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IgE anti Hepatitis B virus surface antigen antibodies detected in serum from inner city asthmatic and non asthmatic children.

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ABSTRACT

Viral hepatitis type B (HBV) is a public health concern, but hasn't been linked to asthma. Immunoglobulin (Ig) G is involved in HBV immune responses; less is known about IgE antibodies (Abs) against HBV in asthma. Given the importance of HBV, we sought to determine whether HBV vaccine contributes to asthma in children, by stimulating specific IgE production. Total IgE, IgE- or IgG- anti- HBVs Abs were studied in vaccinated pediatric asthmatics and nonasthmatics. We found: (1) total IgE was higher in asthmatics (2) total IgE didn't correlate with IgE anti- HBVs; (3) IgE anti-HBVs did correlate with IgG-anti HBVs in all subjects; (4) IgE- and IgG- HBVs Abs were similar in both groups; (5) IgE-or IgG anti-HBVs Abs didn't correlate with age. Our findings indicate that HBV vaccination induces IgE responses in asthmatics and non asthmatics.

Key Words: Hepatitis B Virus surface antigen, immunoglobulin E (IgE), asthma

Abbreviations used:

HBV: Hepatitis B Virus

HBVs : Hepatitis B Virus surface antigen

Abs: antibodies

IgG: Immunoglobulin G

IgE: Immunoglobulin E

AR: allergic rhinoconjunctivitis

AD: atopic dermatitis

ELISA: enzyme linked immunosorbent assay

TBS: tris buffered saline

TMB: 3, 3', 5, 5'-tetramethylbenzidine

HIV: human immunodeficiency virus

M. pneumoniae: Mycoplasma pneumoniae

C pneumonia: Chlamydia pneumoniae

HAV: Hepatitis A Virus

INTRODUCTION

Hepatitis B Virus (HBV) is an infectious disease that affects two billion people worldwide¹. It has been reported that 360 million people are chronically infected and 600,000 people will die each year from HBV-related liver disease or hepatocellular carcinoma^{1,2}. Clinically, the disease might present with neurological, dermatological, or rheumatological manifestations³. In order to reduce disease burden, the HBV vaccine was first introduced in the United States in 1981, and is now one of the most used vaccines in the world², and has been included in the routine vaccination schedule for infants and children².

Over the past two decades, increased occurrence of allergic diseases including asthma, atopic dermatitis, allergic rhinitis, food hypersensitivity and other immune disorders have been reported⁴. Strachan, *et al.* was the first to describe the “hygiene hypothesis” in that infections and unhygienic contact in childhood may confer protections against hay fever, allergic rhinoconjunctivitis (AR) and atopic dermatitis (AD)⁵. Others have explained the hygiene hypothesis based on the fact that Th1 responses, induced by certain microbial infections, can counterbalance allergen-induced Th2 responses⁶⁻⁹. Some viral infections (i.e. measles, Hepatitis A) may inhibit allergic disease¹⁰ and may be associated with lower IgE levels¹¹⁻¹², decreased IgE sensitization¹³, and asthma¹⁴.

Although several studies have reported associations of chronic HBV infections with atopic dermatitis, asthma, AR, asthma, and atopy¹⁰, less is known whether vaccination with HBV vaccine, has any effect on diseases associated with altered IgE regulation or specific IgE responses. Thus, IgE responses to HBV vaccine might play an important role in contributing to allergic inflammation in humans with asthma.

The current investigation was carried out to assess the relative role and prevalence of humoral immune responses to HBV vaccine, by analyzing serum specific anti-HBVs IgG-, or IgE- antibody levels in asthmatic children without exacerbation. The results of this study suggest that HBV vaccination induces IgE responses in both asthmatic and non asthmatic children, and that long-term persistence of IgE anti-viral responses might contribute to protective immunity in certain populations.

MATERIALS AND METHODS

Study Population.

Study subjects were recruited among patients at the Pediatric Asthma and General Pediatric Clinics at Kings County Hospital Center (Brooklyn, NY, USA). Subject inclusion criteria included: physician's diagnosis of asthma or current clinically defined persistent asthma symptoms¹⁵, or both, and allergic rhinoconjunctivitis. Subjects were nonsmokers and had no chest infections or antibiotic use in the previous 6 weeks. All subjects were human immunodeficiency virus (HIV) negative. Exclusion criteria included history of autoimmune disorders or immunodeficiency, recent use of systemic corticosteroids (less than 30 days), or immunotherapy, personal or family history of cigarette smoking, and incomplete follow-up. Control subjects were recruited from an outpatient pediatric practice (Brooklyn, NY), and defined by absence of asthma and/or atopy, based on clinical criteria¹⁶. HBV vaccine titer levels were confirmed by positive anti-HBV IgG antibody levels (0.0623-0.076 Ab index borderline; >0.076 Ab index, positive) (ELISA). The protocol was approved by the SUNY Downstate Medical Center Institutional Review Board, and the procedures followed were in accordance with institutional guidelines involving human subjects.

Peripheral blood (15 mL) was obtained from asthmatic patients (N=20) and non asthmatic controls (N=13) (males/females; ages 1-20 years old), from Kings County Hospital Center (Brooklyn, NY) and from an outpatient pediatric practice (Brooklyn, NY). Asthmatic subjects (48%) received daily treatment with a controller medication.

HBV Vaccine.

Subjects were vaccinated intramuscular (IM) with Recombivax HB Hepatitis B vaccine (Recombinant) Pediatric/Adolescent formulation (Merck Sharp & Dohme Corp, Whitehouse Station, NJ). Each 0.5 mL dose contains 5 mcg of HBV surface antigen. The vaccination regimen consisted of 3 doses of vaccine given according to the following schedule: first dose: at elected date, second dose: 1 month later, third dose: 6 months after the first dose. Blood samples for this study were collected >5 years after completion of primary HBV vaccine series.

Immunoglobulin determination.

Total Serum IgE. Blood was collected and IgE levels were determined in serum using the UniCap Total IgE fluoroenzyme immunoassay (Pharmacia and Upjohn Diagnostics) performed according to the manufacturer's recommendations (Reference range for healthy serum: 20-100 IU/mL). All tests were performed in the Clinical Diagnostic Laboratory at SUNY Downstate Medical Center (Brooklyn, NY).

HBV vaccine serum antibody detection: ELISA.

- a. IgG.** Serum IgG antibodies to HBVs were determined by enzyme linked immunosorbent assay (ELISA) (Abnova Corporation, Taiwan; Fisher Scientific, Springfield, NJ) according to manufacturer's recommendations. Data are reported as Ab Index. (Ranges for HBV Ab IgG: Negative <0.06; borderline: 0.0623-0.076; Positive: > 0.076).
- b. IgE.** The presence of IgE anti-HBVs antibodies was determined by a modification of ELISA using IgG HBV ELISA kits (Abnova Corporation; Fisher Scientific). Briefly, samples were directly added (100 ul) to the microwell plates pre-coated with HBV

antigen and incubated for 1h RT. Goat polyclonal anti-human IgE (100 ul) (ICN Biomedicals, Aurora, OH), diluted 1:100 in TBS wash buffer (Tris Buffered Saline-0.05% Tween) was added to each well, and incubated for 1 hr. The wells were washed 3X in wash buffer. Rabbit anti-goat peroxidase-labeled antibody (ICN Biomedicals), diluted 1:1000 in washing/diluting buffer, was then added to each well and incubated for 1 hour. The wells were washed again 3X in washing/diluting buffer, and developed in 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution (100 ul) (Bio-Quant) for 10 min. The reaction was stopped by adding 1N H₂SO₄ (100 ul). Samples were run in duplicate. The plates were read using an automated microplate reader (Model Elx800; Bio-Tek Instruments, Winooski, VT); optical density (O.D.) measurements were read at 450 nm. For determination of HBV IgE, data are reported as O.D. values. Final O.D. value reported was subtracted from chromagen blank O.D. value (background). Data are reported as O.D. value. (Range: positive cutoff value >2.3 OD.)

Statistical methods.

Immunoglobulin levels are presented as median +/- standard deviation. Spearman tests were used to non-parametrically correlate levels of anti-HBVs IgG and IgE in all subjects. Kruskal-Wallis tests were used to non-parametric compare immunoglobulin levels between asthmatics and non-asthmatics.

Anti HBVs antibodies were dichotomized using predefined assay thresholds (IgG: 0.069) and differences of positivity were assessed in asthmatics compared with non-asthmatics using Chi-Square tests. All data and statistical analysis were performed with

SAS software version 9.2 (SAS Institute, Cary, N.C.). A two-sided *P*-value <0.05 was taken to indicate statistical significance for all tests.

RESULTS

1. Subjects.

A total of 33 subjects were recruited into the study; 20 were asthmatic patients and 13 were non-asthmatic controls. 59% of all subject were male, 41% were female; Mean age of subjects was 13.6 ± 9.0 years. Asthmatic subjects had significantly higher total serum IgE antibody levels than non-asthmatic subjects (234 ± 220 , 74 ± 95 , respectively; $P=0.004$).

2. Correlation between total serum IgE levels and specific IgE anti -HBVs antibodies.

Total serum IgE levels and specific IgE anti HBVs antibody levels did not correlate between asthmatic and non asthmatic subjects (Pearson correlation, $R=0.185$, $P=0.31$) (Figure not shown).

3. Correlation between levels of specific IgE- and IgG anti-HBV antibodies.

There was a moderate positive correlation between specific IgE and IgG anti HBVs antibodies in asthmatic and non asthmatic subjects (Pearson correlation, $R=0.662$, $P=0.007$) (Fig 1).

4. Immune responses to HBV vaccine are similar according to asthma status.

However, levels of specific IgE-and IgG-anti HBVs antibodies were similar in patients with and those without asthma (Mann Whitney U Test, $P=0.238$, 0.762 , respectively) (Fig 2).

5. Prevalence of specific- IgE and IgG anti HBVs antibody responses.

HBV-vaccine responders were selected according to positive IgG titer status. In patients with asthma and non asthmatic subjects there were no significant differences

between age groups in prevalence of specific IgE- or IgG- anti-HBVs antibodies (Fisher Exact Test ($P=0.86$) (Data not shown). However, there was a trend to higher IgG and IgE- HBVs levels in children younger than 10 years of age (1.70 +/- 1.50, 3.0 +/-0.56, respectively) compared with older children (1.03 +/- 1.10, 2.5 +/- 0.45, respectively) that did not reach statistical significance (Mann Whitney U Test) (Table 1).

DISCUSSION

This is the first study to demonstrate that (1) total serum IgE and specific-IgE anti-HBVs antibody levels did not correlate in asthmatic and non asthmatic subjects; (2) there was a moderate correlation between specific IgE- and IgG- anti HBVs antibody levels; and (3) there was no difference in prevalence of specific IgE- or IgG- anti HBVs antibodies according to age strata.

HBV is a noncytopathic virus¹⁰; immune responses against HBV are responsible for viral clearance and pathogenesis of the disease during infection¹⁷. It has been reported that an increase in IL-12 production, along with induction of Th1 cytokines (IFN- γ , IL-2), is required for immune control over the replication of HBV¹⁸. The anti-HBV antibody to Hepatitis B infection is long lasting, and is associated with lifetime immunity¹⁹. However, vaccination with HBV vaccine leads to the development of anti-HBVs abs, and this protects most normal individuals against HBV infection²⁰. This vaccination might induce Th1 type immune responses, and thus might reduce atopy²¹. However, studies of Destefano, *et al.*, have demonstrated a weak association with asthma in infants receiving Hepatitis B vaccine²². In contrast, studies of Koh, *et al*, reported that the presence of anti-hepatitis B surface antibody produced by a natural HBV infection or vaccination might be inversely associated with atopy in young adults²⁰.

7-15% of people who receive HBV vaccination have low or no response to the vaccine²³, especially neonatal infants born from HBV-positive mothers, and dialysis patients with renal failure²⁴⁻²⁵. In those studies, the immune response was blunted, and the level of anti-HBV antibodies declined a shorter time after immunization than

previously observed in immunocompromised subjects, who also have poor antibody responses to the HBV vaccine²⁴⁻²⁵. However, overall, the HBV vaccine is effective in preventing HBV infection and from the time the vaccine was introduced, the HBV infection rate has decreased²⁶.

As has been demonstrated in previous studies, increased antibody titers against HBV viral proteins are sufficient for protection against infection²⁷; both humoral and cell mediated responses are necessary to confer protection against antigens, which elicit antigen specific humoral and/or cell-mediated immune responses in the host²⁷. Long-term protection after hepatitis B vaccination is dependent on the persistence of a strong immunologic memory response²⁸. Detection of circulating Hepatitis B surface antigen – specific memory B cells has been reported in HBV-vaccinated subjects or patients who recovered from previous HBV infection²⁸⁻²⁹. Interestingly, the number of Hepatitis B antigen-specific memory B cells remains significant 14, 16, 15, and 12 years after vaccination³⁰. Thus, this fact may highlight and explain the maintenance of HBVs anti-IgG and IgE-specific responses (>5 years post HBV vaccination) observed in our study. However, in those studies HBVs anti-IgE responses were not assessed³⁰. Of notable interest, individuals that lost their anti-Hepatitis B seropositivity still had immunologic T cell memory, and these T cells were able to trigger anti-Hepatitis B production of B cells activated by revaccination²⁸. However, in our study, Hepatitis B surface antigen-specific memory B cells were not studied.

Data from the present study demonstrate that prevalence of specific IgE- or IgG-anti-HBVs antibodies did not correlate with age. As has been demonstrated in previous studies, in healthy infants, children, and adults, adherence to Hepatitis B vaccination

schedules results in a protective concentration of anti-Hepatitis B antibodies ($\geq 10\text{mIU/mL}$) in 90-100% of vaccinated subjects^{2,31}. However, studies of Liao, *et al.* observed that long-term IgG antibody titer levels in HBV vaccinated children in China (5-15 post vaccination), were still considered protective in approximately 50% of subjects³².

According to the introduction of the universal Hepatitis B virus vaccination for infants³³ all children and adolescents in our study (up to the age of 21), would be expected to have received HBV vaccine. In our NYC community population acute infection with wild-type Hepatitis B infection is low at a rate of 1.5 per 100,000 people³⁴, vaccination rates are high, and the seropositivity rates are similar to large prospective studies³². Therefore, our results most likely reflect direct immune responses to HBV vaccination.

Adults over the age of 40, as well as immunosuppressed people are less likely to develop protective antibody titer concentrations^{2, 35-36}. However, the HBV vaccine is a very immunogenic one, thus, post vaccination serology testing (in the United States) is usually not indicated². Existing data are consistent with the conclusion that HBV vaccine is both safe and effective in children³⁷. However, IgE-mediated local reaction from the recombinant HBV vaccine has been reported³⁸.

There are few clinical studies related to total serum IgE levels or specific IgE anti-HBVs levels in asthma patients immunized with HBV vaccine or their long-term immunological memory responses to the vaccine. Studies of Masten, *et al* demonstrated that HBV- immunized asthmatic children demonstrated an increased amount of Th2 mediated serum IgE anti-Hepatitis B antibody at 180 days post vaccination, compared

with non-asthmatic children, but had comparable amounts of IgG1, IgG2, IgG3, IgA and IgM anti-Hepatitis B antibody, as well as lymphocyte IFN- γ , IL-4, and IL-5³⁹; further, no significant differences were reported in antibody isotype or cytokine production between asthmatic subjects receiving treatment with budesonide or nedocromil, compared with placebo³⁹. Total serum IgE levels were not studied. The authors concluded that the observed Th2 bias may be largely genetic and that children with asthma responded to Hepatitis B immunization as well as non asthmatic children with the major isotypes of anti-Hepatitis B antibody³⁹. Thus, our results that there were moderate correlations between IgE and IgG anti-HBVs responses in asthmatic patients are in agreement with the aforementioned studies for asthmatic children, but contrary to the findings in non asthmatic children. Whether this difference lies in the humoral specificity of the response or different demographics remains to be determined. However, our findings suggest a broader role for IgE in that IgE might protect against viral infection, and also gives further support for the association between IgE and specific-IgE viral responses, regardless of asthma status. To this end, the presence and persistence of IgE anti-Hepatitis A Virus (HAV) antibodies have been described⁴⁰. In those studies serum levels of IgE anti-HAV antibodies increased with booster vaccinations suggesting a direct relationship of IgE anti-virus responses and viral antigen exposure⁴⁰.

In the current study, there was no significant correlation between total serum IgE levels and specific IgE levels in the asthmatic patients, compared with non asthmatic subjects. However, this finding is contrary to our earlier findings that total serum IgE levels, but not specific IgE levels, were increased in asthmatic children with *M. pneumoniae*⁴¹. A possible explanation may be a difference in the antigenic structure of

the *M.pneumoniae* compared with the Hepatitis B Virus, as well as the nature of infection (intracellular vs. extracellular).

Our data raise the possibility of an interplay between specific IgE antibodies and the HBV vaccine antigen. It is currently unknown how HBVs specific IgE and B cell memory responses relate to protective immunity and how these markers compare with IgG or IgM titer levels or assay sensitivity and specificity. However, in our present studies of IgE anti-HBV we demonstrate that specific IgE is present in both subjects with either asthma or no asthma, thus indicating a different role or physiologic function for IgE in the context of vaccination than in airway disease and infection.

Several limitations to our study should be acknowledged. This study had a small study size, which is usually associated with decreased power and increased type II error. In order to confirm the findings of this pilot study, future larger studies are necessary in order to reveal whether there are significant correlations between IgE and the HBV vaccine antigen. However, in our patient population, vaccination rates are high (>95%). Strengths of our study are that (1) our findings are relevant in addressing a possible relationship between IgE and the immune response to the HBV vaccine, and (2) this model may have value in determining a potential role for IgE in asthmatic and non asthmatic children immunized with HPV vaccine, as well as other vaccines, and how IgE mediates innate host protection against extracellular bacterial or viral infections. The mechanism by which viral infections elicit specific IgE responses *in vivo* requires further study.

In conclusion, our data demonstrate that both specific IgG- and IgE- anti-HBVs responses are key factors in maintaining sustained HBV antibody expression after HBV vaccination, and contribute to immune regulation in the inflammatory environment without adverse outcomes in children with asthma. Specific IgE responses may provide further insight into persistent viral memory responses, and should be taken into account when designing future vaccines, in terms of safety and efficacy.

Legend to Figures**Figure 1. Correlation between levels of specific IgE- and IgG anti-HBV antibodies.**

Asthmatic and non asthmatic subjects. Subjects selected based on IgG positivity (Pearson correlation, $R=0.662$, $P=0.007$).

Figure 2. Immune responses to HBV vaccine are similar according to asthma status.

Specific IgE-and IgG-anti HBVs antibody levels were similar in asthmatic patients (N=20) and non asthmatic (N=13) subjects (Mann Whitney U Test, $P=0.238$, 0.762 , respectively)

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Figure 1

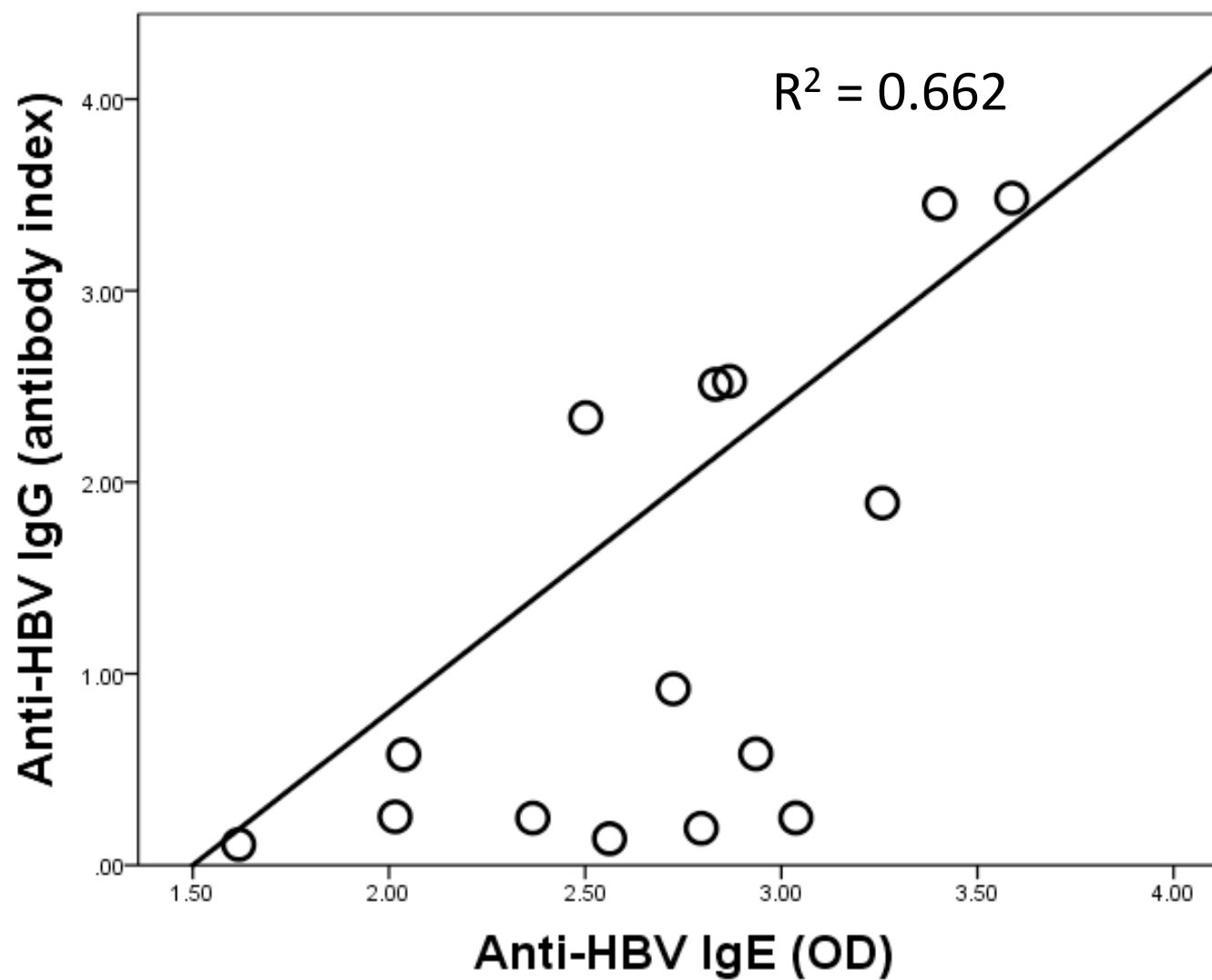


Figure 2

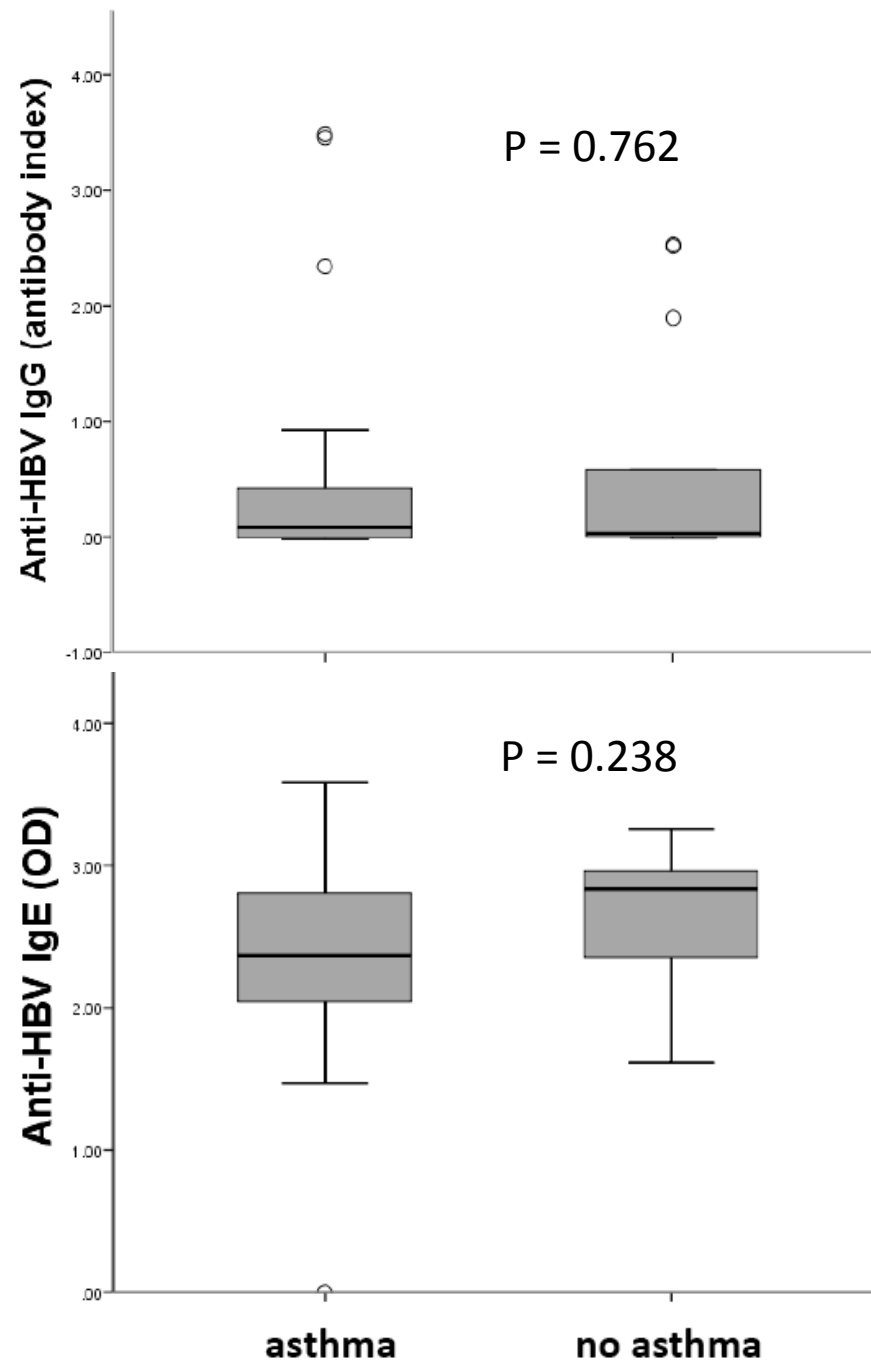


Table I. Levels of specific IgE-and IgG-anti HBVs antibodies according to age group

Subject age	*Mean IgG (Ab index)	*Mean IgE (OD value)
≤ 10 years	1.70 +/- 1.50	3.0 +/- 0.56
> 10 years	1.03 +/- 1.10	2.5 +/- 0.45

*Mann Whitney U Test: ≤10 years vs. >10 years ($P>0.05$)