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Original article

Hepatitis B virus infection and its associated risk factors in pregnant women in Upper Egypt: A cross-sectional study

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ABSTRACT

Background: Hepatitis B virus (HBV) is transmitted from mother to child during labor. This work aimed to assess the frequency of hepatitis B surface antigen (HBsAg) seropositivity among pregnant women in Upper Egypt, to identify factors associated with virus acquisition, and to assess the predictors of anti-HBe seropositivity in pregnancy. **Methods:** This cross-sectional research was done on 300 pregnant women attending the Antenatal Care Unit of Sohag University Hospital from April 2022 to February 2023. Participants answered a survey about demographic information, HBV vaccination, and risk factors for transmission. Blood samples were tested for HBsAg. Positive samples were tested for transaminases, hepatitis B e antigen (HBeAg), anti-HBe, and viral load. **Results:** Frequency of HBsAg seropositivity was 5.33%. HBV DNA was detectable in 56.25%, median of 437 IU/ml (48-719 IU/ml); all were HBeAg negative, anti-HBe was positive at 25%. The only statistically significant difference between HBsAg-positive and negative cases was the husband's HBV infection (p = 0.005). The only statistically significant difference between anti-HBe positive and negative cases was quantitative HBsAg. One variable was significant using the univariate regression analysis (husband's HBV infection); there was no need to do multivariate regression analysis, so the husband's HBV infection was the only supposed risk factor for the mother's HBsAg positivity. **Conclusions:** The frequency of HBsAg seropositivity in pregnant women is high in Upper Egypt, denoting the importance of HBV screening during pregnancy to decrease the risk of maternofetal transmission. The detection of anti-HBe in the sera of pregnant women should be interpreted cautiously.

Introduction

Life-threatening and preventable hepatitis B virus (HBV) causes liver infections. HBV is responsible for an estimated 296 million cases of infection and 820,000 fatalities globally, primarily from cirrhosis and hepatocellular carcinoma [1]. It is estimated that between 1.5% and 9.5% of pregnant women have an HBV infection [2,3].

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HBV is predominantly transmitted from mother to child during labor [4]. In terms of maternofetal transmission, a great viral load is the most critical risk factor [5]. Hepatitis B antigen (HBeAg) seropositivity, HBV S variant, and risks of premature delivery are additional variables [6].

In Egypt, guidelines for managing HBV in pregnant women have been established by the National Committee for Control of Viral Hepatitis. These guidelines address different approaches for HBV screening, vaccination, treatment, delivery and newborn management, and follow-up of pregnant women with HBV infection [7]. Many strategies exist for reducing the possibility of maternofetal transfer. Antenatal screening with hepatitis B surface antigen (HBsAg) for all pregnant women should be the first step. Infants born to mothers who test positive for HBV should be passively and actively vaccinated throughout the first twelve hours of their lives. Finally, beginning in weeks 24-28 and continuing for 12 weeks following delivery, pregnant women with great viremia (≥200,000 IU/mL) should be administered antiviral prophylaxis [8].

Many authors reported a significant association of quantitative HBsAg and HBV viral load [9,10]. In addition, there is evidence from several sources that correlate HBeAg seropositivity to an elevated viral load [11]. The detection of Hepatitis B e antibodies (anti-HBe) in the circulation of chronic HBV patients indicates suppression of viral replication [12]. This explains the proposed protective role of anti-HBe against maternofetal transmission [13-15]. Thus, this work aimed to assess the frequency of HBsAg seropositivity among pregnant women in Upper Egypt, to identify factors associated with virus acquisition, and to assess the predictors of anti-HBe seropositivity in pregnancy.

Material and methods

Study area

Participants were recruited from the Antenatal Care Unit of Sohag University Hospital.

Study design and ethical considerations

This cross-sectional research was done on 300 pregnant women attending the Antenatal Care Unit of Sohag University Hospital from April 2022 to February 2023 after obtaining ethical approval from the Scientific Research Ethical Committee, Faculty of Medicine, Sohag University (IRB: Soh-Med-22-03-33). Clinical Trials registration number: NCT05442372. We included all pregnant women who attended the antenatal care and agreed to participate in the study during the study period.

Data collection procedure

Participants answered a brief survey that included demographic information, history of HBV vaccination, and potential risk factors for HBV transmission such as previous operations, dental procedures, tattooing, blood transfusion, family history of HBV infection, and HBV infection of the husband.

Blood sample collection and analysis

Blood samples were aspirated from pregnant women on both plain (for serum isolation) and EDTA-supplemented (for plasma isolation) blood-collecting tubes under complete aseptic conditions. Samples were centrifuged at 5000 rpm for 10 minutes and then stored at -20 °C for further analysis. We investigated HBsAg with a Cobas e411 analyzer (F. Hoffmann, La Roche, Basel, Switzerland) that uses electrochemiluminescence immunoassay technology. The software generated the results on its own. The measuring range was 50–13,000 IU/mL, lower detection limit: of 0.5 IU/ml. All positive samples were investigated for alanine transaminase (ALT) and aspartate transaminase (AST) by the Cobas c311 chemical analyzer (Roche Diagnostic GmbH, Mannheim, Germany) (measuring range: 5 - 700 U/L), HBeAg (cut off value: 1, lower detection limit: ≤ 0.3 IU/ml), and anti-HBe by the Cobas e411 analyzer (F. Hoffmann, La Roche, Basel, Switzerland) (cut off value: 1, lower detection limit: ≤ 0.2 IU/ml), and quantitative detection of HBV deoxyribonucleic acid (DNA) by Rotor-GeneQ real-time polymerase chain reaction (PCR) (QIAGEN, Hilden, Germany) (Analytical sensitivity: 0.02 IU /micro L).

Statistical analysis

- Statistical package for social sciences (IBM-SPSS), version 25 (IBM-Corporation, Chicago, USA; August 2017) was used for statistical data analysis.
- Data were expressed as mean, standard deviation (SD), number and percentage. Mean and SD were used as descriptive value for quantitative data.
- Student t-test was used to compare the means between two groups and Mann-Whitney test was used instead of student t-test for non-parametric data to compare medians rather than means between the two groups.

- Pearson Chi-square test was used to compare percentages of qualitative variables, and Fisher's exact test was used instead for non -arametric data.

- Pearson correlation test was used to compare two quantitative variables. The value of (r) is explained in the following figures:

  - r < 0.2-0.4 ➔ weak correlation
  - r between 0.4-0.7 ➔ moderate correlation
  - r > 0.7 ➔ strong correlation

- Receiver Operating Characteristics curve (ROC curve) analysis was done to assess the predictive value of both quantitative HBsAg and PCR for positive anti-HBe

- Univariate followed by multivariate binary logistic regression analysis was done to assess the possible predictors for both HBsAg positivity, and anti-HBe antibodies seropositivity.

- For all these tests, the level of significance (*p*-value) was considered significant if <0.05 and highly significant if <0.001.

Results

The mean age of the studied population was 27.85±5.78 years, with a range of 17–42 years. The mean gestational age was 30.65±8.78, with a range of 6–40 weeks. The only statistically significant variance amongst HBsAg-positive and negative cases was the husband's HBV infection (*p* = 0.005). **(Table 1).** As shown in **figure (1)**, HBsAg seropositivity was found in 5.33% (16 cases) of the study population. Among HBsAg-positive cases, HBV DNA was detectable in 9 cases (56.25%), with a median of 437 IU/ml (range 48-719 IU/ml), and quantitative HBsAg had a median of 525 IU/ml (range 370-2850 IU/ml). All HBsAg-positive cases were HBeAg negative, while anti-HBe was positive in four cases (25%). Mean ALT and AST of HBsAg-positive cases were 11.13±2.53 IU/L and 17.81±8.22 IU/L, respectively **(Table 2).**

On comparing anti-HBe positive and negative cases, we found that quantitative HBsAg was the only highly statistically significant difference between both groups (*p* <0.001) **(Table 3)**.

As only one variable was significant using the univariate regression analysis for predictors of HBsAg seropositivity (husband's HBV infection), there was no need to do multivariate regression analysis, and so the husband's HBV infection was the only supposed risk factor for the mother's HBsAg positivity **(Table 4)**.

According to **table (5)**, none of these variables (age, gestational age, booster dose, operation, dental procedures, blood transfusion, tattoo, family history of HBV, husband's HBV infection, quantitative HBsAg, PCR, ALT, and AST) could be considered a predictor of positive anti-HBe among cases with positive HBsAg. There was no need to do multivariate regression analysis.

Table 6 displayed that the only significant association was found amongst AST levels and each of the quantitative HBsAg and ALT levels (*p*= 0.020, <0.001 respectively), with a positive and moderate correlation between AST and quantitative HBsAg and a strong correlation between AST and ALT.

According to **figure (2)**, HBsAg cut-off levels above 1635 IU/ml were associated with positive anti-HBe with a sensitivity of 100% and a specificity of 91.7%.

PCR cannot be used as a predictive test for positive anti-HBe with a non-significant relationship **(Figure 3).**

Table 1. Demographic data of the study population.

Item		**Total (N=300)**	**HBsAg-positive cases (N=16)**	**HBsAg-negative cases (N=284)**	***P* value**
Age	Mean±SD Median(range)	27.85±5.78 27(17-42)	30.31±5.43 28.5(22-40)	27.71±5.78 27(17-42)	0.080(NS)
Gestational age	Mean±SD Median(range)	30.65±8.78 34(6-40)	33.00±5.05 34.5(21-38)	30.51±8.9 34(6-40)	0.084(NS)
Booster dose	No. (%)	17(5.7%)	2(12.5%)	15(5.3%)	0.224(NS)
Operation	No. (%)	125(41.7%)	8(50%)	117(41.2%)	0.487(NS)
Dental procedures	No. (%)	95(31.7%)	6(37.5%)	89(31.3%)	0.606(NS)
Blood transfusion	No. (%)	35(11.7%)	4(25%)	31(10.9%)	0.088(NS)
Tattoo	No. (%)	2(0.7%)	0	2(0.7%)	1.000(NS)
Family history of HBV	No. (%)	9(3%)	1(6.3%)	8(2.8%)	0.433(NS)
Husband HBV infection	No. (%)	2(0.7%)	1(6.3%)	1(0.4%)	0.005*

HBV: hepatitis B virus, NS: not significant, SD: standard deviation, * significant

Table 2. Laboratory data of HBsAg-positive cases (N=16).

Item		**Value**
Quantitative HBsAg (IU/ml)	Mean±SD Median (range)	1194±1070 525 (370-2850)
PCR (IU/ml)	Mean±SD Median (range) Positive Under-detectable	433.11±233.22 437 (48-719) 9 (56.25%) 7 (43.75%)
HBeAg	Positive Negative	0 16 (100%)
Anti-HBe	Positive Negative	4 (25%) 12 (75%)
ALT (IU/L)	Mean±SD Median (range)	11.13±2.53 10 (10-18)
AST (IU/L)	Mean±SD Median (range)	17.81±8.22 15 (10-40)

ALT: alanine transaminase, Anti-HBe: hepatitis B e antibody, AST: aspartate transaminase, HBeAg: hepatitis B e antigen, HBsAg: hepatitis B surface antigen, PCR: polymerase chain reaction, SD: standard deviation.

Table 3. Comparison between anti-HBe positive and negative cases.

Item		Anti-HBe positive cases (N=4)	Anti-HBe Negative cases (N=12)	*P* value
Age	Mean±SD	31.00±8.29	30.08±4.60	0.781 (NS)
	Median (range)	31.5 (22-39)	28.5 (25-40)	
Gestational age	Mean±SD	30.75±6.08	33.75±4.71	0.410 (NS)
	Median (range)	32.5 (22-36)	35.5 (21-38)	
Booster	No. (%)	1 (25%)	1 (8.3%)	0.450 (NS)
Operation	No. (%)	1 (25%)	7 (58.3%)	0.569 (NS)
Dental	No. (%)	1 (25%)	5 (41.7%)	0.551 (NS)
Blood transfusion	No. (%)	1 (25%)	3 (25%)	1.000 (NS)
Tattoo	No. (%)	0	0	-
Family history of HBV	No. (%)	0	1 (8.3%)	1.000 (NS)
Husband HBV infection	No. (%)	0	1 (8.3%)	1.000 (NS)
Quantitative HBsAg (IU/ml)	Mean±SD Median (range)	2745±0.12 2270 (2590-2850)	676.7±625.5 500 (370-2640)	**<0.001****
PCR (IU/ml)	Mean±SD Median (range)	484.3±248.5 566 (132-672)	392.2±240.4 420 (48-719)	0.591 (NS)
ALT (IU/L)	Mean±SD Median (range)	10.25±0.50 10 (10-11)	11.42±2.88 10 (10-18)	0.443 (NS)
AST (IU/L)	Mean±SD Median (range)	21.00±3.56 20.5 (18-25)	16.75±9.16 14 (10-40)	0.389 (NS)

ALT: alanine transaminase, Anti-HBe: hepatitis B e antibody, AST: aspartate transaminase, NS: not significant, PCR: polymerase chain reaction, ** Highly significant.

Table 4. Predictors of HBsAg seropositivity.

Item	Univariate regression analysis			Multivariate regression analysis		
	Odd's ratio	**CI of Odd's**	**P value**	**Odd's ratio**	**CI of Odd's**	***P* value**
Age	1.078	0.990-1.174	0.085	1.078	0.984-1.182	0.107
Gestational age	1.042	0.968-1.121	0.278	1.044	0.961-1.134	0.307
Booster dose	2.562	0.533-12.317	0.240	2.250	0.401-12.619	0.357
Operation	1.427	0.521-3.911	0.489	1.153	0.390-3.407	0.797
Dental procedures	1.315	0.463-3.729	0.607	0.873	0.282-2.708	0.814
Blood transfusion	2.720	0.826-8.955	0.100	2.815	0.785-10.093	0.112
Tattoo	1.000	0.999-1.001	1.000	1.000	0.999-1.001	0.999
Family history of HBV	2.300	0.270-19.604	0.446	1.703	0.096-30.169	0.716
Husband HBV infection	18.867	1.125-316.534	0.041*	16.071	0.581-44.506	0.101

*Significant, CI: confidence interval.

Table 5. Predictors of Anti-HBe seropositivity among HBsAg-positive cases.

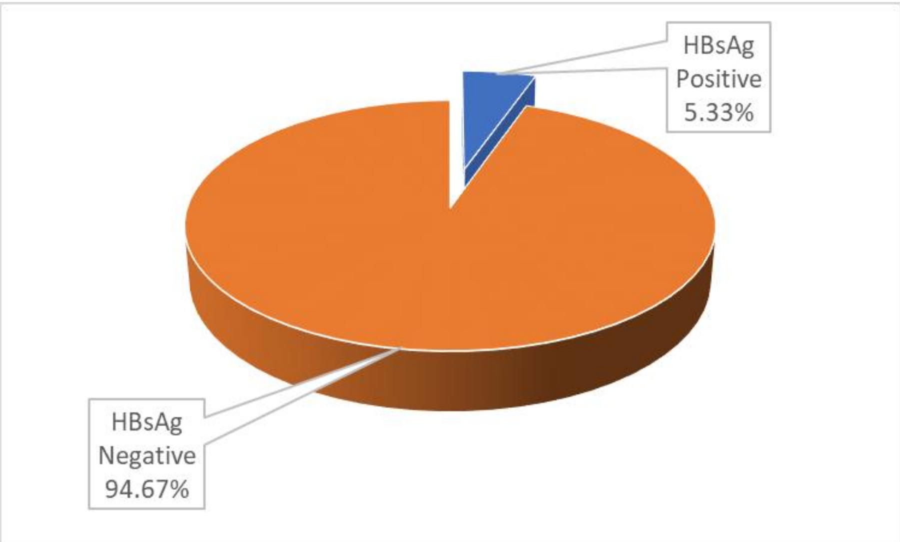
Item	**Univariate regression analysis**		
	Odd's ratio	**CI of Odd's**	***P* value**
Age	1.033	0.835-1.279	0.763
Gestational age	0.893	0.719-1.111	0.311
Booster dose	3.667	0.173-77.552	0.404
Operation	0.238	0.019-3.011	0.268
Dental procedures	0.467	0.037-5.903	0.556
Blood transfusion	1.000	0.073-13.644	1.000
Tattoo	0.333	0.033-3.987	0.057
Family history of HBV	0.999	0.998-1.003	1.000
Husband HBV infection	1.000	0.997-1.004	1.000
Quantitative HBsAg	0.680	0.005-8.40	0.417
PCR	1.002	0.996-1.008	0.539
ALT	0.675	0.204-2.239	0.521
AST	1.063	0.929-1.217	0.375

Table 6. Correlation statistics.

		Quantitative HBsAg	PCR	Age	Gestational age	ALT
PCR	R	0.177				
	P	0.649				
Age	R	0.132	-0.077			
	P	0.626	0.844			
Gestational age	R	-0.076	-0.416	0.092		
	P	0.779	0.266	0.111		
ALT	R	0.178	-0.162	0.430	0.099	
	P	0.510	0.677	0.097	0.714	
AST	R	**0.573**	0.164	0.209	-0.035	0.794
	P	**0.020**	0.674	0.437	0.897	<0.001

P: P value, R: Pearson Correlation coefficient.

Figure 1. Distribution of the study population according to HBsAg seropositivity.



HBsAg: hepatitis B surface antigen

Figure 2. ROC curve analysis for quantitative HBsAg as a predictor for positive anti-HBe.

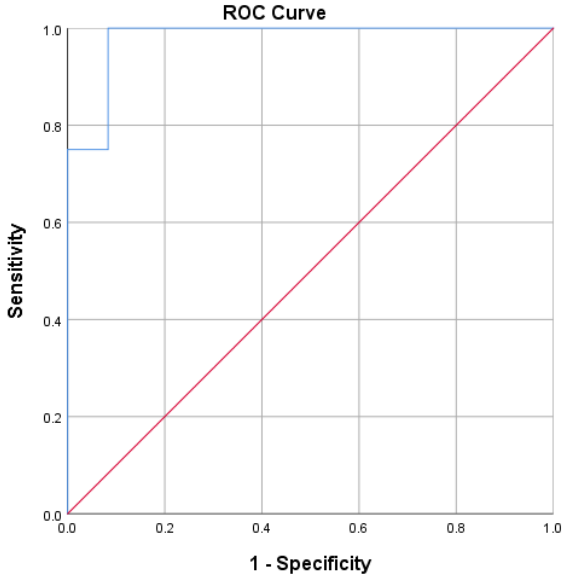
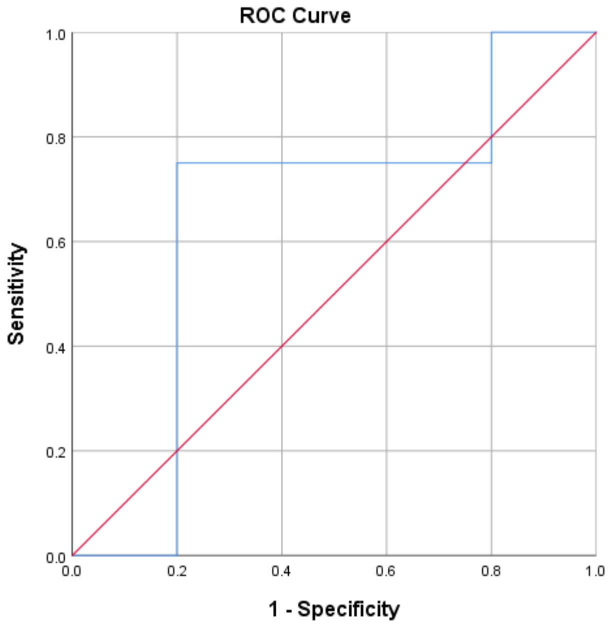


Figure 3. ROC curve analysis for PCR as a predictive for positive anti-HBe.



Discussion

The current cross-sectional study displayed that the mean age of the studied population was 27.85±5.78 years, with a range of 17–42 years. The mean gestational age was 30.65±8.78, with a range of 6–40 weeks. There were 9 women (3%) with no family history of HBV. There were 2 women (0.7%) with no husband HBV infection.

This was consistent with **Fomulu et al.** [16] who stated that the study involved 959 participants, whose ages ranged from 15 to 43 years, with an average age of 27.6 ±5.2 years. Our cases' average gestational age was 29.2 weeks, with a minimum of 6 and a maximum of 42 weeks.

Our results demonstrated a moderate frequency (5%) of HBsAg seropositivity in pregnant women. A similar percentage among pregnant women in Northeast Egypt was reported by **Kisk et al.** [17]. Also, the pooled prevalence of hepatitis B infection among pregnant women in Africa was found to be intermediate-high (6.77%) [18]. In addition, **Wondmeneh et al.** [19] reported that an intermediate endemic level of HBV infection (2–7%) was observed among pregnant women in Africa. However, there was significant heterogeneity across studies. Lower values were reported from Nigeria [20] and Saudi Arabia [21]. On the contrary, higher values were reported from Kenya [22] and Pakistan [23]. **Kumar et al.** [24]

reported that in the Asia–Pacific region the prevalence of chronic HBV infection in pregnant women varies significantly depending on the region. The variability of HBsAg seropositivity in literature could be explained by diverse cultural backgrounds and sexual practices [25].

We found that the only statistically significant variable among HBsAg-positive and HBsAg-negative cases was the husband's HBV infection. Moreover, regression analysis showed that the husband's HBV infection was the only supposed risk factor for the mother's HBsAg seropositivity. However, we did not find a statistically significant variance between HBsAg-positive and HBsAg-negative cases concerning age, gestational age, booster dose, operation, dental procedures, blood transfusion, tattoo, or family history of HBV, and these factors were not supposed to be risk factors for the mother's HBs Ag positivity.

In the same vein, our results agreed with **Rabiu et al.** [26], who detected that there was no statistically significant variance between anti-HBe positive and negative cases concerning age, previous blood transfusion, previous dental surgery, and the presence of tattoos. In addition, **El Ghitany et al.** [27] reported that tattoos were not supposed to be a risk factor for positive HBsAg. Also, a meta-analysis carried out by **Wondmeneh et al.** [19] reported that the pooled effect size of the included studies indicated non-statistically significant association between tattoos and HBV infection.

On the other hand, our results contrast with **Kinfe et al.** [28], who observed that dental procedures, history of surgery, and history of tattooing were among the factors associated with HBsAg seropositivity in pregnant Ethiopian women. Moreover, **Luksamijarulkul et al.** [29] stated that tattooing was a significant risk factor for HBsAg positivity in pregnant women from Thailand. The difference in safety measures of these procedures between the two localities could explain this contrast. Our results also contrast with **Khalil et al.** [30], who reported that the prevalence of a family history of HBV among HBV-positive participants was 51%, which was substantially higher than that of HBV-negative patients (13.6%) (p = 0.001). Moreover, they found a significant relationship between previous surgical procedures and the presence of HBsAg (p = 0.009).

Another finding of the current study is the significantly higher levels of HBsAg in anti-HBe positive pregnant women. Moreover, we found that HBsAg at cut-off levels above 1635 IU/ml was associated with positive anti-HBe with high sensitivity and specificity (100% and 91.7% respectively). This finding could be explained by the high frequency of the pre-core and core promoter mutants in the Middle East [31, 32]. Chronic HBV patients infected with these mutants will have negative HBeAg in their serum, along with positive anti-HBe despite active viral replication [9].

According to our results, none of these variables (age, gestational age, booster dose, operation, dental procedures, blood transfusion, tattoo, family history of HBV, husband's HBV infection, quantitative HBsAg, PCR, ALT, and AST) could be considered a predictor of positive anti-HBe among cases with positive HBsAg.

We found that the only significant correlation was found between AST levels and each of the quantitative HBsAg and ALT levels, with a positive and moderate correlation between AST and quantitative HBsAg and a strong correlation between AST and ALT.

Along with our results, **Cerrah et al.** [33] reported that there were significant relationships amongst the HBsAg DNA level and ALT. In addition, **Fazaa et al.** [34**]** reported that a positive correlation was found between HBV-DNA loads and ALT and AST.

Conclusions

The frequency of HBsAg seropositivity in pregnant women is high in Upper Egypt, denoting the importance of HBV screening during pregnancy to decrease the risk of maternofetal transmission. The detection of anti-HBe in the sera of pregnant women should be interpreted cautiously.

Abbreviations

ALT: alanine transaminase, anti-HBe: hepatitis B e antigen, AST: aspartate transaminase, DNA: deoxyribonucleic acid, HBeAg: heptitis B e antigen, HBsAg: hepatitis B surface antigen, HBV: hepatitis B virus, PCR: polymerase chain reaction.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Scientific Research Ethical Committee, Faculty of Medicine, Sohag University (IRB: Soh-Med-22-03-33). Clinical Trials registration number: NCT05442372. Written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Availability of data and material

The required data and materials were all available.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MAE collected participants' data. EMM analyzed and interpreted the patient data. RFM and NA performed the laboratory workup. OOA and AM were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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