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Hepatitis B vaccination failure in children with diabetes mellitus?

The debate continues

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Key words: hepatitis B virus, vaccine, type 1 diabetes mellitus, children

Background: The aim of our study was to evaluate the presence of specific antibodies against HBsAg in diabetic children (IDDM) previously vaccinated against hepatitis B virus.

Results: 46 of 110 diabetic children (41.8%) and 16 of 100 healthy controls (16%) were found not to have anti-HBs antibodies ($p < 0.0001$). The mean antibody titer was found significantly-lower ($p < 0.0001$) in IDDM children than healthy controls. No correlation was found between antibody titer, age, duration of disease and HbA1c. We did not find any difference of gender, age, years of onset of the disease and metabolic control, between diabetics with anti-HBs antibodies and those without.

Patients and Methods: 110 diabetic children were retrospectively studied and 100 healthy controls were recruited. In all patients surface antigen, HBV core IgG, antibodies against HBV "e" antigen and quantitative HBV surface antibodies were detected. In 45 patients molecular typing of HLA alleles was performed.

Metabolic control was evaluated as mean glycated hemoglobin (HbA1c) and all patients were compliant to insulin therapy.

Conclusions: Our data confirm the reduced seroprotection rate for HBV vaccination in diabetics. However it remains poorly clarify the real clinical significance of this result. In our study no diabetic children showed markers of HBV infection.

Introduction

In healthy people the current vaccine against hepatitis B virus (HBV) is very effective, only 4–10% of immune-competent subjects fails to elicit protective levels of antibodies to recombinant hepatitis B surface antigen (HBsAg) after the standard three doses of HBV vaccination schedule.[1,2] In addition the immunological memory seems to persist beyond 10 y after vaccination, even if antibody levels are no longer detectable.[3]

It is known that some specific pediatric populations (i.e. patients with chronic renal insufficiency, cirrhosis or under dialysis)[4,5] do not elaborate an immune response after HBV vaccination. Moreover, some genetic pathologies, such as celiac disease, are also identified as risk factors for non-responsiveness to HBV vaccine.[6,7]

Currently the exact mechanism of an impaired immunological response in healthy people is poorly understood, but, since it is known that the immune response to HBV vaccine is largely determined by the presentation of the immunogenic peptides via HLA-DR and DQ molecules, it has been suggested that the presence of DR3;DQ2 and DR7;DQ2 haplotypes predisposes to a lower response to vaccines.[8-11]

Type I Diabetes Mellitus (IDDM) is a clinical condition that influences the cellular and humoral immune system,[6,12] as that the immunological response to HBV vaccine could be less optimal than in non diabetic individuals. Since several studies have clearly demonstrated that both HLA-DQ and HLA-DR influence IDDM susceptibility,[13,14] this HLA profile could represent the link between the IDDM and non-responsiveness to HBV vaccine.

This low sero-conversion rate found in IDDM patients could represent a significant health problem, with respect to the actual prevalence of IDDM in Europe,[14] considering also that these patients have several risk factors for enhancing hepatitis B virus exposure, including self-monitoring, blood glucose, intravenous and subcutaneous insulin injections and repeated hospitalizations. Thus, IDDM non responders patients could constitute an important reservoir, that is potentially capable of modifying the endemics of HBV disease, without protection against HBV complications, such as cirrhosis and hepatocarcinoma.

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Table 1. Demographic characteristics, HBs antibody titer and number of responders or non-responders between diabetic patients and healthy controls

	Diabetic Patients	Controls	p-value
Number	**110**	**100**	
Sex (M/F)	75/35	56/44	ns*
Age (years) (mean ± SD)	13.67 ± 4.9	13.06 ± 4.3	ns**
Mean antiHBs titer (mean ± SD)	58 ± 112.9	266.49 ± 335.85	0.0001**
Responders/Non Responders	64/46	84/16	0.0001*

ns, not significant; *Fisher's exact test; **Mann-Withney U test.

Table 2. Demographic characteristics, duration of the disease and therapeutic compliance in responders and non responders

	Iddm -Responders	Iddm Non Responders	p-value
Total Number	**64**	**46**	
Sex (M/F)	35/29	27/19	ns*
Age (years) (mean ± SD)	13.63 ± 5.3	13.74 ± 4.4	ns**
Time (years) Onset-sample (years) (mean ± SD)	5.91 ± 4.1	6.35 ± 4.3	ns**
Hb1Ac (%) (mean ± DS)	7.83 ± 1.07	7.85 ± 0.86	ns**

Ns, not significant; *Fisher's exact test; **Mann-Withney U test.

The aim of our observational study was to evaluate the specific antibody presence against HBsAg (anti-HBs) in children with IDDM, after three doses of intramuscular HBV vaccine, according to the standard national calendar, administered at 3, 5 and 11 mo of life respectively, and to state the presence of serological markers of HBV infection in those without protective antibodies.

Results

A tabulation of gender, age, number and percentage of responders, HBs antibody titer of the studied groups is summarized in **Table 1**.

We found that 75 of 110 IDDM patients (68%) were male, the mean age was 13.6 ± 4.9 SD and the mean HBsAb titer was 58.5 ± 112.9 SD.

No difference with respect to gender and age was found between IDDM patients and healthy controls. On the contrary an highly significant difference ($p < 0.0001$) of the mean antibody titer was found between IDDM patients and healthy controls.

According to the quantitative HBsAb measurements, 46 of 110 IDDM patients (41.8%) and 16 of 100 control subjects (16%) were found to have not anti-HBs antibodies against HBsAg and this difference was highly significant ($p < 0.0001$).

No significant correlation was found between antibody titer and age of patients, duration of disease and HbA1c.

When we compared diabetic children with anti-HBs antibodies with those without anti-HBs antibodies we did not find any significant difference with respect to gender, age, years of onset of the disease and metabolic control, assessed by HbA1c values (**Table 2**).

Table 3 summarizes the results obtained by molecular typing of HLA alleles on 45 of the 110 diabetic patients enrolled in the study. Among these patients, 25 responded to the vaccine for hepatitis B virus, while 20 did not develop an adequate antibody response.

None of our non responders IDDM patients showed the presence of serum anti-HBc or HBsAg.

Discussion

In literature previous studies described a reduced efficacy of hepatitis B virus vaccination both in chronic diabetic adults[15,16] and in children.[17]

Recently, a meta-analysis of current literature underlined the role of IDDM in determining an impaired response to HBV vaccine, finding a lower percentage of HBV vaccine responders among IDDM patients with risk factors for poor response to vaccines, such as long-term dialysis and chronic renal insufficiency,[18,19] when compared with the same group of high risk patients without IDDM. Nevertheless, the cause of this lower IDDM patients' response to HBV vaccine still remains unknown.

A previous study leaded on healthy people stated a genetic predisposition to hepatitis B virus vaccine non responsiveness.[3,20] It is known that the humoral response to vaccines is influenced by allelic variants, which differ in their ability to bind and present antigens to T lymphocytes. This may be linked to defects in the immune response on the antigen uptake, processing and presentation, as well as T cell suppression and lack of type 2 helper T cell response, necessary for B cells production of anti HBs antibodies.[21]

HLA system is critical for the control of the immune response, since it applies in the mechanisms of immune recognition of all foreign substances that keep in contact with the immune system. Therefore it is likely that individuals expressing specific HLA molecules may respond abnormally to immunization. With this regard, the expression of HLA-DQ2 seems to be associated with a poor antibody production in response to the HBV vaccine, but also the -DR3 and -DR4 alleles seem to be associated with a failure in the response to the vaccine.[9-11]

Since more than 90% of diabetic patients expresses one or both DR3/DQ2, DR4/DQ8 haplotypes, this HLA profile could justify by the unresponsiveness to HBV vaccine in diabetic patients.[9]

In our study the molecular typing of HLA alleles was performed in only 45 of 110 diabetic patients and it should be noted that all the 5 patients expressing HLA DR3/DQ2-DR4/DQ8 haplotype did not show the presence of anti-HBs antibodies. It is well established that this haplotype is associated with the highest risk of developing diabetes and this finding seems particularly important to further support the relationship between some specific genetic patterns and failure in response to hepatitis B virus vaccine in diabetic patients. Douvin and coll highlighted the same relationship between HLA-DQ2 and low

response to the vaccine in IDDM patients.[22] In our study it seems that this hypothesis could be confirmed in the patients tested for HLA type, nevertheless as the impact of the large number of patients not tested for HLA type can represent a limitation of this hypothesis, we cannot formulate a definitive conclusion.

Moreover, the lag time between the onset of the disease and the evaluation of antibody levels could be considered a risk factor that influences the low response to HBV vaccination. However our study showed that defects in HBV vaccine response does not seem to be related with the timing of the disease. Moreover, in our study, as in that one of Douvin and coll,[22] there was no relationship between metabolic control of the disease, assessed by HbA1c measurement and failure in vaccine response.

However other studies found a good response to HBV vaccine. Marseglia and coll[23] demonstrated both a successful immune response to a recombinant HBV vaccine and, 4 y later, a long-term persistence of anti-HBs protective levels in the same young IDDM children.[24]

In a study leaded on 299 diabetic subjects, Halota and coll demonstrated a protective anti-HBs titer in 98.7% of them after vaccination, and they suggested that patients with diabetes react to HBV vaccination similarly to healthy population.[25] Similar findings were described in further literature data.[26-28] Thus, these contradictory results justify the debate on responsiveness to HBV vaccine in IDDM patients and on long-term efficacy of the vaccine, this matter remaining still open. On this regard, as in our study we performed an evaluation of the seroprotection rate after more than 10 y from the last vaccine dose, the lag time between the vaccine and the analysis of anti HBV antibodies could represent an important limitation, linked to the retrospective analysis of the anti-HBV seroprotecion rate. As a matter of fact, in other studies the major issue is that anti-HBs levels < 10 mIU/ml are equated with "non-response" to vaccine, in this setting of testing many years after hepatitis B vaccination, although responsiveness to hepatitis B vaccination is usually determined by testing within 2–6 mo after receipt of the third vaccine dose. However in our study we found that, even if a cut-off interval had passed between vaccination and dosage of anti HBs antibodies, these latter was significantly lower in IDDM patients than in control group, considering that in both groups the cut-off interval was the same. In our study no patient was affected by HBV infection, as the presence of serum anti HBc or HBsAg was detected in none of non responders.

Since the immunological memory seems to persist beyond 10 y after the vaccination, even when antibody levels are no longer detectable,[29] our data reinforce the hypothesis that a solid immunologic memory can be assessed in previously vaccinated subjects, even in absence of anti-HBs antibodies.

With respect to this, additional observations are required in children with long-standing diabetes, in order to establish the loss of vaccine efficacy and to identify the need and timing of booster doses. Currently there are no protocols or guidelines on the management of non-responders to recombinant hepatitis B virus vaccine. The intradermally (ID) administration of the vaccine could be an alternative solution to pursuit an higher concentration of the recombinant vaccine.

Table 3. HLA haplotypes of diabetic patients vaccinated for HBV

HLA haplotype	N° Of Iddm Patients	Iddm Patients-Non Responders
DR3/DQ2	21	9
DR4/DQ8	8	1
DR3/DQ2–DR4/DQ8	5	5
DR3/DQ2–DR7/DQ2	4	1
DR7/DQ2	3	1
DR4/DQ8–DR12/DQ7	1	1
DR3/DQ2–DR5/DQ7	1	1
DR7/DQ2–DR5/DQ8	1	0
DR4/DQ8–DR7/DQ2	1	1

With this regard it is important to mention that the ID subministration has already been successfully tested in celiac disease (CD) patients who do not respond to conventional IM vaccination.[30,31]

Patients and Methods

Patients. A total of 110 patients affected by type 1 diabetes mellitus (75 males and 35 females), aged between 2 and 23 y (mean age 13.67 y ± 4.92 SD), were retrospectively studied. Diabetic patients were consecutively recruited, as they were hospitalized for the first time or, instead, they were followed up in our Unit. Type I diabetes was diagnosed on the basis of hyper-glycemia, absolute insulin dependence and positivity to specific Isle Cell Antibodies (ICA) and Glutamic Acid Decarboxylase Antibodies (GADA). The patients were receiving human insulin, using two or three injections daily. Metabolic control was evaluated as mean glycated hemoglobin (HbA1c) and all patients were compliant to insulin therapy. Since 1982, in all patients recombinant hepatitis B virus vaccine (Engerix B, GlaxoSmith&Kline) had been administered in 10 μg doses, at the age of 3, 5 and 11 mo by three intramuscular injection into the frontlateral leg muscle. Vaccination records were obtained from parents and reviewed in all the cases, to ensure the completeness of childhood vaccination.

All diabetic patients included in the study had completed HBV vaccination at least 6 mo before the enrollment and had been vaccinated before the onset of diabetes. We excluded patients that had not completed the standard schedule for HBV vaccination, with associated autoimmune disorders or performing immune suppression therapy.

In all patients it was assessed the time between the onset of the disease, the age of vaccination and the beginning of our study. None of IDDM patients and control subjects had a previous history of hepatitis B virus infection. Only in 45 patients (36 males and 9 females, aged between 5 and 23 y, mean age: 16.33 ± 4.62 SD) molecular typing of HLA alleles was performed. As control group, 100 healthy subjects (56 males and 44 females) aged between 3 and 22 y (average age: 13.06 ± 4.34 SD), were recruited. All these patients had received the same HBV immunization schedule of diabetic patients. Informed consent was obtained from parent's patients and controls.

Methods. In diabetics and in healthy control group, 4 ml of venous peripheral blood were obtained. Samples were then centrifugated for 8 min to 3,500 turns and serum samples immediately stored at -30°C, before performing the assessment of antibody titer.

In all patients and healthy subjects, surface antigen (HBsAg), HBV core antibody IgG (anti-HBc IgG), antibodies against hepatitis B virus "e" antigen (anti-HBe) and quantitative HBV surface antibodies (anti-HBs) were detected many years after the last vaccine dose (mean: 12.5, ±SD: 42 y). Anti-HBs antibodies were measured by enzyme-linked immune-adsorbent assay by using commercially available assay (hepanostica anti-HBs, bioMerieux, Netherlands) and titer was reported in international units per liter. The cut-off value for a negative qualitative anti-HBs antibody test was 10 mIU/mL and patients with anti-HBs titer >10 IU/ml were considered responsive. In 45 patients (including 36 males and 9 females, aged between 5 and 30 y, mean age: 16.33 ± 4.62 SD) was performed molecular typing of HLA alleles (class I and II) by microlymphocytotoxicity and DQ-α and DQ-β chain molecular analysis by polymerase chain reaction. HbA1c levels were measured with the immunochemical method DCA 2000 (considering as normal range values under 48 mmol/mol). In all patients, organ and non organ-specific autoantibodies were tested by passive hemoagglutination test or by indirect immunofluorescence.

Statistical analysis. Differences between groups, as continuous variables, have been evaluated with the Mann-Whitney test and those presented as categorical variables with the Fisher test.

The Mann-Whitney test was used to compare age, titer level of HBsAb, and number of years between the onset of diabetes in IDDM patients and healthy control and to compare age and Hb1Ac values between diabetic responders and non responders.

The Fisher's exact test was used to compare the number of "failed response" between diabetics and control group, male and female patients between diabetic group and healthy controls and between diabetic responders and non-responders.

A p-value < 0.05 was considered to be statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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