# Markedly decreased antibody titers against hepatitis B in previously immunised children presenting with juvenile idiopathic arthritis

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## Abstract

### Objectives

*Hepatitis B is a vaccine preventable disease with intermediate endemicity in Greece. Patients with juvenile idiopathic arthritis (JIA) on immunomodulating therapy are prone to infection or reactivation of hepatitis B virus (HBV). The aim of this study is to define the immune status against HBV in children newly-diagnosed with JIA.*

### Methods

*Case-control prospective study including 89 JIA patients and 89 controls matched for age and gender. Eighty-nine JIA patients were included in the study (22 males), with a mean age of 6.8 years. Sera were tested for hepatitis B surface antigen, hepatitis B core antibody, and anti-HBs. Patients with anti-HBs titers ≥10 IU/L were considered immune. Data were analysed with SPSS 18.0 version.*

### Results

*In the JIA group 55% were HBV immune (anti-HBs level ≥10IU/L) while in the control group 92% were immune against HBV ($p<0.001$). Antibody levels in the patient group were significantly lower compared to the control group. The mean concentration of anti-HBs levels in JIA patients was 18.3 IU/L versus 82.6 IU/L in the control group ($p<0.001$).*

### Conclusion

*Antibody titers against HBV in fully vaccinated JIA patients due to start treatment are significantly lower compared to matched healthy children in this study. Diagnosis of JIA and older age were associated with the absence of protective antibodies. Although there is no evidence to support the introduction of a booster HBV dose in healthy children who mount low antibody response following immunisation, further studies are required to address this question in patients with JIA.*

**Key words**

juvenile idiopathic arthritis, hepatitis B vaccination, hepatitis B infection, immunosuppressive treatment

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## Introduction

Hepatitis B virus (HBV), is a highly contagious and potentially life threatening virus. Infection may have detrimental consequences such as cirrhosis, liver failure and hepatocellular carcinoma. Hepatitis B is a vaccine preventable disease with intermediate endemicity in Greece (1). In our country, the prevalence of HBsAg carriers in children aged 2–15 years is 0.6%, while 4.5% of children older than 12 months show evidence of past HBV infection (2). National vaccination against HBV was introduced in 1998; however around 10% of the population fail to mount strong immune response. Although vaccination against HBV is mandatory approximately 5% of the population aged less than ten is unvaccinated (3). Additionally, immigration from high endemicity countries such as Africa and the Middle East has changed the prevalence of HBV infection in Greece (4). The majority of juvenile idiopathic arthritis (JIA) patients receive immunosuppressive or biologics' treatment which renders them more susceptible to infections including HBV infection or reactivation (5), when compared to healthy population. The aim of this study is to evaluate immune responsiveness against HBV in fully vaccinated children presenting with JIA prior to initiation of any treatment and compare them to matched healthy children.

## Methods

*Patient population and setting*

Prospective single centre case-control study including 89 newly-diagnosed JIA patients and 89 healthy controls matched for age and gender, followed up at "P&A Kyriakou" Children's Hospital, which is an academic, tertiary referral centre for paediatric rheumatology, in Athens, Greece. The study was approved by the hospital's ethics and research committee. We enrolled our study population from January 2011 until January 2012. The cohort included children up to 16 years of age with a confirmed diagnosis of JIA, as defined by the International League of Associations for Rheumatology 2001 criteria (6), who have completed three doses of Hepatitis B vaccine at the time of diagnosis. The control group consisted of healthy (previously unhospitalised) children matched for age and gender to the patient group, who underwent routine Otolaryngology (ENT) procedures in the same institution. Demographic data including ethnic origin, age, gender, past medical history and vaccination history were documented. Information regarding the type of vaccine given (single or combined vaccine) was also recorded as this was indicated by the patients' health records (official documentation in the Child Health Booklet). Excluded from the study were children with an underlying primary immunodefiency, children who had received chemotherapy or immunomodulating treatment including steroids for a period longer than 6 weeks and within six months to the time of enrolment, as well as children who had received a blood transfusion. We also excluded patients who had not completed the primary vaccination schedule at the time of diagnosis, which according to the national vaccination programme, includes three doses of the HBV vaccine given at 2, 4 and 6–18 months of age. The exclusion criteria applied for both the patient and the control group. The primary outcomes were the measurement of anti-HbS titers in the patient group and the percentage of patients with antibody concentrations above the seroprotection level, followed by comparison of these findings against the measurements of the control group.

*Specimen collection*

Blood samples were collected prior to the initiation of treatment. Sera were tested for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), and hepatitis B surface antibody (anti-HBs). Antibodies were measured using indirect immunofluoresense assay (IFA). Quantative measurements of antinuclear antibodies (ANA), rheumatoid factor (RF), immunoglobulins IgM, IgE, IgA and IgG with subclass analysis, as well as full blood count with differential were also recorded. Patients with anti-HBs titers ≥10 IU/L were considered immune. ANA titers >1/80, and RF >20 IU/ml were considered positive.

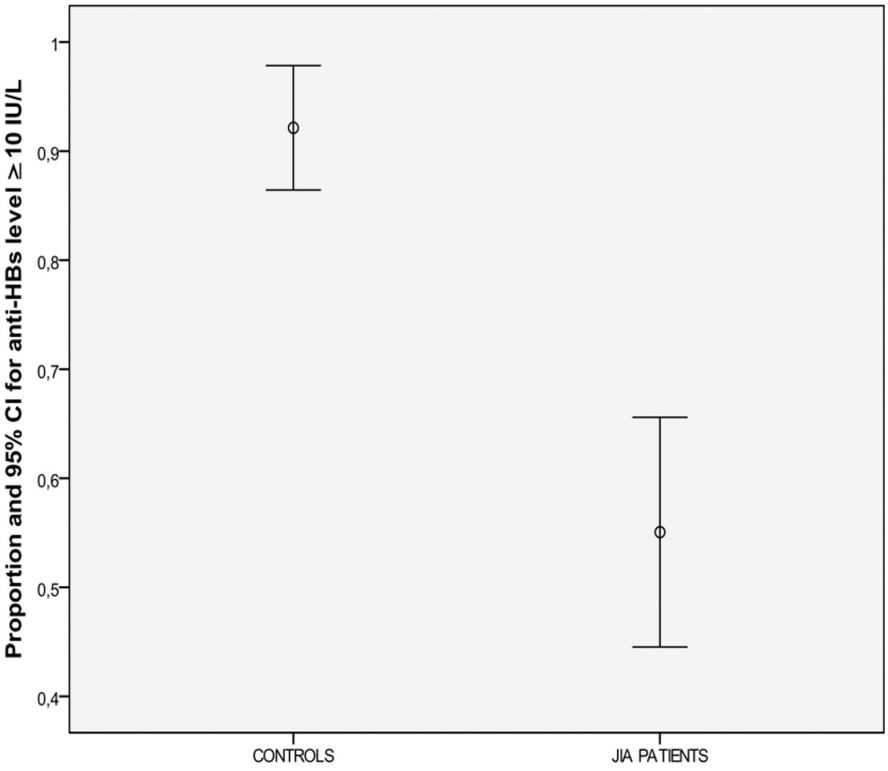


**Fig. 1a.** Proportion and 95% confidence intervals for anti-HBs levels >10IU/L in the patient and the control group.

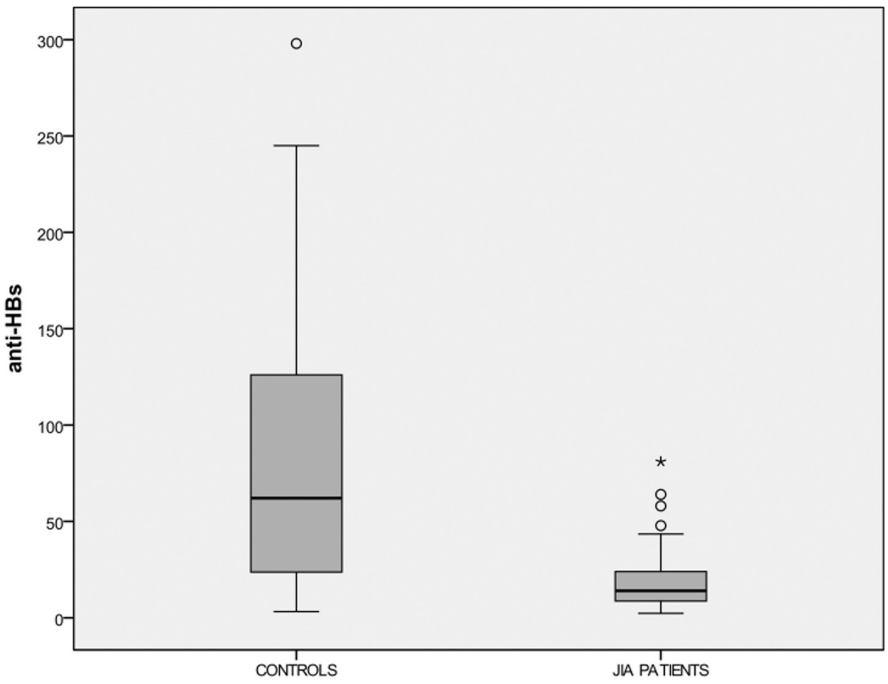


**Fig. 1b.** Anti-HbS titers for the patient and the control group including median and interquantile range (IQR).

*Statistical analysis*

For the comparison of mean antibody concentrations between the two groups the independent Student's *t*-test was used. Chi-square test was used for the comparison of proportions. In order to explore differences between the patient and the control group conditional logistic regression analyses were performed. Odds ratios with their 95% confidence intervals were computed from the results of the conditional logistic regression analyses. *P*-values reported are two-tailed. Statistical significance was set at 0.05 and analysis was conducted using SPSS, version 18.00 (SPSS Inc, Chicago, IL).

## Results

A total of 122 patients were diagnosed with JIA in our institution, of whom 27% (33) did not meet the inclusion criteria. One patient had IgA deficiency, one patient had received a blood transfusion, fourteen patients were not fully vaccinated at the time of enrolment and in seventeen patients data were missing from the Child Health Booklet. Eighty-nine JIA patients participated in the study (22 males), with a mean age of 6.8 years (range 1.5–13 years). The control group consisted of 89 healthy children matched for age and gender with the JIA group, with a mean age of 6.7 years (range 1.5 to 13 years). All subjects were Caucasian (73% Greek, 10% Albanian, 17% Eastern European origin). The JIA subtypes included were oligoarticular JIA (37%), polyarticular JIA (28%), enthesitis related arthritis (25%), and psoriatic JIA (10%). All participants were vaccinated against HBV in infancy and early childhood (3 doses). The mean time from last vaccine dose to time of enrolment was 3 years (range 9 months to 11 years) for the patient group and 3.4 years (range 1 to 9.5 years) for the control group. None of the patients or the controls was positive for HBsAg or anti-HBc. Twenty-one patients (24%) were ANA positive and 2(2.4%) were RF positive. The time period between final vaccination and blood collection was similar in both groups. The proportion of JIA patients with evidence of HBV immunity was significantly lower than their healthy counterparts. In the JIA group 55% (49/89) were HBV immune (anti-HBs level ≥10 IU/L) while in the control group 92% (82/89) were immune against HBV. Conditional logistic regression analyses showed that healthy subjects are more likely to have anti-HBs level ≥10 IU/L (OR=17.50, 95% CI: 4.21–72.76, $p<0.001$). The proportion of subjects with anti-HBs

**Table I.** The demographic characteristics in the patient and the control group.

| Characteristics | Patient group | Control group | *p*-value |
|---|---|---|---|
| Total number of participants | 89 | 89 | Non significant (NS) |
| Mean age (years) | 6.8 (range1.5–13 years) | 6.7 (range 1.5–13 years) | 0.858 |
| Gender | | | |
| female | 77 (75%) | 77 (75%) | NS |
| male | 22 (25%) | 22 (25%) | |
| Type of disease/procedure | Oligoarticular JIA: 33 (37%)<br>Polyarticular JIA: 22 (25%)<br>Enthesitis related arthritis 22 (25%)<br>Psoriatic JIA: 12 (13%) | Tonsillectomy: 28 (31%)<br>Adenoidectomy: 16 (18%)<br>Foreign body removal:6 (7%)<br>Myringotomy: 39 (44%) | Not applicable |
| ANA positive | 22 (24%) | 0 | NS |
| RF positive | 2 (2.4%) | 0 | NS |
| Mean anti-HBs concentration | 18.9IU/L | 83.2IU/L | < 0.001 |
| Median concentration (IQR) | 14 (9–23) | 62 (24–126) | |
| Number of patients with HBs>10 IU/L | 49 (55%) | 82 (92%) | <0.001 |
| Number of patients with HBs<10 IU/L | 40 (45%) | 7( 8%) | |

Percentages are shown in brackets. Comparison showed a statistically significant difference in the absence of protective anti-HbS titers between the JIA group and the control group (level of significance *p*-value <0.05).

level ≥10 IU/L along with 95% confidence interval in both groups is shown in Figure 1a. Antibody levels in the patient group were significantly lower compared to the control group. The mean concentration of anti-HBs levels in JIA patients was 18.9 IU/L (range: 4.5–69 IU/L) *versus* 83.2 IU/L (range: 3.4–245 IU/L) in the control group (Fig. 1b). Conditional logistic regression analysis showed that healthy subjects are accompanied with greater concentration of anti-HBs with OR=1.10 (95% CI: 1.04–1.16, $p<0.001$) (Table I). Diagnosis of JIA and older age were statistically significant risk factors associated with the absence of protective antibodies. Sixty two (73%) children in the patient group and 68 (78%) in the control group received three single vaccine doses while the remaining either received a combination of hexavalent and single doses. The type of vaccine (single *vs*. hexavalent) given in infancy did not affect adequate antibody response. No association between ANA positivity and HBV immunity status was found. Finally, no differences in seroprotection rates amongst the different JIA subtypes were shown.

## Discussion

In this prospective controlled study, anti-HBS antibody titers of therapy-naïve JIA patients were compared against healthy controls matched for age and gender. Results show that JIA patients demonstrate a significantly lower titer of anti-HBs antibodies (18.9IU/L *vs*. 83.2 IU/L for the controls). Furthermore, a high percentage (45% *vs*. 8% for controls) of JIA patients was found to have anti-HBs titers bellow the "protective" level of 10IU/ml, thus theoretically rendering them susceptible to HBV infection. To our knowledge, this is the first study to suggest that vaccinated children who develop autoimmune diseases lack seroprotection against HBV, prior to commencement of treatment.

The present study is in accordance with a recently published retrospective cross sectional study from the Netherlands assessing the immune status against diphtheria, tetanus, measles, mumps and rubella in children with JIA (7). The aforementioned study showed decreased vaccine-specific geometric mean antibody concentrations (GMC) against the majority of the pathogens tested (mumps, rubella, diphtheria, tetanus) while it showed increased GMC for measles in children with JIA compared to healthy population.

The majority of patients with JIA receive long-term immunosuppressive treatment often combined with biologics. B cell depletion observed in patients under immunosuppressive treatment is a well documented factor leading to antibody depletion. Immunity against HBV is severely affected in patients receiving immunosuppressive treatment, rendering these patients susceptible to HBV infection (8). Although these data cannot be directly applied to the subpopulation of children with autoimmune diseases, they require careful consideration. Additionally, although the standard of care for children with JIA has improved, there is always the potential risk of HBV exposure. Earlier studies have shown that the majority of previously unvaccinated children on disease-modifying anti-rheumatic drugs have an adequate response to hepatitis B vaccination (9); hence it may be a very effective and affordable way of avoiding the risk of future infection. It is not clear however if a booster dose would be sufficient in children already immunised, as a recent Cochrane review was unable to identify studies that assess the benefit of booster vaccination in the prevention of HBV in healthy children (10). On the other hand, although the literature is limited there are sufficient data which show that biologics' treatment may further reduce antibody production thus underlining the need for preemptive management in this group of patients (11).

Patients with autoimmune diseases occasionally exhibit certain primary im-

munodeficiencies most commonly selective IgA deficiency or IgG subclass deficiency, which could partly provide an explanation for low titers. This subpopulation of patients was excluded from our cohort. None of our patients was lymphopenic, however we did not perform any further testing to assess B cell functional defects. JIA is a lymphocyte-mediated autoimmune disease with abnormality in the adaptive immune system. Self-antigens set off reactive T cells including Th1 cells with production of pro-inflammatory cytokines. Defective Th1 reaction has been repeatedly reported in HBV vaccine non-responders, rendering this hypothesis challenging for further insight (12).

## Conclusion

This study has certain weaknesses. The major weakness is the relatively small number of controls compared to the patient group. However, patient and control group were matched for age and gender. Our study was not powered enough to bring up differences amongst the different JIA subtypes, and it did not include any patients with systemic JIA. Finally, although baseline immunology tests were carried out, further studies on B cell function were not performed.

In conclusion, our study showed that a significant percentage of JIA patients due to start immunomodulating treatment do not have protective antibody levels against HBV. This could be attributed to the immune dysregulation noted in patients with autoimmune diseases. Larger studies are needed to explore this hypothesis. Although there is no evidence to support the introduction of a booster HBV dose in healthy children who mount low antibody response following immunisation (13), further studies are required to address this question in patients with JIA.

## Key messages

- Children with autoimmune diseases show evidence of immune dysregulation.
- Antibodies against HBV in therapy naïve JIA patients are significantly decreased when compared to healthy children.
- A significant proportion of children with JIA due to start immunosuppressive treatment have reduced anti-HBs levels.

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