Role of Gluten Intake at the Time of Hepatitis B Virus Vaccination in the Immune Response of Celiac Patients

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Several studies have reported an inadequate response to hepatitis B virus (HBV) vaccination in patients affected by celiac disease (CD). Unfortunately, the causes of this impaired response are unknown (1–8).

In the general population, it is recognized that several factors influence the production of protective levels of antibodies against HBV after the standard immunization. Well-known modifiers include age, obesity, smoking, drug abuse, alcoholism, infections, immune suppression, and the route of vaccination (9, 10). Additionally, hepatitis B vaccine nonresponsiveness, due to the presence of specific human leukocyte antigen (HLA) genotypes, has been described (11–13).

Celiac disease (CD) is an HLA-associated immunological disease, and for this reason, a genetic predisposition as a possible cause of a lower grade of immunization to recombinant hepatitis B vaccines has been considered (4, 5). In fact, HLA-DQ2 status may predispose CD patients to fail to develop immunity after hepatitis B vaccination through a Th2 response that is inadequate for B-cell differentiation and the formation of memory B cells (5). In contrast, several studies have hypothesized gluten intake as a cause of failed immunity at the time of vaccination. Gluten might be implicated because both hepatitis B surface antigen (HBsAg) protein fragments and gliadin peptides bind to HLA-DQ2 molecules and induce proliferation of T lymphocytes. Competition between the proteins may result in defective antibody production (6–8).

The aim of our study was to evaluate the HBV vaccination response in relation to gluten exposure status in a series of CD patients and healthy controls.

MATERIALS AND METHODS

The study population consisted of CD patients born after 1980 and vaccinated as infants or as 12-year-old adolescents according to the Italian vaccination program. Patients were consecutively recruited from the Celiac Disease Centre of the University of Naples Federico II in Italy from September 2010 to May 2012. In the study population, the recombinant hepatitis B vaccine (Engerix-B; GlaxoSmithKline, Belgium) was administered according to the Italian vaccination program: 3 doses of 10 μg each are given at the ages of 3, 5, and 11 months by intramuscular injection to infants vaccinated at birth and 3 doses of 20 μg each are given at 0, 1, and 6 months to adolescents. The date of commencement of the gluten-free diet (GFD) for each CD patient was also confirmed.

In accordance with gluten exposure status at the time of vaccination, we considered three groups: group A (exposed to gluten), including patients vaccinated as 12-year-old adolescents (the CD diagnosis was established after vaccination); group B (not exposed to gluten), including patients vaccinated as 12-year-old adolescents on a gluten-free diet at the time of vaccination; and group C (infants), including patients vaccinated at birth. The response of celiac patients to hepatitis B vaccination was compared to that of healthy subjects, i.e., those in the control group (group D). This study included 163 celiac patients (group A, 57 patients; group B, 46 patients; and group C, 60 patients) and 48 controls (group D). An inadequate response to hepatitis B immunization was present in 43.9% of patients in group A, 34.8% of patients in group B, 58.3% of patients in group C, and 8.3% of patients in group D (group A versus group D, P < 0.001; group B versus group D, P = 0.002; group C versus group D, P = 0.001) (no significant difference for group A versus group B and group A versus group C was evident). Our data suggest that gluten exposure does not influence the response to hepatitis B immunization and that the human leukocyte antigen probably plays the main immunological role in poor responses to hepatitis B-vaccinated celiac patients.
the case of undetectable HBsAb, an arbitrary value of 0.5 mIU/ml was assigned to enable the calculation of antibody geometric mean concentrations (GMCs). Analysis of variance (ANOVA) was performed with and without adjustment for covariates. Natural log transformation of HBsAb was used in the ANOVA. A P value of ≤0.05 was considered significant. On the basis of previous studies, we estimated that a sample size of 38 subjects per group would offer 80% power to detect a 30% difference in HBV vaccination outcomes between the CD group members and the controls (assuming a minimum 90% response to HBV vaccination in the general population).

RESULTS

This study included 163 CD patients (group A, 57 patients; group B, 46 patients; and group C, 60 patients) and 48 controls (group D). All subjects were found to be HBsAg negative; HLA-DQ2 was present in 92.6% of the CD patients and 25% of the controls.

As shown in Table 1, gender was not significantly different among groups (P = 0.97). A significant difference was evident between age at the time of testing and the interval of time from vaccination to testing (P = 0.001 and P < 0.001, respectively). CD patients vaccinated as infants (group C) were younger than other subjects and showed a longer interval from vaccination to testing.

We observed an inadequate antibody response to hepatitis B immunization in all celiac patients compared to that in the controls (Table 2): 43.9% of the patients in group A, 34.8% of the patients in group B, 58.3% of the patients in group C, and 8.3% of the controls in group D. Geometric mean concentrations (GMCs) of HBsAb were significantly different between the CD groups and our control group among groups pre- and postadjustment (Table 4). The results were also adjusted for the following covariates: years from vaccination (model a) and the age at HBsAb testing and the age at vaccination (model b). Both the titers of HBsAb and the prevalence of people with concentrations of <10 mIU/ml were different among groups pre- and postadjustment (Table 4). The results were also statistically significant when the ANOVA models were reanalyzed after the exclusion of group C (the sole group vaccinated at birth) (F > 8.56, P < 0.001).

DISCUSSION

In typical healthy populations, 4 to 10% of vaccine recipients fail to produce protective levels of antibodies to the HBV vaccine after standard immunization (10). In contrast, in the subjects who have an efficacious response, HBsAb seroprotection persists at least 10 years; thus, a booster dose is not necessary until 10 years after the primary immunization (14, 15).

Recently, our group reported that 31.4% of CD patients and 8.3% of controls showed HBsAb concentrations of <10 mIU/ml approximately 11 years after the primary vaccination. Moreover, CD patients with HBsAb concentrations of ≥10 mIU/ml showed GMCs lower than those of controls (3). Adishali et al. (4) found that 68% of adults with CD were responsive to HBV vaccination; this percentage was significantly lower than that in the general population (100%). They also found no statistically significant difference in the prevalence of HBsAb concentrations of <10 mIU/ml between patients vaccinated at 12 years as adolescents who were exposed to gluten at the time of vaccination (group A) and patients vaccinated at the same age who were on a GFD at the time of vaccination (group B). Instead, there was a significant difference in the prevalence of HBsAb concentrations of <10 mIU/ml when group B was compared with the group vaccinated as infants (group C) (Table 3). In general, group C had a higher prevalence of inadequate response to hepatitis B vaccination when defined as HBsAb titers of <10 mIU/ml and lower levels of GMCs, compared with groups A and B.

Table 4 shows the results of two ANOVA calculations that compared the serum concentrations of HBsAb and the prevalence of people with HBsAb concentrations of <10 mIU/ml. The results were also adjusted for the following covariates: years from vaccination (model a) and the age at HBsAb testing and the age at vaccination (model b). Both the titers of HBsAb and the prevalence of people with concentrations of <10 mIU/ml were different among groups pre- and postadjustment (Table 4). The results were also statistically significant when the ANOVA models were reanalyzed after the exclusion of group C (the sole group vaccinated at birth) (F > 8.56, P < 0.001).

**TABLE 1 Main features of the population under scrutiny**

| Feature                               | Group A (exposed to gluten) (n = 57) | Group B (not exposed to gluten) (n = 46) | Group C (infants) (n = 60) | Group D (controls) (n = 48) | P   |
|---------------------------------------|-------------------------------------|-----------------------------------------|---------------------------|-----------------------------|-----|
| Female (%)                            | 86                                  | 65                                      | 78.3                      | 75                          | 0.97|
| Age at HBsAb testing (median [IQR]) (yr) | 25 (23, 27)                        | 27 (25, 28)                             | 19.5 (18, 20)             | 23 (21, 26.5)               | 0.001b|
| Time interval between vaccination and testing (median [IQR]) (yr) | 13 (11, 15)                        | 15 (13, 16)                             | 19.5 (18, 20)             | 10 (9, 13)                  | <0.001b|

Note: IQR, interquartile range (25th and 75th percentiles).

Kruskal-Wallis analysis.

**TABLE 2 HBsAb concentrations in celiac patients (group A, 57; group B, 46; group C, 60) and in controls (48) vaccinated against hepatitis B**

| HBsAb titer     | Comparison group (no. [%]) | Controls (no. [%]) | P   |
|-----------------|----------------------------|--------------------|-----|
| Group A vs controls<br>&lt;10 mIU/ml | 25 (43.9) | 4 (8.3) | &lt;0.001 |
| 10–100 mIU/ml | 14 (24.5) | 9 (18.8) | 0.17 |
| &gt;100 mIU/ml | 18 (31.6) | 35 (72.9) | &lt;0.001 |
| Group B vs controls<br>&lt;10 mIU/ml | 16 (34.8) | 4 (8.3) | 0.002 |
| 10–100 mIU/ml | 20 (43.5) | 9 (18.8) | 0.009 |
| &gt;100 mIU/ml | 14 (21.7) | 35 (72.9) | &lt;0.001 |
| Group C vs controls<br>&lt;10 mIU/ml | 35 (58.3) | 4 (8.3) | &lt;0.001 |
| 10–100 mIU/ml | 18 (30) | 9 (18.8) | 0.131 |
| &gt;100 mIU/ml | 7 (11.7) | 35 (72.9) | &lt;0.001 |

**TABLE 3 HBsAb concentrations in celiac patients**

| Group | No. (%) of patients with an HBsAb concn of: |
|-------|-------------------------------------------|
|       | &lt;10 mIU/ml | 10–100 mIU/ml | &gt;100 mIU/ml |
| A     | 25 (43.9) | 14 (24.5) | 18 (31.6) |
| B     | 16 (34.8) | 20 (43.5) | 10 (21.7) |
| C     | 35 (58.3) | 18 (30) | 7 (11.7) |

P &lt; 0.05 for group A versus group B for HBsAb &lt;10–100 mIU/ml, for group A versus group B for HBsAb &gt;100 mIU/ml, and for group B versus group C for HBsAb &lt;10 mIU/ml.
that 50% of the patients who had an autoimmune disease along with CD were more likely to be unresponsive to the HBV vaccine, emphasizing the involvement of genetic factors in vaccine failure. In a separate study, Noh et al. (5) reported that 13 of the 19 adult patients who were either homozygous or heterozygous for HLA-DQ2 did not respond to an HBV vaccine. However, several other authors have shown that HLA-DQ alleles may play a primary role in responsiveness to HBV vaccine. Nemes et al. (6) found that the percentage of seroconversion (95.5%) in a group of patients vaccinated after the initiation of dietary treatment was similar to that in healthy individuals. Furthermore, 36 out of the 37 nonresponders in this study showed seroconversion after the administration of a booster dose of vaccine. Ertem et al. (7) demonstrated that the HBV vaccine in children with CD who were compliant with the GFD was not different from that in the healthy population. In a paper by Ertem et al. (8), HBsAb positivity was significantly higher in celiac patients who were compliant with the GFD than in those who were noncompliant.

However, although we confirmed the deficient response of CD patients to HBV immunization, we did not confirm a favorable role for a GFD in the vaccination response. Our data indicate that approximately 35% of the patients vaccinated while on a GFD did not show a protective titer of HBsAb. Patients in group C (vaccinated in infancy) had lower levels of protective HBsAb and a greater percentage of nonresponders than patients in groups A and B (vaccinated as adolescents). This observation, however, could be the result of a longer period of time from vaccination to measurement of the HBsAb dosage level (16).

In conclusion, our data suggest that immunological factors and/or genetics, rather than gluten exposure by itself, are involved in the impaired response to HBV vaccination in CD patients. Hence, CD may represent a useful model for investigating the factors involved in the immune response to HBV vaccination. Although evidence that lower HBsAb titers correspond to a higher risk of HBV infection in the CD population is limited, reevaluation of antibody titers, with the potential for revaccination for HBV, should be a part of the follow-up protocol for adult CD patients.

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