

Seroprevalence of Syphilis, HBV and Its Associated Risk Factors Among Pregnant Women Attending at Saint Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia

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Research

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Abstract

Background: Syphilis and Hepatitis B Virus (HBV) share similar modes of transmission. Their occurrence during pregnancy has an adverse effect both on the fetus and pregnant women. The aim of this study was to determine the seroprevalence and associated risk factors of syphilis and HBV among pregnant women.

Methods: A cross-sectional study was undertaken 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. Chromatographic kits were used to detect the presence of Hepatitis B surface antigen (HBsAg) and antibody against syphilis infection from the serum of study participants. Data were analyzed by SPSS version 22. Bivariate and multivariable analysis was employed to identify factors associated with seroprevalence of syphilis and HBV. Variables with a *p*-value of < 0.05 were considered as cut point to determine a significant association.

Results: 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 HBV infection.

Conclusion: In the study the seroprevalence of HBV and syphilis is moderate; having multiple sexual partners was found to be significantly associated with the prevalence of HBV infection.

Background

Syphilis and Hepatitis B Virus (HBV) share a similar mode of transmission. Pregnant women experiencing unattended syphilis and HBV infection may face adverse outcomes which can happen both to the fetus and the pregnant women. 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 [1, 2].Syphilis during pregnancy can cause stillbirth, abortion, premature delivery, and malformation [1]. 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) [2].

The prevalence of syphilis varies from place to place; it ranges from 0.02–4.5% in developed countries [3]. A high prevalence of syphilis (18%) among pregnant women was reported from Africa. Globally, close to 10 million people are infected with syphilis worldwide. Most of the syphilis cases are seen in Asia and Africa [5]. Over 90% of these infections occur in resource-limited countries [6]. The prevalence of syphilis among pregnant women in Africa region was estimated to be 2 % [7]. 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 [8].

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 [9]. 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 [10]. Approximately, 56% of the populations in Africa have evidence of past exposure to HBV infection. The seroprevalence of hepatitis B surface antigen (HBsAg) in Africa ranges from 6–20% [11].

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

Since HBV is found in body fluids it can be transmitted both horizontally and vertically [9]. 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 infected mothers to neonates during delivery. Transmission via close contact (horizontal) among children is also commonplace in areas of high and intermediate occurrence. HBV transmissions in low-endemic 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, 10-20% 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 [16–18]. A study from Ethiopia indicated that 75% of newborns born from HBV infected mother were positive for HBsAg [14]. The rate for chronicity is much lower (< 5%) among the adult population; however, it may reaches 90% in infected newborns [19, 20].

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 [21].

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.

Methods

Study design and period

This is a cross-sectional study was conducted at Saint Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia from July to September 2019. 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. 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 [22], 3% degree of precision, and 5% none-respondent rate. Finally, the calculated sample size was 300. Participants were recruited using systematic random sampling technique.

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 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) following the manufacture's instruction. Known positive and negative control samples were run to control the quality of HBsAg kits. Syphilis seropositivity was tested using a rapid plasma reagin (RPR) (Zhejiang Orient Gene Biotech Co., Ltd) following manufacture instruction. Reactive sample were confirmed by using *Treponema palladium* hemaggultinaion assays (TPHA (Omega Immuterp TPHA, UK)

Data quality control

The questionnaire was pretested before use in the actual study on 5% of the total sample size. 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. The methods used for the detection of antibodies to *T. pallidum* and HBsAg were checked by known positive and negative controls. Quality control inbuilt in the kit was also used.

Data analysis

Data were entered and analyzed by Epi data Version 3.1 and SPSS software version 22 respectively. Descriptive statistics were used to determine the seroprevalence of syphilis and HBV. 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 analysis model. Variables with a *p*-value of < 0.05 were considered as cut point to determine a significant association.

Results

Sociodemographic and gestational profile

In this study, out of 300 pregnant women approached, 290 participated with a response rate of 96.7%. The majority of participants were in the age group of 26–34; most of them were house-wives (Table 1).

Table 1

| Sociodemographic and gestational characteristics of pregnant women | |
|--|--|
| at Saint Paul Hospital Medical College, Addis Ababa, Ethiopia (N = 290). | |

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

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 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).

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 | Category | Serostatus <i>T.pallidun</i> | | Bivariate Anal | Bivariate Analysis | | Multivariate Analysis | |
|-------------|------------------------|---------------------------------|---------------------------|-----------------------|--------------------|----------------------|-----------------------|--|
| | | Reactive n (%) | Non- reactive n (%) | COR(95%CI) | p-value | AOR(95%CI) | p- value | |
| Age in year | S | | | | | | | |
| | 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 | status | | | | | | | |
| | 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 | | | | |
| Educationa | l status | | | | | | | |
| | 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 | | | |
| | | 1 (2.4) | 40 (97.6) | 1 | | | | |

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

| Variables | Category | tegory Serostatus for <i>T.pallidum</i> | | Bivariate Anal | Bivariate Analysis | | Multivariate Analysis | |
|----------------------------|------------------|--|---------------------------|--------------------------|--------------------|-----------------------|-----------------------|--|
| | | Reactive n (%) | Non- reactive n (%) | COR(95%CI) | p-value | AOR(95%CI) | p- value | |
| | < 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 | tus | | | | | | | |
| | 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 | | | | |
| Gestationa | l age | | | | | | | |
| | 1st trimester | 2 (3.8) | 50 (96.2) | 1 | | | | |
| | 2nd trimester | 2(2.9) | 68 (97.1) | 1.36(0.19– 9.98) | 0.762 | - | - | |
| | 3rd trimester | 3 (1.8) | 165 (98.2) | 2.20 (0.36- 13.54) | 0.395 | - | - | |
| Multiple se | xual partners | | | | | | | |
| | Yes | 1(14.3) | 6(85.7) | 1.32(0.16- 11.20) | 0.800 | - | - | |
| | No | 51(18.0) | 232(82.0) | 1 | | | | |
| Previous pr | regnancies | | | | | | | |
| | 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 | - | - | |
| COR: crude 1: reference | | R: adjusted o | dds ratio, CI: (| confidence inter | val, n: num | ber, ETB: Ethiop | ian Birr | |

| Variables | Category | | Serostatus for <i>T.pallidum</i> | | Bivariate Analysis | | Multivariate Analysis | | |
|---|---------------------|-------------------|-------------------------------------|----------------------|--------------------|------------|-----------------------|--|--|
| | | Reactive n (%) | Non- reactive n (%) | COR(95%CI) | p-value | AOR(95%CI) | p- value | | |
| | \geq 3 births | 0 | 28 (96.6) | 1 | | | | | |
| History of a | 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 trans | sfusion | | | | | | | | |
| | 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 | | | | | | | | | |

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 | Category | Category HBV status E | | Bivariate Ana | alysis | Multivariate Analysis | |
|-------------|------------------------|-----------------------|-------------------|----------------------|-------------|-----------------------|-------------|
| | | Positive n (%) | Negative n (%) | COR (95%Cl) | p- value | AOR (95%Cl) | p- value |
| Age in year | S | | | | | | |
| | 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 | ı | | | | | | |
| | 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 | | | |
| Educationa | al status | | | | | | |
| | 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 in | come in ETH | | | | | | |

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

| Variables | Category | HBV status | | Bivariate Analysis | | Multivariate Analysis | |
|--------------|------------------|-------------------|-------------------|----------------------|-------------|-----------------------|-------------|
| | | Positive n (%) | Negative n (%) | COR (95%Cl) | p- value | AOR (95%Cl) | p- value |
| | < 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 stat | tus | | | | | | |
| | Married | 12(4.3) | 268 (96.7) | 2.48(0.29- 21.2) | 0.406 | - | - |
| | Single | 1(10.0) | 9 (90.0%) | 1 | | | |
| Gestationa | l age | | | | | | |
| | 1st trimester | 3 (5.7) | 50 (94.3) | 1 | | | |
| | 2nd trimester | 4 (5.8) | 65 (94.2) | 98(0.21- 4.56) | 0.974 | - | - |
| | 3rd trimester | 6(3.6) | 162 (96.4) | 1.6(0.39- 6.71) | 0.506 | - | - |
| Multiple se | xual partner | | | | | | |
| | Yes | 6(11.5) | 46(88.5) | 4.3(1.38- 13.40) | 0.012 | 3.99(1.20- 13.38) | 0.025 |
| | No | 7(2.9) | 231(97.1) | 1 | | | |
| Number of | previous pregnar | ncies | | | | | |
| | 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 | - | - |
| | \geq 3 births | 1 (14.3) | 6(85.7) | 0.28 (0.03- 2.99) | 0.290 | - | - |
| History of a | abortion | | | | | | |
| - | odds ratio, AOR: | adjusted od | ds ratio, CI: co | nfidence interv | al, n: num | ber, ETB: Ethiop | oian Birr |

| Variables | Category | HBV statu | HBV status | | Bivariate Analysis | | Multivariate Analysis | |
|--|-------------------|-------------------|-------------------|----------------------|--------------------|----------------|-----------------------|--|
| | | Positive n (%) | Negative n (%) | COR (95%Cl) | p- value | AOR (95%Cl) | p- value | |
| | 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 I | 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 | | | | | | | | |

Discussion

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%) [13, 23–25], Nigeria (0.6%) [26], Cameron (1.7%) [27], and Tanzania (1.6%) [28]. The finding of the current study is lower than study conducted in other parts of Ethiopia (3.7%) [29], Tanzania (7.3%) [30], Nigeria (9.9%) [31], and South Sudan (22.1%) [32]. The variation observed can be due to the laboratory methods used for the diagnosis, sample collection period and the absence or presence of an effective prevention strategy in the respective countries. For instance, the screening of syphilis is not regularly performed in all government health facilities found in Ethiopia due to various reasons. Moreover, screening of syphilis is not performed in the majority of private health facilities providing antenatal care in Ethiopia. Our finding of syphilis seroprevalence among pregnant women (2.4%) is comparable to finding reported from Gondar, Ethiopia (2.9%) [33], Benin (2.5%)[34], and Zambia (2.2%) [35].

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%) [36], Dessie, Ethiopia, (4.9%), and Arba-Minch, Ethiopia (4.3%) [38]. In contrast to the present study, the high prevalence was reported from Southern Ethiopia (6.1–7.2%) [13, 22], and Eastern Ethiopia (6.9%) [39]. On the other hand, the low 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%) [41], Taiwan (15.5%) [42], Uganda (11.8%) [43], Kenya (9.3%) [44], Ghana (7.7%) [45], and Sudan (7.5%) [46]. It is high compared to finding reported from Eritrea (3.2%) [47] and pooled prevalence from Iran (1.2%) [48]. The comparable finding was reported from Laos (5.4%) [49] and Ethiopia (4.7%) [50]. Additionally, the finding of this study is in line with a pooled prevalence reported from Ethiopia (4.7%) [2].

In this study, none of the factors were significantly associated with seroprevalence of syphilis. Having multiple sexual partner was significantly associated with seroprevalence of HBV (p = 0.025), similar finding was reported Ethiopia [50]. Even though it is not significantly associated (p > 0.05) high seroprevalence of syphilis was found among pregnant women who were married.

Conclusions

In this study, moderate seroprevalence of syphilis and HBV among pregnant women was found. Among factors assessed only multiple sexual partners was significantly associated with seroprevalence of HBV infection.

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

Declarations

Consent for publication

Not applicable.

Availability of data and materials

All necessary data are available within the paper.

Ethical approval and consent to participant

The study was approved by the Institutional Review Board of College of Medicine and Health Sciences, Hawassa University (Ref No IRB/205/11) and Saint Paul's Hospital Millennium Medical College (Ref No pm23/368). An official permission letter was obtained from the study site. The objectives, expected outcomes, benefits, and risks of the study were explained for study patricians. They have also informed that participation in the current study voluntary and they can quit anytime they want. Data was collected after written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki.

Authors' contributions

YAW Conceived and designed the study, execution of the study, laboratory work, data acquisition data analysis and interpretation, article preparation.
MMA Conceived and designed the study, data analysis and interpretation, article preparation. All authors reviewed and agreed on the final version of the article before submission.

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Competing Interests

The authors declare no competing interest.

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