

## Evaluation of hepatitis B virus antibodies in serum in vaccinated children at Tishreen University Hospital in Syria

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**Abstract:** Background: This study was done to evaluate the level of serum antibody titers in children vaccinated for HBV at Tishreen University Hospital, and to compare the titers of anti-HBs between the different age groups. This study also aimed to evaluate the relation between the infant feeding type and the response to the vaccine.

Materials and Methods: A total of 170 children aged 1-12 years including 73 girls and 97 boys were gathered from pediatric department in Tishreen University Hospital. All the children in this study have undergone hepatitis B vaccine program according to the national standards (3 doses at 0, 1, 6 months after birth). The study sample was distributed into three groups according to age as follow: Group1 included children aging [1-4] years, Group2 included children aging [5-8] years, and Group3 included children aging [9-12] years. Plasma anti-HBs titers were measured. In the first age group, it was studied the response rate, and effects of both gender and infant feeding type on immune response to the vaccine. between response due to vaccination and that due to past infection.

Results: Children who had a titer higher than 10 miu/mL were considered as responders. Our results revealed that the response rate was (81.1%). No significant difference was observed in antibody concentration between groups 1 and 2 ( $P=0.14$ ). However, significant statistical difference was observed between groups 2 and 3 ( $P=0.0009$ ), and between groups 1 and 3 ( $P=0.01$ ). There was no significant difference between girls and boys regarding the response rate ( $P=0.65$ ). On the other hand, the statistical analysis showed that there was no relation between the infant feeding type and the response rate. ( $P=0.96$ ).

Conclusion: The response rate to HBV vaccine in our study was 81.1%, which is less than the expected international rate (90-95%). Significant relation was observed between age and waning of antibody titer. However, no statistically significant difference was observed between type of infant feeding and response to HBV vaccine.

**Keywords:** Hepatitis B virus, HBV vaccine, anti HBs, response rate.

## تقييم مستويات الأجسام المضادة لفيروس التهاب الكبد B في دم الأطفال الملقحين في مشفى تشرين الجامعي في سوريا

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المستخلص: الهدف: تقييم مستويات الأجسام المضادة لفيروس التهاب الكبد B في دم الأطفال الملقحين في مشفى تشرين الجامعي، ومقارنة مستوى هذه الأجسام المضادة بين الفئات العمرية المختلفة، وتقييم تأثير نوع الإرضاع على الاستجابة للقاح. أهمية ومبررات البحث: لايزال يشكل التهاب الكبد البائي مشكلة صحية عالمية على الرغم من إدخال لقاحه في برامج التلقيح الوطنية، إضافةً إلى نسبة الإزمان العالية التي ترافق الإصابة بالفيروس في الطفولة الباكرة والمضاعفات الخطرة الناتجة عن إزمان المرض كتنشع الكبد وسرطانة الخلية الكبدية التي تنتهي بالوفاة. من هنا تأتي أهمية الوقاية من المرض عن طريق التأكد من فعالية اللقاحات وتقييم مستويات الأجسام المضادة للفيروس في الدم نظراً لعدم وجود دراسات سابقة في سوريا تدرس مستوى الأجسام المضادة والاستجابة للقاح.

الطرق: شملت الدراسة 170 طفل (73 أنثى و97 ذكر) من الأطفال الملقحين المراجعين لقسم الأطفال في مشفى تشرين الجامعي بين 2019/12/1 و 2020/12/1. حيث تم إجراء اختبار Anti HBs. كما تم تقسيم الأطفال حسب العمر إلى ثلاث مجموعات، المجموعة الأولى وهي الأطفال الذين تتراوح أعمارهم من [1-4] أعوام بينما تشمل المجموعة الثانية الأطفال الذين تتراوح أعمارهم بين [5-8] أعوام، وتشمل المجموعة الثالثة الأطفال الذين تتراوح أعمارهم بين [9-12] أعوام وذلك لحساب متوسط تراكيز الأضداد anti-HBs لديهم ومقارنتها بين هذه المجموعات. تم تقييم الاستجابة للقاح في المجموعة العمرية الأولى على اعتبار أن تركيز الأضداد يتناقص بشكل ملحوظ بعد سن الخامسة. تمت دراسة عيارات الأضداد Anti-HBc للعينات الإيجابية، وذلك للتأكد من إيجابية الأضداد ناجمة عن التلقيح وليس عن إصابة سابقة بالفيروس.

النتائج: أظهرت الاختبارات إيجابية الأجسام المضادة في 47 عينة من 58، أي أن نسبة الاستجابة للقاح بلغت 81.1%. حيث اعتبر تركيز الأجسام المضادة أعلى من 10ميلي وحدة دولية/ميلي ليتر مؤشر لوجود استجابة للقاح. لم يلاحظ وجود فرق هام إحصائياً في نسبة الاستجابة بين الذكور والإناث (P-value=0.65). في حين كانت الفروقات هامة إحصائياً عند مقارنة تراكيز الأجسام المضادة بين المجموعتين العمريتين الثانية والثالثة (p-value = 0.0009) وكذلك بين المجموعتين الأولى والثالثة (p-value = 0, 01) بينما لم توجد فروق هامة إحصائياً عند مقارنة تراكيز الأضداد بين المجموعتين الأولى والثانية (p-value = 0, 14). وعند دراسة تأثير نوع الإرضاع على الاستجابة للقاح لم نشاهد وجود فرق هام إحصائياً في الاستجابة بين أنواع الإرضاع (p-value=0.96). الخلاصة: نسبة الاستجابة من الإصابة بالتهاب الكبد البائي 81.1% وهو أقل من النسبة العالمية المتوقعة والتي تبلغ 90-95% كما لوحظ تناقص عيار الأجسام المضادة مع زيادة عمر الطفل.

الكلمات المفتاحية: لقاح التهاب الكبد البائي، أضداد المستضد السطحي، الأطفال، الاستجابة

## INTRODUCTION.

Despite the application of a universal vaccination program against hepatitis B virus (HBV), this pathogen continues to be responsible for important morbidity and mortality all over the world.<sup>[8]</sup> It has been an international health problem causing nearly one million deaths every year.<sup>[10]</sup>

The most common routes of HBV infection are still the perinatal transmission from mother to child, the transfusion of blood and its products, and the unsafe sexual activities. The age at which infection takes place affects significantly the course of the infection. In fact, about 90% of neonates infected from HBV positive mothers develop chronic infection.<sup>[7]</sup> The prevalence of the disease is still high in some parts of the world (e.g. the sub-Saharan Africa and East Asia,) where more than 8% of the adult population are chronically infected.<sup>[14, 4]</sup> In contrast, the incidence of HBV infection is between 0.1% and 0.7% in Northern, Western, and Central Europe.<sup>[9]</sup> According to the global review of hepatitis B and C in 2013, the Syrian Arab Republic is classified as having high prevalence of hepatitis C and low—inter-mediate prevalence of hepatitis B.<sup>[1]</sup>

Since the advent of HBV vaccine, the active immunization has been the most effective measure to control and prevent HBV infection. Indeed, a percentage of 70-90% of vaccinated individuals develop a protective response against the virus.<sup>[3]</sup> HBV vaccination triggers antibody response, and antibody to hepatitis B surface antigen (anti-HBs) levels of  $\geq 10$  miu/mL are usually regarded as seroprotective.<sup>[11]</sup>

In 1993, Syria included hepatitis B vaccine in the national vaccination program, and the World Health Organisation (WHO) recommended vaccination schedules was adopted. This program includes three doses of vaccine (at 0, 1, and 6 months after birth and the first dose being administered within 24 hours of birth)<sup>[6]</sup>.

Hepatitis B vaccines are currently available in different combinations preventing not only from HBV but also from other diseases (e.g. diphtheria, tetanus, pertussis, and poliomyelitis).<sup>[15]</sup> Various studies have reported that the response rate can be affected by different factors like the number of injections, site of injection, the use of adjuvant materials, obesity, diabetes, gender.<sup>[16]</sup> There is no previously published data on the evaluation of the response rate to hepatitis B vaccine in Syria. That is why, the present investigation was carried out.

## Materials and Methods.

### Ethics:

All samples were obtained after informed consent had been given; as patients were under age, approval was obtained from parents.

### Samples:

This cross-sectional study was conducted on 170 children aging [1-12] years in Tishreen University Hospital-Lattakia from December 2019 to December 2020. All enrolled children have received three doses of recombinant hepatitis B vaccine at (0, 1, and 6 months after birth). Children who received immunosuppressive treatments, HBIG, HBV booster dose, blood or blood products were excluded from this study. Besides, children who complain of chronic diseases were also not included in the study. Five milliliters of venous blood were taken from each child by venipuncture. The sera samples were separated by centrifugation and then stored at  $-20^{\circ}\text{C}$  until the test was performed.

### Evaluation of HBs-Ab titers

All of the sera samples were tested for the presence of HBV antibodies using the commercial kit "HBsAb ELISA" (Biorex, India) according to the manufacturer's instructions. The tests were conducted at least one month after the third dose of the vaccine. The results were categorized into three groups as follow: 1) infants with antibody titers  $\leq 10$  miu/mL were considered as non-responders, 2) infants with antibody titer between 10 and 100 miu/mL were considered as poor responders. 3) infants with antibody

titer  $\geq 100$  miu/mL were considered as good responders. Samples that tested positive for Hbs-Ab were also analyzed for the presence of HBc-Ab, which is a marker of HBV infection by using "HBcAb ELISA kit" (Biorex, India) according to the manufacturer's instructions.

### Statistical analysis:

All quantitative variables were reported as mean  $\pm$  standard deviation, and percentage was used for qualitative variables. Chi-Squared test was used to evaluate the goodness of fit between categorical variables. T-test was used to determine the mean differences between pairs of groups. Variable correlation was evaluated by spearman correlation coefficient. Statistical analysis was performed using SPSS 21.0 and significance was regarded at p-Value  $<0.05$ .

### Results.

The titration of HBs-Ab of the first age group was revealed that 47 children out of 58 (81.1%) had antibody titers  $\geq 10$  miu/mL, while 11 children (18.9%) had antibody titers  $<10$  miu/mL.

The results were showed that there is no significant difference between girls and boys regarding the response rate ( $P = 0.65$ ).

**Table (1) The response rate to hepatitis B vaccine in the first age group based on gender**

Variations				
	antibody titers $\geq 10$	antibody titers $<10$	Total (%)	p-value
Boy	43(81.1%)	10(18.9%)	53(100)	0.65
Girl	4(80%)	1(20%)	5(100)	
Total	47	11	58	

The titers was analyzed for HBs-Ab in the studied groups. The mean antibody titers in groups 1, 2, and 3 were  $282.7 \pm 68$  miu/mL,  $167.7 \pm 20$  miu/mL and  $82.6 \pm 14$  miu/mL, respectively.

No significant difference was observed in mean antibody titers between groups 1 and 2 ( $P=0.14$ ). However, a significant statistical difference was observed between groups 2 and 3 ( $P=0.0009$ ), and between groups 1 and 3 ( $P=0.01$ ).

**Table (2) Antibody titers in the three groups according to age**

Variations	Mean antibody titer
Group1 :[1-4]years	$282.7 \pm 68$
Group2 :[5-8]years	$167.7 \pm 20$
Group3 :[9-12]years	$82.6 \pm 14$
p-value (Group1 and Group2)	0.14
p-value (Group2 and Group3)	0.0009
p-value (Group1 and Group3)	0.01

Additionally, the impact of feeding type on the response rate in the first age group was also evaluated. Our results revealed that there was no effect of the type of feeding on the response rate to HBV-vaccine ( $P = 0.96$ ). The levels of response rate to vaccination was similar in the three age groups.

**Table (3) The response rate to hepatitis B vaccine in the first age group based on the type of infant feeding**

Variations				p-value
	antibody titers $\geq 10$	antibody titers $< 10$	Total (%)	
Breast feeding	24(80%)	6(20%)	30(100)	0.96
Formula feeding	13(81%)	3(19%)	16(100)	
Mixed feeding	10(83%)	2(17%)	12(100)	

Samples positive for HBs-Ab were analyzed for the presence of HBcAb in order to exclude a possible past HBV infection. The samples were negative for HBcAb, confirming that positive HBs-Ab is the direct result of HBS vaccination.

## Discussion.

This study investigated for the first time in Syria, the efficiency of Hepatitis B vaccination since its implementation in 1993.

Results obtained from this study revealed that 81.1% of studied children had an antibody titer higher than 10 miu/ml, which is lower than the expected international rates. The study sample was divided into 3 groups as mentioned earlier, and it was observed that the mean titer of antibodies decreased over time. There was no statistical differences observed between gender or infant feeding type and the response to the vaccine. Lee et al showed in their study that 73.8% of children had antibody titers higher than 10 miu/ml, which was lesser than the results in our study. The titers showed fluctuating decreases. After 7 years of age, 50% of vaccinated children tested negative for HBsAb, and HBsAb titers reached their lowest levels by 14 years of age. The difference of response between girls and boys was statistically significant ( $p = 0.032$ ).<sup>[6]</sup> Sanou et al reported that 61.6% of children responded to the vaccine. They observed that 81% of children with anti-HBs titer  $< 10$  miu/ml were over 18 months of age suggesting a decline of anti-HBs titer with time.<sup>[12]</sup> Shindano et al showed that seroprotection was achieved in 84.5% of infants, and also showed that neither age nor sex associated with the response rate.<sup>[13]</sup> In China, Li et al reported that 79.3% of children had an anti-HBs level higher than 10 miu/mL, a result that agrees with our findings. By comparing anti-HBs titers of different age groups. They concluded that the older age group had lower level of HBs-Ab, which is consistent with our results. Li. et.al also found that the CD4+ T cell levels of the non-responder group were significantly lower than those of the high responder group, whereas CD8+ T cell and Tfh cell levels of the non-responders were significantly higher than the responders.<sup>[7]</sup>

The reason for this low level of response to hepatitis B vaccine in our study is not well defined. One hypothesis could be malnutrition, which is likely to be present in a large proportion of syrian children due to difficult socio-economic situation caused by the current unstable conditions in Syria.

Studies have revealed that the nutritional status of children could have an impact on antibody response to vaccination.<sup>[12]</sup> Unfortunately, data on nutritional status were not available making the confirmation of this hypothesis uncertain.

Studies have shown that there are many factors that influence the response to the vaccine, such as sex, age, weight, genetics, and immunocompetency of individuals.<sup>[5, 2]</sup> In addition, type of vaccine, the number of injections, the use of adjuvant materials, and storage conditions of vaccine are known to play a role in the differences in immunogenicity of the hepatitis B vaccine.<sup>[16]</sup> Each of these factors should be evaluated separately in order to define its role in the response to the vaccine.

## Conclusion.

Our results revealed that the response rate to HBV vaccines in children in Latakia-Syria was 81.1%, this is lower than the expected global ratios. All the samples that were positive for HBsAb were negative for HBeAb; this emphasizes that the cause of HBsAb positivity is the vaccine and not a previous infection. There was no significant difference of HBsAb titers between girls and boys. By comparing the antibody titers among age groups, we could conclude that antibody titers were declining over time. We found as well that the type of feeding does not affect HBsAb titers.

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