



Original Article

The prevalence of Hepatitis B Surface Antigen (HBsAg) among pregnant women admitted to one public Hospital in Damascus, Syria

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ABSTRACT

Background: Chronic Hepatitis B (CHB) affects 240 million people worldwide and is responsible for 686000 deaths annually due to its major complications. The World Health Organization (WHO) reported Syria as intermediate in the prevalence of CHB among the general population. However, no previous data was published about HBsAg-prevalence among pregnant women in this country.

Objective: To determine the prevalence of Hepatitis B Surface Antigen (HBsAg) among the pregnant women admitted to one of the two main centers of obstetrics and gynecology in Damascus, Syria.

Methods: A cross-section study included 794 participants who were evaluated using a pretested structured questionnaire and then were screened for HBsAg using the fourth generation of ELISA.

Results: Six of all participants (0.75%) had a positive test for HBsAg. Hepatitis B vaccine has been introduced to the National Immunization Program for newborn and infants in Syria since 1994. We classified the recruited pregnant women into two age groups: (≤ 21) and (>21) years old. This classification correlates to the year of introducing the vaccine. All positive HBsAg tests were among the women older than 21 years (1.13%, $p=0.186$). The multivariate analysis using log-regression test showed that only the previous knowledge about Hepatitis B virus (HBV) was found to be a negative factor regarding the presence of HBsAg ($P=0.003$).

Conclusion: The prevalence of HBsAg among the pregnant women admitted to an obstetrics and gynecology center in Damascus, Syria was 0.75%. The study emphasizes the importance of raising the level of awareness about HBV in the Syrian society with the need to conduct further studies in high-risk groups to determine precise risk factors of transmitting HBV infection.

Key words: Hepatitis B; Hepatitis B Surface Antigens; Pregnancy; Syria

BACKGROUND

Chronic Hepatitis B (CHB) affects 240 million people worldwide, and is responsible for 686.000 deaths annually due to its major complications; hepato-cellular carcinoma and cirrhosis.¹ Hepatitis B virus (HBV) consists of a core that contains partially double-stranded circular DNA and DNA polymerase enzyme with several antigens: Hepatitis B surface antigen (HBsAg), core antigen (HBcAg) and envelope antigen (HBeAg).² According to HB e Ag status, two types of virus were described: wild and mutant, the latter is the most common type in Syria according to a study published in 2010.^{2,3} HBV is transmitted by blood, other body fluids and organ transplantation. However, vertical transmission (from infected mother to newborn) around delivery is considered to be a major

source of infection.⁴ Regarding factors that influence mother-to-infant transmission, pregnant women with a high viral load and positive e-antigen without using any prophylaxis nor treatment, have up to 90% likelihood to transmit HBV to their newborns. This means that children of these women have a 90% chance of becoming chronic carriers (while chronic state develops only in 5% of adults), and are subsequently at a high risk of developing complications in the early childhood.^{1,5,6} Nevertheless, it is important to notice that vaccine alone can provide about 60% protection against newborn infection, while using vaccine in addition to the Hepatitis B Immune Globulin (HBIG) can increase the protection up to 90%.⁶ Searching medical literature, numerous studies have described the prevalence of HBV infection among pregnant women in many countries worldwide. A systematic review from Nigeria reported that the prevalence

of HBV in women attending antenatal clinics was 14.1%.⁷ In China and India this prevalence was 7.5% and 2.9% respectively.^{8,9} On the other hand, the prevalence of HBV infection among pregnant women in the Arab-countries has been reported to range between 1% in Qatar and 10.8% in Yemen and it has been shown that prevalence rate varies according to the degree of development of health facilities.¹⁰ Up till now, there is no published data about the prevalence of HBV in pregnant women in Syria, but the national guidelines for management of CHB (which are similar to WHO classification) released 2003, listed Syria as intermediate in endemicity of HBV infection, reporting a prevalence of 3-6% (information from national bank of blood transfusion and pre-marriage clinics).¹

OBJECTIVES

We conducted this study to assess the prevalence of HBsAg among pregnant women admitted to one public obstetrics and gynecologic hospital: al Zahrawi, in Damascus, Syria.

METHODS

Study design, setting and population:

This study was conducted by the Department of Gastroenterology at the Syrian Private University (SPU) as a prospective cross-sectional study from August to December 2015.

All pregnant women admitted for delivery to al-Zahrawi hospital were recruited into the study during the study period. This hospital is one of the two main public hospitals of obstetrics and gynecology in Damascus.

During 2015, the hospital offered free services for about 3500 pregnant women admitted for delivery.

The study was approved by the Ethics Commission of the Medical Faculty at SPU. An informed consent form has been obtained from each subject prior to its recruitment.

Data collection and sampling procedures:

The researchers were trained on study procedures: the way to introduce the study to the patient, getting the informed consent, taking blood samples and how to deal appropriately with them. Besides, the questionnaire was pilot-tested in ten pregnant women in order to assess questions, time to answer and responsiveness of recruited participants (Table 1).¹¹ To ensure the safety of the researchers who collected the blood samples, all three doses of hepatitis b vaccine were already taken. After obtaining the written informed consent, a questionnaire about personal data and risk factors of HBV infection was completed.

Laboratory Procedures:

Blood samples were immediately taken to the laboratory located in the same hospital where plasma was separated then stored frozen at (-20)°C (Table 1).¹¹ Each group, consisting of 90 samples, was transported to the central laboratory of the Ministry of Health where samples were screened for HBsAg. It used AMP PLATOS R 496 - MICROPLATE READER. The

fourth generation of Enzyme-linked Immunosorbent Assay (ELISA-ULTRA Version, Dia.Pro Diagnostic Bio Probes, SAG1ULTRA.CE) was the technique used to detect the HBsAg. It has a specificity of >99.5% and sensitivity of 100%. Moreover, the Laboratory of Al-Assad Hospital was the reference for quality control because it is used as the central reference laboratory for other labs in Damascus. Five randomly chosen control samples from each group were used for the quality control. For women who tested positive a re-test was done. It used the (ARCHITECT Abbott, i2000 immunoassay analyzer, with ARCHITECT Abbott (HbsAg 2G22, HBeAg 6C32, and anti-HBe 6C34) Kits.

Data analysis:

Data were entered in Microsoft Excel 2010 then exported for analysis in SPSS Statistics (IBM SPSS Statistics Version 20.0.0.0, 2011). Prevalence of HBsAg was the ratio between HBsAg positive samples and total sample collected. We used proportions, mean age to describe data. Besides, Fisher exact test, relative risk values were calculated to assess association between risk factors and HBsAg positivity. P value of ≤ 0.05 is considered statistically significant.

RESULTS

Study Participants:

The study initially included 800 participants, six of whom declined to proceed at a later stage, within effect there were 794 participants whose ages ranged between 14-43 years. The mean age was 25.45 \pm 6.3 years. The predominant age group was 20-30 years old (59% of general population). The majority of participants 679 (85.5%) had completed their elementary school. 755 (95.1%) were housewives and 508 (64%) were multipara (Table 2).

Prevalence of HBV and HBeAg positivity:

The HBsAg was found to be positive in only 6 patients (0.75%). Two of them could be eventually followed up to perform a further serological investigation. Both had negative HBeAg, one of the two had normal liver enzymes titers. (Table 3). The research team lost the connection with the other four patients.

HBV Risk Factors:

The following risk factors were questionnairized: Previous knowledge of HBV, previous history of jaundice, family history, HBV vaccine, HBV-antibody titers, blood transfusion, history of surgery during last 4 years or more, tattooing, hemodialysis, diabetes mellitus, drugs (steroids), hematological disorders (Thalassemia, sickle cell anemia and hemophilia), mutual personal items (razors, toothbrush, clippers) and previous HBV tests. The multivariate analysis using log-regression test showed that only the previous knowledge about Hepatitis B virus (HBV) was found to be a negative factor for regarding the presence of HBsAg (P=0.001). No demographic factor was found to be statistically significant or has its influence on HBV positivity (Table 5).

Table 1: Sampling and screening procedures

<u>Sampling and safety rules:</u>	
-	Hand Hygiene: washed hands with water and soap then single-use towel, or if not apparently contaminated, used alcohol rub.
-	Used non-sterile gloves for one time.
-	Used alcohol swabs for disinfecting vein puncture area.
-	Used tourniquet.
-	Use of single- use needle.
-	For recapping needle: the one-hand scoop technique.
-	Discarded used needle, gloves and cotton swabs in sharps container.
-	Blood samples were labeled with numbers, then kept upright in holder.
<u>Plasma separation:</u>	
-	Blood samples were collected in tubes containing heparin 2.5 ml.
-	Centrifugation was done for five minutes.
-	Plasma was separated using 1000 µg micropipette.
-	Plasma was stored frozen in 1000 µg-ependrofs.
<u>Screening for HBsAg:</u>	
-	Washed the plate once.
-	A1* was kept empty for blank calibration.
-	150 µm of plasma, control and calibrator.
-	100 µm of conjugate: turning from yellow into reddish pink.
-	Preservation for 120 minutes at 37c.
-	Washed for five times.
-	Preserved in dark place at room temperature for 30 minutes: positive samples turn into blue.
-	100 µm of reaction stopper: color was turned into dark yellow.
-	Results were read on wavelength of 450-620 nm.

†A1 is the first position on the plate

Table 2: Demographic characteristics of participants of the survey about HBV prevalence among pregnant women

Characteristic	N*	Percentage (%)
AGE (years)		
21≤	264	33.2
21>	530	66.8
Education		
None	25	3.1
Elementary	679	85.5
High-school	49	6.2
University	41	5.2
Employment		
Unemployed	755	95.1
Employed	29	3.7
Student	10	1.3
Child birth		
0	286	36
≥1	508	64

DISSCUSION

The recruited patients in the present study came mainly from the Damascus countryside and North-Eastern provinces of Syria. This cross-sectional study was conducted to assess the prevalence of hepatitis B surface antigen (HBsAg) among a group of pregnant women admitted to al-Zahrawi hospital. It was found to be 0.75%. HBV vaccine was added to the National Program for vaccination in Syria in 1994. Therefore, we classified the patients into two age groups, the first group was 21 years old or younger while the second group older than 21 years (born before or after 1994). All positive results (presence of HBsAg) were among women aged older than 21 years who have not received HBV vaccine (1.12%, p=0.186), this may emphasize the efficacy of vaccine in preventing HBV (Table 5). Therefore, we recommend the preservation of administrating the vaccine, especially in light of the challenges which Syria has been facing since 2011. This recommendation correlates to the guidelines of the American Association for the Study of Liver Diseases (AASLD) that emphasize the role of vaccination as a corner stone in the prevention of HBV infection.¹²

According to the results of this study, we have initial estimation to provide precise amount of Hepatitis B Immune Globulin (HBIG) for infected pregnant women. In spite of its higher cost, the evident protection value of HBIG in preventing transmission of HBV from infected mother to her child is up to 90% when administered with HBV vaccine within the first 12 hours postpartum, then completed with second and third doses of vaccine at months 1 and 6 postpartum(12). On analyzing data, 531 (66.87%) have not received HBV vaccine. 755 (95.08%) of the participants were housewives, so they were at a low risk of infection with HBV in relation to their occupation. This finding included participants in whom the results of the HBsAg test were positive (Table 4). We noticed marked lack of knowledge about HBV and its transmission routes. Due to current conditions in Syria (conflict and demographic dislocations and displacement), we have succeeded to communicate with only two patients of the overall positive patients, and found them to have negative HBeAg; One with normal liver enzymes titers, and the other with high enzyme titers. A hepatologist followed up these patients (Table 3). Because of the limited number of positive results the multivariate analysis failed to show an independent risk factor for the HBV infection. The overall prevalence rate was lower compared to neighboring countries: In Jordan and Lebanon 4.30% and 2.90% respectively^{13,14} and in Turkey 1.5%.¹⁵ This would be of noteworthy particularly that the ongoing war in Syria since 2011 should have resulted in substandard health and medical conditions (blood transfusion, surgery...) characteristic of conflict zones. We recommend conducting further multi-centric studies with a large number of patients to determine precise risk factors for transmitting HBV infection and assess the knowledge about hepatitis B in Syria, especially that there is no previous published data about the knowledge of hepatitis B except for one study that was conducted among medical students at the Syrian Private University (SPU).¹⁶

Table 3: Follow up of positive patients

	Anti-HBeAg	HBeAg	HBsAg	ALT (GPT) (U/l)		AST (GOT)(U/l)	
				Reference range	Lab value	Reference range	Lab value
Positive	Negative	Positive	First patient	37	Upto 31	37	0 - 32
Positive	Negative	Positive	Second patient	7		15	

Table 4: Risk factors associated with HBV positivity among pregnant women

Risk factor		Number	Positives	Percentage (%)	95% [CI%]	Risk Ratio (RR)	Fisher exact p-value
Previous knowledge about hepatitis B	no	786	4	0.5	[0.01 , 1.1]	65.167	0.001
	yes	8	2	25	[0 , 55]*		
Jaundice history	no	782	6	0.77	[0.16 , 1.38]	0	1.00
	yes	12	0	0	-----		
Family history	no	789	5	0.63	[0.08 , 1.19]	39.2	0.037
	yes	5	1	20	[0 , 55.1]*		
HBV Vaccine	no	531	6	1.13	[0.23 , 2.03]	0	0.186
	yes	263	0	0	-----		
antibodies titration	no	794	6	0.76	[0.15 , 1.36]	-----	-----
	yes	0	0	0	-----		
Blood Donation	no	769	6	0.78	[0.16 , 1.4]	0	1.00
	yes	25	0	0	-----		
Blood Transfusion	no	742	6	0.81	[0.16 , 1.45]	0	1.00
	yes	52	0	0	-----		
Blood Transfusion (during last 4 years)	no	766	6	0.78	[0.16 , 1.41]	0	1.00
	Yes	28	0	0	-----		
Surgery	no	495	3	0.6	[0 , 1.29]*	1.662	0.678
	yes	299	3	1	[0 , 2.13]*		
Surgery (during last 4 years)	no	606	4	0.66	[0.02 , 1.3]	1.618	0.631
	yes	188	2	1.06	[0 , 2.53]*		
Tattoo	no	775	6	0.77	[0.16 , 1.4]	0	1.00
	yes	19	0	0	-----		
Hemodialysis	no	792	6	0.76	[0.15 , 1.4]	0	1.00
	yes	2	0	0	-----		
Diabetes Mellitus	no	790	6	0.76	[0.15 , 1.36]	0	1.00
	yes	4	0	0	-----		
Corticosteroids	no	790	6	0.76	[0.15 , 1.36]	0	1.00
	yes	4	0	0	-----		
Thalassemia	no	791	6	0.76	[0.15 , 1.36]	0	1
	yes	3	0	0	-----		
Sickle cell Anemia	no	789	6	0.76	[0.15 , 1.36]	0	1.00
	yes	5	0	0	-----		
Hemophilia	no	794	6	0.76	[0.15 , 1.36]	----	----
	yes	0	0	0	-----		
Shared personal sharp items	no	4	0	0	-----	0	1.00
	yes	790	6	0.76	[0.15 , 1.36]		
Intravenous Drugs	no	794	6	0.76	[0.15 , 1.36]	-----	----
	yes	0	0	0	-----		
Previous liver test	no	789	4	0.51	[0.01 , 1]	130.83	0.00
	yes	5	2	40	[0 , 82.9]*		

Table 5: Prevalence of HBV in relation to socio-demographic and obstetrical characteristics of pregnant women

Risk factor	Number	Positives	Percentage (%)	95% [CI%]	Risk Ratio (RR)	Fisher exact p-value
Age (years)						
≤21	264	0	0	0	0	0.186
>21	530	6	1.13	[0.23 , 2]		
Education						
≤Elementary	704	6	0.85	[0.17 , 1.5]	0	1.00
>Elementary	90	0	0	0		
Employment						
Unemployed	765	6	0.78	[0.16 , 1.4]	0	1.00
Employed	29	0	0	0		
Childbirth						
0	286	0	0	0	0	0.093
≥1	508	6	1.18	[0.24 , 2.1]		

List of abbreviations: HBsAg: Hepatitis B Surface Antigen; HBV: Hepatitis B virus; HBcAg: hepatitis B core antigen; HBeAg: hepatitis B envelope antigen; HBIG: Hepatitis B Immune Globulin; WHO: World Health Organization; SPU: Syrian Private University; RR: Risk Ratio; CI: confidence interval.

Ethical approval: Committee of Ethics of Syrian private university (SPU). Reference number of approval: 2/4 Date of approval: 2/8/2015

Competing interests: The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions: NI conceived and designed the study and the questionnaire, reviewed, participated in writing, participation in data analysis corrected the manuscript, and followed up the positive pregnant patients. TA and HH participated equally in collecting and preparing blood samples, collecting data, participation in data analysis, drafting the manuscript and contributed in communicating with the positive participants. All authors read and approved the final manuscript.

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