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Effect of obligatory Hepatitis B vaccination program on the prevalence of occult hepatitis B among pregnant women in Egypt: A cross sectional study

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Abstract

Hepatitis B virus (HBV) infection is a global health problem. HBV is of intermediate endemicity in Egypt. "Occult" HBV (OBI) indicates replication of HBV-DNA in the liver of individuals with negative serum HBsAg**.** This study aimed to determine the prevalence of OBI among pregnant women in Egypt and to compare this prevalence among HBV vaccinated and unvaccinated women (received obligatory vaccination). This cross-section study included 474 pregnant women in the third trimester divided in two groups. Group I: (n=247) assumed received obligatory hepatitis B vaccination and group II: (n=227), did not receive HBV vaccination. Study participants were screened for HBsAg, anti HBs, anti HBc total, anti HBc IgM, HBeAg, anti HBe, HCV Ab, and HIV Ab by immunoassays and HBV-DNA by Real-Time PCR. Anti HBs was detected in 65 (13.7%) of pregnant women, 36 (14.6%) in the vaccinated group and 29 (12.8%) in the unvaccinated group. The anti HBs levels were significantly higher in the unvaccinated group. HBc Ab showed positive results in 6 cases (2.4%) in the vaccinated group, and 14 cases (6.2%) in unvaccinated group. HBcAb and/or HBsAb were detected in 72 (15.1%) of pregnant women, 39 (15.8%) in the vaccinated group and 33 (14.5%) in the unvaccinated group. HBV-DNA was detected only in one vaccinated pregnant woman. HB vaccination program in Egypt, since 1992 affected the frequency of OBI in pregnant women (p=0.04). In conclusion, HBV infection may persist lifelong in the hepatocytes even when viral functions are suppressed, HBsAb and anti-HBc-positive individuals are present. The levels of HBsAb were higher in unvaccinated pregnant women compared to vaccinated pregnant women. HBV infection in OBI pregnant women may not transmit to the new-born.

Keywords: Occult Hepatitis B; Hepatitis B vaccination; Pregnant women; Immunization

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Introduction

Hepatitis B virus (HBV) infection is a major public health issue, causing high mortality and disease burden worldwide. In 2015, the World Health Organization (WHO) estimated that 257 million people were living with chronic HBV

infection.[1] Although the preventive vaccine and anti-viral treatments that arrest disease progression and reduce liver cancer risk are available, hepatitis B is still a major public health problem all over the world.[2]

Egypt is considered by the WHO as an intermediate area as regards the epidemiology of HBV. The WHO goals include 90% complete coverage of HBV vaccination, a reduction in the prevalence of HB surface antigen (HBsAg) in children < 5 years of age to 0.1% and an improvement in the rates of treatment to 80% by 2030. In Egypt, the HBV vaccination program was applied in 1992 with a schedule of 2, 4, and 6 months of age, while routine screening of pregnant women for HBsAg was not applied[3]. Recently, the Ministry of Health and Population stated that the programme attained great success by vaccinating a large number of target groups, which included 2.04 million newborns (the zero dose) during the first 24 hours of birth, in addition to 6.2 million children under 6 months of age.[4] Between 1980 and 2007, studies indicated that the prevalence of overt HBV infection in Egypt was 6.7% in the general population, 11.7% in Upper Egypt, 4.6 in Lower Egypt, and 4% in pregnant women.[5] In 2015, a cross-sectional study, observed that 1.4% of general Egyptian population were positive for HBV, with a 1.9% prevalence in males and a 1.1% prevalence in females.[6] Abo-Salem et al., 2014,[7] also reported that HB infection is of intermediate endemicity among pregnant women in the Shebin El-Kom district of Menoufia governorate, whereas Elkadeem et al., 2021,[8] found that the prevalence of HBsAg in pregnant females was 3.39%.

Chronic hepatitis B (CHB) infection is characterized by the detection of HBsAg and viral genomic materials in the serum, resulting from active replicative and transcriptional activities. In contrast, occult HBV infection (OBI) refers to a condition where replication competent HBV-DNA is present in the liver, in the presence or absence of HBV-DNA in the blood, in individuals with negative serum HBsAg. In OBI, the HBV-DNA, in the form of episomal covalently closed circular DNA (cccDNA), is in a low replicative state owing to host immune or epigenetic control. Therefore, when serum HBV-DNA is detectable, it is invariably in a low viremia range (i.e., <200 IU/L) and may only be intermittently detected.[9]

Serology of OBI can be classified as either seropositive or seronegative. Seropositive OBI accounts for 80% of all OBI cases,[10] where antibody to HBV core antigen (anti-HBc) and/or antibody to HBsAg (anti-HBs) are detectable in the serum. Conversely, the absence of both antibodies in seronegative OBI leaves serum HBV-DNA as the only detectable marker, making the diagnosis more challenging since the probability of detecting positive serum HBV-DNA is highest in individuals who are anti-HBc positive and anti-HBs negative.[11]

Mother-to-child transmission is commonly associated with HBV infection in high-intermediate endemic areas. Pregnant women are at high risk of transmitting HBV to their offspring.[12] About 95% of neonates infected with HBV at birth are at high risk of developing CHB and 15%–40% of them are at risk of developing cirrhosis and liver cancer.[12] Prevention of mother-to-child transmission of HBV is one of the five core strategies for global HBV elimination by 2030.[13] There is little information about OBI in pregnant women, and there is no data about the prevalence of OBI among pregnant women in Egypt. Therefore, this study aimed to determine the prevalence of OBI among pregnant women in Egypt and to compare this prevalence among HBV vaccinated and unvaccinated women (received obligatory vaccination).

Subjects and Methods

Study participants: This cross-sectional study recruited 500 pregnant women. However, 26 were excluded as 5 women had HCV infection, 2 were HBsAg positive, 10 refused to participate and 10 had history of blood transfusion. The remaining 474 pregnant women were included in the study. They were either from the Obstetric Outpatient Clinic or women admitted to the Obstetrics and Gynecology Department of Women's Health Hospital, Assiut University, Egypt during the period between January 2017 through December 2020. All women visited or admitted to the hospital in the third trimester of pregnancy were invited to participate. On the

other hand, we excluded patients diagnosed with HCV, HIV and/ or overt HBV infections.

The study protocol was ethically reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Assiut University (approval dated September 2016). Informed written consents were obtained from all study participants before included in the study.

A personal interview was conducted with each participant, personal and demographic data, and other relative obstetrics and medical history were collected. Participants were then allocated to one of two groups according to their date of birth. Group I (vaccinated): included 247 individuals who were born in 1992 and after, they were supposed to have received obligatory HBV vaccination as part of their scheduled vaccinations in accordance with the Egyptian vaccination schedule. Group II (unvaccinated): included 227 women, born before 1992 when HBV vaccination was not a part of the routine vaccinations in the country (Figure 1).

From each participated women a venous blood sample (8 ml) was collected under complete aseptic conditions into a plain sterile vacutainer tube. Serum was obtained after centrifugation and divided into aliquots for assessment of hepatitis B markers, liver function tests and HBV-DNA real time polymerase chain reaction (RT-PCR). In addition, umbilical cord blood (2 ml) was collected under complete aseptic conditions into a plain sterile vacutainer tube and serum was collected for detection of HBV-DNA RT-PCR.

HBsAg, anti HBs, anti HBc total, anti HBc IgM, HBeAg, anti HBe, HCV Ab, and HIV Ab were performed using a chemiluminescent microparticle immunoassay by an automated analyzer (ARCHITECT i1000sr immunoassay analyzer, Abbott Diagnostics, USA), according to the manufacturer's instructions.

Liver function tests were performed using an automated blood chemistry analyzer (Siemens Advia 1800 Chemistry Analyzer, GmbH, Germany), according to the manufacturer's instructions. The isolation and purification of DNA from serum was performed by using commercial kits (Bosphore viral DNA extraction spin kit, lot no. XVR032 supplied by Anatolia®, Turkey) according to the to the manufacturer's instructions. For all participants, HBV-DNA viral load was detected using commercial PCR kits [lot number: 1,40724, GeneProof Hepatitis B Virus (HBV) and the Cobas AmpliPrep Cobas TaqMan 48 (CAP/CTM)], according to the manufacturer's instructions, on a thermal cycler (7500 Fast Real-Time PCR System, Applied Biosystems®, USA).

Statistical Analysis

The IBM Statistical Package for the Social Sciences (SPSS) Version 21·0 was used for data analysis. Descriptive statistics means, standard deviations, medians, and percentages were calculated. Test of significance: chi-square/Fisher's exact test was used to compare the difference in the distribution of frequencies among different groups. The U test was calculated to test the mean/median differences between the data sets (parametric/non-parametric). An independent t-test was used for the comparison of means among groups to correlate between variables. A p value of <0·05 was considered significant.

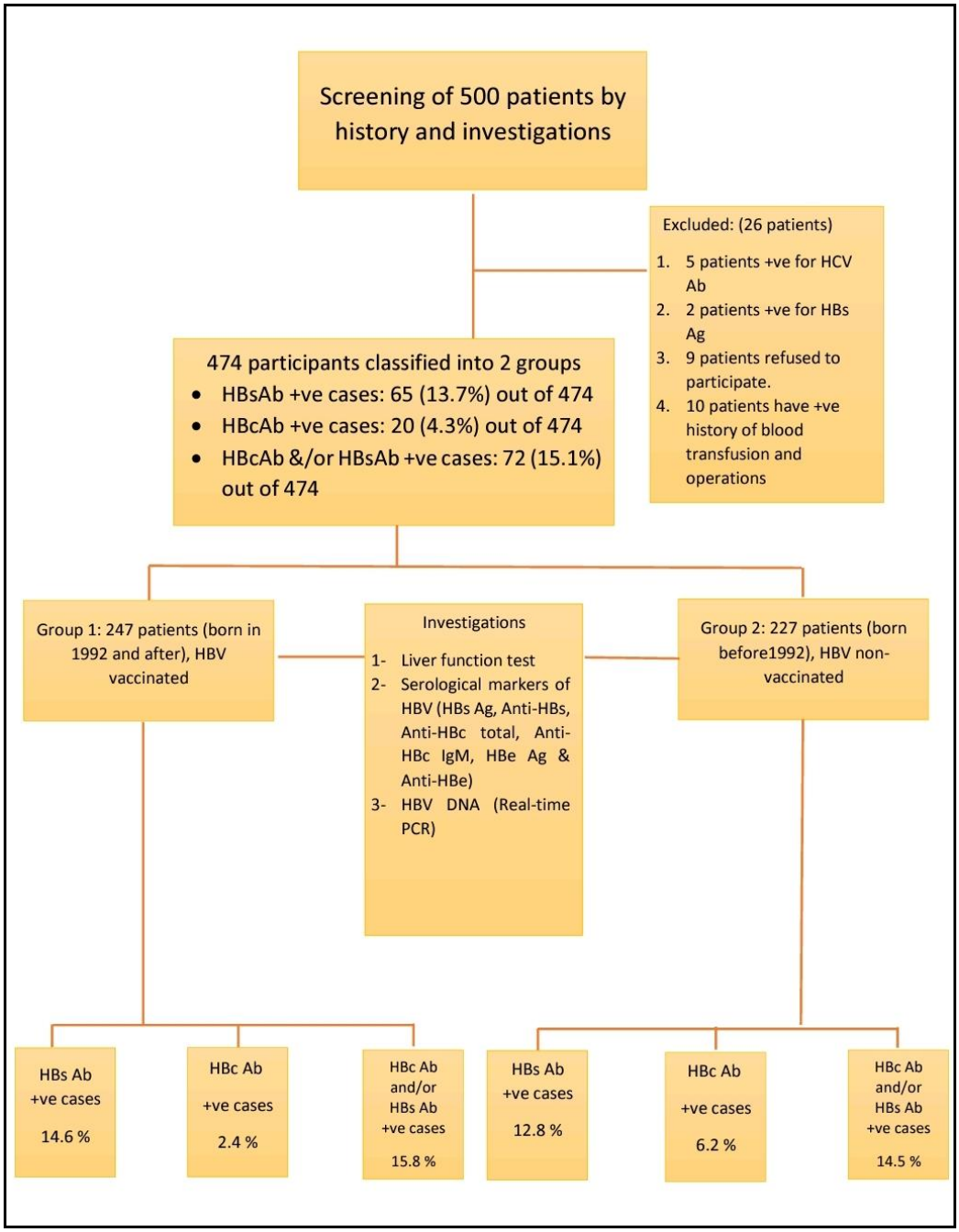

Figure 1. Study flowchart.

Results

The study included 474 pregnant women. They were classified into two groups, Group I included 247 women, immunized against HBV and Group II included 227 women, unimmunized group. Table 1 shows the results of obstetric data and liver enzymes in pregnant immunized and non-immunized groups.

The total number of vaginal deliveries and caesarean section deliveries were significantly higher in the unvaccinated group compared to the vaccinated group (p<0.001 for each). As regards to hepatitis B markers, HBs Ab were detected in 65 (13.7%) of all pregnant women, with no difference between vaccinated and unvaccinated groups, 36 (14.6%) and 29 (12.8%), respectively.

Table 1. Comparison of obstetric data and liver enzymes among vaccinated and unvaccinated pregnant women.

Parameter	Vaccinated (Group I) (n=247)	Unvaccinated (Group II) (n=227)	*p*-value
		Mean ± SD Median (Range)	
Gestational Age/weeks	38.23 ± 1.9 38 (27 - 42)	38.26 ± 2.1 38 (28 - 42)	NS
Previous Deliveries			
Total No.	1.45 ± 0.1 1 (0 - 5)	2.93 ± 0.1 3 (0 - 8)	< 0.001
Vaginal delivery	0.74 ± 0.1 0 (0 - 5)	1.58 ± 0.1 1 (0 - 8)	< 0.001
Caesarean section	0.71 ± 0.1 0 (0 - 4)	1.36 ± 0.1 1 (0 - 7)	< 0.001
ALT	14.88± 6.7 10 (2 – 205)	13.64 ± 11.1 11 (3 - 158)	NS
AST	23.98 ± 19.9 18 (5 - 226)	22.·37 ± 14.1 20 (6 - 134)	NS
ALP	131.09 ± 61.7 121 (15 - 381)	133.27 ± 66.9 124 (12 - 593)	NS

P > 0.05 is not significant (NS).

The levels (mIU/ml) of HBs Ab showed a statistically significant increase in the unvaccinated group when compared to the vaccinated groups (*p*=0.027). HBc Ab were positive in 20 (4.3%) of pregnant women, with a statistically significant increase in the unvaccinated group 14 (6.2%) than in the vaccinated group 6 (2.4%) (*p*=0.043). HBcAb and/or HBsAb were detected in 72 (15.1%) of pregnant women, with no difference between the vaccinated group 39 (15.8%) and the unvaccinated group 33 (14.5%).

HBc IgM, HBe Ag were not detected in the studied pregnant women. HBe Ab was detected in 9 (1.2%) of pregnant women, but no difference was observed between the vaccinated group 2 (0.8%) and the unvaccinated group 7 (3.1%). HBV-DNA was detected only in one vaccinated pregnant woman (Table 2). PCR testing for babies' cord blood samples were all negative for HBV-DNA.

Table 2. Comparison of hepatitis B virus (HBV) markers and HBV-DNA in the vaccinated and unvaccinated pregnant women.

Parameter	Vaccinated (Group I) (n=247)	Unvaccinated (Group II) (n=227)	*p*-value
HBsAb			
Non-reactive	211 (85.4%)	198 (87.2%)	NS
Reactive	36 (14.6%)	29 (12.8%)	
HBsAb Level (mIU/ml)			
Mean ± SD	137.95 ± 178.1	278.22 ± 144.4	0.027
Median (Range)	30 (10 - 1000)	59 (12 - 1000)	
HBcAb			
Non-reactive	241 (97.6%)	213 (93.8%)	0.043
Reactive	6 (2.4%)	14 (6.2%)	
HBcAb and/or HBsAb			
Non-reactive	208 (84.2%)	194 (85.5%)	NS
Reactive	39 (15.8%)	33 (14.5%)	
HBc IgM			
Non-reactive	247 (100%)	227 (100%)	-------
Reactive	0 (0%)	0 (0%)	
HBe Ag	247 (100%)	227 (100%)	-------
Non-reactive	0 (0%)	0 (0%)	
HBe Ab			
Non-reactive	245 (99.2%)	220 (96.9%)	NS
Reactive	2 (0.8%)	7 (3.1%)	
PCR	246 (99.6%)	227 (100%)	NS
Reactive	1 (0.4%)	0 (0%)	

P > 0.05 is not significant (NS).

Discussion

The present work investigated the prevalence of occult HBV infection among pregnant women in Egypt and compared the effect of the obligatory birth HB vaccination schedule on the prevalence of OBI in pregnancy. To the best of our knowledge, such work has not been conducted among Egyptian population before. Vertical HBV transmission may occur in the OBI setting,[14] so screening for maternal HBsAg may be insufficient and should be supplemented with neonatal immune prophylaxis.[15]

Our data showed that the frequency of positive total HBc Ab in the studied cases was 4.2%, and the frequency was statistically significantly increased in unvaccinated group when compared to the vaccinated group. Our results are consistent with those reported by Mbangiwa et al., 2018,[16] and Ali et al., 2020,[17] they found that the prevalence of OBI in was 6·6% and 5% in pregnant women, respectively. Moreover, Chang et al., 2010[14] also reported an increase in frequency of OBI cases among pregnant women which could be explained by immunosuppressive status of pregnancy. Georgiadou et al., 2009[18] studied OBI in subjects with autoimmune liver diseases. They reported that the risk of HBV infection reactivation can be explained if immunosuppression permits

viral replication, represses the function of immune cells, and the response of the immune system is exaggerated, leading to cellular injury. Pregnancy is also associated with disturbed cellular immunity. In particular, the maternal Th1 immune response may be suppressed during normal pregnancy to avoid rejection of the fetus, resulting in a predominant Th2 phenotype that allows the pregnancy to continue. While this Th2-dominant phenotype at the maternal–fetal interface allows fetal survival, the presence of anti-inflammatory cytokines such as interleukin (IL)-4 and IL-10 may predispose to an increased risk of infection, including reactivation of latent viruses, such as HBV Chang et al., 2010.[14]

However, the presence of anti-HBc is not an ideal marker for the diagnosis of OBI, it is recommended to be used as a surrogate marker whenever an HBV-DNA test is not available to identify potential seropositive OBI individuals, such as in cases of blood, tissue, or organ donation, or in cases of patients undergoing immunosuppressive therapy Raimondo et al., 2008.[19] In addition, anti-HBc determination is useful, even when HBV-DNA is available, because of the possibility of intermittent viremia. Our findings, of negative viremia and the presence of anti-HBc, could also be explained by the characterization of chronic occult infection by periods of transient HBV viremia alternating with periods in which the viral DNA is undetectable in the serum. In such cases, not all anti-HBc positive individuals are positive for HBV-DNA. In addition, the absence of this antibody does not rule out OBI (seronegative OBI) as suggested by Urbani et al., 2010.[20]

Moreover, Weber, 2006,[21] Ocana et al., 2011,[22] also attributed the occurrence of OBI to a mutant HBV gene S (encoding for HBsAg) and the production of modified HBsAg that cannot be detected by widely used commercial tests. They also reported that the viremic levels were comparable with those observed in patients with normal HBV infection.

In the present study, HBs Ab was detected in 13.7% of pregnant women, 14.6% from the vaccinated group and 12.8% from the unvaccinated group. The levels of HBs Ab showed a statistically significant increase in the unvaccinated group when compared to the vaccinated groups. HBc Ab and/or HBs Ab were detected in 17·1% of pregnant women, 15·8% in the vaccinated group and 14·5% in the unvaccinated group. These findings agreed with those reported by Ali et al., 2020,[17] who found that the frequency of anti HBs was 13·2% in pregnant women and with data of Mak et al., 2020,[23] who reported that the serum markers were used to define different OBI types, which can be classified as seropositive or seronegative. In seropositive anti-HBc and/or anti-HBs were detectable in the serum.

A study by Norouzirad et al., 2014,[24] reported that the potential problem of HBV immunization was that vaccine-induced anti-HBs Ab titers diminished to low or undetectable levels with age. This observation may explain the cause behind the statistically lower HBs Ab level in the vaccinated group when compared to the unvaccinated group in our study. Moreover, the detection of these antibodies depended on the sensitivity, the analytical and clinical specificity of the tests used as suggested by Pondé, 2019.[25]

Data from this study showed that HBe Ag was negative in all the studied groups. These findings agreed to those observed by Bremer et al., 2009,[26] who reported that OBI individuals lack hepatitis B virus "e" antigenemia. In the present study, HBV-DNA was detected only in one case (0.21%). The detection of HBV-DNA-PCR was performed by two different analyzers for confirmation, the 7500 Fast Real-Time PCR System (the detection limit was up to 36.9792 IU/ml) and the Cobas AmpliPrep Cobas TqMan 48 (CAP\CTM) system (the detection limit was up to 20 IU/ml). This finding is explained by Hollinger and Sood 2010,[27] who reported that the viral load detection limit in pregnant women with seropositive OBIs was very low (20 IU/mL in nearly half of the cases) and approaching the HBV-DNA detection limit of 5 IU/mL indicated OBI. Also, this finding is supported by findings of Ali et al. 2020,[17] who reported that the frequency of OB ranged from 0% to 0·24% when using Roche CobasTaqman for HBV-DNA detection. They attributed such observation to underestimation of HBV-DNA detection in

serum because its detection in liver biopsy was the best way for diagnosing OBI. But liver specimens are not easily available as liver biopsy is an invasive procedure. Moreover, the Food and Drug Administration (FDA, USA) has not permitted the use of a standardized and validated assay for detection of HBV-DNA in liver tissues. The detection limits of HBV-DNA in our study agreed with those of a study by Abd Allah et al., 2021,[28] who tested 65,211 blood donations in Assiut university hospitals and found that the HBV-DNA frequency by nucleic acid testing technique (reactive/seronegative) for HBV was 36 (0·05 %).

Moreover, OBI is characterized by the persistence of HBV cccDNA in the nucleus of infected hepatocytes. It is generally believed that the detectability of HBsAg in individuals with OBI, despite cccDNA persistence, is due to the suppression of viral replication as a result of epigenetic or immune control of gene expression Raimondo et al., 2019.[9] Furthermore, Hedayati-Moghaddam et al., 2020[29] reported that the prevalence of OBI varied significantly across areas and patients' categories, with rates estimated at 0.06% among blood donors regardless of anti-HBc status, 7.90% among anti-HBc positive blood donors, 2.49% among hemodialysis patients, 4.44% among HIV-positive patients, and 7.76% among HCV-positive patients.

In the present study, babies' cord blood samples tested by PCR were HBV-DNA negative. These findings agreed to those of a study by Kwon et al., 2008,[30] who found that by using two different HBV-DNA detection methods, cord blood samples from pregnant women with OBI were negative for HBV-DNA. Also, Khamduang et al., 2013,[31] reported that regarding mother-child transmission of OBI, there are no documented cases of vertical transmission from an OBI mother resulting in chronic infection in the child, possibly in part because of concomitant vaccination.

As regards to serum levels of AST, ALT, and ALP, no difference was detected between the vaccinated group to the unvaccinated group. Our results agreed with those reported by Mbangiwa et al., 2018,[16] who found that there was no significant difference in AST, ALT, and ALP levels between the different groups of pregnant women (chronic HBV, occult HBV, chronic or occult HBV and HBV-Negative). Our findings also agreed with those reported by Ramaty et al., 2014,[32] who attributed the normal liver enzymes to a very low or non-replicative state of HBV within liver cells and the presence of an intermittent increase in the quality and magnitude of host immune responses against HBV infection.

In conclusion, the HBV vaccination program, implemented in Egypt since 1992, significantly affected the frequency of OBI in pregnant women. The level of anti-HBs Ab was higher in the unvaccinated pregnant compared to vaccinated pregnant women. Anti-HBs Ab titer fades gradually by time after HBV vaccination and may decline below the protective level by time. HBV infection in the occult form in pregnant women may not transmitted to the newborn.

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Author Contributions

AME, OMS designed the idea and tools of the study. RAY, DTK performed the laboratory work. SKS, DTK made the statistical analysis. OMS examined the patients. RAY, HAA collected the samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was ethically reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Assiut University (approval dated September 2016).

Informed consent

Informed written consents were obtained from all study participants before included in the study.

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