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Study of Immune Response in Infants of Non-Gestational Diabetic Mothers

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Abstract

Background
Infants of diabetic mothers (IDMs) have significantly greater risk for spontaneous abortion, stillbirth, congenital malformations and perinatal mortality and morbidity but whether maternal DM affects the neonatal innate immune system is unknown. We aimed to assess the immune response of infants of diabetic mothers (IDMs) with non-gestational DM for Hepatitis B vaccine and to compare them with those of healthy mothers.

Materials and Methods
At a prospective case control study in Minia University Hospital for Children, 150 neonates were included in this study; divided into 2 groups, Group I included 75 infants of diabetic mothers (IDMs) and Group II: Included 75 apparently healthy infants of apparently healthy mothers. These neonates received Hepatitis B virus vaccine during the first 24 hours of age and at 1 and 6 months of age, Hepatitis B virus surface antibodies, Hepatitis B virus envelope antibody (HBe Abs), and Hepatitis B core antibody (HBc Abs) were measured at 9 and 12 months of age.

Results
Results showed that at 9 months of age, Hepatitis B surface antibodies (HBs Abs) titers were significantly higher in healthy neonates compared to IDMs group ($p < 0.001$); while at 12 months of age they were comparable ($p=0.118$). Negative correlation between HBs antibodies levels at 9 months and Aspartate Aminotransferase (AST), Alanine Transferase (ALT), Glycated Hemoglobin (HbA1c) and the duration of maternal diabetes ($p < 0.001$).

Conclusion
According to the results, delayed immune response for hepatitis B vaccine was present in infants of non-gestational diabetic mothers compared to healthy neonates reflecting the effect of diabetes on the immune response of IDMs. They became immune at 12 months of age while normal healthy neonates became immune at earlier age.

Key Words: Diabetic, Egypt, Infants, Immune Response, HB vaccine.

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1- INTRODUCTION

The current widely used Recombinant hepatitis B surface antigen (rHBsAg) vaccines are a viral subunit produced by yeast that has been transfected with a plasmid that contains the *S* gene (codes for HBsAg) either as a single preparation or in combined form (1). Long-term protection against hepatitis B virus (HBV) infection depends on the persistence of strong immunological memory (2). A course of three vaccine injections are given; the first dose is given within the first 24 hours of life; the second dose is at least one to two months (minimal interval of four weeks) after the first dose and the third dose administered at the sixth month (at least eight weeks after the second dose and at least 16 weeks after the first dose) (3).

Hepatitis B vaccine is very effective in preventing Hepatitis B virus infection. After receiving all three doses, Hepatitis B vaccine provides greater than 90% protection to infants, children, and adults immunized before being exposed to the virus (4). To evaluate the response to vaccination following the course of the three doses, a blood test may be taken after an interval of 1-4 months to establish if there has been an adequate response, which is defined as an anti-hepatitis B surface antigen (anti-Hbs) antibody level above 10-12 mIU/ml (5).

Infants of diabetic mothers (IDMs) have significantly greater risk for spontaneous abortion, stillbirth, congenital malformations and perinatal mortality and morbidity but whether maternal DM affects the neonatal innate immune system is unknown (6, 7). The aim of this study was to assess the immune response in infant of diabetic mothers with non-gestational diabetes mellitus and to compare between them with those of healthy mothers for Hepatitis B vaccine which is given during the first day of life and at 1 and 6 months of age (3).

2- MATERIALS AND METHODS

This is a prospective case control study. This study was carried out in Neonatal Outpatient Clinic, Minia University Hospital for Children, and the primary vaccination centers of Ministry of Health, Minia governorate, Egypt, during the period from October 2014 to December 2017.

2-1. Study population

This study was conducted on 150 neonates (77 males and 73 females). These neonates received Hepatitis B virus vaccine during the first day of life and at 1 and 6 months according to Egyptian schedule of vaccination (3). All neonates were classified into two groups:

- **Group I:** Included 75 infants of diabetic mothers (IDMs) born for mothers suffering from non-gestational DM.
- **Group II:** Included 75 apparently healthy infants of apparently healthy mothers.

2-2. Ethical Approval

The goal and methodology of the study were described to all neonates' parents after approval of Ethical Committee Pediatric Department, Faculty of Medicine, Minia University, Egypt. Both verbal and written consents were taken from the parents in the study. The potential benefits and inconveniences of all aspects of the study were clearly stated to the parents. All neonates were chosen according to the following inclusion and Exclusion and Inclusion criteria:

2-2-1. Inclusion criteria

Full term neonates (gestational age 37-42 weeks), and infants of diabetic mothers born for mothers suffering from non-gestational DM and negative for Hepatitis B core antibodies.

2-2-2. Exclusion criteria

Preterm neonates, infants of preeclamptic mothers, neonates with sepsis, non-physiological jaundice, congenital anomalies or ischemic encephalopathy, neonates with any liver disease, mothers with gestational diabetes, neonates with positive hepatitis B core antibody (anti-HBc), organomegaly, or any abnormalities during clinical examination. All neonates were subjected to: Full history taking (personal history, obstetrical history, postnatal history, familial history of chronic disease, and the duration of mothers' diabetes in the IDM group) and Clinical examination.

2-3. Laboratory investigations

2-3-1. Sampling

About 4 ml of venous blood were taken from each neonate under complete aseptic conditions and divided into two tubes. The first tube contained Ethylenediaminetetraacetic acid (EDTA) for evaluating complete blood count (CBC) 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 to evaluate the other laboratory parameters.

2-3-2. The laboratory investigations included

Complete blood count (CBC) was determined using automated cell counter Sysmex, NE, TAO, Medical Incorporation, Ono, Japan. Liver enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which were assayed by colorimetric method using commercial kits. Kidney function: Urea and Creatinine which were determined by colorimetric method using commercial kits. Glycated Hemoglobin (HbA1c) was assessed by using resin column chromatography. Kit contents were supplied by TECO DIAGNOSTICS, California, USA.

2-3-3. The immune response tests

All of neonates received Hepatitis B virus vaccine during the first day of life and at 1 and 6 months. The immune response was assessed after 3 and 6 months of the last dose of HBV vaccination i.e. at 9 months and 12 months of age. 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 virus surface antibody (HBs Abs):**
- It was assayed by Human anti-S Antigen of Hepatitis B virus antibody (HBs Abs) ELISA Kit DEIA002, produced by Creative Diagnostic NY, USA.
- **Hepatitis B virus envelope antibody (HBe Abs):**
- It was assayed by Human anti-E Antigen of Hepatitis B virus antibody (Anti-HBe Abs) ELISA Kit DEIA004, produced by Creative Diagnostic NY, USA.
- **Hepatitis B core antibody (HBc Abs):**
- It was assessed by Human anti-Core Antigen of Hepatitis B virus antibody (Anti- HBcAb) ELISA Kit DEIA005, produced by Creative Diagnostic NY, USA by ELISA technique.

2-4. Statistical analyses

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 24.0. Descriptive statistics were done for parametric quantitative data by mean, standard deviation (SD), and minimum and maximum of the range, and median for non-parametric quantitative data, while they were done for categorical data by number and percentage. Analyses were

done for parametric quantitative data between the two groups using independent samples t-test, and for non-parametric quantitative data using Mann Whitney test. Analyses were done for non-parametric quantitative data within each group using Wilcoxon signed rank test. Analyses were done for qualitative data using Chi square test (if number per cell > 5), and Fisher exact test (2×2 tables). Correlation between two quantitative variables was done by using Pearson's correlation coefficient and for C-reactive protein CRP using Spearman's rho correlation.

Correlation coefficient ranges from (0-1): weak (r=0-0.24), fair (r=0.25-0.49), moderate (r=0.5-0.74), strong (r=0.75-1). Simple logistic regression analysis was used to assess the prediction probability of the HBs abs in diagnosis of DM. Receiver Operating Characteristic curve (or ROC curve.) analysis of HBs abs was used to determine the AUC, optimal cutoff, sensitivity, specificity, PPV, NPV and accuracy in diagnosis of DM. The level of significance was taken at (P- value < 0.05)

3- RESULTS

The aim of this study was to assess the immune response in infant of diabetic mothers with non-gestational diabetes mellitus and to compare between them with those of healthy mothers for Hepatitis B vaccine which is given during the first day of life and at 1 and 6 months of age. There were no significant differences between the studied groups about the demographic data (sex, weight, maternal age and mode of delivery) and the laboratory data as shown in (**Tables 1 and 2**); there were no significant differences between the two groups regarding the levels of Hb, WBCs, and platelets. Also, there were no significant differences between cases and controls in AST, ALT, urea and creatinine concentrations **(Table.3).** Hepatitis B surface antibodies (HBs Abs) titer (measured at 9 months of age) was significantly higher in controls as compared to cases (median levels were 15.0 mIU/ml and 4.0 mIU/ml respectively, p <0.001). However, Hepatitis B envelope antibodies (HBe Abs) there was no significant difference between the two groups (p = 0.533). At 12 months of age, there were no significant difference between cases and controls about hepatitis b surface antibodies (median levels were 14 for cases and 15 for controls, p=0.118). There was a significant difference between the levels of the HBsAb of IDM measured at 9 and 12 months (mean levels were 4 mIU/ml and 14 mIU/ml respectively, p<0.001) (**Table.4**).

HBs Abs had significant negative correlation with liver enzymes AST and ALT (r = -0.65, r = -0.73 respectively p<0.001). It has a strong negative correlation with both the duration of maternal DM and HbA1c (r = -0.907 and r = -0.948 respectively, p<0.001). However, it had a positive correlation with urea concentration; while, no significant association was found between HBs Abs and weight, age of mother, platelet count or HBe antibodies **(Table 5)**.

ROC curve analysis of Hepatitis B surface antibodies (HBs Abs) in prediction of immunity against Hepatitis B virus showed that at a cutoff point of ≥11 (mIU/ml), HBs Abs titer have a high sensitivity (80.0%) and high specificity (93.3%) with positive predictive value of 92.3 % and negative predictive value of 82.4 % and accuracy of 86.7% **(Table.6, and Figure.1)**

Table-1: Demographic data of studied groups.

Variables	Group (I), Cases (n=75)	Group (II), Control (n=75)	P- value
Gender Male Female	 34 (45.3%) 41 (54.7%)	 32 (42.7%) 43 (57.3%)	0.724
Weight (kg) Range Mean ± SD	 (2.4-4.5) 3.4 ± 0.4	 (2.5-4.7) 3.5 ± 0.5	0.178
Maternal Age (year) Range Mean ± SD	 (19-38) 28.1 ± 5.0	 (20-37) 27.2 ± 4.9	0.267
Mode of delivery SVD CS	 24 (32.0%) 51 (68.0%)	 27 (36.0%) 48 (64.0%)	0.605
Duration of maternal DM Range Mean ± SD	 (1-13) 6.7±3.3	 - -	-

SVD: spontaneous vaginal delivery; CS: cesarean section; DM: diabetes millets; SD: standard deviation.

Table-2: The complete blood count between studied groups.

Variables	Group (I), Cases (n=75)	Group (II), Control (n=75)	P- value
HB (g/dl) Range Mean ± SD	 (7.5-17) 11.6 ± 1.9	 (1.6-16) 11.7 ± 2.3	0.771
WBCs ($\times 10^3$ / mcL) Range Mean ± SD	 (3-15) 7.4 ± 2.9	 (4-11.2) 7.7 ± 1.7	0.470
Platelets ($\times 10^3$/ mcL) Range Mean ± SD	 (16-390) 230.8 ± 73.4	 (20-460) 239.1 ± 97.1	0.559
HbA_{1c} Range Mean ± SD	 (5.5-10) 7.8±1.3	 -	-

HB: Hemoglobin; WBC: White blood Cell; HbA1c: Glycated Hemoglobin; SD: Standard deviation.

Table-3: Liver enzymes and kidney function of studied groups.

Variables	Group (I), Cases (n=75)	Group (II), Control (n=75)	P- value
AST (U/L) Range Mean ± SD	 (15-45) 29.4 ± 5.8	 (18-43) 28.8 ± 6.3	0.544
ALT (U/L) Range Mean ± SD	 (15-47) 31.9 ± 6.1	 (20-45) 30.4 ± 6.5	0.147
Urea (mg/dl) Range Mean ± SD	 (7.3-21.3) 12.0 ± 3.9	 (6-17) 12.3 ± 2.9	0.593
Creatinine (mg/dl) Range Mean ± SD	 (0.5-2.2) 1.2 ± 0.3	 (0.1-2.4) 1.1 ± 0.4	0.105

AST: Aspartate Aminotransferase; ALT: Alanine Transferase; SD: Standard deviation.

Table-4: Hepatitis B surface antibodies (HBs Abs) at the 9th and the 12th months, Hepatitis B envelope antibodies (HBe Abs) in studied groups.

Variables	Group (I), Cases (n=75)	Group (II), Control (n=75)	P- value
HBs Abs *(mIU/ml)* (At 9 Months) Median Range Mean ± SD	 4.0 (1-18) 6.1±4.9	 15.0 (11-19) 14.8±2	<0.001
HBe abs *(S/C.O)* Range Mean ± SD	 (2-10) 5.91±2.15	 (2-11) 6.12±2.02	0.533
HBs Abs *(mIU/ml)* (At 12 Months) Median Range Mean ± SD	 14 (12-17) 14.1±1.3	 15.0 (11-19) 14.8±2	0.118 <0.001*

* Between HBSAbs of cases at 9 month and HBsAb of cases at 12 months; S/CO: S (individual optical density) / cut off.

Table-5: Correlation between Hepatitis B surface antibodies (HBs Abs) at 9 months and other variables in cases group.

Correlations	(r)	P- value
Weight	-0.02	0.894
Age of mother	-0.04	0.714
Duration of DM	-0.907	<0.001
HB	0.34	0.003
WBCs	0.22	0.062
Platelets	-0.07	0.528
AST	-0.65	<0.001
ALT	-0.73	<0.001
Urea	0.85	<0.001
Creatinine	-0.27	0.018
Maternal HbA1c	-0.948	<0.001
HBe abs	-0.041	0.725

DM: Diabetic mother; HB: Hemoglobin; WBC: White blood Cell; HbA1c: Glycated Hemoglobin; HBe antibodies: Hepatitis B envelope antibodies; AST: Aspartate Aminotransferase; ALT: Alanine Transferase; SD: Standard deviation.

Table-6: The ROC curve analysis of Hepatitis B surface antibodies (HBs Abs) in prediction of immunity against Hepatitis B virus.

Item	Optimal cutoff (mIU/ml)	AUC	P-value	Sensitivity	Specificity	PPV	NPV	Accuracy
HBs abs	≥11	0.891	<0.001	80.0	93.3	92.3	82.4	86.7

AUC: Area Under the Curve; PPV: positive predictive value; NPV: Negative predictive value.

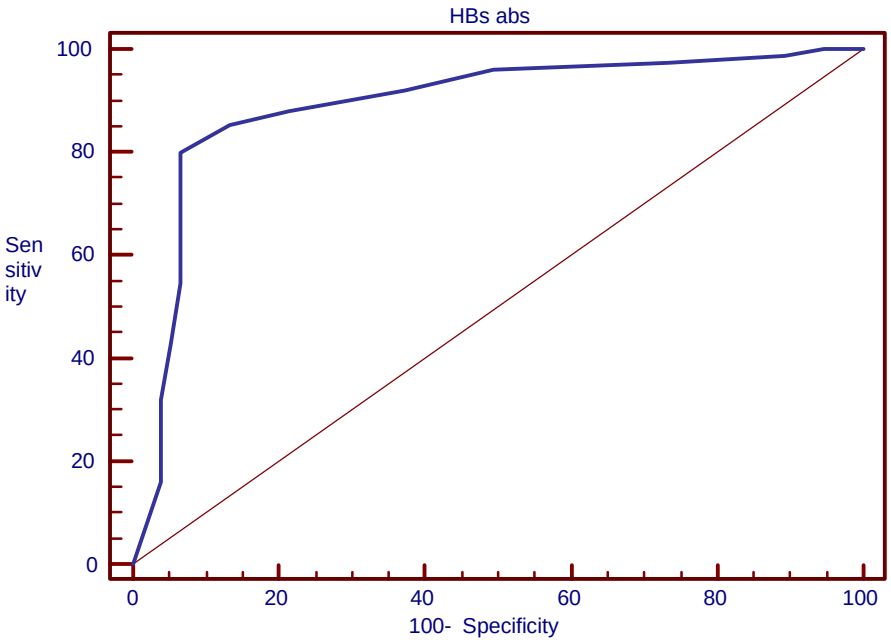

Fig.1: ROC curve analysis of HBs Abs in prediction of immunity against Hepatitis B virus. Hepatitis B surface antibodies (HBs Abs).

4- DISCUSSION

Hepatitis B Virus (HBV) infection is a major global health problems. It has been estimated that up to 2 billion individuals have evidence of exposure to HBV and an estimated 350 million persons worldwide are chronically infected with HBV (8). The acute hepatitis B causes liver inflammation, vomiting, jaundice and, rarely, death and chronic hepatitis B may eventually cause cirrhosis and liver cancer (9). Vaccination is the main preventive method against hepatitis B infection and now many countries routinely vaccinate infants against hepatitis B (10). Several studies showed that good glycemic control reduces neonatal complications (11, 12; and still diabetes in pregnancy is associated with adverse fetal and maternal outcomes even after adequate antenatal care and glycemic control (13). Despite the scientific interest, there were no studies reported on immune response of infants of diabetic mothers suffering from non-gestational diabetes mellitus; but, it has been reported that there is a high prevalence of Type 2 diabetes among HCV-infected patients with chronic hepatitis and there is growing evidence indicating that HCV is associated with Type 2 diabetes (14). Also, Hepatitis B virus vaccination is also recommended by the U.S Centers for Disease Control and Prevention to unvaccinated adults with diabetes mellitus due to higher rates of infection and progression to cirrhosis than in the general population (15). The present results revealed that there was no significant difference between the two groups regarding sex distribution, weight of newborns, age of mothers and mode of delivery and we beloved that this is important to ensure the homogenization of the studied groups to get accurate results from the comparison between groups. The present results showed that Hepatitis B surface antibodies (HBs Abs) was significantly ($p<0.01$) higher in control group (16.0 mIU/ml) as compared to cases group (4.0 mIU/ml) at 9 months of age, but at 12 months, there was no significant difference between the two groups about HBsAb.

HBsAb was negatively correlated with the maternal HbA1c and the maternal duration of diabetes, also it was negatively correlated with the liver enzymes. It is well known that the presence of Hepatitis B surface antibody is generally interpreted as indicating recovery and immunity from hepatitis B virus infection. Hepatitis B surface antibody also develops in a person who has been successfully vaccinated against hepatitis B. Also, a positive or reactive Hepatitis B surface antibody (HBs Abs) test result (> 10-12 mIU/mL) indicates that a person is protected against the hepatitis B virus and this means that the person is "immune" and protected against the hepatitis B virus (5). These results indicated that infants of healthy non diabetic mothers "with positive Hepatitis B surface antibody status 16.0 mIU/ml" are immune and protected against the hepatitis B virus infection while infants of diabetic mothers with HBs Abs 4.0 mIU/ml (< 10-12 mIU/mL) were not immune or protected against the hepatitis B virus infection and the their vaccinations did not get a successful result (16) at 9 months of age while the normal immune response of healthy infants to HB vaccine was reached at 9 months of age.

This is the first study to evaluate the immune response of infants of diabetic mothers and compare them with those of normal healthy mothers for Hepatitis B vaccine. These results might be explained by that infants of diabetic mothers had immunological abnormities which can cause these results and the mechanisms underlying these results are unknown and should be further explored. Roll et al., (1994) investigated the impact of diabetic mothers on the maturation of the immune system in their neonates (17). They found that the infants of diabetic mothers the percentages of total lymphocytes (T and B lymphocytes) were significantly decreased compared to the neonates of healthy mothers. They concluded that there were changes in children of diabetic mothers that may reflect a cellular immune reaction to the maternal environment, characterized by both an abnormal metabolic state and persisting autoimmunity in the affected mother. Diabetes mellitus can alter the placental and neonatal immune systems (18, 19). Mrizak et al., (2014) reported that GDM was associated with increased expression of interleukin (IL)-6, toll-like receptor (TLR) 4, and transforming growth factor beta mRNA in the placenta (18). While Atègbo et al., (2006) showed elevated levels of T helper type 1 cytokines, IL6 and TNF-α but low levels of IL-10 in the serum of macrocosmic babies born to GDM mothers (19).

However, because of the paucity of studies that focus on the interactions between the immune system and DM in pregnancy, whether and how maternal DM affects the neonatal immune system remains unclear. Rajeev and Petrova, (2005) studied the neutrophil functional activity in the cord blood of IDMs. They found that maternal diabetes leads to impairment of cord blood neutrophil motility and post phagocytic bactericidal capacity (20). Sakika et al. stated that maternal DM induces excessive inflammatory activation in neonates via a TLR5- or TLR1/2-mediated innate immune response leading to excessive production of IL-8 and TNF-α (7).

In a recent study conducted in china by Li et al., (2106**)** they studied the relationship between positivity for hepatitis B surface antibody (HBsAb) and diabetes mellitus (16). They found that HBsAb-positive status is associated with a low rate of diabetes and better metabolic status. Similar findings were found by Khalili and Sanyal, (2016) (22). Also, Demir et al., (2008**)** investigated the prevalence of occult HBV infection among hepatitis B core antibody (HbcAb) +/- hepatitis B surface antibody (anti-HBs) positive Type 2 diabetes mellitus patients (23).

They found that the prevalence of occult HBV infection is higher in diabetics compared with healthy controls and this may contribute to the increased prevalence of primary hepatocellular carcinoma in diabetics. Li et al., (2016) reported that HBsAb positive state in subjects may indicate better innate and/or acquired immune regulation against HBV infection (21). In addition, patients with diabetes have decreased efficiency in developing anti-HBV antibodies after vaccination (24). However, the association between HBsAb positive status and the prevalence of diabetes remains unclear. A previous study showed that HBV infection is strongly associated with diabetes[25]. It has also been reported that the incidence of gestational diabetes is related to HBV infection (26).

Disturbances in cellular innate immunity play a role in the pathogenesis of the increased prevalence of infections in DM patients. In general, a better regulation of the DM leads to an improvement of cellular function. A second important mechanism is the increased adherence of the microorganism to diabetic cells. Furthermore, some microorganisms become more virulent in a high glucose environment (27). Kawaguchi et al., (2007) reported that patients with diabetes mellitus have infections more often than those without DM and one of the possible causes of this increased prevalence of infections is defects in immunity (28).

Different disturbances such as low complement factor 4, decreased cytokine response after stimulation in humeral innate immunity have been described in diabetic patients. Concerning cellular innate immunity most studies show decreased functions (chemotaxis and phagocytosis) of diabetic polymorphonuclear cells and diabetic monocytes/macrophages compared to cells of controls. Furthermore, some microorganisms become more virulent in a high glucose environment. Another mechanism which can lead to the increased prevalence of infections in diabetic patients is an increased adherence of microorganisms to diabetic compared to nondiabetic cells. Eduardo et al., (2012) studied the transfer of maternal immunity to newborns of diabetic mothers; they determined antibodies levels, superoxide release, phagocytosis and bactericidal activity of phagocytes (29). They stated that diabetic mothers had lower IgA and IgG levels in colostrum and lower IgG and IgM levels in blood than non-diabetic mothers. Newborns of diabetic mothers have an immature immune system and are compatible with the immunological profile of newborns of normoglycemic mothers.

The hyperglycemia alters IgG transfer across the placenta and decreases immunoglobulin levels in maternal blood and colostrum. Also, Morceli et al., (2011) reported that the reduction in immunoreactive proteins production may be related to changes in the metabolism of carbohydrates, lipids, and proteins, as well as in various organ systems caused by the hyperglycemic status of pregnant women (30). Wijsman et al. studied the relationship between higher glucose concentrations and low cytokine response. They found a significant negative association between glucose concentrations and cytokine response capacity ($p<0.05$) (31).

High glucose levels limit and irregulate neutrophil synthesis, cytosolic calcium in polymorphonuclear leukocytes (PMNs) increases in the presence of hyperglycemia and is inversely proportional to the occurrence of phagocytosis in patients with diabetes. High levels of cytosolic calcium inhibit the synthesis of adenosine triphosphate (ATP), which is essential for phagocytosis. The ability of PMN leukocytes to mobilize to the site of infection and stimulate of apoptosis is negatively impacted as well.

Hyperglycemia causes other undesirable changes in the function of the immune system such as decreased complement response, leukocyte adherence and bactericidal activity (28). Targher et al. found that soluble intracellular adhesion molecule-1 (sICAM-1) activity were markedly higher (P< 0.01 or less) in diabetic patients than in healthy controls and has a significant positive correlation with both HbA1c and duration (32). Also, Liberatore et al. stated that lower IgG and complement 4 levels in uncontrolled diabetic patients were detected. Both C4 and IGg values were negatively correlated with HbA1c (33). According to Hostetter, hyperglycemia can be responsible for a reduced C3 activity as the glucose may serve as an acceptor molecule for C3, by formation of stable glucose-C3, through glycosylation (34).

Metabolic control might influence the humoral response and Ig synthesis. It is also possible that impaired intracellular glucose metabolism leads to reduced metabolic activity and consequently, less protein synthesis (35). In our study, the results of the ROC curve analysis of HBs Abs in prediction of immunization against Hepatitis B virus infection revealed that, in a cutoff point of ≥11, HBs abs have a high sensitivity (80.0%) and high specificity (93.3%) with positive predictive value of 92.3 % and negative predictive value of 82.4 % and accuracy of 86.7%.

These results agreed with many authors. It is well known that the protective efficacy of hepatitis B vaccination is directly related to the development of antibody to Hepatitis B surface antigen and the efficacy of HBV vaccines has been demonstrated in clinical trials involving several groups which showed complete protection in persons who developed anti-HBs concentration of more than 12 mIU/ml following vaccination (36-38).

5- CONCLUSION

Infants of non-gestational diabetic mothers had a delayed immune response for hepatitis B vaccine compared to healthy neonates of born to healthy mothers reflecting the effect of non-gestational diabetes on the immune response of IDMs. They became immune at 12 months of age while normal healthy neonates became immune at earlier age.

6- ABBREVIATIONS

WBC: White blood Cells.
AST: Aspartate Aminotransferase.
ALT: Alanine Transferase.
HbA1c: Glycated Hemoglobin.
HBe antibodies: Hepatitis B envelope antibodies.
HCV: Hepatitis C Virus.
GDM: Gestational Diabetes Mellitus.
TNF-α: Tumor Necrotizing Factor Alfa.
TLR5- or TLR1/2-mediated: Toll- like Receptor 5 or 1/2-mediated.
C3: Complement 3.
C4: Complement 4.
IgA: Immunoglobulin A.
IgG: Immunoglobulin G.
IgM: Immunoglobulin M.
PPV: Positive Predictive Value.
NPV: Negative Predictive Value.

7- AUTHORS' CONTRIBUTIONS

RA and AE conceived the study, carried out its designing, coordinated the implementation, helped to perform the statistical analysis and drafted the manuscript. MA was responsible for interpretation of laboratory data of patients and revision of the manuscript. All authors contributed equally in this work and read and approved the final manuscript.

8- CONFLICT OF INTEREST: None.

9- ACKNOWLEDGMENTS

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