Original Research Article

Sero-prevalence and risk factors for hepatitis A virus infection among pregnant women at the Samandin Medical Center, Ouagadougou, Burkina Faso.

ABSTRACT

Aims: This study determined prevalence and possible risk factors associated with HAV infection and compared Immunochromatography (IgG) rapid point of care test with standard ELISA test for diagnosis.

Study design: Cross sectional epidemiological survey.

Place and Duration of Study: The study was carried out at the Samandin Medical Center (Ouagadougou, Burkina Faso), from July 2017 to December 2017.

Methodology: A total of 180 pregnant women were recruited at the Samandin Medical Center. Anti-HAV IgM and IgG detection tests were performed using AccuDiag™ HAV ELISA kit (Calabasas, CA, USA) and SD BIOLINE HAV IgG/IgM Rapid Diagnostic Test (Gyeonggi-do, Korea).

Results: IgG anti-HAV antibodies (AccuDiaG Elisa) were found with a prevalence of 88.9% (160/180; CI95% [84.3 – 93.5]) and Anti-HAV IgM were 1.1% (2/180; CI95% [0.0 – 2.6]). Of the 160 AccuDiag HAV IgG positive samples, 44.4% (80/180; CI95% [37.2 – 51.7]) tested positive using SD BIOLINE Diagnostics HAV IgG. Regarding risk factors, 86.9% (86/99) of the IgG positive women were illiterate; 90.7% (39/43) lived mostly in peripheral areas; 89.2% (74/83) were household workers and 88.6% (93/105) used fountain water.

Conclusion: Our results demonstrated poor agreement between the assays tested, which are consistent with previous reports demonstrating significant variability between HAV ELISA and RDT HAV. Moreover, the detection of HAV specific IgM antibodies in two asymptomatic pregnant women signaled the current circulation of HAV in this area.

Keywords: Hepatitis A Virus, pregnant women, socio-economic and demographic conditions, Burkina Faso

1. INTRODUCTION

Formerly known as infectious hepatitis, hepatitis A is caused by the hepatitis A virus [1]. Hepatitis A virus (HAV) belongs to the genus *Hepatovirus* of the family of *Picornaviridae* [2, 3]. It is one of the most common infectious etiologies of acute hepatitis worldwide. Each year, more than 10 million cases are identified. In addition, the epidemiology of this virus, is closely associated with unsafe water, food contaminated with the feces from an infected person, poor sanitation and personal hygiene, sexual partner of an acute hepatitis A infected person, and close physical contact with an infectious person that traveled to endemic areas without being immunized or that injected drugs [4-6].

The endemicity of HAV in the world differs according to geographical area as shown by the prevalence studies of anti-HAV antibodies [7]. It is estimated that most children (90%) will be infected with HAV before ten years of age in developing countries with very poor sanitary conditions and hygienic practices. However, HAV infection is clinically silent in children under three years of age, but is usually symptomatic in adults [8].

The clinical diagnosis of current or recent HAV infection is evoked by the presence of IgM anti-HAV antibodies [9], which can be detected immediately after infection and remain detectable for about 6 months. A healed infection can be determined by serum HAV IgG antibodies, which are detectable soon after the onset of symptoms and bring about long-term or even lifelong immunity [10].

Despite the large body of data on HAV worldwide, little information is available regarding HAV infection during pregnancy. Among the few published cases, a high frequency of obstetric complications including contractions and preterm deliveries have been reported in symptomatic forms of hepatitis A during the second and third trimesters of pregnancy [11]. There are also numerous cases reported of vertical HAV transmission, utero complications and neonatal hepatitis [12-14].

In Burkina Faso, the lack of good hygiene and low socioeconomic status expose the population to enteropathogens [7, 15], and from these HAV is shown to be endemic [16]. However, the few studies performed with different commercial HAV IgG enzyme immunoassays have shown large variability in seroprevalence [16]. This study aimed to examine the serodetection of HAV by two analytical methods to identify the associated factors with HAV infection among pregnant women and to help inform the HAV vaccination policy.

2. MATERIALS AND METHODS

2.1 Ethical considerations

Ethical approval was obtained from Ethics Committee for Health Research by the decision N° 2014-12-138. The study participants received information about the study, after which they signed a written informed consent.

2.2 Study design and topics

This is a descriptive prospective study that took place from July 2017 to December 2017, in the laboratory of Samandin Urban Medical Center (Baskuy Sanitary District). We recruited 180 pregnant women from 16 to 49 years that attended prenatal clinics. After recruitment, the study protocol was explained to each woman and written informed consent was obtained. Women who agreed to participate received a pre-test questionnaire to obtain information on demographic characteristics and risk factors, including age, gender, place of residence, education level, job, food regime (outside), water source, medical history and diet.

2.3 Collection and processing of samples

Blood samples (5 ml) were collected in dry tubes without anticoagulant and centrifuged at 3000 round per minute (RPM) for 10 min at 25°C. Sera were collected in cryotubes and stored at -20 ± 5°C until serological analysis. The sera were tested for anti-HAV IgG using the enzyme-linked immunosorbent assay, (AccuDiag™ HAV IgG DIAGNOSTIC AUTOMATION, Calabasas, CA, USA). AccuDiag HAV-IgG is a qualitative immuno-competitive serological assay that calculates a result based on

signal-to-cutoff ratio S/C.O.). Specimens with S/C.O. values > 1.00 were considered negative and S/C.O. values ≤ 1.00 were considered positive for anti-HAV IgG. S is the individual absorbance (OD) of each specimen and Cut-off value (C.O.) is the mean absorbance value of the three negative controls times 0.5. The sensitivity of the test for measuring IgG antibodies was reported to be 100% and the specificity was reported as 100% according to the manufacturer's manual. All serum specimens tested for IgG were tested for IgM using AccuDiag™ HAV IgM DIAGNOSTIC AUTOMATION ELISA Kit, (Calabasas, CA, USA**).** AccuDiag HAV IgM ELISA is a qualitative immuno-capture serological assay and the results are calculated as shown above. Positive results must have a value of S/C.O.≥1, where S is the individual absorbance (OD) of each specimen and Cut-off value (C.O.) is the mean absorbance value of the three negative controls × 2.1. Negative results must have S/C.O. <1 and specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Our results obtained by ELISA were subsequently compared with SD BIOLINE HAV IgG/IgM Rapid Diagnostic Test. The sensitivity of this assay is 97.6% and the specificity is 98.0% according to the manufacturer's manual. In brief, the HAV IgG/IgM rapid test is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to Hepatitis A virus in human serum or plasma. To determine the HIV Status of pregnant women, we performed HIV serology on all samples using rapid tests (RDTs): HIV (SD BIOLINE HIV-1/2 3.0).

2.4 Statistical Analysis

Logistic regression analyses were carried out to determine which variables were significantly associated with detection of HAV antibodies. The lower and upper limits of the 95% confidence interval (CI) for a proportion were also calculated.

3. RESULTS

3.1 Socio-demographic characteristics of study participants

The average age of the study population is 26.3 ± 0.9 years. Regarding the level of education, 45% of participants completed primary, secondary or higher education; 55% were illiterate. Of the hundred and eighty (180) pregnant women, 53.9% were official workers and 46.1% were household workers (Table 1). Concerning the source of drinking water, 41.7% of the pregnant women used tap water supplied by the national office of water and sanitation (ONEA) and 58.3% used fountain water. 76.1% of the study population resided in the urban area and only 23.9% lived in the peripheries of Ouagadougou (Table 1).

According to their activities, the pregnant women had a diversified diet out of the home consisting of fruit, vegetables, meat and other (rice and maize cake). Fruits and vegetables were eaten according to the season. The prevalence of HIV among pregnant women was 2.2% (4/180; CI95% [0.1 – 4.4]).

Table 1. Socio-demographic characteristics of study participants (N = 180)

CHARACTERISTICS	NUMBER	PERCENTAGE (%)
AGE		
[16-25[	**81**	**45.0%**
[25-36[	**86**	**47.8%**
≥ 36	**13**	**7.2%**

LEVEL OF EDUCATION		
EDUCATED	**81**	**45%**
UNEDUCATED	**99**	**55%**
PROFESSION		
OFFICIAL WORKERS	**97**	**53.9%**
HOUSEHOLD WORKER	**83**	**46.1%**
LOCALITY		
SUB URBAN	**43**	**23.9%**
URBAN	**137**	**76.1%**
SOURCE OF WATER SUPPLY		
TAP	**75**	**41.7%**
FOUNTAIN	**105**	**58.3%**
FOOD REGIME (OUTSIDE)		
FRUITS AND VEGETABLES	**58**	**32.2%**
PORK MEAT (GRILL)	**61**	**33.9%**
OTHER	**61**	**33.9%**
GYNECOLOGICAL ANTECEDENTS		
ABORTION	**2**	**1.1%**
BORN DEAD	**0**	**0.0 %**
HIV STATUS		
POSITIVE	**4**	**2.2%**
NEGATIVE	**176**	**97.8%**

3.2 Seroprevalence and comparison of HAV antibody test results in pregnant women.

Using the rapid detection test (RTD, SD Bioline) the IgG HAV seropositivity was 44.4% (80/180; CI95% [37.2 – 51.7]) while using the ELISA test (AccuDiag), it was 88.9% (160/180; CI95% [84.3 – 93.5]) (Table 2). Of the 160 AccuDiag HAV IgG reactive samples, 80 were reactive with the SD BIOLINE IgG Anti-HAV IgG immunochromatographic assay. There was poor agreement between these assays (Table 2). All AccuDiag HAV IgG negative samples were also negative with SD BIOLINE IgG Anti-HAV IgG assay (Table 2). Based on these results we considered AccuDiag HAV ELISA as the reference diagnostic test for the diagnosis of HAV. Thus, the diagnostic efficacy of SD Bioline test when compared with ELISA showed a positive predictive value (PPV) and negative predictive value (NPV) of 44.4% and 100%, respectively.

Table 2: Comparison of IgG HAV antibody test results using RDT SD BIOLINE and Diagnostics AccuDiag HAV ELISA kits

		SD BIOLINE IgG Anti-HAV		
		Positive	Negative	Total
AccuDiag HAV IgG	Positive	80 (44.4%)	80 (44.4%)	160 (88.9%)
	Negative	0 (0.0%)	20 (11.1%)	20 (11.1%)
	Total	80 (44.4%)	100 (55.5%)	180

In our 180 pregnant women population, 5 women were IgM HAV positive (2.8%; CI95% [0.4 – 5.2]) using RDT (SD Bioline) while by the ELISA test (AccuDiag), only 2 were IgM anti-HAV positive (1.1%; CI95% [0.0 – 2.8]). The two AccuDiag HAV IgM positive samples were positive with SD BIOLINE IgM Anti-HAV antibody assay. Of the 5 SD BIOLINE Anti-HAV IgM positive samples, 3 tested negative for HAV IgM on AccuDiagIgM Anti-HAV (Table 3). Positive predictive value (PPV) and Negative predictive value (NPV) were 100% and 98.3%, respectively, according to the diagnostic efficacy of SD Bioline test when compared with ELISA.

Table 3: Comparison of test results between the AccuDiag HAV IgM ELISA and RDT HAV IgM SD BIOLINE Diagnostics

		SD BIOLINE IgM Anti-HAV		
		Positive	Negative	Total
AccuDiag HAV IgM	Positive	02 (1.1%)	00 (0.0%)	02 (1.1%)
	Negative	03 (1.7%)	175 (97.2%)	178 (98.9%)
	Total	05 (2.8%)	175 (97.2%)	180

3.3 Population stratification and risk factor analysis

HAV seropositivity was high among women below 25 years of age (90.1% (73/81)) relative to pregnant women over 36 years of age (84.6% (11/13)), , followed by the 25-36 year group (88.4% (76/86)) (Table 4). 90.7% (39/43) of the positive pregnant women resided in peripheral areas of the city and 88.3% (121/137) lived in the urban center. Regarding the level of education, 91.4% (74/81) of educated and 86.9% (86/99) of uneducated women were positive for IgG anti-HAV. In addition, 89.2% (74/83) of positive pregnant women were household workers and 88.6 (93/105) used fountain water. No risk factors were statistically evident of infection association (Table 4). The prevalence of HIV among pregnant women was 2.5% (4/180; CI95% [0.1 – 4.9]). All the HIV positive women were co-infected with HAV ($p < 0.05$).

Table 4: Prevalence of HAV antibodies according to population stratification and risk factors

Population stratification	*IgG anti HAV Prevalence using ELISA*	*OR (95% CI)*	*p-Value*
Age			
[16-25[	90.1% (73/81)	1.7 [0.2 – 7.8]	0.8234
[25-36[	88.4% (76/86)	1.4 [0.2 – 6.2]	
+ de 36	84.6% (11/13)	-	
Level of education			
Educated	91.4% (74/81)	1.6 [0.6 – 4.4]	0.3404
Uneducated	86.9% (86/99)	-	
Profession			
Household worker	89.2% (74/83)	1.1 [0.4 – 2.7]	0.9158
Official workers	88.7% (86/97)	-	
Food regime (outside)			
Fruits and vegetable	84.4% (49/58)	-	
Pork meat	95.1% (58/61)	3.5 [1 – 16.7]	0.1436
Nothing	86.9% (53/61)	1.2 [0.4 – 3.5]	
Locality			
Urban	88.3% (121/137)	-	0.6653
Suburbanl	90.7% (39/43)	1.3 [04 – 4.7]	
Source of water supply			
Tap	89.3% (67/75)	1.1 [0.4 – 2.9]	0.8726
Fountain	88.6% (93/105)	-	

4. DISCUSSION

This study aimed to determine the prevalence of HAV infection through two different detection methods, while exploring the risk factors associated with this infection in Samandin Urban Medical Center (Ouagadougou).

4.1 Seroprevalence of HAV antibody in pregnant women.

In this study, a poor concordance of test results between two commercial HAV IgG/IgM assays (AccuDiag ELISA and SD BIOLINE immunochromatographic assay) was observed. Only 50% (80/160) of AccuDiag HAV IgG positive specimens were positive with the SD BIOLINE Diagnostics HAV IgG. The AccuDiag ELISA test was more sensitive than the RDT SD BIOLINE ($p < 0.001$). This observation is similar to a study conducted by Traore et al, which reported a low prevalence of IgG anti-HAV with the SD BIOLINE kit [16]. HAV IgM assays also showed poor agreement between the two commercial kits. In our study, both specimens that were positive with the

AccuDiag HAV IgM assay were positive with the SD BIOLINE HAV IgM assay. However, three SD BIOLINE HAV IgM positive specimens were negative with the AccuDiag HAV IgM assay, demonstrating poor agreement between these assays. Although rapid diagnostic tests are more practical, ELISA tests where large numbers of specimens are tested, seem to provide results that are more reliable, and should be of most use in routine diagnostic laboratories. However, the diagnostic efficacy of SD BIOLINE test when compared with AccuDiag ELISA suggests that RDT SD BIOLINE can be used as a screening test and all positive specimens must be confirmed using AccuDiag ELISA for both IgG and IgM. Moreover, the rapid test does have great utility in peripheral health settings where relatively few specimens are processed [17]. The present study showed an incidence of 1.1% of HAV infection with ELISA in pregnant women. The results of this study supported by the study of Traore et al. (2%), which indicated that the incidence of infections is relatively high and that there is a risk of major outbreaks if vaccination is not continued [7, 18].

4.2 Coinfection between HAV antibodies and HIV

In this study, 2.2% (4/180; CI95% [0.1 – 4.4]) of pregnant women were positive HIV. All positive cases had IgG anti-HAV antibodies and all were found among household workers. A previous study showed prior HIV infection may predispose populations to HAV infection and this coexistence carries a high risk of morbidity and mortality for pregnant women [19]. The positive association old markers of exposure to HAV and HIV infection may suggest epidemiological and/or pathogenic links.

4.3 Prevalence of HAV antibodies and risk factors

When analyzed according to age groups, the prevalence of IgG anti-HAV (ELISA) ranged from 84.6 - 90.1% among pregnant women between 16 to 45 years old, which is similar to high prevalences of HAV observed in west African countries [16, 20]. These high prevalences could be related to the source of water supply and sanitation, and individual social characteristics of those who did not change behaviors toward the prevention and awareness of the disease. In addition, young adults appear to be the most exposed to the external environment thereby exposing them to the virus. In this study, the prevalence of HAV infection among women living in unsanitary and underserved areas (Peripheral, 90.7%) was higher than women living in serviced areas (Center, 88.3%) (table II). Of the 180 pregnant women, 58.3% used fountain water for drinking, washing vegetables and fruit, washing kitchen utensils, which was emphasized as a risk factor for HAV in other studies [20, 21]. However, in our study, no socioeconomic variables such as educational level, nor environmental variables such as access to safe water were significantly associated with HAV, although the local environmental conditions theoretically favour the transmission of enteric pathogens. Moreover, the overall level of knowledge about hepatitis A was generally poor among the pregnant women.

In our study we found no statistically significant result when the medical history was explored. Nevertheless, Israeli researchers claim that the occurrence of acute hepatitis A in a pregnant woman is associated with high rates of gestational complications and premature delivery [22]. A few cases of mother-to-child transmission of hepatitis A that is responsible for neonatal hepatitis or in utero complications [12] have also been published, but which we have not explored and which could be a subject of future research.

5. CONCLUSION

This study revealed a poor concordance of test results between the AccuDiag ELISA and SD BIOLINE immunochromatographic commercial assays. Moreover, the detection of HAV specific IgM antibodies in the asymptomatic pregnant woman, is highly suggestive of the possibility of a vertical HAV transmission. Future studies should try to understand the difference in the analytical sensitivity of these two anti-HAV assays.

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