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Incidence of Low Seroimmunity to Hepatitis B Virus in Children with Inflammatory Bowel Disease: A Single Center Experience

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Conflict of Interest
The author has no financial conflicts of interest.

ABSTRACT

Purpose: Immunosuppressive therapy is frequently administered to patients with inflammatory bowel disease (IBD), which may make them more susceptible to infections like hepatitis B.
Methods: A cross-sectional study was conducted on patients aged 5–18 years diagnosed with IBD who visited a gastroenterology clinic along with controls who were the same age as the patients with IBD and were healthy overall. A logistic regression analysis using the independent variables of age, sex, race, disease phenotype, surgery, and medications and the dependent variable of adequate hepatitis B surface antibody (HBsAb) titers (>10 mIU/mL) was performed on quantitative serum HBsAb titers.
Results: The study enrolled 62 patients, including 37 males and 25 females. Crohn's disease, ulcerative colitis, and indeterminate colitis were diagnosed in 16, 22, and 24 patients, respectively. Thirty-nine patients were taking corticosteroids at the time of the study, 42 were taking immunomodulators, and four were taking biologics. Compared to 44.7% of the control group, 9.3% of the patients had protective titers. Only 12 out of 62 patients had HBsAb titers greater than 10 million IU/mL. None of the patients who received biologics or corticosteroids and 3.2% of those who received immunomodulators were found to be seroimmuned.
Conclusion: The younger patients had the highest titers. Patient-specific factors that may impact these low titers include the length of the patient's illness and the use of immunosuppressants.

Keywords: Inflammatory bowel disease; Hepatitis B surface antibody

INTRODUCTION

Once considered rare in the East, inflammatory bowel disease (IBD) is now recognized to be an emerging entity in the region. East or West, the clinical features and treatment options for IBD are the same; however, the exact pathogenesis or the initiating events may differ [1]. IBD refers to ulcerative colitis (UC) and Crohn's disease (CD), which are chronic inflammatory conditions of the gastrointestinal tract. Additionally, patients may initially be categorized as having indeterminate colitis or IBD-unclassified (IBD-U).

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Patients with IBD often receive immunosuppressive therapy, which may make them vulnerable to a variety of infections, including hepatitis B virus (HBV). They may also be at an increased risk of hepatitis B infection due to the potential for blood transfusions and surgical interventions [2]. We focus on HBV specifically, as it is a vaccine-preventable disease.

HBV is a global public health issue, with >350 million people chronically infected [3]. Primary infection with HBV can lead to a chronic carrier state. HBV is a vaccine-preventable infection and was added to the routine infant immunization schedule in Egypt in 1992.

Data showing a higher prevalence of HBV infection in patients with IBD are lacking; however, reactivation and fulminant hepatitis have been reported [4]. These documented cases followed the use of infliximab in combination with systemic steroids and/or immunomodulators [5].

MATERIALS AND METHODS

This study aimed to identify the incidence of lower hepatitis B surface antibody (HBsAb) titers and determine which patient factors may be associated with lower HBsAb titer in pediatric patients with IBD who presented to the gastroenterology clinic in the new child hospital (Abo El Reesh Hospital) Cairo university together with age- and sex-matched controls from the outpatient clinic free of any chronic disease. The study was approved by the Ethical Committee of the Pediatric Department at Cairo University.

Sixty-two children were recruited regardless of the disease activity. They were recruited consecutively between February 2019 and April 2020 and were confirmed to have completed their full series of hepatitis B vaccinations (0, 2, 4, and 6 months).

Participants in the study group were aged 5–16.5 years, and 30 age- and sex-matched children aged 5–13.5 years were included as controls. Patients who did not receive a full hepatitis B vaccine series were excluded.

Quantitative serum HBsAb titers were measured, and logistic regression analysis with age, sex, race, disease phenotype, surgery, and medications as independent variables and adequate HBsAb titers (>10 mIU/mL) as a dependent variable was performed.

After reading the study information sheet and having the study explained to patients and their parents, patients were asked on their clinic visits to provide informed consent to participate. Data were collected from patients and recorded on a patient's data collection sheet.

Blood samples for HBsAb titers levels were collected in addition to the other routine blood work such as a complete blood count (including hemoglobin level and white blood cell and platelet counts), erythrocyte sedimentation rate, C-reactive protein, serum albumin liver enzymes, stool analysis, and occult blood in the stool.

HBsAb titer was measured using a DiaSorin antibody to HBsAb (anti-HBs) enzyme-linked immunosorbent assay (ELISA) kit for quantitative determination of anti-HBs in serum samples.

Principle of the test

The method for quantitative anti-HBs determination is a direct, non-competitive sandwich assay based on the ELISA technique.

Specimen collection and preparation

Three milliliters of blood were collected in sterile plane tubes and allowed to clot. The serum was separated from the clot as soon as possible and stored at −20℃ till the time of assay.

Assay procedure

Blank wells containing chromogen/substrate only, controls, and anti-HBs calibrators were done in all runs with each plate of patient specimens.

The absorbance of specimens was measured with a photometer at 450/630 nm and 405/630 nm within 30 minutes of adding the blocking reagent.

Results were automatically calculated after selecting the suitable protocol using the four-parameter mathematical method (Rodbard function). The final HBs concentration was derived directly from the calibration curve.

Interpretation of results

Anti-HBs concentration >10 IU/L is indicative of the resolution of a past infection or positive response to vaccination. In both cases, acquired immunity to type B viral hepatitis may be assumed. Antibody concentrations <10 IU/L are indicative of the absence of acquired immunity, as a level of 10 IU/L is considered as the lower limit of protection.

Statistical methodology

Data was entered on the computer using the 'Microsoft Excel 2010 (Microsoft)' for Windows. Data was then transferred to the IBM SPSS Statistics for Windows, Version 23.0 (IBM Co.) for statistical analysis. Quantitative variables were presented as range, mean, standard deviation (SD), median, and interquartile range (IQR) (when SD is more than the mean half). Qualitative variables were presented as frequency and percentages. Comparisons of different variables in various groups were done using the student t-test. The Chi-square ($X2$) test was used to compare the frequency of qualitative variables among different groups. The $p>0.050$ indicated an insignificant test, while $p<0.050$ indicated a significant test. Sensitivity was calculated as (true positive/true positive+false negative)×100. Specificity was calculated as (true negative/true negative+false positive)×100. A graphic presentation of the results was also performed.

RESULTS

The present study included 62 children with IBD as the patient group and 30 children as the control group. The patient group included 37 (59.6%) males and 25 (40.4%) females. They were aged 60–198 months with a mean age of 102.2±41.5 months. The control group included 16 (53.3%) males and 14 (46.7%) females; they were aged 60–160 months with a mean age of 101.7±33.4 months. Their z-score mean weight and height for age were −1.82±1.58 and −2.43±1.77, respectively; this may be due to the multifactorial etiology of growth failure in patients with IBD.

In the patient group, 24 (38.7%) children were diagnosed with IBD-U, while 22 (35.5%) were diagnosed with IBD-UC, and the least frequent diagnosis was IBD-CD (25.8%).

The age-adjusted weight and height z scores showed no statistically significant differences comparing the IBD subtypes. The mean weight-for-age z score in the IBD-UC, IBD-CD, and IBD-U groups was −2.15±1.4, −2.09±2.04, and −1.3±1.3, respectively (p=0.058). The mean height-for-age z score in the IBD-UC, IBD-CD, and IBD-U groups was −2.4±1.9, −2.8±2.1, and −2.2±1.4, respectively (p=0.460). This may be attributed to the fact that growth failure is a critical concern in childhood-onset IBD and that patients with IBD-CD have some degree of weight loss at the time of diagnosis, which may be the only presenting symptom.

The z-score mean weight and height for age in the controls were −0.48±1.12 and −0.69±0.98, respectively. The age-adjusted weight and height z scores were statistically significantly different between the patients and controls regarding the mean age for weight z score (p<0.001); there was a significant difference between the patients and controls regarding the mean age for height z score (p<0.001).

The IQR of the duration of the disease in the patient group was 8.8–36 months, while the IQR of time between vaccinations till diagnosis was 18.5–93 months. This may be attributed to the fact that the younger the patients, the higher the HBsAb titers. The immunogenicity and effectiveness of vaccines are influenced by the immune status of the recipient, which may be affected by the number of years after the primary vaccination series, an individual's underlying medical condition, nutrition, and the use of medications that may alter the individual's immune response to vaccines.

A significant difference was found comparing the duration of the disease among different subtypes (p=0.001). IQR was 11.75–33, 19–60, and 4.25–20.75 months for patients with IBD-UC, IBD-CD, and IBD-U, respectively. No significant difference was found comparing the time since diagnosis to vaccination for different IBD subtypes (p=0.630).

IQR was 17–82.5, 16.25–100, and 21.5–95 for patients with IBD-UC, IBD-CD, and IBD-U, respectively.

Regarding clinical presentation, bleeding per rectum was the most frequent presenting symptom (74.2%), diarrhea was the second most frequent (51.6%), followed by abdominal pain (29%), while hematemesis (6.5%), vomiting (4.8%), and melena (1.6%) were the least frequent.

Regarding received medications, all the patients received mesalazine, while 67.7%, 62.9%, and 6.5% of the patients received azathioprine, steroids, and biological treatment, respectively.

Moreover, 81.3%, 72.7%, and 41.7% of the patients with IBD-CD, IBD-UC, and IBD-U received steroids, respectively. A significant difference was found comparing the different IBD subtypes regarding steroid treatment (p=0.020). Similarly, 81.8%, 81.3%, and 45.8% of patients with IBD-UC, IBD-CD, and IBD-U received azathioprine, respectively. A significant difference was found comparing the different subtypes regarding azathioprine treatment (p=0.014). Moreover, 18.8% and 4.5% of patients with IBD-CD and IBD-UC received biological treatment, respectively. A significant difference was found comparing the different subtypes regarding biological treatment (p=0.050).

Regarding relapse following discontinuation of steroid therapy, 74.2% of the patients were steroid non-relapsers with no or only one relapse; however, 10% of the patients had relapses twice, and 9.7% of the patients had three or more relapses. There was no significant difference comparing the different subtypes regarding the number of steroid relapses (*p*=0.200). Specifically, 58.3%, 27.3%, and 18.8% of patients with IBD-U, IBD-UC, and IBD-U had no relapses, respectively.

However, 56.3%, 43.8%, and 29.2% of patients with IBD-UC, IBD-CD, and IBD-U had one relapse, respectively. Similarly, 25%, 22.7%, and 4.2% of patients with IBD-CD, IBD-UC, and IBD-U had relapses twice, respectively. Moreover, 12.5%, 9.1%, and 8.3% of patients with IBD-CD, IBD-UC, and IBD-U had three or more steroid relapses, respectively.

IQR was 4.75–24, 1.25–23, and 0–5.75 months in patients with IBD-UC, IBD-CD, and IBD-U, respectively. The different subtypes were not significantly different regarding the duration of azathioprine intake (*p*=0.011).

Regarding the patient group in this study, 75.8% (47/62) had HBsAb titer <10 mIU/mL, and 24.2% (15/62) had titer >10 mIU/mL. According to different IBD subtypes, the IQR ranged between 5 and 9.68 mIU/mL, 3.8 and 8.45 mIU/mL, and 5.4 and 26.6 mIU/mL for IBD-UC, IBD-CD, and IBD-U, respectively.

Regarding the control group, all children (100.0%) received their full vaccination schedule. After testing their HBsAb titer, 53.3% had titer >100 mIU/mL, and none of them had HBsAb titer <10 mIU/mL (immunized) with IQR ranging between 27.25 and 206.75 mIU/mL.

A low HBsAb titer was found among the patients' group, with a mean titer level of 61.1±238.3 mIU/mL compared to the control's mean titer of 140.9±189.7 mIU/mL. A highly significant difference between the two groups was found regarding HBsAb titer (*p*<0.001).

Regarding the age of patients as a risk factor for a lower titer, the older the age of the patient, the lower the titer (*p*=0.040); titer levels of <10, 10–100, and >100 mIU/mL were observed for the mean age of 108.1±41.44, 78.55±37.32, and 99±38.41 months, respectively.

Regarding the growth of the patient group, there was no statistically significant difference between growth failure and lower titer (weight z score: −1.9±1.7, height z score: −2.51±1.86; *p*=0.510, 0.48, respectively).

Regarding disease subtypes as a risk factor for a lower titer, 75.8% (47/62), 17.7% (11/62), and 6.45% (4/62) of the patients had titer <10, 10–100, and >100 mIU/mL, respectively (*p*=0.320) (**Table 1**). Low titer <10 mIU/mL was found in 87.5% (14/16), 81.8% (18/22), and 62.5% (15/24) of patients with IBD-CD, IBD-UC, and IBD-U, respectively (*p*=0.241).

Table 1. Descriptive data of HBsAb titer and subtypes among patient groups (n=62)

Parameter	<10 mIU/mL (n=47)	10–100 mIU/mL (n=11)	>100 mIU/mL (n=4)
IBD-UC (22)	18	3	1
IBD-U (24)	15	6	3
IBD-CD (16)	14	2	0
p-value		0.320	

HBsAb: hepatitis B surface antibody, IBD-UC: inflammatory bowel disease ulcerative colitis, IBD-U: inflammatory bowel disease unclassified, IBD-CD: inflammatory bowel disease Crohn's disease.

Table 2. Comparison between the disease duration and time since vaccination till diagnosis for different HBsAb titer subtypes for patients' group (n=62)

Parameter	Disease duration (mo)	Time since vaccination till diagnosis (mo)
<10 mIU/mL (n=47)	29.34±22.1 (11–40)	60.1±41.9 (21–103)
10–100 mIU/mL (n=11)	9.27±6.6 (3–12)	48±41.7 (17–92)
>100 mIU/mL (n=4)	27.25±37.9 (6.5–65.75)	48±35.3 (18–84)
p-value	0.005	0.640

Values are presented as mean±standard deviation (interquartile range).
HBsAb: hepatitis B surface antibody.

Table 3. Comparison between the different medical treatments among HBsAb titer subtypes in patients' group (n=62)

Parameter	Steroids	Azathioprine	Biologics
<10 (n=47)	34 (72.4)	38 (80.9)	4 (8.5)
10–100 (n=11)	5 (45.5)	2 (18.2)	0 (0.0)
>100 (n=4)	0 (0.0)	2 (50.0)	0 (0.0)
p-value	0.007	<0.001	0.500

Values are presented as number (%).
HBsAb: hepatitis B surface antibody.

Regarding disease duration as a risk factor for lower titer, HBsAb titer <10 mIU/mL was associated with a longer disease duration of 29.34±22.1 months (IQR, 11–40 months; p=0.005) (**Table 2**).

Regarding received medications as a risk factor for lower HBsAb titer, 34/47 patients who received steroids had titer <10 mIU/mL (p=0.007), while 38/47 patients who received azathioprine had titer <10 mIU/mL (p<0.001). The longer the duration of azathioprine intake, the lower the HBsAb titer <10 mIU/mL (p=0.001) (**Table 3**).

DISCUSSION

The risk of HBV infection is increased in patients with IBD due to immune-suppressive medications and other risk factors related to infection, such as prior blood transfusions, surgery, and endoscopy during IBD [6]. Despite an increased risk for this infection, many patients with IBD are not being vaccinated appropriately [7].

Regarding the age of the study participants, the mean age was 102.2±41.5 months in the study group and 101.7±33.4 months in the control group. This was in agreement with Watts et al. [8], who documented the same age group for patients with IBD in their study (14.2 years), while the mean age of the participants in the study by Moses et al. [9] was 12.6±3.2 years. Regarding the age of patients as a risk factor for a lower titer, the older the age of the patient, the lower the titer (p=0.040).

Regarding the sex distribution of the studied children, 60% and 40% of the participants in the study group were male and female, respectively. Males accounted for 77.3%, 50%, and 50% of participants in IBD-UC, IBD-CD, and IBD-U groups, respectively. This was dissimilar to a previous study [8] in which 49.1% of the participants were males, and 50.9% were females; however, it was similar to a previous research [9], which reported that 60% of their patients were males. Musa et al. [10] reported that the male-to-female ratio in their study was 110:182, 85:121, and 0:2 in patients with IBD-CD, IBD-UC, and IBD-U, respectively.

Regarding this study, 35.5%, 25.8%, and 38.7% of the children had IBD-UC, IBD-CD, and IBD-U, respectively, compared to Watts et al.'s [8] report of 64.7%, 27.6%, and 7.8% children, respectively. Moses et al. [9] reported that 91% and 9% of the children in their study had IBD-CD and IBD-UC, respectively.

In this study, the IQR of the disease duration was 8.8–36 months, with a mean duration of 25.7±19.7, 38.8±24.2, and 16.9±20.1 months in patients with IBD-UC, IBD-CD, and IBD-U, respectively, with high statistical significance between the IBD subtypes (p=0.001). HBsAb titer was <10 mIU/mL with a longer duration of the disease, 29.34±22.1 months (IQR, 11–40 months; p=0.005). Musa et al. [10] reported in their study that a mean disease duration of 154±138, 107±106, and 4±4 months in patients with IBD-CD, IBD-UC, and IBD-U, respectively.

In this study's patient group, no association was found between the time since vaccination till diagnosis for different IBD subtypes (p=0.630), and this was in agreement with Watts et al. [8], who found no association between interval from diagnosis and hepatitis B seroimmunity, and the overall number of patients with disease activity <1 year was low. IQR of the time from the initial vaccination to when anti-HBs titers were checked in this study ranged between 18.5 and 93 months, and the mean duration was 57.2±41.1 months (p=0.630). This was in agreement with Moses et al. [9], who found that the mean duration was 13.3±3.8 years for immuned patients and 12.6±4.3 years for non-immuned patients. The difference between the two groups was also not significant.

Regarding medical treatment, 62.9% of the patient group with IBD received steroids, 67.7% received immunomodulators, 6.5% received biological treatment, and all the patients received mesalazine. Approximately 81.3%, 72.7%, and 41.7% of patients with IBD-CD, IBD-UC, and IBD-U received steroids, respectively. A significant difference was found comparing the different IBD subtypes regarding steroid treatment (p=0.020). A significant difference was found comparing the different subtypes regarding azathioprine treatment (p=0.014). Biological treatment was received by 18.8% of patients with IBD-CD and 4.5% with IBD-UC. A significant difference was found comparing the different subtypes regarding biological treatment (p=0.050).

Regarding received medications as risk factors for lower HBsAb titer, 34/47 patients who received steroids had titer <10 mIU/mL (p=0.007).

Thirty-eight of 47 patients who received azathioprine had titer <10 mIU/mL (p<0.001). The longer the duration of azathioprine intake, the lower the HBsAb titer <10 mIU/mL (p=0.001). No immunosuppressive therapy was predictive of an immune response compared to immunomodulators (risk ratio [RR], 1.33; 95% confidence interval [CI], 1.08–1.63) or anti-tumor necrosis factor-α (RR, 1.57; 95% CI, 1.19–2.08) [11]. Immunosuppressive therapy for IBD did not influence the vaccine response [12]. In multivariable analysis, treatment with infliximab (adjusted odds ratio [OR], 17.642; 95% CI, 8.514–33.937) and azathioprine (adjusted OR, 3.344; 95% CI, 1.653–9.145) were the only factors associated with weaker response to HBV vaccination. The response rate to the standard HBV vaccination in patients with IBD is low, mainly in those treated with infliximab and/or azathioprine [13].

Moses et al. [9] reported that 53% of their study participants had immunity to HBV, defined as an anti-HBs level ≥10 mIU/mL.

Watts et al. [8] found that 60% of patients aged 5–10 years had protective titers versus 22–27% in the older groups. Of their 116 patients, only 33% had HBsAb titers >10 mIU/mL. Seroimmunity was reported in 20%, 27%, and 24% of the patients who received corticosteroids, immunomodulators, and biologics, respectively. Musa et al. [10] reported that of their 220 patients screened, 51% of the patients with IBD were found to be non-immuned against HBV.

Watts et al. [8] reported that no association was found between any clinical variables and the presence of low HBsAb titers, with the exception of age; children aged <10 years were much more likely to have adequate hepatitis B seroimmunity (OR, 4.56; p=0.040). There was no significant association with the mode of immune suppression: corticosteroids (p=0.880), immunomodulators (p=0.190), and biologics (p=0.260), and they found that the majority (>70%) of the patients with IBD had low titers against HBV. Titers were highest in the younger patients. Bruce et al. [14] reported in a study done on immunocompetent individuals that 51% of the patients had HBsAb levels <10 mIU/mL 30 years after a primary vaccination series.

Conclusion

Disease duration was significantly longer in IBD-CD than in IBD-UC and IBD-U. The older the age of the patient, the lower the titer. The longer the disease duration, the lower the titer. Immunosuppressive (steroids and azathioprine) therapy was associated with lower HBsAb titer.

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