



Original Article

The Delayed Immune Response of Infants of Diabetic Mothers to Hepatitis B Vaccine: A prospective Case-Control Study

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Abstract

Background: Infants of diabetic mothers (IDMs) have a significantly greater risk for perinatal morbidity, mortality, and altered immune response.

Aim of work: To assess the immune response of IDMs for hepatitis B virus (HBV) vaccine and to compare them with infants of non-diabetic mothers as a control group.

Patients and Methods: 150 neonates met the inclusion criteria; 100 IDMs and 50 infants' non-diabetic mothers as controls. All neonates who met the inclusion criteria received HBV vaccine during the first 24 hours of age and at 1 and 6 months of age according to the WHO guidelines. Furthermore, hepatitis B surface antigen (HBsAg), hepatitis B virus surface antibodies (anti-HBs), and hepatitis B virus core antibodies (anti-Hbc) were measured at 9, 12, and 24 months of age.

Results: IDMs had significantly higher birth weight when compared to infants of the non-diabetic mothers (3.6 ± 0.4 kg and 3.03 ± 0.5 kg, P-value 0.01). There was a significant difference between the response to anti-HBs between case group and control group at 9 months 7 ± 12 mIU/dL versus 20 ± 23 mIU/dL (P-value < 0.0001). However, anti-HBs antibodies levels follow-up at 12 and 24 months showed no significant differences between the case and control groups.

Conclusion: At 9 months of age, post HBV vaccination, there was a delayed immune response for HBV vaccine was present in IDM compared to infants of non-diabetic mothers. However, IDM became immune at follow-up at 12 and 24 months of age.

Key words: Infants of diabetic mothers, IDM, Immune response, Hepatitis B vaccine.

Introduction

In 1963, Blumberg et al. [1] discovered "Australia Antigen" now known as hepatitis B surface antigen (HBsAg). This protein was later discovered to be a component of the virus that causes "serum hepatitis" (hepatitis B virus). Chronic hepatitis B virus (HBV) infection represents a major global health problem, affecting about 257–291 million people worldwide and is related with severe clinical complications, including chronic liver diseases and hepatocellular carcinoma. Chronic HBV infection affects 1.59 million people in the United States of America [2]. Egypt is moderately endemic for HBV, where the prevalence of HBsAg is estimated to be 1.2-1.6% among the general population

The mainstay of HBV prevention is the HBV vaccine. In 1992, the World Health Organization (WHO) recommended the implementation of universal childhood vaccination worldwide [2]. The HBV vaccine protects against infection and its

consequences such as liver cirrhosis and hepatocellular carcinoma. As a result, it is the first vaccine against cancer, preventing chronic liver diseases, and decreased maternal-fetal viral transmission occurring at birth [4]. A course of three vaccine injections was given; the first dose is given within the first 24 hours of life; the second dose is at one month and the third dose was administered at the sixth month [5].

Long-term protection against HBV infection depends on immunological memory [6]. The complete vaccination series induces protective antibody levels in more than 95% of immunocompetence infants, children, and young adults [2]. However, little information is known about the strength and duration of immunological memory of HBV vaccine in immunocompromised individuals including premature infants [7], human immunodeficiency virus [8], diabetes mellitus (DM) type 1 [9], and others. IDM had immunological abnormalities when compared to infants born to healthy

mothers, such as lower total lymphocytes [10], increased expression of interleukin (IL)-6, and transforming growth factor beta (TGF- β) [11]. However, it is unknown how maternal DM affects the neonatal immune response to vaccinations. Therefore, this study aimed to compare the immune response of IDM to neonates of healthy mothers after given HBV on the first day of life and at 1 and 6 months of age and follow up immunological response to HBV vaccine at 9, 12, and 24 months of neonatal age.

Methods

The current clinical study was performed at the neonatal intensive care unit (NICU) in the Pediatrics Department, Faculty of Medicine, Minia University, Egypt in cooperation with the Department of Microbiology, Faculty of Medicine, Minia University, Egypt, and Pediatrics Department, Faculty of Medicine, Sohag University, study period from January 2017 to June 2019. Local ethical approval for the study was obtained from the Research Committee

of the Faculty of Medicine at Minia University. Written informed consent was obtained from all parents of the children.

We included all IDMs (full term or preterm more than 34 weeks) born during the study period. Exclusion criteria included mothers with positive HBs antigen, the infant did not receive three full course of HBV vaccine, IDMs with preterm delivery less than 34 weeks or 2kg, major congenital malformation, severe perinatal asphyxia, twins, or erythroblastosis fetalis. One hundred IDMs met the inclusion criteria during the study period. Another 50 newborn infants of non-diabetics mothers delivered during the study period considered as a control group. Both the cases and the control groups were drawn from the same population characteristics. Mother's HBsAg screen result was mandatory before enrolled in the study. DM type 1, type 2 and gestational diabetics were diagnosed according to the American Diabetes Association [12]

as the following I) a fasting blood glucose ≥ 126 mg/dL. II) Blood glucose level ≥ 200 mg/ dL, 2 hours after a 75 gram oral glucose intake. Gestational diabetics are diagnosed in pregnant women with no previous history of DM before pregnancy [13]. Furthermore, follow-up glycated HB (HbA1C) was done in the third trimester for diabetic mothers included in the study.

All neonates who met the inclusion criteria received HBV vaccine at 0, 1, 6 months of age according to the Egyptian schedule of vaccination [14] and were subjected to full maternal and neonatal history taking including age and sex, gestational age, mode of delivery, APGAR score, neonatal admission, duration of diabetes and medications used for control. Detailed general and local examinations were done for all included neonates and measurements as weight, length, and vital sign were recorded.

Furthermore, a volume of 2 ml of venous blood was drawn from each neonate

before discharge under complete aseptic conditions and divided into two tubes. The first tube contained EDTA for CBC which done by (Roche HITACHI Cobas C-311 Auto-Analyzer System) and the second tube was left in the incubator for 30 min, centrifuged at 3000 rpm for 10 min and then the separated serum was collected for liver enzymes, urea, and creatinine which measured by colorimetric method using commercial kits.

The immune response was assessed at 9, 12, and 24 months of infant age after a full course of HBV vaccine taken as schedule. Each time, the collected samples were left to clot for two hours at room temperature or overnight at 4°C and then centrifuged for 15 minutes at 1000 rpm. Samples were stored at -20°C or -80°C then used for the assessment of hepatitis B surface antigen (HBsAg), hepatitis B virus surface antibody (Anti-HBs), and hepatitis B core antibody (Anti-HBc). These measurements each

time was done by ELISA Kit DEIA002, Creative Diagnostic NY, USA.

Ethical approval and consent to participate

Local ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, Mina University, Egypt. Written informed consents were obtained from all parents of the children.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 21 under windows 7 operating system. Quantitative data were represented as mean, standard deviation, median, and range. Data were subjected to student t-test to compare means of two groups. When the data were not normally distributed, Mann-Whitney's test was applied. Qualitative data were presented as number and percentage and compared using either the Chi square test or Fisher's exact test. Probability level (P-

value) was assumed significant if less than 0.05.

Results

In total, 150 neonates met the inclusion criteria and were included in this study. In which 100 IDMs as a case group and 50 neonates of non-diabetes mothers a control group. There was a significant difference between the weight of the case group compared to the control group (3.6 ± 0.4 kg and 3.03 ± 0.5 kg, P-value 0.01). However, there were no other significant differences in maternal or neonatal differences between the case group and control group, even for maternal age (P-value = 0.26), mode of delivery duration (P-value= 0.43), as shown in Table 1. Furthermore, IDMs had higher hemoglobin (17.4 ± 1.3) gram/dL and hematocrit ($55.5 \pm 16.4\%$) compared to control group hemoglobin (15.5 ± 2.0) gram/dL and hematocrit ($46 \pm 17.2\%$), (both P-value 0.01). Moreover, as shown in Table 2, no significant difference between the case and control group as

regards other CBC parameters, AST, ALT, urea, and creatinine concentrations. Hepatitis B virus serology after HBV vaccination: Comparison between cases and control groups regarding hepatitis B virus serology follow-up after vaccination, there was a significant difference between the response to anti-HBs between the case group and control group at 9 months 7 ± 12 mIU/dL versus 20 ± 23 mIU/dL (P-value < 0.0001). However, anti-HBs follow-up at 12 and 24 months have shown no significant differences between the case and control group as shown in Table 3. Furthermore, no cases in the case group or control group had HBsAg or anti-HBc positive initially at 9 months or during follow-up at 12 and 24 months of age (Table 3).

Discussion

The immune response of infants to different vaccines is still a question especially in immunocompromised children [15]. IDM had some immunological abnormalities which could affect their immunity [10].

In this study, we found that the immune response of IDM after a full course of HBV vaccine by measuring anti-HBs was significantly lower at 9 months when compared to an infant of non-diabetic mothers. However, IDM developed a protective immunity by the increased titer of anti-HBs at 12, 24 months of age. Furthermore no positive HBs antigen or anti-HBc between cases and control groups was present. Moreover, IDM had a higher birth weight, hemoglobin, and hematocrit level when compared to the control group.

Spontaneous abortion, stillbirth, congenital malformations, and perinatal mortality and morbidity are all substantially higher in IDMs [16]. The present results revealed that there was no significant difference between groups regarding sex distribution, maternal age, mode of delivery, and Apgar score. We believed that this is important to ensure the homogenization of the studied groups to get accurate results from the comparison between groups. However,

we found that birth weight, hemoglobin, and hematocrit were significantly higher in IDMs compared to the control group. These data agree with other reports about fetal macrosomia and polycythemia in IDMs due to neonatal hyperinsulinemia [17].

HBV vaccine is highly effective in preventing HBV infection and is recommended for all infants, all adolescents, and adults at risk for HBV infection [18]. WHO recommended vaccine schedules for hepatitis B protection at birth that provide a birth dose will avoid most perinatal infections and provide early protection from horizontal transmission [19, 20]. It is well known that the presence of anti-HBs is generally interpreted as indicating recovery and immunity from HBV [19, 21]. Anti-HBs also develop in a person who has been successfully vaccinated against HBV. A positive anti-HBS test result (> 10 mIU/mL) indicates that a person is immune against HBV [15]. In this study except at 9 months after

vaccination, follow-up at 12 and 24 months, anti-HBs were above (>10 mIU/mL) in case and control group.

The possible explanation about the impaired immunity of IDMs to HBV vaccine early after vaccination at 9 months had different suggestive theories. Roll et al. [10] studied the impact of diabetic mothers on the maturation of the immune system in their neonates. They found that in IDMs, percentages of total lymphocytes were significantly decreased compared to neonates of non-diabetes mothers. However, according to Mrizak et al. [11] gestational diabetes can alter the placental and neonatal immune systems. They found an increased expression of interleukin (IL)-6, toll-like receptor (TLR) 4, and transforming growth factor-beta mRNA in the placenta. Another study by Atègbo et al. [22] found an elevated levels of T helper type 1 cytokines and low levels of IL-10 in the serum of macrocosmic babies born to gestational diabetic mothers.

Moreover, Mehta, and Anna [23] studied the impaired neutrophil functional activity in the cord blood of IDMs. Franca et al. [24] found that diabetic mothers had lower IgA and IgG levels in colostrum and lower IgG and IgM levels in the blood than non-diabetic mothers. These finding suggested that hyperglycemia changes antibody production in pregnant women and may decrease immunoglobulin production and this can affect negatively in the immunity of IDMs. This may explain the early impaired immunity to HBV vaccine in IDMs at 9 months of age. In agreement with our result, Abd el Aziz et al. [25] reported that infants of non-gestational diabetic mothers had delayed response to HBV vaccine at 9 months of life.

According to Lee et al. [19] at 12 months of life follow-up after HBV vaccine, 94% of children were surface antibody-positive (anti-HBs ≥ 10 IU/L) but negative for anti-HBc, suggesting that, they had been successfully vaccinated and were uninfected. A small proportion

(1%) had no evidence of response to the vaccine. These agree with our result as we found that at 12 and 24 months both IDMs and infant of non-diabetics mothers had a significant level of anti-HBs with negative HBs antigen and anti-HBc. However, the HBV vaccine did not, in general, give a 100% protection against HBV infection as children with anti-HBs < 10 IU/L rose from 1% in the first year after vaccination to 4% in years 2–4, and up to 25% in year 7 or later [6]. Therefore the risk of infection with positive anti-HBc rose to 2.7% by year 7 after vaccination [21]. In this study, we did not found any positive cases in IDMs or control group as all infants had anti-HBc and HBs antigen negative initially and at follow-up.

The limitation for this study was as follows: we measured anti-HBs, HBs antigen, and anti-HBc at 9, 12, and 24 months, prolonged follow-up should be done in further research. We include only IDM with their mother HBs antigen

negative, further researches include positive HBs antigen mother needed.

Conclusions

Our results suggested that the immune response of IDM after a full course of HBV vaccine by measuring anti-HBs was significantly lower at 9 months when compared to the infant of non-diabetic mothers. However, there was no significant difference in anti-HBs between the case and control group at the age of 12, 24 months. Furthermore no positive HBsAg or anti-HBc cases between the case and control group. Moreover, IDM had a higher birth weight, hemoglobin, and hematocrit level when compared to the control group.

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Author's contributions

AMA, GTS, AME, RAM equally contributed in the study concept, design, supervision, methodology, statistical analysis and data collection. RAM wrote the first manuscript. MSM performed the investigations and laboratory workup and wrote the first draft of

the manuscript. All authors reviewed and approved the final manuscript for publication.

Conflict of interest

Authors declare they have no conflict of interest

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Abbreviations

Anti-HBc: Hepatitis B core antibodies; Anti-HBs: Hepatitis B virus surface antibodies; DM: Diabetes mellitus; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; IDMs: Infants of diabetic mothers; WHO: World health organization.

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Table (1): Comparison between studied groups regarding the demographic data

Variable	Case group (n=100)	Control group (n=50)	P value
Sex			
Male	45 (45.0%)	21 (42.0%)	0.70
Female	55 (55.0%)	29 (58.0%)	
Weight (kg)			
Mean \pm SD	3.6 \pm 0.4	3.03 \pm 0.5	0.01*
Maternal age (year)			
Mean \pm SD	28.1 \pm 5.0	27.2 \pm 4.9	0.26
Mode of delivery			
SVD	32 (32.0%)	18 (36.0%)	0.60
CS	68 (68.0%)	32 (64.0%)	
Gestational age Weeks (Mean \pm SD)	36 \pm 3.3	37 \pm 4.4	0.12
APGAR score			
1 Min (Median (range))	8 (7-9)	8 (6-9)	0.8
5 Min (Median (range))	10 (7-10)	10(8-10)	0.75
Preeclampsia (Yes)	30 (30%)	14 (28%)	0.90
PROM >18 hours (Yes)	10 (10%)	6 (12%)	0.76
HBA1C			
Median and range	6.5(5.5-9.6)		
Median	7.1 \pm 1.6		
Type of diabetes			
Gestational diabetes	53 (53%)		
Type 1 diabetes	12 (12%)		
Type 2 diabetes	35 (35%)		

*significant;

Table (2): Comparison between studied groups regarding the laboratory data.

Variable	Group (I) Cases (n=100)	Group (II) Control (n=50)	P value
HB (g/dl) Mean \pm SD	17.4 \pm 1.3	15.5 \pm 2.0	0.01*
Hematocrit (%)	55.5 \pm 16.4	46 \pm 17.2	0.01*
WBCs $\times 10^3$ U/L Mean \pm SD	7186 \pm 3330	7842 \pm 2146	0.161
Platelets $\times 10^3$ U/L Mean \pm SD	230.8 \pm 73.4	239.1 \pm 97.1	0.559
AST (U/L) Mean \pm SD	25.2 \pm 4.9	23.9 \pm 4.7	0.111
ALT(U/L) Mean \pm SD	29.1 \pm 4.9	28.1 \pm 5.2	0.182
Urea (mg/dL) Mean \pm SD	21.9 \pm 3.78	20.9 \pm 3.85	0.853
Creatinine (mg/dL) Mean \pm SD	0.67 \pm 0.17	0.65 \pm 0.11	0.531

**significant*

Hb: hemoglobin; WBC: white blood cells; AST: Aspartate transaminase; ALT: Alanine transaminase

Table (3): Comparison between cases and control groups regarding hepatitis B virus serology follow up after vaccination.

Item	HBs antigen	Anti-HBs	Anti-HBc
9 months			
Case group (N=100)	Negative	7±12	Negative
Control group (N=50)	Negative	20±23	Negative
P value		< 0.0001	
12 months			
Case group (N=97)	Negative	235±110	Negative
Control group (N=47)	Negative	265±45	Negative
P value		0.07	
24 months			
Case group (N=94)	Negative	138±95	Negative
Control group (N=45)	Negative	165±60	Negative
P value		0.08	

NB: HBs antigen: Hepatitis B surface antigen; Anti-HBs Hepatitis B surface antigen antibodies; Anti-HBc: Hepatitis B core antibodies.

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