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#### Seroprevalence of Syphilis and HBV infection among Pregnant Women at Saint Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia

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#### Abstract

Hepatitis B virus (HBV) infection is a global public health problem, even though its prevalence is disproportionately high in low- and middle-income countries. Mother-to-child transmission is a major route of HBV transmission in endemic areas. This study aimed to assess the seroprevalence of HBV, Syphilis and their determinant among pregnant women. A crosssectional study was conducted from July to September 2019 in 290 pregnant women at Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Gestational and socioeconomic data were collected using a questionnaire. Serum Hepatitis B surface antigen (HBsAg) and antibody against syphilis infection was tested using a rapid diagnostic test. Data were analyzed by using Statistical Package for Social Sciences (SPSS) software version 22. Multiple logistic regression analysis was done to identify the independent risk factors of HBV and syphilis serostatus at p-value of < 0.05. Two hundred ninety of the total 300 pregnant women participated in the study resulting in a response rate of 96.7%. Seroprevalence of HBV and syphilis infection were found to be 4.5 % and 2.4 % respectively. Having multiple sexual partner [AOR=3.99, 95%Cl= 1.20-13.38, p=0.025] was significantly associated with Hepatitis B virus infection. An intermediate sero-prevalence of hepatitis B virus was detected among participants in our study area. Having multiple sexual partners was found to be significantly associated with the prevalence of HBV infection. Therefore; implementing strategies for routine screening of women for HBV and syphilis during antenatal care would be critical.

#### Introduction

Hepatitis B virus (HBV) is a major global public health problem affecting millions of people across the world. Pregnant women experiencing unattended syphilis and HBV infection may face adverse outcomes which can happen both to the fetus and the pregnant women<sup>1</sup>. Syphilis and HBV infection are mainly transmitted via close contact such as blood transfusion, sexual intercourse, from mother to the newborn during and after birth.<sup>2</sup> Syphilis during pregnancy can cause stillbirth, abortion, premature delivery, and malformation. <sup>1</sup> Newborns infected with HBV, in most cases acquired from mother, at an early age have a high chance of developing the chronic liver disease (CLD) which may lead to liver cirrhosis and hepatocellular carcinoma (HCC). <sup>2</sup> The prevalence of syphilis varies from place to place; it ranges from 0.02% to 4.5% in developed countries. <sup>3, 4</sup> An estimated 36 million people are infected with syphilis worldwide, with 12 million new infections

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are reported every year, and of which 2 millions are pregnant women. <sup>5</sup> Over 90% of these infections occur in resource-limited countries.<sup>6</sup> The prevalence of syphilis among pregnant women in Africa region was estimated to be 2 %. <sup>7</sup> In 2016, the World Health Organization (WHO) proposed a strategy to control and prevent sexually transmitted infections (STI). The goal was to reduce the burden of syphilis by about 90% and congenital syphilis to less than 40 cases per 100,000 live births. <sup>8</sup>

Approximately two billion people have serological evidence of HBV infection worldwide. In the year 2015, 3.5% of the world's populations were carriers of HBV infection. Every year greater than 20 million people are infected with HBV. About 1.34 million deaths occurred due to HBV infection. The majority of deaths were due to CLD or cirrhosis induced by chronic infections.<sup>9</sup> The burden of HBV infection is high in Africa, carrying the second-highest number of chronically infected people. More than 65 million HBV carriers live in Africa mainly in Sub-Saharan Africa with about 50 million CLD.<sup>10,11</sup>

The seroprevalence of HBsAg among pregnant women in Ethiopia ranges from 3% to 6.1%. <sup>12, 13</sup> Moderate prevalence, 3% to 7%, of HBV was reported from Ethiopia. <sup>14</sup>A systematic review reported an overall prevalence of 7.4% HBV among the general population in Ethiopia. <sup>15</sup>

Since HBV is found in body fluids it can be transmitted both horizontally and vertically. <sup>9</sup>HBV modes of transmission differ depending on the degree of endemicity. In areas where there is a high seroprevalence of HBV, the transmission is mostly vertical: from during infected mothers to neonates delivery. Transmission via close contact (horizontal) among children is also commonplace in areas of high and intermediate occurrence. HBV transmissions in lowendemic areas occur during adolescence and early maturity and are mostly associated with high-risk behaviors comprised of unsafe sexual contact and drug use through injection. 9 Approximately, 10% to 20% of HBsAg-positive pregnant women transmit HBV to their babies usually during birth or soon after birth. Mothers who are positive for both HBsAg and HBeAg have about 100% chance of transmitting HBV to their newborns. Over 85-90% of newborns infected at an early age will eventually become chronic carriers of the virus.<sup>16-18</sup> A study from Ethiopia indicated that 75% of newborns born from HBV infected mother were positive for HBsAg.<sup>14</sup> The rate for chronicity is much lower (<5%)

among the adult population; however, it may reaches 90% in infected newborns. <sup>19, 20</sup>

Minimizing vertical transmission of HBV is key in reducing the disease burden in the African continent. An effective strategy for reducing the incidence of chronic HBV infections involves maternal screening along with post-exposure prophylaxis (both active and passive immunization) immediately after birth for newborn born to HBsAg-positive mothers.<sup>21</sup> Since pregnant women who are positive for syphilis and HBV are at increased risk of transmitting to their babies, determining the magnitude of syphilis and HBV and its predictors is required to inform policy-makers and health care providers so as to facilitate evidence-based interventions. Moreover, HBV infection poses a heavy burden on the health care system because of the costs of treatment of the liver in countries where HBV is highly endemic. The aim of this study was to determine the seroprevalence of syphilis and HBV and associated factors among pregnant women.

#### **Materials and Methods**

#### Study design and period

A cross-sectional study was conducted at Saint Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia from July to September 2019. Monthly more than 830 pregnant women visit antenatal care clinic of SPHMMC for routine follow-up.

#### **Eligibility criteria**

Pregnant women who visited SPHMMC for antenatal care during the study period, those who were in any gestational age, and those who were willing to participate in the study were included. However, pregnant women who were on active labor were excluded from the study.

#### **Study variables**

#### **Dependent variables**

Seroprevalence of syphilis and HBV

#### **Independent variables**

Sociodemographic factors such as age, and educational status, income; gestational factors such as gestational age, history of natural abortion, number of sexual partners, history of stillbirth.

#### Sample size and sampling technique

The sample size was determined using a single population proportion formula, 95% level of confidence, the prevalence of Hepatitis B virus 7.2% reported from southern Ethiopia, <sup>21</sup> 3% degree of precision, and 5% none-respondent rate. Finally, the calculated sample size was 300.

#### **Sampling technique**

Systematic random sampling technique was used to recruit 300 study participants. Considering a three months of study period, an average estimate of 1425 pregnant women were expected to visit the antenatal care unit according to hospital plan and the past three months performance document review.

To decide the interval (k) estimated total number of ANC attendant was divided to allocated sample size of the study, ie, K=N/n, where N=total number of ANC, n= required sample size and K= sampling interval.

Thus  $k=1425/300=4.75 \approx 5$ . The first study subject was selected by a lottery method and then every 5<sup>th</sup> woman was invited to participate in the study until the required sample size was obtained.

#### **Data collection**

Sociodemographic (age, occupation, educational status, residence, monthly income, marital status), gestational characteristics (gestational age, number of previous pregnancies, history of abortion, history of stillbirth, history of neonatal death) and risk factors (blood transfusion and multiple sexual parents) were captured by nurse using a semi-structured questionnaire via interview. After written consent was obtained, five milliliters of venous blood was collected from each study participants.

The serum was separated by centrifugation at 3000 rpm for 5 minutes and tested for HBsAg rapid test (Zhejiang Orient Gene Biotech Co., Ltd) and syphilis seropositivity was tested using a rapid plasma reagin (RPR) (Zhejiang Orient Gene Biotech Co., Ltd).

Reactive sample were confirmed by using *Treponema* pallidum hemaggultinaion assays (TPHA (Omega Immuterp TPHA, UK) following the manufacture's instruction.

#### **Rapid HBsAg screening test**

Rapid HBsAg screening test has a sensitivity of 99.8% and specificity of greater than 99.7 %.<sup>22</sup> It is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum. The membrane was precoated with anti HBsAg antibodies on the test band region and anti-mouse antibodies on the control band region. During testing, the serum sample reacts with the dye conjugate (mouse anti HBsAg antibody colloidal gold conjugate) that was coated on the test strip. Then, the mixture by capillary action reacts with anti-HBsAg antibodies on the membrane and generates a red band. Presence of this red band indicates a positive result while its absence indicates a negative result.

#### **Data quality control**

Initially, the questionnaire was prepared in English then it was translated to Amharic and finally translated back to English by different translators to check its consistency of the questionnaire. Before the actual data collection, pretest was conducted on 5% of the sample size at Tikur Anbessa Specialized Hospital to ensure the validity and reliability of the survey tools. The result was used to correct the questionnaires. Standardized procedure was strictly followed during the blood sample collection, storage, and analytical process. Positive and negative controls were run according to the test manual and quality assurances were followed. HBsAg positive participates serum was double-checked using ELISA in the referral laboratory located in the city.

#### Data analysis

Data were entered and analyzed using version 22 of the Statistical Package for Social Sciences (SPSS). Descriptive statistics were used to define the characteristics of syphilis and HBV participants. The association between dependent and independent variables was checked using bivariate analysis. Variables with a p-value of less than 0.25 were further analyzed using the multivariable regression analysis model. Variables with a p-value of < 0.05 were considered as cut point to determine a significant association.

#### **Results and Discussion**

#### Sociodemographic and gestational profile

Two hundred ninety of the total 300 women participated in the study resulting in a response rate of 96.7%. The majority of participants were in the age group of 26-34 and more than half (67.2%) them were house-wives. Almost all (96.6%) of the study participants were married. Nearly half (45.5%) of the study participants had income per month  $\geq$ 2000 Ethiopian Birr and most of them were urban residence (Table 1).

#### Seroprevalence and associated factors

Among 290 participants included, 7(2.4%) were positive for the antibody produced to *T. pallidum*; 13(4.5%) were positive for HBsAg. Among those positive for hepatitis B surface antigen, the majority of participants of them (92.3 %, 61.5%, 53.8 %), 46.2%, 46.1) and were married, housewife, age between 26-34 years, had multiple sexual partners and history of abortion respectively.

Among factors assessed, only multiple sexual partners were significantly associated with seroprevalence of HBV infection. Other factors were not significantly associated with seroprevalence of syphilis and HBV infection (Tables 2 and 3).

Syphilis and HBV infection during pregnancy can cause serious problems among the fetus and newborns. Several efforts have been made in Ethiopia to reduce transmission of syphilis and HBV to newborns before and during birth; however, the problem is not totally controlled. As part of this, we aimed to determine the current prevalence of syphilis and HBV infections among pregnant women.

In the current study, the seroprevalence of syphilis among pregnant women was detected to be 2.4%. Our finding is high compared to the seroprevalence of syphilis among pregnant women reported from other parts of Ethiopia (0-1.8%), <sup>13, 23-25</sup> Nigeria (0.6%), <sup>26</sup> Cameron (1.7%), <sup>27</sup> and Tanzania (1.6%). <sup>28</sup> The finding of the current study is lower than study conducted in other parts of Ethiopia (3.7%), <sup>29</sup> Tanzania (7.3%), <sup>30</sup>Nigeria (9.9%), <sup>31</sup> and South Sudan (22.1%). <sup>32</sup>

Table.1 Sociodemographic and gestational characteristics of pregnant women at Saint Paul Hospital Medical College,
Addis Ababa, Ethiopia
(N-200)

		(1N-290).		
Variable	Categories	Frequency n (%)		
	18-25	101 (34.8)		
Age in years	26-34	169 (58.3)		
	35-43	20 (6.9)		
	Housewife	195 (67.2)		
Occupational status	Private Business	55 (19.0)		
	Government Employee	40 (13.8)		
Marital status	Married	280 (96.6)		
	Single	10 (3.4)		
	No formal education	29 (10.0)		
	Primary school	123 (42.4)		
Educational status	Secondary school	81 (27.9)		
	College and above	57 (19.7)		
	Urban	249 (85.9)		
Residence	Rural	41 (14.1)		
	≤500	20 (6.9)		
Monthly income in ETB	501-1000	58 (20.0)		
	1001-1500	29 (10.0)		
	1501-2000	51 (17.6)		
	≥2000	132 (45.5)		

ETB: Ethiopian Birr

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## **Table.2** Seroprevalence of syphilis infection and associated risk factors among pregnant women attending the antenatal clinic of Saint Paul hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019

(N=290)

Variables		Serostatus for <i>T.pallidum</i>		Bivariate Analysis		Multivariate Analysis		
	Category	Reactive	Non-reactive	COR (95%CI)	<i>p</i> -value	AOR (95%CI)	<i>p</i> -value	
		n (%)	n (%)					
Age in years								
	18-25	3(3.0)	98 (97.0)	1				
	26-34	3(1.8)	166 (98.2)	2.56 (0.42-15.56)	0.309	-	-	
	35-43	1(5.0)	19 (95.0)	0.28 (0.04-1.77)	0.274	-	-	
			Occupation sta	atus				
	Housewife	`4 (2.1)	191 (97.9)	1.22 (0.13-11.25)	0.858			
	Private business	2 (3.6)	53 (96.4)	0.68 (0.06-7.76)	0.756	-	-	
	Government employee	1 (2.5)	39 (97.5)	1				
			Educational st	atus				
	No formal education	3(10.3)	26 (89.7)	1				
	Primary school	2 (1.6)	121 (98.4)	0.16 (0.02-1.56)	0.113	0.19 (0.02-2.10)	0.175	
	Secondary school	1 (1.2)	80 (98.8)	1.08 (0.96-12.17)	0.950	1.62 (0.13-20.69)	0.710	
	College and above	1(1.8)	56 (98.2)	1.43 (0.09-23.32)	0.802	2.13 (0.12-39.38)	0.612	
Residence							-	
	Urban	6 (2.4)	243 (97.6)	1.01 (0.12-8.63)	0.991			
	Rural	1 (2.4)	40 (97.6)	1				
			Monthly income	in ETB				
	<500	1(4.8)	20 (95.2)	0.15 (0.009-2.56)	0.192	0.35 (0.01-8.48)	0.517	
	501-1000	3(5.2)	54 (94.8)	0.14 (0.01-1.36)	0.09	0.16 (0.01-1.90)	0.147	
	1001-1500	1(3.3)	29 (96.7)	0.22 (0.01-1.36)	0.294	0.51 (0.02-11.07)	0.666	
	1501-2000	1 (1.9)	50 (98.1)	0.39 (0.02-6.27)	0.502	0.37 (0.02-6.40)	0.494	
	>2001	1 (0.8)	130 (99.8)	1				
			Marital stat	us				
	Married	5 (2.5)	275 (97.5)	13.75 (2.30-81.9)	0.004	7.77 (0.99-60.50)	0.052	
	Single	2(22.2)	8 (77.8)	1				
Gestational age								
	1 <sup>st</sup> trimester	2 (3.8)	50 (96.2)	1				
	2 <sup>nd</sup> trimester	2(2.9)	68 (97.1)	1.36 (0.19-9.98)	0.762	-	-	
	3 <sup>rd</sup> trimester	3 (1.8)	165 (98.2)	2.20 (0.36-13.54)	0.395	-	-	
Multiple sexual partners								

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	Yes	1(14.3)	6(85.7)	1.32 (0.16-11.20)	0.800	-	_	
	No	51(18.0)	232(82.0)	1				
Previous pregnancies								
	No birth	4 (4.7)	82 (95.3)	0.73 (0.80-6.83)	0.784	-	-	
	1-2 Births	3 (1.1)	173 (98.9)	0.36(0.27-35.20)	0.364	-	-	
	$\geq 3$ births	0	28 (96.6)	1				
History of abortion								
	Yes	4 (3.6)	108 (94.4)	3.30 (0.50-10.45)	0.283	-	-	
	No	3 (1.7)	175(98.3)	1				
Blood transfusion								
	Yes	1 (2.0)	48 (98.0)	2.16 (0.74-9.39)	0.32	-	-	
	No	6 (2.2)	235 (97.8)					

COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, n: number, ETB: Ethiopian Birr, 1: reference

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# Table.3 Seroprevalence of HBV infection and associated risk factors among pregnant women attending the antenatal clinic of Saint Paul hospital Millennium Medical College, Addis Ababa, Ethiopia, 2019 (N=290).

Variables		HBV status		Bivariate Analysis		Multivariate Analysis			
	Category	Positive n (%)	Negative n (%)	COR (95%CI)	<i>p</i> -value	AOR (95%CI)	<i>p</i> -value		
Age in years									
	18-25	4 (4.0)	97 (96.0)	1					
	26-34	6 (3.6)	16 (96.4)	1.12 (0.31-4.07)	0.863	0.93 (0.25-3.52)	0.913		
	35-43	3 (15)	17 (85.0)	0.23(0.05-1.14)	0.072	0.24 (0.05-1.28)	0.095		
			Occupation		•				
	Housewife	8 (4.1)	185 (95.9)	1.23(0.25 - 6.02)	0.798				
	Private business	3 (5.4)	52(94.6)	0.91(0.14 - 5.73)	0.922				
	Government employee	2 (4.8)	40(95.2)	1					
			Educational stat	us					
	No formal education	1 (3.4)	28 (96.2)	1					
	Primary school	7 (5.4)	116 (96.6)	0.59 (0.07-5.00)	0.630				
	Secondary school	1 (1.2)	80 (98.8)	2.86 (0.17-47.22)	0.463				
	College and above	4(7.0)	53 (93.0)	0.47 (0.05-4.44)	0.512				
	Residence								
	Urban	11 (4.4)	238 (95.58)	1.11(0.24-5.20)	0.895				
	Rural	2 (4.88)	39 (95.12)	1					
			Monthly income in	ETH					
	<500	1(4.7)	20 (95.3)	0.47(0.05-4.73)	0.521	0.47(0.04-4.49)	0.532		
	501-1000	6(10.5)	51(89.5)	0.19(0.05-0.83)	0.036	0.21(0.05-1.02)	0.064		
	1001-1500	1(3.3)	29(96.7)	0.68(0.07-6.77)	0.742	0.87(0.08-9.20)	0.909		
	1501-2000	2 (3.9)	49 (96.1)	0.57(0.09-3.54)	0.550	0.536(0.08-3.44)	0.511		
	>2001	3 (2.3)	128 (97.7)	1					
			Marital status						
	Married	12(4.3)	268 (96.7)	2.48(0.29-21.2)	0.406	-	-		
	Single	1(10.0)	9 (90.0%)	1					
			Gestational age	9					
	1 <sup>st</sup> trimester	3 (5.7)	50 (94.3)	1					
	2 <sup>nd</sup> trimester	4 (5.8)	65 (94.2)	98(0.21-4.56)	0.974	-	-		
	3 <sup>rd</sup> trimester	6(3.6)	162 (96.4)	1.6(0.39-6.71)	0.506	-	-		
Multiple sexual partner									
	Yes	6(11.5)	46(88.5)	4.3(1.38-13.40)	0.012	3.99(1.20-13.38)	0.025		

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	No	7(2.9)	231(97.1)	1			
Number of previous pregnancies							
	No birth	4(4.5)	85 (95.8)	1			
	1-2 Births	8 (4.1)	186 (95.9)	1.10 (0.32-3.72)	0.886	-	-
	≥3 births	1 (14.3)	6(85.7)	0.28 (0.03-2.99)	0.290	-	-
History of abortion							
	Yes	7 (6.3)	105 (93.7)	1.9 (0.63-5.84)	0.256	-	-
	No	6 (3.4)	178 (96.6)	1			
History of blood transfusion							
	Yes	3 (6.1)	46(93.9)	1.51 (0.40- 5.70)	0.545	-	-
	No	10 (4.1)	231 (95.9)	1			

COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, n: number, ETB: Ethiopian Birr, 1: reference

The variation observed can be due to the laboratory methods used for the diagnosis and the absence or presence of an effective prevention strategy in the respective countries. Moreover, screening of syphilis is not performed in the majority of private health facilities providing antenatal care in Ethiopia. Our finding is comparable to finding reported from Gondar, Ethiopia (2.9%), <sup>33</sup> Benin (2.5%), <sup>34</sup> and Zambia (2.2%). <sup>35</sup> In the present study, the seroprevalence of HBV among pregnant women (4.5%) was moderate. A similar finding was reported from Northwest Ethiopia (4.4%)<sup>36</sup>, Dessie, Ethiopia, (4.9%), and Arba-Minch, Ethiopia (4.3%).<sup>38</sup> In contrast to the present study, higher prevalence was reported from Southern Ethiopia (6.1-7.2%),<sup>13,22</sup> and Eastern Ethiopia (6.9%). <sup>39</sup> On the other hand, the lowest prevalence was reported from Southern Ethiopia (2.3%)  $^{40}$  and Addis Ababa, Ethiopia (3%).  $^{14}$ 

The seroprevalence of HBV we found in the current study is lower than a report from Gambia (9.2%), <sup>41</sup> Taiwan (15.5%), <sup>42</sup> Uganda (11.8%), <sup>43</sup> Kenya (9.3%), <sup>44</sup>Ghana (7.7%), <sup>45</sup>and Sudan (7.5%). <sup>46</sup> It is high compared to finding, reported from Eritrea (3.2%) <sup>47</sup> and pooled prevalence from Iran (1.2%). <sup>48</sup> The comparable finding was reported from Laos (5.4%) <sup>49</sup> and Ethiopia (4.7%). <sup>50</sup> Additionally, the finding of this study is in line with a pooled prevalence reported from Ethiopia (4.7%). <sup>2</sup> This difference in estimates might be attributed to the time gap between study periods, geographical setting of study population and difference in sample size of the study.

In this study, none of the factors were significantly associated with seroprevalence of syphilis. Furthermore, this study revealed that the probability of acquiring HBV infection was higher among pregnant women with multiple sexual contacts. The odd of having HBsAg was at most 4 times higher among pregnant women who had a history of multiple sexual partners compared to their counterparts. This finding is consistent with previous study reported from Eastern Ethiopia<sup>11</sup> Uganda, <sup>43</sup> Ghana, <sup>44</sup> and Northwest Ethiopia.<sup>50</sup> The possible justification could be due to the fact that HBV might be found in blood, semen and other body fluids that are exchanged during sexual contact. For instance; women who had multiple sexual partners are more likely to have sexual contact that increases the risks of HBV and other sexually transmitted infections.

In this study, moderate seroprevalence of HBV among pregnant women was found. Among factors assessed

only multiple sexual partners was significantly associated with seroprevalence of HBV infection.

Screening of pregnant women for HBV irrespective of the basis of risk factors and intensified prevention targeting this group may reduce mother to child transmission of HBV infection.

Health education programs on the mode of HBV transmission, high-risk behaviors, and methods of prevention should be instituted during antenatal care clinics at health facilities.

#### **List of Abbreviations**

HBV: Hepatitis Virus, STI: Sexual transmitted infection, HBsAg: Hepatitis B surface Antigen, SPHMMC: Saint Paul's Hospital Millennium Medical College, HCC: Hepatocellular carcinoma, CLD: Chronic liver disease

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