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Major Article

Hepatitis B and asymptomatic malaria coinfection in Sub-Saharan African immigrants: epidemiological and clinical features of HBV infection

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Abstract

Introduction: Here, we conducted an epidemiological study of hepatitis B virus (HBV) mono-infected and asymptomatic malaria/HBV coinfected immigrants and further discussed the possibility of malaria disease modifying the clinical presentation of HBV infection. **Methods:** A total of 195 African immigrants were examined for HBV infection or coinfection with HBV and asymptomatic malaria. HBV infection was diagnosed using serological tests and confirmed by PCR; furthermore, we performed a pan-*Plasmodium*-specific-nucleic-acid-sequence-based-amplification (NASBA) assay to detect asymptomatic malaria infection. The stage/grade of the liver disease was determined using echotomography and elastometry. **Results:** PCR-NASBA results confirmed that 62 of 195 subjects (31.8%) were positive for *Plasmodium* infection, whereas 41 of 195 subjects (21%) tested positive for HBV chronic hepatitis (HBV-DNA positive). Among the HBV-positive subjects, 26 (63.4%) of them were mono-infected patients (Group A), whereas 15 (36.6%) patients had HBV chronic hepatitis and asymptomatic malaria coinfections (Group B). The HBV-DNA median levels were 1.4×10^5IU/mL in HBV-mono-infected patients and 2.0×10^5IU/mL in coinfected patients. Echotomography and hepatic elastometry presented similar findings for both groups of patients. **Conclusions:** Coinfected patients seem to present with the same clinical symptoms of the liver disease as HBV mono-infected patients.

Keywords: HBV-asymptomatic malaria coinfection. Sub-Saharan African immigrants. HBV clinical features.

INTRODUCTION

Malaria is one of the most widespread infectious diseases in the world. According to the current World Health Organization (WHO) estimates, over 3.2 billion people (41% of world's population) remain exposed to this disease. In 2015, there were 214 million new cases of malaria and 438,000 deaths worldwide, and most likely, *Plasmodium falciparum* was the causative agent[1]. In contrast, following exposure to malaria infection, people living in disease-endemic countries tend to have milder symptoms when infected (semi-immunity) with sub-microscopic levels of parasitemia. These individuals may not feel ill at all or present the typical features of malaria (asymptomatic disease)[2]; this clinical feature was also observed in African immigrants arriving in European countries[3,4].

Most of the malaria cases are found in Sub-Saharan Africa [(SSA): 88%)], followed by South-East Asia and Central and South America[1]. In these countries, other infectious diseases are present as endemic infections, including hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections. Consequently, there are several geographical areas, mainly in SSA, where the endemicity of malaria and HBV overlap despite having different transmission routes[5-7]. In tropical areas, HBV is highly contagious from one infected individual to another and sometimes can present the same route of transmission of Plasmodium (by blood-to-blood contact, sharing needles, and blood transfusions). Therefore, it is not rare that the parasitic and viral infections may be present in the same individual, influencing each other from a clinical perspective.

Previous studies of malaria/HBV coinfection are scant with a focus primarily on the epidemiological aspects, while the mutual interactions between the two pathogens, HBV and malaria (symptomatic or asymptomatic disease), remain poorly understood. Lately, it has been suggested that since these two infections share some of their developmental stages within the liver, the co-occurrence of infections possibly influences the progression of HBV infection and is associated with the natural history of both diseases[8,9].

In the last twenty years, there has been a migratory flow of the human populations from developing to industrialized countries (USA and West Europe), and this is accompanied by

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increased spread of some infectious diseases [HIV, tuberculosis (TB), viral hepatitis, and asymptomatic malaria] as well their coinfections.

Here we report a study on the epidemiology of asymptomatic malaria/HBV coinfection in a cohort of SSA immigrants. Comparing the coinfected with HBV mono-infected individuals, we evaluated whether and how malaria infection may affect the clinical presentation of HBV infection.

METHODS

Patients

Blood samples were collected from 195 asymptomatic immigrants who were temporary guests in a refugee camp managed by the Italian Red Cross and located in Borgo Mezzanone (Foggia, Italy). Later, the collected samples were screened for infectious diseases. First, we evaluated the prevalence of *Plasmodium* species in this population of immigrants[10], followed by investigating for the stage of HBV-infection in HBV mono-infected, hepatitis B surface antigen (HBsAg) and/or hepatitis B virus- deoxyribonucleic acid (HBV-DNA) positive (Group A) or HBV/asymptomatic malaria coinfection (Group B). No subject with hepatitis B virus/hepatitis C virus/hepatitis delta virus/ human immunodeficiency virus (HBV/HCV/HDV/HIV) viral coinfection was recruited in our study. All patients were screened for HBV markers, and none reported vaccination against HBV infection. Additionally, the prevalence of a possible asymptomatic infection with *Plasmodium* species was assessed in the patients. All guests in the camp were verbally informed about the purpose of the study and invited to participate. Subsequent recruitment was on a voluntary basis with no special sampling criteria. The study was reviewed and approved by the local Chief of the Red Cross, and a written informed consent, translated in four languages (English, French, Arabic, and Italian), was obtained from each enrolled subject. All study procedures were conducted in accordance with the Helsinki Declaration (1964, amended in 2008). At enrollment, all study participants were interviewed using a questionnaire to collect the baseline demographic, clinical, and socioeconomic information, and assess their previous exposure to malaria and/or HBV. All enrolled subjects also received a full clinical examination and, if necessary, were treated according to the stage of their liver disease.

The mean age of the subjects was 25.1±5.7 years (range: 16-40 years), and most of them (162, 83.1%) were men including few teenagers and older adults. It was difficult to recruit potential female candidates because they refused to undergo clinical examination. Ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood samples were labeled and temporarily stored for a maximum of 24 hours in a freezer bag before transporting them to the laboratory, where the samples were stored at -80°C and later processed for isolating nucleic acids and determining HBV genotype.

Using nucleic acid sequence-based amplification (NASBA) Pan-*Plasmodium*-specific NASBA-targeting-18S-*rRNA* (GenBank accession N° M19172.1), the prevalence of a possible asymptomatic infection with *Plasmodium* species was assessed on an IQ5 Real-Time analyzer.

HBV markers (HBsAg/HBsAb) were measured by commercial immunoassay methods (Abbott-AuszymeMc, Abbott Laboratories, North Chicago, IL, USA). Hepatitis HBeAg and its antibody were detected by radioimmunoassays (HBeAg/antiHBe immune-radiometric DiaSorin, Vercelli, Italy). A commercially available kit was used to detect HBV-DNA (Qiamp Viral RNA, Qiagen, Chatsworth, CA). For the determination of HBV genotypes, HBV-DNA was extracted, and genotypes were determined by the INNO-LiPA HBV-Genotyping assay (Innogenetics NV, Gent, Belgium)[11]. The correct HBV genotype was established by consulting an interpretation chart showing probe reactivity patterns for each genotype. Serum alanine-amino-transferase (ALT) level was quantified by ultraviolet-enzymatic assay (normal range 0-40IU/L).

All patients underwent both abdomen echo-tomography (Esaote, Genova, Italia) and elastometry (Echosens, Fibroscan 502), and their hepatic biochemical, epidemiological, and virological parameters were recorded. All patients with or without biochemical and/or virological activity for HBV infection refused to undergo hepatic biopsy. For the evaluation and classification of severity of liver disease [inactive HBV carriers, mild or moderate/severe chronic hepatitis, cirrhosis or hepatocarcinoma (HCC)], the following parameters were considered: serological, virological, abdominal echography, and hepatic-elastometry. The disease degree was determined through the analysis of additional parameters. For the diagnosis of HCC, an abdominal echography was performed on the patient.

Statistical analysis

Results are expressed as the mean and standard deviation (SD). The chi-square test was used to compare categorical variables (sex, positivity for HBV markers, coinfection with HBV/Malaria, HBV genotypes, and stages of liver disease). Continuous variables (age and ALT levels) were compared by Student's t-test for independent samples. The level of significance was set at a p-value <0.05. Data were analyzed by Stata 10MP software for Mac OS X.

RESULTS

All enrolled participants came from SSA countries (54.3% from East Africa, 35.9% from West Africa, and 10.8% from Central Africa) (**Table 1**), and they were in Italy for a mean period of 54 days (range: 19-121 days). Of 195 subjects, 41 (21%) presented markers of HBV chronic hepatitis (HBV-DNA positive). Among HBV-positive patients, 26 (63.4%) were mono-infected patients (Group A), whereas 15 (36.6%) patients (Group B) had HBV chronic hepatitis and asymptomatic malaria coinfection (diagnosed by PCR-NASBA). Among the individuals affected by asymptomatic malaria infection, polymerase chain reaction (PCR) tests detected the presence of gametocytes of *P. falciparum* in 24/62 (38.7%) samples. Microscopic examination confirmed the positive NASBA results in 14 cases. *P. falciparum* was observed in 13 samples, and one sample was positive for *P. ovale* (**Table 1**).

Clinical features of HBV mono-infected (26) and HBV/malaria coinfected (15) chronic hepatitis patients are presented in **Table 2**. Groups A and B had 19/26 (73.1%) and 11/15

(73.3%) anti-HBe positive patients, respectively; all other subjects presented HBV-wild (HBeAg+) infection, highlighting the homogeneity of the two patient groups (p>0.05).

HBV virological findings

HBV-DNA viremia was detected in both patient groups without a significant difference (p=0.05). Overall, the median levels of HBV-DNA viral load were 1.4×10^5UI/mL in all patients with HBV-infection alone and 2.0×10^5 in coinfected patients; however, there was no significant difference between the two groups (p=0.05). No anti-HBc positive individuals in either group showed the presence of detectable HBV-DNA (occult hepatitis).

For 23 HBV-DNA positive patients, genotype distribution was not significantly different between the groups (p=0.03); patients were classified into genotypes A, D, and E. Among genotypes A and D, there were no significant differences (p=0.03); on the contrary, a significant difference existed between these genotypes and genotype E (p<0.01).

Serological and virological tests for liver diseases

Of 26 patients in Group A, 12 (46.2%) presented normal ALT levels and undetectable serum HBV-DNA (<20IU/mL), 3 (11.5%) had persistently normal ALT levels and HBV-DNA detectable by Real-Time PCR (mean level 353.635IU/mL, range

TABLE 1: Baseline characteristics of the patients [N=195 (including 162 males 83.1%)].

	Not infected N (%)	Asymptomatic malaria N (%)	HBV infected N=119/195 (61.0%)	mono-infected N (%)	HBV malaria coinfected N (%)
Malaria infection	133/195 (68.2)				
P. species		62 (31.8)			
P. falciparum		24/62 (38.7)			
HBV infection					46/62 (74.2)
not infected	76/195 (39.0)				
previous HBV			78/195 (40.0)	47/119 (39.5)	31/119 (26.0)
current HBV			41/195 (21.0)	26/119 (21.8)	15/119 (12.6)

N: number of patients**; HBV:** hepatitis B virus; ***P.***: *Plasmdoium.*

TABLE 2: Clinical features of HBV mono-infected and HBV/malaria coinfected chronic hepatitis patients.

	HBV mono-infected N=26 (%)	HBV malaria coinfected N=15 (%)	p-value
HBeAg positive	7/26 (26.9)	4/15 (26.6)	0.05
HBV-DNA detected	14/26 (53.8)	9/15 (60.0)	0.05
HBV-DNA load	1.4×10^5 IU/mL	2.0×10^5 IU/mL	0.05
Genotypes (23 patients)			
E	8/14 (57.1)	5/9 (55.5)	0.05
A	3/14 (21.4)	3/9 (33.3)	0.05
D	1/14 (7.1)	3/9 (33.3)	0.05
Value of ALT			
normal value (<40IU/mL)	12/26 (46.2)	6/15 (40.0)	0.05
elevated mean	89 (SD 31)	127 (SD 31)	0.05
Serum bilirubin	1.5mg/dl	3.4mg/dl	0.03
Splenomegaly detected in 18/41 (43.9%) patients	7/18 (38.9)	11/18 (61.1)	<0.01
Echotomography			
normal	15/26 (57.7)	9/15 (60.0)	0.05
moderate alterations	6/26 (23.1)	3/15 (20.0)	0.05
inomogenous patchy	3/26 (11.5)	2/15 (13.3)	0.05
nodular liver surface	2/26 7.7)	1/15 (6.7)	0.05
Hepatic-elastometry	8.4 (SD 0.5) KPa	8.7 (SD 2.4) KPa	0.05

HBV: hepatitis B virus; **N:** number of patients**; HBeAg:** hepatitis B "e" antigen**; HBV-DNA:** hepatitis B virus-deoxyribonucleic acid; **IU/mL:** international unit per millilitre**; ALT** alanine amino-transferase; **SD** standard deviation; **KPa:** kilopascal.

86.637-1.875.247), and 11 (42.3%) showed elevated ALT levels (mean level 89, SD 31IU/mL, range 73-285) and detectable levels of serum HBV-DNA (mean level 1741677 IU/mL, range 418.768-5.254.468).

Among 15 patients of Group B, 6 (40.0%) presented normal ALT levels and undetectable serum HBV-DNA (<20IU/mL), 3 (20.0%) presented persistently normal ALT levels and HBV-DNA detectable by Real-Time PCR (mean level 556.681IU/mL, range 138.574-2.673.555), and 6 (40%) had elevated ALT levels (mean level 107, SD 31 vs. 127IU/mL, range 92-294) and detectable levels of serum HBV-DNA (mean level 1.954.847 IU/mL, range 839.937-6.893.527).

Liver function tests in patients from both groups revealed elevated transaminase levels (89 SD 31 vs. 127 SD 31IU/mL) whereas a moderate increase in bilirubin (mostly indirect) was observed primarily in the coinfected (especially those suffering from falciparum malaria) than the HBV mono-infected (3.4 vs. 1.5mg/dL) patients; however, the two groups did not differ significantly (p=0.05). Normocytic hemolytic anemia was observed in 61/195 (31.3%) cases, and leucopenia due to a decrease in granulocytes and lymphocytes was confirmed in 96/195 (49.2%) subjects. Leucopenia was significantly associated with Group B (p<0.01). The hematological, biochemical, and virological results were similar in HBeAg positive and anti-HBe positive patients.

Clinical examinations

Following physical examination of all patients from both groups, 18/41 (43.9%) cases of HBV infection were found to have splenomegaly and hepatomegaly. Of these 18 patients, there were 7 (38.9%) and 11 (61.1%) patients from Groups A and B, respectively. Splenomegaly and hepatomegaly were significantly associated with Group B (splenomegaly: p=0.05; hepatomegaly: p=0.01). A significant association was found between 18S NASBA positivity and splenomegaly (p<0.01), hepatomegaly (p<0.01), and leucopenia. The stages of liver disease and hepatic fibrosis were also analyzed by echo-tomography and elastometry.

Diagnosis by imaging

Echo-tomography showed the following results for Group A (26) vs. Group B (15): normal findings of hepatic texture in 15 (57.7%) and 9 (60%) patients of Groups A and B, respectively; modest alterations of the echo-texture with ultrasound attenuation in 6 (23.1%) and 3 (20%) subjects of Groups A and B, respectively; hepatomegaly, inhomogeneous patchy, and diffuse increased echogenicity in 3 (11.5%) and 2 (13.3%) patients of Groups A and B, respectively; changes in the shape of the liver, inhomogeneous echo-texture, and irregular-nodular liver surface delineation in 2 and 1 patients from Groups A and B, respectively. Intrahepatic vessels were indistinct (echo coarse pattern). Lymph nodes were sometimes detected in the hepatoduodenal ligament. None of our patients presented ultrasound patterns of HCC.

Following the hepatic-elastometry examination, the mean value of liver elasticity was found to be 8.4±0.5 kPa and 8.7 SD 2.4 kPa for HBV mono-infected and coinfected patients, respectively (normal value of liver s*tiffness* (KPa): <4.5-5.5). Both echo-tomography and hepatic-elastometry showed no significant difference between the two groups of patients (**Table 2**).

The evaluation of the severity of liver disease showed that 12/26 (46.2%) patients with chronic HBV infection alone were HBV inactive carriers vs. 6/15 (40%) of coinfected patients, 3/26 (11.5%) vs. 3/15 (20%) presented a phase of immune-tolerance, 6/26 (23.1%) vs. 3/15 (20%) had mild chronic hepatitis, 3/26 (11.5%) vs. 2/15 (13.3%) had moderate/severe chronic hepatitis, and 2/26 (7.7%) vs. 1/15 (6.7%) had cirrhosis. All the stages of liver disease were substantially and statistically similar between the two groups (p=0.05). Of 5 patients with moderate/severe hepatitis or cirrhosis, 3 of them had HBV wild-type infection, while 2 patients were anti-HBe positive (p=0.03).

DISCUSSION

Malaria and HBV infections are common in several tropical areas, and the endemicity of these infections overlaps frequently. Clinically, the malaria infection may arise in various ways. Asymptomatic malaria infection and full-blown illness with typical febrile paroxysms are extreme points in the wide spectrum of clinical diseases caused by malaria parasites. Naturally acquired clinical immunity, parasite load and virulence, host's age, and genetic factors are all likely to modulate the clinical expression and severity of malaria. Many people from malaria-endemic countries may have acquired semi-immunity with sub-microscopic levels of parasitemia, resulting in less serious to no manifestation of disease (asymptomatic malaria)[12-14]. This was also reflected in our study as the frequency of semi-immune subjects was low (31.8%). Thus, we evaluated the epidemiology of HBV coinfection/malaria in a cohort of African immigrants with asymptomatic malaria and investigated whether and how this clinical form of malaria may affect the clinical expression of HBV infection. Among our malaria patients, chronic B hepatitis was detected in 24%, although their numbers were very small.

Comparing the rate of prevalence of coinfection among subjects from three continents (Africa[6], Asia[15], and South America[16]), it is evident that data differ significantly between continents; further, the clinical outcomes closely associate with geographical areas presenting different epidemiological patterns of two microorganisms and clinical features of malaria infection. For patients with different forms of chronic B hepatitis and anti-HBc subjects, increased number of coinfected individuals (around 45-50%) was reported in two studies conducted in SSA. However, in the same country (Nigeria), significant differences in the prevalence of coinfection have also been observed[6,17]. This is probably due to a decreasing trend of malaria and HBV infections, as reported by WHO.

In various studies conducted in Brazil, considerably low rates of coinfection were observed both in the general population and those at risk, highlighting the prevalence of both forms of HBV infection[7,9].

A completely different situation has been described in Asia where the prevalence of coinfection varies based on the geographical location (Vietnam 23.8%, Nepal 0%)[5,15].

Our data fall in the middle of the range obtained from the abovementioned studies and match closely with those from the study conducted in Africa. It must be reminded that most of the patients included in our study were immigrants from SSA.

Many studies have been conducted to identify migration-associated diseases[18,19] in industrialized countries with high levels of immigration, including those with a high prevalence of HBV infection; however, there are no completed or ongoing studies related to coinfections with malaria and hepatitis B. Thus, we believe that this is the first or one of the first studies examining this global health problem.

The interaction between two pathogens has been studied by several researchers, and as both infections share an intrahepatic stage in their life cycles, their interaction has been hypothesized to occur at both immunological and cellular levels. Intriguingly, both pathogens may also utilize common receptors during the hepatocyte invasion[20]. Malaria hepatitis is characterized by elevated levels of serum bilirubin and ALT (>3 n.v.) as well as a histologic liver picture that shows malarial pigment, swollen hepatocytes, inflammatory infiltrate, and centrizonal necrosis. These characteristics are usually described in patients with *P. falciparum* infection and also in some cases of infection by *Plasmodium vivax*[21,22]. In recent years, both experimental and clinical studies have demonstrated how the malarial infection can determine the clinical presentation and evolution of HBV infection[5,7,14].

Interestingly, it was reported that intrahepatic HBV replication and gene expression are inhibited in mice infected by the malaria species *Plasmodium yoelii*[8]. Actually, this parasitic infection causes an intrahepatic inflammatory response characterized by the influx of natural killer cells, macrophages, T cells, along with the induction of IFNγ, IFNα/β, TNFα, and iNOS proteins in the liver. Probably, these cytokines inhibit HBV replication; however, it appears that IFNγ and IFNα/β are essential to suppress HBV gene expression and replication in the liver. The antiviral effect of malaria is likely to be triggered by phagocytosis of infected erythrocytes by Kupffer cells, leading to their activation and production of chemokines. Subsequently, these cytokines recruit NK, NKT, and malaria-specific T cells, and together, these cells likely produce inflammatory cytokines to eliminate HBV from the hepatocytes[8].

These data suggest that malaria infection might influence the presentation and clinical evolution of HBV infection in coinfected humans. Some studies seem to confirm this hypothesis; a study in Papua New Guinea investigated the interactions in the coinfected individuals and showed that patients with the advanced stages of malaria infection had the lowest prevalence of hepatitis B infection[23]. Another study reported that viremia in chronic hepatitis B infection appears to fluctuate in concert with *P. falciparum* infection[24], suggesting that the amount and type of intrahepatic inflammatory cytokines induced by malaria infection may influence the course of HBV infection in humans. Further, a study performed in a Vietnamese hospital showed that patients with cerebral malaria had a slightly greater risk of registering positive serology for the HBV surface antigen[5]. Another study on Gambian children with severe malaria reported significantly increased HBV viral load in children with parasitemia than those with HBV infections alone, suggesting that increased viremia in individuals with severe malaria was likely due to decreased HLA expression[25].

Our findings, obtained by comparing a group of subjects coinfected vs. a group of patients HBV mono-infected, do not reflect the above data as the coexistence with *Plasmodium* does not seem to significantly affect the clinical course of HBV infection. Comparing the numerical data of the two patient groups, we demonstrated that 24.2% of coinfected vs. 19.8% of mono-infected subjects exhibited a clinical picture of chronic hepatitis. Although there was a greater percentage difference between coinfected vs. mono-infected (50.0% vs. 39.8%), anti-HBc positivity data was not significant between the two groups. Clinical chemistry findings, virological effects, and diagnostic imaging (ultrasonography and elastometry) were substantially similar in the two study groups, without any evidence of Plasmodium-induced effects on the clinical evolution of HBV infection.

The only statistically significant association was found between 18S NASBA positivity (for Group B) and splenomegaly ($p<0.01$), hepatomegaly ($p<0.01$), and leucopenia. One type of morbidity associated with malaria is hepatomegaly, a condition that is mostly resolved within two weeks of treatment. Malarial splenomegaly represents one of the leading causes of massive splenomegaly in malaria-endemic countries[26]. It is caused by an aberrant immune response to chronic antigenic stimulation in subjects exposed for a long time to malaria parasites, as observed during acute malaria that usually resolves after clearance of infection[27].

The regression of splenomegaly occurs with similar kinetics to the resolution of hepatomegaly; however, chronic hepatosplenomegaly can occur with persistent malaria infections[27]. Consistent with previous findings in patients from Brazil[7,9,12,14] and Africa[6,17,25,26], we observed a situation of persistent malaria infection in our patients.

Our data suggest that, in SSA immigrants with asymptomatic malaria and HBV coinfection, the parasitic pathogens do not appear to significantly affect the course of virus B infection. Plasmodium-infected individuals with active or previous HBV infection are more likely to be asymptomatic, present with lower parasitemia, and have decreased inflammatory cytokine profile (asymptomatic malaria presented a reduced IFN-c/IL-10 ratio[20]). Though the number of subjects examined was relatively small in this observational cross-sectional study, there was no clear evidence to demonstrate that the clinical status of underlying hepatitis B-related liver disease is affected during malaria infection. However, given the increasingly frequent arrival of SSA immigrants (with increased risk of HBV/occult malaria coinfection) in our country, this study offers some valuable insights for the clinicians and researchers working on this interesting topic. Some of our patients (73/195) are still being followed, but so far, we have not found any change with respect to their hepatic parameters, including both virological and serological responses.

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Conflict of interest

The authors declare that there is no conflict of interest.

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