HBS Ag, 481 patients were subjected to anti-HCV Antibody. We found in the study population 155 patients were positive for HBSAg, 20 positive for anti-HCV Antibody and 13 patients co-infected with HBSAg/ anti-HCV Antibody.

Seroprevalence

Regardless of the distribution of risk factors, the prevalence of viral hepatitis B and C varies by study population, country, and even region. We observed a seroprevalence of 10.06% of HBV in the patients included in our study. According to the literature, among blood donors, the prevalences of HBV and HCV are respectively 18.0% and 9.2% in Burkina Faso [5] and 13.1% and 1.4% in Mali [13]. Among health care workers in Mali, they are 12.1% and 0.6%, respectively. Laboratory tests that differ in sensitivity and specificity may also contribute to observed differences between studies [14].

Regarding hepatitis C, the seroprevalence observed in this study was 0.9% in patients visiting the Mali Hospital during this period. The low prevalence could be explained by the mode of contamination, the two main ones being intravenous drug addiction and history of transfusion. This latter method has been hampered since 1991 due to systematic screening and viral inactivation measures in the preparation of bloodderived products [15]. HVB/HCV co-infection was 0.84%, this result is slightly higher than that of Kpossou AR et al., 2019 [16], which reports a prevalence of 0.2%, but lower than that of Ballo PL et al., 2017 [17], which finds 3% prevalence. This variation could be explained by the large size of our sample compared to these previous studies. Our data confirm the observations of the WHO, according to which Mali is located in an area of low and high endemicity respectively for HCV and HBV [8].

Socio-epidemiological aspects

The average age of patients carrying the Ag HBS was 38 ± 17 years and that of the carriers of the anti HCV antibody was 41.77 ± 17.86 years. These results are similar to those of Kodjoh N et al., 2015 [18] and Kpossou et al., 2019 [16], whose mean ages of subjects carrying HBSAg were respectively 36.4 ±11.2 years and 39,7±13 years old. The average age of HCV antibody-positive subjects in our study was lower than those reported by Kodjoh N et al., 2015 [18] and Kpossou AR et al., 2019 [16], who found 54.6± 11.3 years and 59.3±14.3 years. The most affected age group in our series was the young adult class 21 to 39 years old that is 47.47% of all cases, this result is similar to but higher than that of Ballo P et al., 2017 [17], who finds in his study a young adult age group but of a different proportion, that is 20.9%. This observation is bitter, because young people, the most active age group in our society, occupying the first rank of infection facilitates the spread of the virus and would present a public health problem in Mali. However, it should be emphasized that if the achievement of the young adult remains a characteristic, it is not exclusive and all ages can be affected.

Thus, in our study we noticed that the positive HBS Ag mainly affected young adult subjects that the positive anti HVC Antibody mainly affected the elderly. This remark is justified by the mode of transmission of HBV, which is mainly perinatal, while HCV is mainly transmitted parenterally [19]. Transmission through the blood and during treatment seems to predominate according to the studies reported in the literature [20] compared to the use of drugs injected intravenously, more described in Western countries [20].

We noted a female predominance in our study with a sex ratio of 1.69, but a high male HBSAg positivity of 5.45% was observed compared to the female sex, with 4.61%. However, this strong female predominance in our study is explained by the high attendance of health structures in terms of care. Consequently, according to the study conducted by Kpossou AR *et al.*, 2019 [16], the male predominance was mainly noted in subjects with positivity for Ag HBS, with 71.2%. The same observation was found by Kodjoh N *et al.*, 2015 [18], which indicate a male positivity of 58.82%. This difference could be related to the choice of sampling according to the inclusion criteria selecting only HBSAg- positive cases in their studies, which is contrary to ours.

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Indeed, the presence of certain risk factors for contamination such as social constraints such as polygamy, consanguineous marriage. At this stage, the male sex is more vulnerable to HBV than the female sex. Our results even lower than previous studies [21, 22], report a male HBV frequency of 76.6% and 64.8% respectively. Even if the male tropism of HBV is a well-known reality, this pathology presents a male face in sub-Saharan Africa. Contrary to our results, the study by Koné O *et al.*, 2015 [23], reports an 85% female predominance positive for HBSAg.

Our results even lower than previous studies [21, 22], report a male HBV frequency of 76.6% and 64.8% respectively. Even if the male tropism of HBV is a well-known reality, this pathology presents a male face in sub- Saharan Africa. Contrary to our results, the study by Koné O *et al.*, 2015 [23], reports a female predominance of 85% positive for HBSAg.

Most of our patients requesting HBsAg analyzes came from outpatient clinics, and were the layer most affected by this infection with 7.40%. This result is contrary to that of Sidibé S *et al.*, 2022 [8], the majority came from the Infectious Diseases department with 62.5%, followed by medicine with 36.5%. This difference is linked to the absence of an infectious disease service in the algorithms of the consultation and hospitalization services of the hospital in Mali.

The "isolated anti-HBc Antibody" serological profile is reflected by the presence of antibodies directed against HBc Ag as the sole serological marker of HBV infection. Anti-HBc Antibody is the first antibody produced during HBV infection and persists throughout life. It is associated with HBs Ag during the acute and chronic phase of hepatitis B virus infection and with anti-HBs Antibody in the event of recovery. It has been shown that anti-HBc Antibody can be present in isolation and in the absence of other serological markers. This "isolated anti-HBc Ac" serological profile is difficult to interpret, especially since it can correspond to several situations of acute or chronic infection or post-infectious immunity. Thus, several explanations could be given for the absence of detection of HBsAg and that of anti-HBs Antibody in the presence of anti-HBc Antibody [27].

Out of 43 patients' sera analyzed, the Anti HBc Ac was positive in 8 HBsAg positive patients, with 0.52% against Berrajah-Fki *et al.*, 2012 [27], found in his study on these 160 serums of patients analyzed, 141 were positive for anti-HBc Ac, with 88.12. This difference may be related to the type of serological markers because his study was exclusively on isolated anti-HBc Antibody.

This study reported in the sera of 5 patients, 0.13% was positive for Anti HBs Antibody. On the

other hand, we observe in the study by Berrajah-Fki *et al.*, 2012 [27], an increase compared to our study with a rate of 43.66% positive for anti-HBs Antibody. During this study on the sera of 18 patients analyzed, the anti-HBe IgG antibody was found positive in 7 patients with 0.45% lower than the result obtained by Danté M *et al.*, 2022 [25], with 68 .7% positive for anti-HBe Antibody.

The analysis of HBe Ag in the sera of 6 patients reported 2 positive cases with 0.13%. In the study by Danté M *et al.*, 2022 [25], out of a total of 115 serum samples, 36 patients were HBeAg positive, with 31.3%. Most of our HBSAg carrier patients did not perform the serological follow-up marker assay, with 5.39%, which is contrary to the result obtained by that of Danté M *et al.*, 2022 [25].

CONCLUSION

Beyond the significant reduction in the prevalence rates of viral hepatitis B and C and primary liver cancer of which they are the main cause, the objective of this study was to highlight the seroprevalence of hepatitis B and C in the Malian population visiting health structures. Hepatitis B remains largely unknown to the general public but also to many medico-social actors. This lack of knowledge represents a major obstacle to prevention and screening. It also has as a corollary the stigmatization of people who are affected, in romantic encounters or at work in particular. A high seroprevalence of HBsAg obtained indicates the spread of hepatitis B in the Malian population.

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6

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