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Multiplex Rapid Test with Acceptable Diagnosis Performance as a Solution to Increase Diagnosis of Hepatitis B and C Viruses in Pregnant Women in an Area of High Prevalence of Both Hepatitis Viruses Associated with HIV

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Abstract

Background and Objective: HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) are very widespread in the world, however, less than 20% of the people affected are diagnosed and treated. This study aimed to determine the prevalence of HIV, HCV and HBV co-infections in pregnant women at Bangui Community University Hospital and the cost of screening. **Methods:** A cross-sectional study involving consenting pregnant women who came for antenatal care was performed. HIV, HCV antibodies and HBV antigens were detected using Exacto Triplex® HIV/HCV/HBsAg rapid test, cross-validated by ELISA tests. Sociodemographic and professional data, the modes of trans-

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mission and prevention of HIV and both hepatitis viruses were collected in a standard sheet and analyzed using the Epi-Info software version 7. **Results:** Pregnant women aged 15 to 24 were the most affected (45.3%); high school girls (46.0%), and pregnant women living in cohabitation (65.3%) were the most represented. Twenty-five (16.7%) worked in the formal sector, 12.7% were unemployed housewives and the remainder in the informal sector. The prevalence of HIV, HBV, and HCV viruses was 11.8%, 21.9% and 22.2%, respectively. The prevalence of co-infections was 8.6% for HIV-HBV, 10.2% for HIV-HCV, 14.7% for HBV-HCV and 6.5% for HIV-HBV-HCV. All positive results and 10% of negative results by the rapid test were confirmed by ELISA tests. The serology of the three viruses costs 39,000 FCFA (60 Euros) by ELISA compared to 10,000 FCFA (15.00 Euros) with Exacto Triplex® HIV/HCV/*AgHBs* (BioSynex, Strasbourg, France). **Conclusion:** The low level of education and awareness of hepatitis are barriers to development and indicate the importance of improving the literacy rate of women in the Central African Republic (CAR). Likewise, the high prevalence of the three viruses shows the need for the urgent establishment of a national program to combat viral hepatitis in the CAR.

Keywords

HIV-HCV-HBV Co-Infection, Multiplex Immunochromatographic Rapid Test, Central Africa, Serology Test Cost, Diagnostic Accessibility

1. Introduction

According to the World Health Organization (WHO), 257 million people have chronic HBV infection, 71 million have chronic HCV infection and around 37 million people are living with HIV [1] [2], or a total of 365 million people worldwide. Co-infection with HIV is a significant risk factor for chronic hepatitis. Chronicity is observed in 10% of adults and 90% of children for HBV, and in 75% to 85% for HCV, respectively [3] [4] [5]. The two hepatitis viruses account for almost all chronic hepatopathies (6 - 10 million). HIV is the cause of 690,000 deaths worldwide [6] compared to 1.4 million for hepatitis, 96% of which are due to HBV and HCV [2] [7]. The epidemic caused by HBV affects mostly the WHO Western Pacific Region and the African Region [2]. Additionally, more than two-thirds of total HIV infections, new HIV infections and HIV-related deaths are recorded in Sub-Saharan Africa [5]. The epidemiology of HBV differs from that of HCV because HBV is generally transmitted in childhood [8] through breastfeeding, and close contact with parents; whereas HCV is transmitted mainly in adulthood through unsafe injections, tattoos, scarification and other direct contact with blood [9]. Mother-to-child transmissions of HIV and HBV are frequently recorded. Transmission of HCV with or without HIV co-infection occurs at a rate of 5% to 7% and 15% to 18%, respectively [10] [11]. Pregnant women have long served as an important study population for HIV

sero-surveillance but have only recently been leveraged as a study population for hepatitis surveillance. WHO considers accelerating the elimination of HBV in mothers and children a major step towards achieving the goals of the WHO Global Hepatitis Strategy [12]. However, one of the main obstacles to hepatitis control is the limited availability of affordable diagnostic hepatitis testing. Few people with viral hepatitis have been diagnosed (9% of HBV-infected people, 22 million, and 20% of HCV-infected people, 14 million) [2].

An example country where these problems of hepatitis diagnosis occur is the Central African Republic (CAR). The CAR is a country of around 6 million people with HIV, HCV and HBV prevalence of 3.5%, 5% and 15% respectively [13] [14] [15] [16]. The CAR has just created its national hepatitis control program. Many children there are infected with viral hepatitis during the first year of their life. Cirrhosis and primary liver cancer (hepatocarcioma), as secondary diseases caused by hepatitis infection are very common among young people in CAR [17]. The diagnosis of HIV is made widely accessible to pregnant women thanks to rapid screening tests carried out systematically and free of charge as part of the program to prevent mother-to-child transmission of HIV. However, the diagnosis of hepatitis, which is based on ELISA tests, is impossible to implement in the countryside due to a severe lack of resources, and is difficult to access even in Bangui due to its high cost. The multiplex rapid test Triplex® HIV/HCV/AgHBs (BioSynex, Strasbourg, France) was evaluated in France [18] and in the Central African Republic [19]. These evaluations showed good virological performances. The test was used for the simultaneous screening of the three viruses in pregnant women or even in all women of childbearing age and appears as a promising tool to strengthen the diagnosis and the prevention of their transmission to children [20]. Exacto Triplex has shown good practicability and high acceptability in the Democratic Republic of Congo for the simultaneous screening of HIV, HBV and HCV [21]. The objective of this study was to determine the value of the Triplex HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France) in the screening of HIV, HCV and HBV co-infections in pregnant women at the Community University Hospital of Bangui, in the CAR.

2. Methods

2.1. Study Site and Design

The study took place at the Bangui Community Hospital, one of the four city's University Hospitals. This hospital houses the country's reference gyneco-obstetrics department, where pregnant women have been recruited as study subjects. This was a cross-sectional study, carried out from March 1, 2018, to September 30, 2018, lasting six months.

2.2. Study Population

The study population consisted of all women who received an antenatal consultation (ANC) during the time of the study and consented to participation. Tak-

ing the prevalence of HBSAg, which is 15% in CAR, the theoretical sample size required to estimate a frequency for an observational study was 196 according to Schwartz's formula [22].

2.3. Data Collection

A standardized questionnaire was used to collect relevant data on the subjects including socio-demographic data (age, education level, and marital status), professional data (occupations encoded in sectors of professional activities), awareness of transmission modes and prevention of HIV/HBV/HCV, as well as serology test results for the 3 viruses.

2.4. Samples Collection, Transport, Pretreatment and Storage before Analysis

The rapid tests were carried out using venous whole blood collected in an EDTA tube. The tubes containing the remainder of each sample were transported to the laboratory in an insulated container triple-packaged. Upon arrival at the laboratory, the tubes were centrifuged and the plasma from each tube transferred into 2 cryovials and preserved by freezing at −80˚C until the end of data collection. The plasma samples preserved in these cryovials were used to carry out the ELISA tests.

2.5. Laboratory Tests

The Exacto® Triplex HIV/HCV/HBsAg rapid diagnostic test (BioSynex, Strasbourg, France) was used for screening of HIV, HBV and HCV. It is a 3rd generation immunochromatographic test which uses the synthetic antigens *gp41* and *gp36* of HIV-1 and HIV-2, the recombinant core antigens and the proteins NS3, NS4 and NS5 of the HCV and the Hepatitis B surface antigen (*HBsAg*) of HBV to simultaneously detect infection with all 3 viruses. Previous evaluation studies done in France showed very high sensitivity and specificity of Triplex HIV/HCV/*HBsAg* and results comparable to the gold standard [18]. The test can be performed on capillary or venous whole blood, serum, or plasma. The quantity of the whole-blood needed to perform the test is 50 µL. The results are read 15 min after depositing the sample and the migration sample. Exacto® Triplex HIV/HCV/*HBsAg* test (BioSynex, Strasbourg) was carried out on site in a box set up in the prenatal consultation service and the results were immediately communicated to the practitioners for pregnant woman care. Exacto® Triplex HIV/HCV/HBsAg test (BioSynex, Strasbourg) contains a strip on which appears a control strip indicating the test has been successfully carried out. The presence of this single band indicates a negative result. Positivity for each virus is materialized by the appearance of a band on the corresponding line [19]. All the positive test results and 10% of the negative results were also subject to cross-validation. Cross-validation tests were carried out at the National Laboratory of Clinical Biology and Public Health which is the HIV National Reference Laboratory, responsible, among other things, for evaluating the performance of diagnostic

tests. Murex® ELISA tests (Diasorin, Dartford DA1 5LR, United Kingdom) for the detection of *HBsAg* (screening and confirmatory), HCV and HIV; the latter combined with Genscreen® ULTRA HIV Ag-Ab (Bio-Rad, Marnes-la-Coquette, France) according to the national HIV diagnosis algorithm were used. Genscreen® ULTRA HIV Ag-Ab (Bio-Rad, Marnes-la-Coquette, France) and Murex® HIV Ag/Ab Combination (Diasorin, Dartford DA1 5LR, United Kingdom) are 4th generation ELISA tests which use HIV-1 and 2 envelope antigens and *anti-p24* antibodies. Murex® HBsAg (Diasorin, Dartford DA1 5LR, United Kingdom) and Murex® anti-HCV (version 4.0) (Diasorin, Dartford DA1 5LR, United Kingdom) are 3rd generation ELISA tests. The former uses mouse monoclonal antibodies specific to different epitopes on the "a" determinant of *HBsAg* and the latter uses highly purified antigens which contain sequences from the core, NS3, NS4 and NS5 regions of HCV. Each test was performed according to the manufacturer's instructions on the Human® Laboratories (Wiesbaden, Allemagne) ELISA chain.

2.6. Statistical Analysis

Results were evaluated with Epi-Info version 7 software (WHO, Geneva & CDC, Atlanta). The mean of each quantitative variable and the proportions of the categories of each qualitative variable were determined.

2.7. Ethical Considerations

The protocol has been approved by the Ethics and Scientific Committee of the Faculty of Health Sciences of the University of Bangui, representing the national ethics committee.

3. Results

During the study period, 150 pregnant women agreed to participate in the study and were included. The participants were aged 15 to 43 with a mean age of 27 (±7) years. Pregnant women aged 15 to 24 were the most represented (45.3%). The high school level was also the most represented education level at 46.7%, and about two thirds of the participants (68.7%) had not reached higher education. Pupils and students were the most representative occupational category. More than half (51.4%) of the participants were without professional occupation (pupils, students, or unemployed and housewife). Only 16.7% worked in formal sector of professional activities. Around two-thirds (65.3%) of pregnant women lived in cohabitation with their partner (Table 1). Regarding awareness of the viral diseases, 18.7% of the subjects were generally aware of the 3 viruses, 31.3% knew that HIV, HBV and HCV are transmitted sexually, and 22.7% knew about the possibility of mother-to-child transmission. Forty-nine pregnant women (32.0%) knew that the 3 viruses are transmitted by blood, including *via* blood transfusion. Condom use was the best-known method of prevention (32.7%) followed by Faithfulness (24.7%). Vaccination against hepatitis B virus was known to prevent transmission of the infection by Hepatitis B virus in 20.7% as shown in Table 2.

Table 1. Sociodemographic characteristics of the 150 pregnant women.

Age (years)	**Number**	**%**
15 - 24	68	45.3
25 - 34	55	36.7
35 years and over	27	18.0
Educational level	**Number**	**%**
No education/primary school	33	22.0
High school	70	46.7
University level	47	31.3
Sector of professional activity	**Number**	**%**
Pupils/students	58	38.7
Informal sector	40	26.7
Public/private sector employees	25	16.7
Unemployed/housewife	19	12.7
Agro-pastoral sector	08	5.3
Marital status	**Number**	**%**
Lives alone	52	34.7
Lives in cohabitation	98	65.3

Table 2. Repartition of 150 pregnant women according to knowledge, viruses transmission modes and prevention measures.

Informed about 3 viruses	**Number**	**%**
Yes	27	18.0
No	123	82.0
Modes of transmission	**Number**	**%**
Blood	48	32.0
Sexual	47	31.3
Saliva (hepatitis B and C viruses)	21	14.0
Mother-to-child	34	22.7
Modes of prevention	**Number**	**%**
Condom	49	32.7
Faithfulness	37	24.7
Abstinence	33	22.0
Vaccination (against hepatitis B virus)	31	20.7

Exacto Triplex® HIV/HCV/HBsAg test was compared with the ELISA tests used as the gold standard. For this, all samples positive by Exacto Triplex® HIV/HCV/HBsAg test for each pathogen taken individually, *i.e.* a total of 84 samples and 10% of the 66 negative samples (6.6) rounded to 7 were tested by ELISA. Samples positive for HIV (18), for HBsAg (32) and for HCV (33) were confirmed by ELISA tests. The negative samples (7) for the 3 viruses were also confirmed by ELISA tests. Samples that showed co-infection with Exacto Triplex® HIV/HCV/HBsAg test (13 for HIV/HBV, 15 for HIV/HCV, 22 for HBC/HCV and 10 for Triple coinfection HIV/HBV/HCV) included in the mono-infected samples and the 7 negative samples were confirmed by ELISA tests. Taking the viruses individually, 18, 32 and 33 samples were tested positive to HIV, HBsAg and HCV respectively. With these numbers of samples tested positive, the prevalence of each virus in pregnant women by Exacto Triplex® HIV/HCV/HBsAg test, confirmed by ELISA tests was 11.8%, 21.9% and 22.2% respectively for HIV, HBV, and HCV. Samples positive for co-infections were first counted in the prevalences for each virus taken individually before being taken into account in coinfections. Thirteen (13), 15, 22 and 20 were positive for double coinfections with HIV/HBV, HIV/HCV, HBC/HCV and Triple coinfection HIV/HBV/HCV. The prevalence of co-infections by Exacto Triplex® HIV/HCV/HBsAg test, confirmed by ELISA tests among women with co-infections was 8.6% for HIV-HBV, 10.2% for HIV-HCV, 14.7% for HBV-HCV and 6.5% for HIV-HBV-HCV (Figure 1). The average prevalence of co-infections was 10.0%. The cost of the HIV, HBV, and HCV serologies by ELISA was 13,000 FCFA (the currency used in the countries of the Economic Community of Central African States or 20 euros per test per virus), is greater than that out of parallel testing of all three viruses by Exacto Triplex® HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France): 10,000 FCFA or 15.00 euros. The cost of the ELISA serology for parallel testing of all three viruses (39,000 FCFA or 60 euros for three tests) equals almost 6 times the cost of the Exacto Triplex® HIV/HCV/*HBsAg* rapid test (BioSynex, Strasbourg, France) (Table 3).

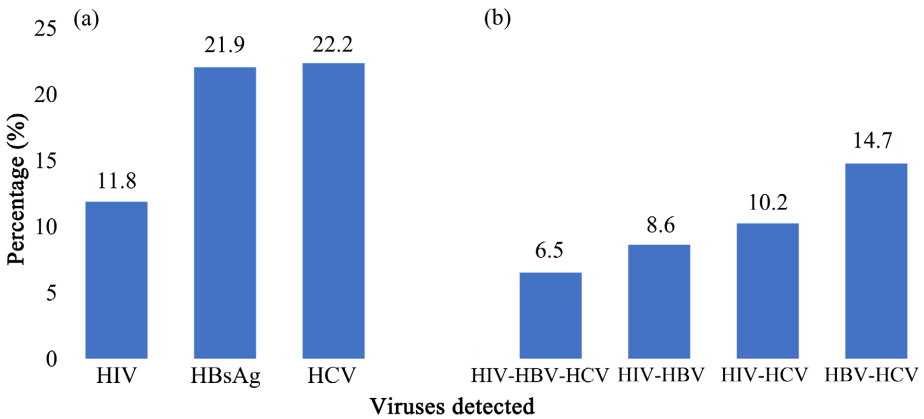

Figure 1. Prevalence of the 3 viruses alone (a) and in co-infection (b) in pregnant women. Samples positive for co-infections were firstly counted in the prevalence for each virus taken individually. The average prevalence of co-infections was 10.0%.

Table 3. Comparison of the results given by Exacto Triplex® HIV/HCV/HBsAg test and ELISA tests.

		ELISA HIV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	18 (72.0)	0 (0.0)	18 (72.0)
	Negative	0 (0.0)	7 (28.0)	7 (28.0)
	Total	18 (72.0)	7 (28.0)	25 (100.0)
		ELISA HCV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	33 (82.5)	0 (0.0)	33 (82.5)
	Negative	0 (0.0)	7 (17.3)	7 (17.9)
	Total	33 (82.5)	7 (17.9)	40 (100.0)
		ELISA HBsAb (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	32 (82.1)	0 (0.0)	32 (82.1)
	Negative	0 (0.0)	7 (17.5)	7 (17.5)
	Total	32 (82.1)	7 (17.5)	39 (100.0)
		ELISA HIV/HBV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	13 (65.0)	0 (0.0)	13 (65.0)
	Negative	0 (0.0)	7 (35.0)	7 (35.0)
	Total	13 (65.0)	7 (35.0)	20 (100.0)
		ELISA HIV/HCV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	15 (68.2)	0 (0.0)	15 (68.2)
	Negative	0 (0.0)	7 (31.8)	7 (31.8)
	Total	15 (68.2)	7 (31.8)	22 (100.0)
		ELISA HBV/HCV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	22 (75.9)	0 (0.0)	22 (75.9)
	Negative	0 (0.0)	7 (24.1)	7 (24.1)
	Total	22 (75.9)	7 (24.1)	29 (100.0)
		ELISA HIV/HBV/HCV (%)		
		Infected	Non infected	Total
Exacto Triplex® HIV/HCV/HBsAg	Positive	10 (58.8)	0 (0.0)	10 (58.8)
	Negative	0 (0.0)	7 (41.2)	7 (41.2)
	Total	10 (58.8)	10 (58.8)	17 (100.0)

4. Discussion

The aim of this study was to determine the value of the HIV/HCV/*HBsAg* Triplex (BioSynex Strasbourg, France) in the screening of HIV, HCV, and HBV co-infections in pregnant women in Bangui. This work reports for the first-time data from a cross-sectional study on co-infections with HIV and Hepatitis B and C viruses in the CAR. The results show that pregnant women aged 15 to 24 were the most affected (45.3%); they were most often high school girls (46.7%), 16.7% worked in formal sector of professional activities; 65.3% living mostly as a couple. Only 18.7% had heard of viral hepatitis, a third of which knew that the 3 viruses were transmitted through blood and sex; the condom was the best-known prevention method (32.7%). The prevalence of infections was 11.8%, 21.9%, and 22.2% respectively for HIV, HBV, and HCV and that of co-infections were 8.6% for HIV-HBV, 10.2% for HIV-HCV, 14.7% for HBV-HCV and 6.5% for HIV-HBV-HCV co-infections. The serologies of the 3 viruses cost 13,000 FCFA (20.00 euros) each and 39,000 FCFA (60.00 euros) for the 3 viruses by ELISA against 10,000 FCFA (15.00 euros) by Exacto Triplex® HIV/HCV/AgHBs (BioSynex, Strasbourg, France). The confirmation of the results of Exacto Triplex® HIV/HCV/HBsAg (BioSynex, Strasbourg, France) by the ELISA tests shows that the rapid tests were reliable and that it could replace the Reference tests. This confirms the results of the previous studies carried out in France and the CAR [18] [19].

The fact that nearly half of pregnant women were under 25 years old and that two thirds had reached only primary education could be related to school dropouts which are a major obstacle to the emancipation of women in resource limited countries like the CAR and to the development of these countries. A pregnant girl in elementary school is expelled from the establishment. While this does not typically happen in high school, mobbing by other (male) students can often cause pregnant students give up attending the establishment. If pregnant students live as a couple (65.3%) this is another reason to drop out of school. In such a situation it is not easy for these women to balance studies, household activities and pregnancy. After childbirth: the duties related to childcare will typically further increase workload. This situation is a serious problem in a developing country where a policy of positive discrimination in favor of women is in place. Even if Sangho, the local language, is also an official language, this low level of education does not give them the ability to obtain work, particularly in the formal sector; the majority of professional activity in the formal sector uses French. This is why only 16.7% were from the informal sector. The vast majority of pregnant women in our series (83.3%) were still studying (38.7%), or unemployed or housewife (12.7%); the others were workers in the informal sector. Pregnancy leads to school dropout, and therefore makes it difficult to undertake training to obtain professional qualification. These results explain and confirm those of the 2019 Multiple Indicator Survey (MICS6) which shows that the literacy rate for women is only 24.9% [23]. Action must be taken to increase the age

of women at first pregnancy, which is likely to improve the literacy rate of women and their ability to obtain work in the formal sector. The most represented sector of activity was that of students (38.7%). This category being educated, knows the importance of prenatal consultations but the probably limited financial resources force them to consult in public establishments. All categories in our sample have little access to information and awareness raising on these diseases within the framework of occupational safety and health, which could explain the high prevalence of these diseases in our sample. These information and education sessions for behavior change are organized and carried out, coordinated and supervised by the Directorate of Occupational Medicine and concern workers in the formal sector, who in our study represent only 16.7% of our sample. There is very little awareness of these conditions at the community level.

This low female literacy rate and the low frequency of those who worked in formal sector may partly explain the lack of knowledge women have about pregnancy and hepatitis [24]. HIV has been well known and has been the subject of awareness since its discovery to date as part of a national control program. However, only 18.7% of pregnant women asked in this study had heard about hepatitis and only one third of those knew the different modes of transmission (blood: 32.0%; sexual: 31.3%); mother-to-child transmission was known by 22.7% of participants. An even lower frequency (17.6%) was found among pregnant women participating in a study in Cameroon [24]. The modes of transmission were better known for HIV among pregnant women in Congo [25]. The best-known prevention method was condom use (32.7%) and the least known was HBV vaccination (20.0%). Knowledge of the modes of transmission and preventive measures of a disease is generally acquired through information from awareness campaigns, organized by a national control program. However, although there is a global viral hepatitis program [12], with a goal of eliminating hepatitis by 2030 [7], a national program to fight viral hepatitis in the CAR is recently created but is not yet fully functional. It is in the process of mobilizing resources to become fully operational. Unlike HIV, there is no awareness campaign against hepatitis. Free vaccination against HBV is included in the expanded vaccination program, however, through this conduit it is only available to children aged 0 to 5. People beyond the age of 5 are not included and therefore have much more difficult access to vaccinations. Even people with the financial resources to afford the vaccine are often uninformed about the virus and therefore do not get vaccinated. The new established national program to fight against viral hepatitis would make it possible to promote access to vaccination for the whole population.

The lack of knowledge on the modes of transmission and prevention would certainly be at least partly linked to the prevalence of each of the three viruses among pregnant women. Firstly, comparison of Exacto Triplex® HIV/HCV/HBsAg test (BioSynex, Strasbourg, France) with ELISA tests used as the gold standard showed that Exacto Triplex® HIV/HCV/HBsAg test (BioSynex, Strasbourg, France) results were confirmed by ELISA tests. These data confirm those pub-

lished in 2022 by a study which evaluated the virological performance of Exacto Triplex® HIV/HCV/HBsAg test (BioSynex, Strasbourg, France), with a specificity of 100% for the 3 viruses and a sensitivity of over 99.0% for HIV, HBV and 96.0% (95% CI [94.0% - 100.0%]) for HCV [19]. The prevalence of HIV, HBV and HCV in pregnant women was 11.8%, 21.9% and 22.2%, respectively. These frequencies exceed the prevalence of each of the 3 viruses in the general population in the CAR: 3.5% for HIV [26], 5% - 8% for HCV [16] and 10% - 15% for HBsAg (HBV) [14] [15]. They are also higher than those of blood donors: 4.72% for HIV, 5.98% for HCV and 8.89% for HBV [16]. In another study of volunteer blood donors over a 5-year period from 2015 to 2019, published in 2021, mean HIV prevalence was 5.6% and as high as 10.8%; HBV prevalence ranged from 15.0% to 20.0%; and HCV prevalence was as high as 9.8%. The average prevalence of co-infections was 9.1% [27]. These results show that even among blood donors, who constitute an educated population, aware of the risk of transmission of the 3 main blood-borne viruses, the prevalence of HIV, HBV and HCV is high, similar to those obtained in our study. The same applies to the average of co-infections prevalence. The prevalence of HCV varies from one country to another and even within the same country [28] [29]. The prevalence of HCV was higher than in sub-Saharan Africa (2.9%) [30]. They are much closer to those of key populations for the first two viruses, a little more studied in CAR than the third: 34.0% for HIV and 19.8% for *HBsAg* (HBV) [31] [32] in CAR and that of hepatitis C in Egypt, the highest in the world [33]. The prevalence of HCV in Benin is 7.4% in Tanguiéta and 1.2% in Cotonou [34]. The prevalence of HIV-HBV co-infections in this study was similar to that of people living with HIV [11] but lower than that of men who have sex with men [31]. The prevalence of HCV in the CAR is higher than in Cameroon (1.9%) and Gabon (2.1%) with significant variations depending on the regions in Gabon [29] [35].

The reference ELISA tests confirmed all the results obtained by the Exacto Triplex® HIV/HCV/*HBsAg* rapid test (BioSynex, Strasbourg, France) which proves the rapid tests' good performance and shows they could be used in the country for the simultaneous screening of the 3 viruses, contingent on confirmation of this trend by a study larger scale study in the CAR. The results of the current study are in agreement with those from the studies already published on Exacto Triplex® HIV/HCV/*HBsAg* performance (BioSynex, Strasbourg, France) [18] and Bangui [19]. It should be noted that the number of samples used in the virological performance evaluation study and in our current study are low: 50 and 33 positive respectively. These results must therefore be considered with reservations and deserve confirmation on a very large sample. If these results are confirmed, it would be a good test for the simultaneous screening of 3 viruses. However, Exacto Triplex® HIV/HCV/*HBsAg* test (BioSynex, Strasbourg, France) has already shown its value in improving adherence to care centers in Sub-Saharan Africa [36], as well as in strengthening disease prevention strategies in resources limited countries among women of childbearing age [20], in mass screening [37]. We could add to these advantages, the screening of the 3 viruses in the

management of blood exposure accidents, whether they are professionals (occupational blood exposure) in the health field or not (sex workers) or sexual but non-professional. Moreover, Exacto Triplex® HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France) is interesting in the blood donations qualification in CAR and probably in other countries with limited resources because these are the 3 viruses targeted by screening in these 2 circumstances. Finally, another important advantage of Exacto Triplex® HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France) is the good affordability of the diagnosis due to the comparatively low price. In our study, the cost of screening by Exacto Triplex® HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France) (10,000 FCFA, or 15.00 euros), was more affordable than that of one of the 3 viruses screened alone and about 6 times more affordable than the combined cost of concurrent screening of all three viruses (39,000 FCFA, or 59.46 euros) by the ELISA reference tests. In the WHO African region, the availability of diagnostic tests is only assured in 44% and 33% of countries for HBV and HCV respectively [38]. Few people with viral hepatitis have been diagnosed (9% of HBV-infected people, 22 million, and 20% of HCV-infected people, 14 million) [2]. The previous inexistence of multiplex and affordable tests and the high cost of screening using the reference tests are partly responsible for the inaccessibility of diagnosis of viral hepatitis. Affordable cost, concurrent screening for all 3 viruses, good performance, ease of use [18] and possibility of storage at room temperature without performance loss are advantages that make Exacto Triplex® HIV/HCV/*HBsAg* (BioSynex, Strasbourg, France) a promising candidate to improve the access of concurrent screening for HIV, HBV and HCV to pregnant women or even the general population, even if there is already a good coverage screening of HIV screening thanks to the adoption of rapid tests [39].

Our study has limitations, linked to the characteristics of rapid tests and the low number of samples. The virological evaluation of Triplex had shown a sensitivity of 96.0% for a number of positive samples of 50 [19]. In our study, the positive tests by Triplex were confirmed by ELISA for a number of 33. These variations could be linked to sampling fluctuations and deserve to be considered with reservation and confirmed on a large sample. The size of this sample should be, according to the Shwartz formula, at least 196 [22] for a clinical study like ours, otherwise 400 for an evaluation of test performance as required by COFRAC [40]. This is why, because of the small sample size, we had not determined the test performances (sensitivity, specificity), especially since the use of ELISA tests only concerned part of the samples for Triplex quality control reasons. Despite these limitations, Triplex allowed us to have an idea of the level of co-infection of HBV and HCV with HIV.

5. Conclusion

The results of this study show that pregnant women generally have a high school girls level, working mainly in the informal sector with a low level of knowledge

and a high prevalence of HIV, HBV and HCV infections, including frequent co-infection. The cost of screening using the Exacto Triplex® HIV/HCV/HBsAg rapid test (BioSynex, Strasbourg, France) is very affordable. Taken together, these results suggest the need to raise awareness among women to increase their age at first pregnancy and their educational attainment and their ability to obtain work in the formal sector. The National Hepatitis Control Program must become quickly and fully operational. In this context, Exacto Triplex® HIV/HCV/HBsAg rapid test (BioSynex, Strasbourg, France), which has numerous advantages, could considerably contribute to improving accessibility to diagnosis and prevention or even to the decentralization of viral screening for hepatitis B and C.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Author Contributions

CBC, NEON, CDMK designed the study; NP, HNK, FSNKV collected clinical data; CBB, CLBG, ASSN did laboratory experimentations; DYG, HDMK did statistical analysis; CBC, NEON, HDMK wrote the first version of the manuscript; all authors have read and approved the final version of the manuscript.

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