

HEPATITIS B VACCINE - PROPOSAL FOR A STANDARDIZED ASSESSMENT OF IMMUNE RESPONSE

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SUMMARY

The authors developed a comparative study of the various methods of assessment of immune response to Hepatitis B vaccine.

Eighty-six health care professionals underwent a vaccination programme with three doses of plasma-derived vaccine against Hepatitis B (H-B-Vax, Merck, Sharp & Dohme) given intra-muscularly.

Assessment of immune response was carried out three months after the end of the programme, by radioimmunoassay (RIA) and enzymeimmunoassay (EIA).

The results showed that the semi-quantitative assessment of Anti-HBs antibodies by RIA or EIA was perfectly comparable to the reference method (quantitative determination of antibodies by RIA).

In view of these findings, the authors suggest a standardization of assessment of immune response to the vaccine, thus permitting correct planning of booster doses and easier comparison between different studies.

KEY WORDS: Hepatitis B; Hepatitis B Vaccine; Anti-HBs; Antibodies.

INTRODUCTION

Infection with Hepatitis B virus (HBV) is very common throughout the world, affecting about 300 million people¹⁰. Severe consequences may result from both acute and chronic forms of the disease and there is currently no satisfactory treatment for the condition⁴. Thus, prophylaxis against the disease is particularly important. This involves a number of measures which should be undertaken by exposed groups. However, the main weapon available against HBV infection is active immunization of at risk subjects.

The availability of safe and efficient vaccines enables infection control programmes to be instituted with the long term aim of eradication of the disease and also, due to the direct relation between Hepatitis B and hepatocarcinoma, protection against cancer¹².

In Brazil, HBV infection may be classified as being endemic to an intermediate degree in most areas, except in the Amazonian region, where it is highly endemic¹. The World Health Organization suggests that in intermediate prevalence areas vaccination should be restricted to risk groups (intravenous drug users, patients with chronic renal failure, male homosexuals and health care professionals, among others)⁵.

In areas of high prevalence, mass vaccination programmes are required for all children, as is occurring in Amazon region¹¹.

The right assessment of immune response to the vaccine is made by quantitative determination of Anti-HBs antibodies, since protection against

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infection is related to the quantity of antibodies formed^{3,9}. The quantification of Anti-HBs antibodies is usually made by radioimmunoassay (RIA), using a standard solution with a known quantity of antibodies to construct a reference curve². However, in current literature the results are often presented in different ways, either through qualitative tests with a positive and negative result, or semiquantitative tests made by RIA or enzymeimmunoassay (EIA), comparing the sample results to the obtained values in negative controls, expressing results as a ratio S/N (sample/negative). Therefore, comparison between different studies is sometimes difficult^{7,16,17}.

The purpose of this study is to assess the immune response to Hepatitis B vaccine and compare the different laboratory methods and the different ways of expressing the results.

MATERIAL AND METHODS

Eighty-six individuals (45 females and 41 males), health care professionals (56 doctors, 16 nurses and 14 laboratory technicians) with ages varying between 17 and 55 years (median of 28 years old) were studied.

Only individuals with negative HBV serology, i.e., HBsAg, Anti-HBs and Anti-HBc negative were included.

Serologic tests were made by enzymeimmunoassay with commercial kits (AUSZYME, AUSAB and CORZYME, Abbott Laboratories, North Chicago, IL).

Three doses of vaccine against HBV (H-B-Vax, Merck, Sharp & Dohme) with 20 micrograms per dose were administered into the deltoid muscle. All vaccines came from the same batch and were kept refrigerated until used. The vaccines were given at 0, 30 and 180 days. Blood samples were obtained for testing 90 days after the third dose.

Quantitative determination of Anti-HBs antibodies: made by RIA, using a standard solution with 1000 UI/L of antibody (La Roche and Co.Ltd., Basle, Switzerland) and a reference curve was constructed with dilutions of antibody corresponding to 100, 50, 20 and 10 UI/L. Serum samples were tested in a previous dilution and the obtained value of counts per minute was compared to the standard curve in order to determine concen-

tration of antibodies in each sample. When necessary, the samples were diluted at 1:10, 1:100 or 1:1000 and submitted to a new assessment.

Semi-quantitative determination of Anti-HBs antibodies: performed by expressing an index, known in current literature as S/N index (sample/negative).

In RIA this index was calculated by a quotient between counts per minute in studied sample and the average count in negative controls (RIA S/N). In EIA this index related the optical density of sample to the average optical density of negative controls (EIA S/N).

Assessment of the intensity of response to vaccine: by both methods (quantitative and semi-quantitative) individuals were classified as **NON-RESPONDERS**, **LOW-RESPONDERS** and **GOOD-RESPONDERS**.

In the quantitative RIA assessment individuals with less than 10 UI/L of antibodies were considered non-responders. Those with levels between 10 and 100 UI/L were low-responders and those with more than 100 UI/L were good responders¹⁵.

In semi-quantitative RIA, non-responders presented an S/N index < 3.0, low-responders an index between 3.0 and 20.0 and good-responders an index greater than 20.0¹⁷.

In the EIA semi-quantitative, non-responders were considered those with S/N index < 2.0; low-responders with an index between 2.0 and 10.0 and good-responders with an index > 10.0¹³.

Statistical method: Mc Nemar's test was used to compare the different tests and 5% ($p < 0.05$) was the level of rejection of the null hypothesis.

RESULTS

Figure 1 shows the results of the quantitative analysis of Anti-HBs. After three doses of vaccine five (5.8%) individuals were considered non-responders, five (5.8%) low-responders and seventy-six (88.4%) were good-responders.

When we compared seroconversion percentage and different levels of response obtained by quantitative method to the results obtained in semi-quantitative tests, whether by RIA or EIA (Tables 1 and 2), no difference was noted.

Table 1
Levels of Immune Response to Hepatitis B Vaccine: Comparison Between Quantitative RIA (QT RIA) and Semi-Quantitative RIA (SQ RIA).

SQ RIA	QT RIA			Total
	NR	LR	GR	
NR	5	2	0	7
LR	0	3	0	3
GR	0	0	76	76
Total	5	5	76	86

NR - non-responders; LR - low-responders; GR - good-responders.

Table 2
Immune Response to Hepatitis B Vaccine: Comparison Between Quantitative RIA (QT RIA) and Semi-Quantitative EIA (SQ RIA)

SQ EIA	QT RIA			Total
	NR	LR	GR	
NR	5	2	0	7
LR	0	3	1	4
GR	0	0	75	75
Total	5	5	76	86

NR - non-responders; LR- low-responders; GR- good-responders.

DISCUSSION

In the studied group we observed a seroconversion percentage after three doses of vaccine of 94.2%. This is similar to the results of other studies^{6,18}.

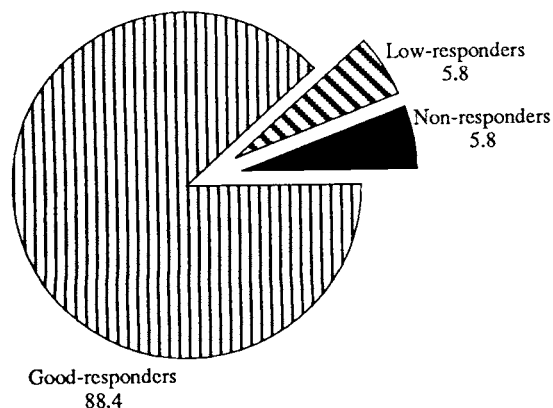


Fig 1 - Levels of Immune Response to Hepatitis B Vaccine

It is important to note that the protection conferred by the vaccine is comparable with plasmatic and recombinant vaccine¹⁴.

When using S/N index to classify different levels of response to the vaccine, the findings were comparable to those found in the quantitative determination of antibodies (Tables 1 and 2), with an easier methodology and at a lower cost.

Classification of response levels to vaccine is of great importance because it is possible to predict the best occasion to carry out booster doses, since many authors have demonstrated that the duration of the immune response is directly dependent on the intensity of response reached at the end of the initial vaccination scheme^{3,9}. Thus, good-responders should receive a booster dose after 5 or 7 years; low-responders after 1 or 2 years and non-responders should be revaccinated immediately⁸.

In conclusion, it is suggested that all persons vaccinated against Hepatitis B should have an assessment of their immunity soon after vaccination, in order to confirm immunity and allow correct planning of booster doses. With this purpose, we suggested the following guidelines:

a. one to three months after the end of the vaccination programme serum samples should be collected to determine Anti-HBs antibodies.

b. Semi-quantitative determination of antibody concentration by radioimmunoassay or enzymeimmunoassay should be performed.

Based on the results of these semi-quantitative tests, booster doses should be administered according to the following scheme⁸:

RIA S/N	EIA S/N	BOOSTER DOSE
<3.0	<2.0	immediatly
>3.0 and <20.0	>2.0 and <10.0	after 1 or 2 years
>20.0	>10.0	after 5 or 7 years

RESUMO

Vacinação contra Hepatite B - Proposta de Padronização da Avaliação da Resposta Imunológica

Os autores realizaram estudo comparativo dos diferentes métodos de avaliação da resposta à vacina contra hepatite B.

Foram estudados 86 indivíduos, profissionais da área de saúde, que foram submetidos a esquema de vacinação com três doses de vacina plasmática contra hepatite B (H-B-Vax, Merck, Sharp & Dohme), aplicadas por via intramuscular.

A avaliação da resposta imunológica à vacina foi realizada três meses após o término do esquema, através tanto de radioimunoensaio como enzimaimunoensaio.

Os resultados obtidos demonstraram que a avaliação semi-quantitativa dos anticorpos anti-HBs por enzimaimunoensaio é perfeitamente superponível ao método de referência, que é a determinação quantitativa de anticorpos por radioimunoensaio.

Tendo em vista estes achados, os autores propõem uma padronização da avaliação da resposta à vacina, com o objetivo de prever a ocasião das doses de reforço e tornar os diferentes estudos comparáveis entre si.

REFERENCES

1. BENSABATH, G. & SOARES, M.P.C. - A Hepatite B Delta em Boca do Acre, Alto Puns. *Mod. Hepat.*, 14:36-40, 1989.
2. COUROUCÉ, A.M. - Quantification of post-vaccination humoral immunity. In: COURSAGET, P. & TONG, M.J. - *Progress on Hepatitis B Immunization*. France, John Libbey Eurotext, 1990. p. 67-72.
3. COURSAGET, P.; YVONNET, B.; CHOTARD, J.; SARR, M.; VINCELOT, P.; N'DOYE, R.; DIOP-MAR, I. & CHIRON, J.P. - Seven-year study of Hepatitis B vaccine efficacy in infants from an endemic area (Senegal). *Lancet*, 2:1143-1145, 1986.
4. DE GROOTE, J.J. - Therapeutic measures after Hepatitis B virus infection: post-exposure prophylaxis. *Postgrad. med. J.*, 63 (Suppl. 2):33-39, 1987.
5. DESAI, H.G. - WHO recommendation for Hepatitis B immunization. *Indian J. Gastroent.*, 5:291, 1986.
6. DIENSTAG, J.L.; WERNER, B.G.; POLK, B.F.; SNYDMAN, D.R.; CRAVEN, D.E.; PLATT, R.; CRUMPACKER, C.S.; OUELLET-HELLSTROM, R. & GRADY, G.F. - Hepatitis B vaccination in health care personnel: safety, immunogenicity and indicators of efficacy. *Ann. intern. Med.*, 101:34-40, 1984.
7. FRANCIS, D.P.; HADLER, S.E. & THOMPSON, J.E. - The prevention of Hepatitis B with vaccine. Report of the Centers for Disease Control multi-center efficacy trial among homosexual men. *Ann. intern. Med.*, 97:362-366, 1982.
8. HOLLINGER, F.B. - Factors influencing the immune response to Hepatitis B vaccine, booster dose guidelines and vaccine recommendations. *Amer. J. Med.*, 87 (Suppl. 3A):36-40, 1989.
9. JILG, W.; SCHMIDT, M.; ZACHOVAL, R. & DEINHARDT, F. - Hepatitis B vaccination: how long does the protection last? *Lancet*. 2:458, 1984.
10. MAYNARD, J.E.; KANE, M.A. & HADLER, S.C. - Control of Hepatitis B by immunization. In: ZUCKERMAN, A.J. - *Viral Hepatitis and Liver Disease*. London, Alen R. Liss., 1988. p. 967-969.
11. MEDEIROS J. L. - Hepatite B no Brasil. *Mod. Hepat.*, 14: 3, 1989.
12. MENDES, T.F. - Vacina contra Hepatite B. *Mod. Hepat.*, 1:3, 1983.
13. MILNE, A.; MOYES, C.D.; WALDON, J.; PEARCE, N.E. & KRUGMAN, S. - Outcome evaluation of New Zeland Hepatitis B immunization programme in children. In: COURSAGET, P. & TONG, M.J. - *Progress on Hepatitis B Immunization*. France, John Libbey Eurotext, 1990. p. 467-473.
14. PAPAANGELOU, G.; ROUMELIOUTOU-KARAYANNIS, A. & TSAKALAKIS, C. - Dose range studies of a Hepatitis B recombinant DNA vaccine: comparison to a plasma vaccine. In: MEETING OF EUROPEAN ASSOCIATION FOR STUDY OF THE LIVER, 23. Leuven, Belgium, 1988. Abstracts, p. 7-8.
15. PEAD, P.J. - Immune response to Hepatitis B vaccination in hospital staff. *Biomed. Pharmacol.*, 40:251-253, 1986.
16. STEVENS, C.E.; ALTER, H.J.; TAYLOR, P.E.; ZANG, E.A.; HARLEY, E.J. & SZMUNESS, W. - Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *New Engl. J. Med.*, 311:490-501, 1984.
17. SZMUNESS, W.; STEVENS, C.E.; HARLEY, E.J.; ZANG, E.A.; OLESZCO, W.R.; ALTER, H.J.; TAYLOR, P.E.; DE VERA, A.; CHEN, G.T.S. & KELLNER, A. - Hepatitis B vaccine. Demonstration of efficacy in a controlled clinical trial in high-risk population in the United States. *New Engl. J. Med.*, 303:834-841, 1980.
18. SZMUNESS, W.; STEVENS, C.E. & HARLEY, E.J. - Hepatitis B vaccine in medical staff of hemodialysis units: efficacy and subtype cross-protection. *New Engl. J. Med.*, 307:1481-1486, 1982.

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