PERSISTENCE OF SPECIFIC IMMUNE RESPONSE AFTER HEPATITIS B VACCINATION IN EGYPT: RELATION TO HLA-DRB1 ALLELES AND SCHEDULE OF VACCINATION

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ABSTRACT

Background: Hepatitis B (HB) is one of the world's major health problems.HB vaccination is the most effective way to prevent transmission of hepatitis B virus (HBV).

Objective: Evaluation of persistence of each of humoral and cellular immunity against HBV after HB vaccination & studying some factors that affect persistence of immune response after HB vaccination including: Human Leukocyte antigens-DRB1 (HLA-DRB1) and Schedule of vaccination.

Methods: The study included three groups with different HB vaccination schedules with time since vaccination ranging from 6 to 16 years. They were investigated for their immunological memory. To evaluate humoral immunity the antibody to hepatitis B surface antigen (anti-HBs) titres were measured. Cellular immunity was determined by enzyme-linked immunosorbent spot assay (ELISpot) to detect HBV specific interferon (IFN)-gamma producing cells.

Results: 65% of participants who displayed unprotected anti-HBs titre were positive HBV specific interferon (IFN)-gamma producing cells and 90% of participants who displayed protective anti-HBs titre were also positive for IFN-gamma producing cells. The most prevalent HLA-DRB1 alleles in those with anti-HBs ≥10 milli-international units per milliliter (mIU/ml) were HLA-DRB1*15.

***Conclusion*:** Primed immune cells to hepatitis B surface antigen (HBsAg) still exist for years even after loss of the protective anti-HBs titre.There is association between HLA-DRB1*15 allele with long term humoral memory to HB vaccine.

Key words: HBV, HB Vaccination, Humoral immunity, Cellular immunity, Human leukocyte antigen.

Abbreviations: Antibody to hepatitis B surface antigen (anti-HBs); Antibody to HB core antigen (anti-HBc); Bacillus Calmette - Guērin (BCG); 5-bromo-4-chloro-3-indolyl-phosphate/ nitroblue tetrazolium (BCIP/NBT); Diphtheria, tetanus, pertussis and oral polio(DTP-OPV); Enzyme-linked immunosorbent assay (ELISA);Enzyme-linked immunosorbent spot assay (ELISpot); Expanded Program of Immunization (EPI); Hepatitis B (HB); Hepatitis B surface antigen (HBsAg);Hepatitis B virus (HBV); Human Leukocyte antigens-DRB1 (HLA-DRB1); Interferon (IFN);Intracellular cytokine staining (ICS); Milli-international units per milliliter (mIU/ml); Peripheral blood mononuclear cells (PBMCs); Phosphate buffered saline (PBS); Phosphate buffered saline- Tween 20 (PBS-T); Phytohaemagglutinin (PHA); Polyvinylidene fluoride (PVDF); Spot forming units (SFCs); World health organization (WHO).

INTRODUCTION

Hepatitis B is one of the world's major health problems . It is estimated that more than 2 billion people are infected with HBV globally, and more than 400 million are chronic carriers. The infection is supposed to be causally related to 1 to 2 million deaths per year worldwide **(1)**. HBV is transmitted through serum and even body fluids such as semen, saliva, sweat, tears, or breast milk.The majority acquire the infection perinatally.However, the currently acknowledged risk factors for infection by the HBV are sexual promiscuity, intravenous drug abuse, blood and derivatives transfusions, hemodialysis, and needle accidents among health-care professionals **(2).**

Neonatal hepatitis vaccination is part of the immunization programme of many countries, both in the industrialized and developing world. HB vaccines are composed of highly purified preparations of HBsAg obtained from the plasma of patients with chronic infection (plasma-derived vaccine) or from yeast or mammalian cells transfected with viral DNA (recombinant vaccine) **(3)**. Universal neonatal and infant HB vaccination has been proven to be highly effective in inducing protective antibodies and preventing perinatal and horizontal transmission of HBV **(4)**. A compulsory vaccination programme against HB infection among infants was started in Egypt in 1992 with coverage rate of about 95% using a yeast recombinant (10µg) and with a schedule of 2,4,6 months of age to coincide with other compulsory vaccines {diphtheria, tetanus, pertussis and oral polio(DTP-OPV)} **(5).** Primary vaccination with a three-dose series of recombinant HB vaccine results in seroprotection (defined as the development of anti-HBs at a concentration greater than 10mIU/mL) in >95% of vaccinated infants and children **(6)**.

Serologic studies have shown that the titre of antibodies against HBsAg drops within the first few years after vaccination and that one-third to one-half of children vaccinated as infants will have titres below 10 mIU/mL by 10–15 years of age. Long-term observational data show that vaccinated individuals even those who have an undetectable titre of HBsAg can mount an amnestic response to contact with HBV **(7)**. Quantification of IFN-gamma expression by T cells is a well-established surrogate test for assessing cellular-mediated immune responses. Several IFN-gamma detection methods have been

developed and are commercially available; all are characterized by specific discriminating features. In particular the enzyme-linked immunosorbent assay (ELISA), ELISpot assay and intracellular cytokine staining (ICS) assay are in widespread use **(8)**.

However, when measuring low-level responses, the ELISpot assay is a better choice due to its lower detection limit with high sensitivity **(9)**. Moreover, it is less expensive to perform, less dependent upon sophisticated instrumentation and better suited to the analysis of frozen samples when limited numbers of cells are available **(10)**. **Lu et al. (2008)** used ELISpot assay to evaluate the memory T cell responses elicited by HB vaccines. A direct correlation between the serological and T cell responses has been shown. They suggested that ELISpot assay is reliable in detecting anti-HBs immunity **(4)**. Short-term immune response to HB vaccine had been closely linked to several HLA alleles. Therefore, it is reasonable to hypothesize that long-term immunological memory to HB vaccination is also influenced by HLA **(8).** The aim of this study was to evaluate the long term humoral and cellular immunity against HBV after HB vaccination & to study some factors that affect persistence of immune response after HB vaccination including: HLA-DRB1 and Schedule of vaccination.

MATERIALS AND METHODS

This comparative study was conducted on 50 HB vaccine recipients.They were divided according to the schedules of vaccination into three main groups: group(I) was 25 healthy adolescents 14-17 years old who had received their three doses of HB vaccine at 2, 4, and 6 months of age according to the World health organization (WHO) Expanded Program of Immunization (EPI) in Egypt, group(II) consisted of 13 health care workers, whose HB vaccination schedule was at 0, 7 and 21 days (accelerated schedule) & their ages ranged from 30-59 years old and group(III) consisted of 12 health care workers, whose HB vaccination schedule was at 0, 1 and 6 months (standard schedule)& their ages ranged from 32-60 years old. Among them 4 (1 in each of group(I) and group(II), 2 in group(III)) with the total antibody to HB core antigen (anti-HBc) +ve and additional 16 participant who did not return in the second visit were excluded from ELISpot & HLA typing. This study was conducted in Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, during the period from 2010 to 2012.

Inclusion criteria: All subjects should have received intramuscular injections of three doses of HBV vaccine. **Exclusion criteria:** History of chronic disorders including (previous infection with HBV, liver failure, end stage renal disease, immunosuppression and/or received corticosteroids and receiving HB immunoglobulin.

All groups were subjected to the following:

1) Full medical history taking. **2)** Collection of blood samples. Blood was drawn aseptically by vein puncture and serum was separated by centrifugation and stored at –70°C.

3) Qualitative determination of total anti-HBc by (Monolisa Anti-HBc PLUS kit Bio-Rad, France) in order to differentiate immunity from naturally occurring HBV infection **(9).**

4) Quantitative determination of anti-HBs titre by competitive ELSA (DiaPro, Milan, Italy) and according to the manufacture instructions. Anti-HBs titres were expressed in mIU/mL **(10)** and were calculated by means of a standard curve calibrated against the WHO reference preparation. Anti-HBs ≥10 mIU/ml was considered according to international standards to be protective against HB infection.

After exclusion of +ve anti-HBc cases and missing participants in second return, then the 15 in Group(I), 8 in Group(II) and 7 in Group(III) were subjected to the following:

1) HBsAg-specific IFN-gamma production by ELISpot assay

Peripheral blood mononuclear cells (PBMCs) were isolated from 5mL heparinized blood samples by density gradient centrifugation and cultured in Roswell Park Memorial Institute (RPMI 1640) medium plus 5% human serum. ELISpot assay was done on freshly isolated PBMCs to measure the secretion of representative cytokine IFN-gamma of the Th1 subset of CD4 cells.The ELISpot human IFN-gamma set pre-coated (Gen-Probe Diaclone, France) was used in accordance with the manufacturer's instructions.To minimize edge effect, the microplate (bottom down) was placed onto a piece of soft aluminum foil (about 4 x 6 inches). Then 100µl of 1x phosphate buffered saline (PBS) was added to every well and the plate was incubated at room temperature for 10minutes.The wells were emptied by flicking the plate over the sink and gently tapping over absorbant paper.Then 50µl of each PBS as negative control, phytohaemagglutinin (PHA) as the positive control and HBsAg were added to each well of a 96 well polyvinylidene fluoride (PVDF) membrane bottom plate coated with a specific IFN-gamma antibody. Subsequently, triplets of $2x10^5$ PBMCs in 50 µl complete medium were

added to each of three wells of negative control, positive control and HBs antigen. Then plate was covered with the lid and the foil was outlined around the edges of the microplate without shaking and incubated at 37°C in a CO2 incubator for 72 hours **(10).** After the incubation, the wells were emptied and 100µl phosphate buffered saline- Tween 20 (PBS-T) solution was added to every well and the plate was incubated at 4°C for 10 minutes.Then the wells were emptied as previously and washed 3x times with 100µl PBS-T Solution.After that 100µl of diluted detection antibody was added to each well and the plate was covered for incubation at room temperature for 1hour 30minutes. Later 100µl of diluted Streptavidin-Alkaline Phosphatase conjugate was placed in every well and the plate was covered for incubation at room temperature for 1hour. Then the wells were emptied as previously and washed 3x times with 100µl PBS-T Solution.The both sides of the membrane were washed 3x under running distilled water, once washing is completed any excess solution was removed by repeated tapping on absorbant paper. Finally 100µl of 5-bromo-4-chloro-3-indolyl-phosphate/ nitroblue tetrazolium (BCIP/NBT) substrate buffer was placed to every well and the plate was incubated for 5-15 min with monitoring spot formation visually throughout the incubation period to sufficient colour development.The wells were emptied and the both sides of the membrane were rinsed 3x under running distilled water and tapped on absorbant paper to remove any excess solution.The plate was allowed to dry completely either at room temperature (60-90 minutes).Then the spots were counted by stereomicroscope as spot forming units (SFCs). Specific spots are round and have a dark center with slightly fuzzy edges as shown in **figure (1)**. Mean numbers of IFN-gamma producing cells and SFCs were calculated from duplicated assays. HBsAg-specific SFCs were calculated by subtracting background SFCs induced by negative well from the total SFCs induced by HBsAg.The ELISpot results were considered positive if there were 2.0-fold more spots than background **(11)**.

2) Genomic DNA extraction for HLA-DRB1 typing: Genomic DNA was extracted from 1 ml peripheral blood in tubes containing EDTA (LOT F100913) using QIAamp DNA Mini kits (QIAGEN, UK) according to the manufacture instructions. HLA typing was performed by polymerase chain reaction followed by detection using sequence- specific oligonucleotides probes as displayed in **figure (2)**.

Ethics approval: the Ethics Committee at the Faculty of Medicine, Zagazig University approved this study.

STATISTICAL ANALYSIS

The data were collected, presented and analyzed using SPSS-PC (version 10) software. Comparisons between groups were done using Mann Whitney test and Kruskal-Wallis test for skewed data. Also, qualitative categories were expressed in the form of frequency and percentage, and comparisons between qualitative categories were done by Chi square test, while fisher exact test was used when there is an observed cell <5. The test results were considered significant when P. value <0.05. While, the test results were considered non-significant when P. value >0.05. Correlation coefficient (r) was calculated to determine correlation of ELISpot IFN-gamma and anti-HBs to the different studied variables. Pearson Correlation was used for normally distributed data, and Spearman's rho Correlation for skewed data. The test results were considered significant when P. value <0.05.

RESULTS

Regarding the time since vaccination, in group(I) ranged from 13 – 16 years with mean value of 14.56 ±1.16 , in group(II) ranged from 6 – 10 years with mean value of (8.15±1.46) and in group(III) ranged from 6 – 10 years with mean value of (9.08±2.3).The median level of anti-HBs in group(I), group(II) and group(III) were 6,32 and 34 respectively.There were statistically significant differences (P<0.05) in anti-HBs level in group I in comparison to group II and group III as displayed in **table (1)**. While there was not significant differences in anti-HBs level betwen group(II) and group(III). We found that 33.3%, 58.3% and 60% in group(I), group(II) and group(III) respectively retained their protective anti-HBs titre ≥10 mIU/ml . There was significant negative correlation between anti-HBs titre and time since vaccination as shown in **figure (3)**.

The cellular immune response to HBs antigen was assessed by IFN-gamma production using the ELISpot assay. Our findings show that 66.7%, 75% and 85.7% in group (I), group (II) and group(III) respectively retained their cell mediated immunity to HBsAg . We found that 65% of participants who displayed unprotected anti-HBs titre were positive for IFN-gamma producing cells and 90% of participants who displayed protective anti-HBs titre were also positive for IFN-gamma producing cells. These results are shown in **table (2)**, respectively. The most prevalent HLA-DRB1 alleles in those with anti-HBs ≥10 mIU/ml were HLA-DRB1*15. These alleles were significantly

higher (P<0.05) in those with Anti-HBs ≥10 mIU/ml than those with anti-HBs <10 mIU/ml. While in those with anti-HBs <10 mIU/ml, the most prevalent HLA-DRB1 alleles were HLA-DRB1*13 and HLA-DRB1*03 as shown in **figure (4)**. There were no significant differences (P>0.05) in HLA-DRB1 broad types between those with ELISpot positive & those with ELISpot negative. These results are shown in **figure (5)**.

FIGURES & TABLES

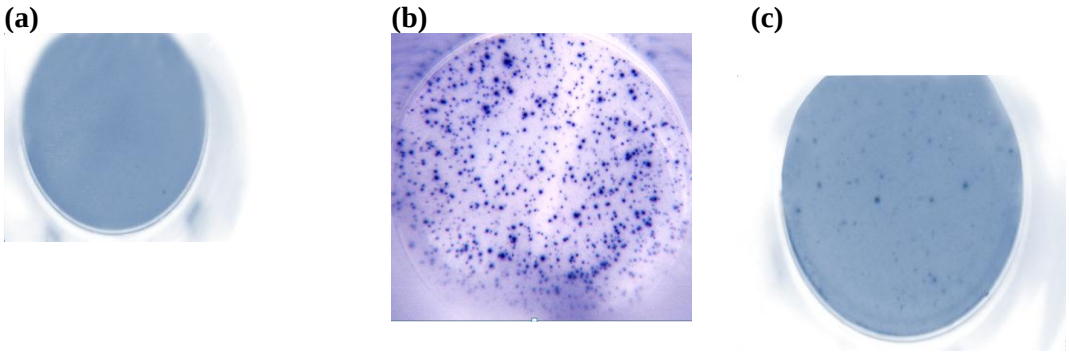

Figure (1): IFN-gamma ELISpot wells
(a) Negative control well
(b) Positive control well
(c) HBs Ag positive well

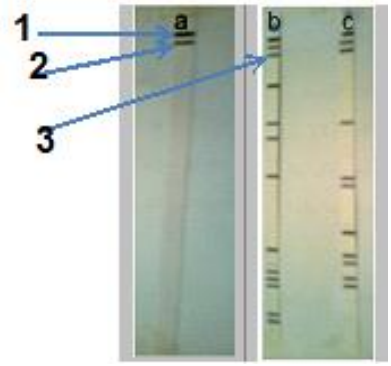

Figure (2): A three strip of HLA DRB1 showing:(١ marker line , **2** conjugate control , **3** HLA DRB control) with **(a)** negative control strip, **(b)** Strip showing DRB1*07, DRB1*13 alleles,**(c)** Strip showing DRB1*03, DRB1*15 alleles.

Table (1): Comparison between the three groups with different schedules of vaccination regarding level of anti-HBs.

Anti-HBs	Group (I)	Group (II)	Group (III)	Kruskal-Wallis test (H-test)	P	Sig
Median	6	32*	34*	-1.991	0.046	S
Min-Max	1-94	3-80	6-94			

Table (2): Comparison of HBs Ag specific IFN-gamma secreting spot- forming cells and the level of anti-HBs.

Criteria	Anti-HBs < 10 mIU/ml	Anti-HBs ≥ 10 mIU/ml
No. of participants	20	10
No. of SFCs positive	13	9
Percent SFCs positive	65%	90%

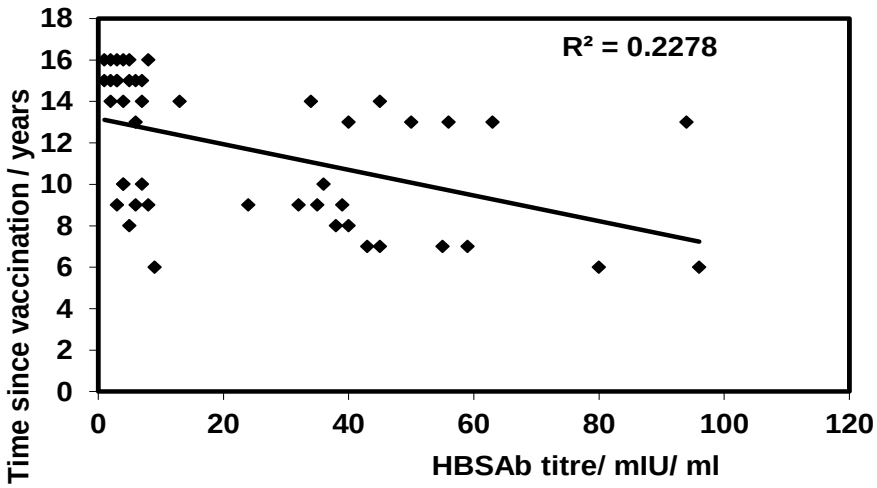

Figure (3): Correlation between anti-HBs titre and time since vaccination.

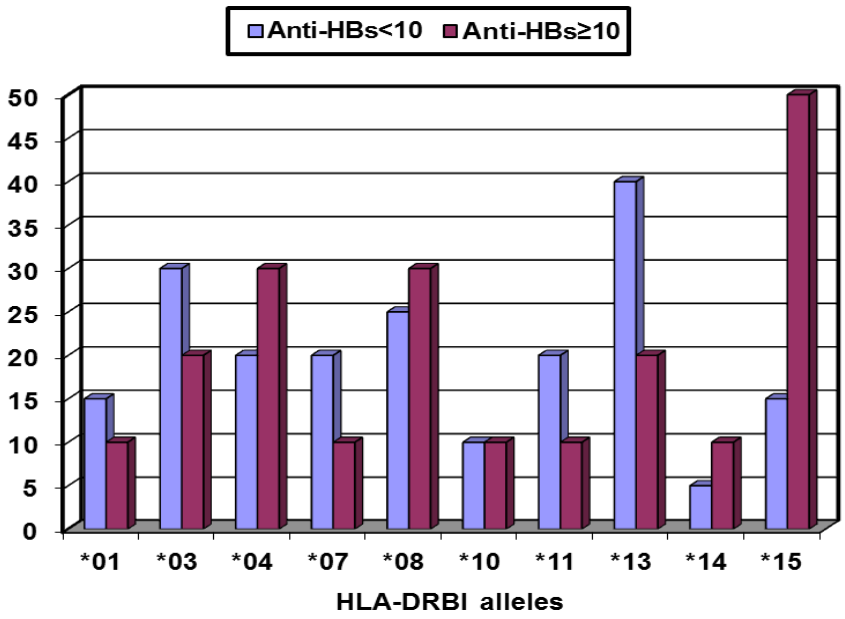

Figure (4): Distribution of DRB1 alleles among population with anti-HBs < 10 mIU/ml & ≥ 10 mIU/ml.

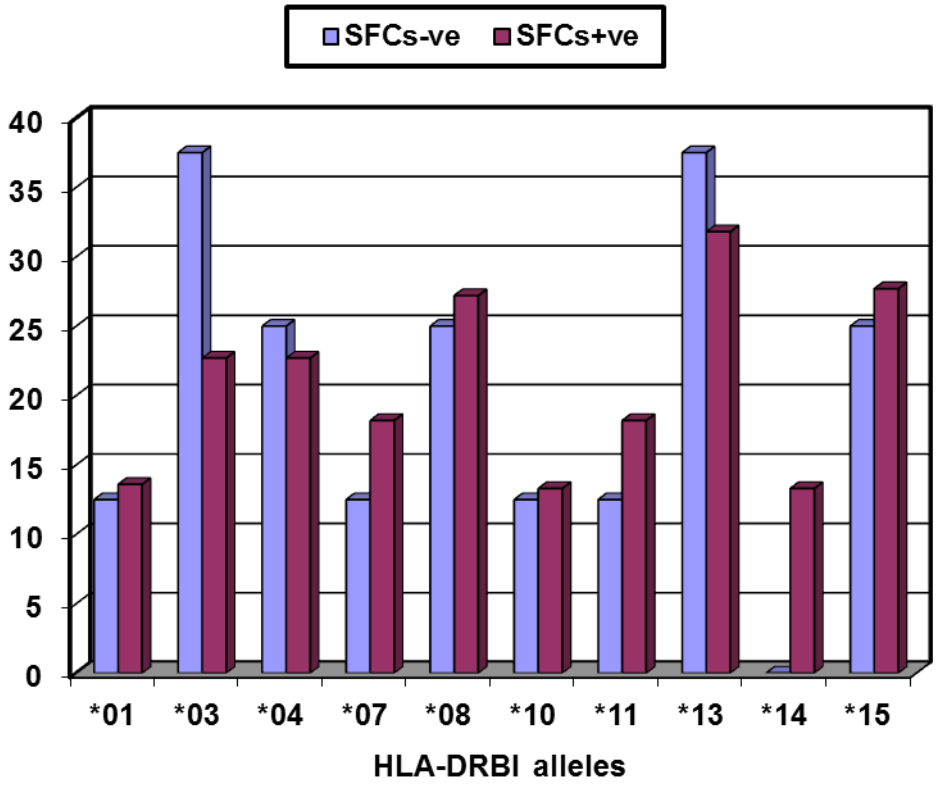

Figure (5): Distribution of DRB1 alleles among population with ELISpot positive and negative.

DISCUSSION

Hepatitis B infection is major public health problem in Egypt. Egypt is considered as a region of intermediate prevalence for HBV infection with reported figure of 4.5% **(13)**. Hepatitis B is preventable with a safe & effective vaccine that has been available since 1982 **(5)**. The results of the present study in adolescents who received HB vaccination at 2, 4, and 6 months of age showed that 33.3% retained their protective anti-HBs titre ≥10 mIU/ml and 66.7% with anti-HBs titre < 10 mIU/ml with mean time since vaccination 14.56 years. Our findings support Egyptian study done by **Afifi et al. (2009)** for evaluation of long term

immunity to HBV among vaccinated children aged 6-11 years according to Egyptian expanded immunizing protocol who reported that, overall seroprotection at 6-11years after immunization was 39.3% **(5)**.Moreover **El Sherbini et al. (2006)** and **El-Sayed et al. (2009)** found that 54% & 48% retain their anti-HBs protective levels ≥ 10 mIU/ml for 10 years since vaccination respectively **(14,15)**. On the other hand, some studies performed on children aged 5-12 years, detected that proportions of children who had protective anti-HBs titre were 81.6%, 95% and 88% as reported by **(16-18)**. There are several possible explanations for the low level of protective anti-HBs observed in our studied adolescents. First, the studied adolescents who received HB vaccination at 2 months of birth as part of routine immunization were not tested for anti-HBs response 1-2 months after last dose. This explanation is supported by **El-Ghandour et al. (1998)** in Ismailia Governorate who noticed that 6.6% of vaccinated infants were poor or non-responders and **Goh et al. (1992)** who found that the persistence of anti-HBs is related to the peak level achieved after completion of the vaccine schedule **(19,20)**. Second, starting the first dose of HB vaccine at birth with neonatal Bacillus Calmette - Guērin (BCG) immunization induced markedly higher primary and memory antibody responses to HB vaccine as concluded by **Ota et al. (2004)**, who reported that BCG administration at time of HB vaccine priming enhances primary infant B and T cell responses to HB vaccine **(21).**

On the other side 58.3% and 60% of our studied health care workers retained their protective anti-HBs level for 6 to 10 years after HB vaccination. In Iranian study, higher rate was observed by **Moghaddam et al. (2004)** who studied anti-HBs level in healthy medical students and hospital residents who received three standard doses of recombinant HBV vaccine **(9)**. Their study revealed that, almost all 98% of vaccinees had protective level of antibody (>10 mIU/mL) at the end of one year. It declined to 94% in 2 – 5 years interval and to 84% in 5 – 10 years after inoculation. In contrast to these results, lower percentage was reported by **Chadha and Arankalle (2000),** who found that only 19% had protective anti-HBs 10 years after vaccination **(22)**. The divergence of the results of these studies compared to our study may be due to genetic variations among populations affecting the produced titer of antibody at the time of vaccination. The persistence of the antibody titer after long time directly depends on the produced titer of antibody at the time of vaccination **(23).** Difference in type of HB vaccine, improper injection of HB vaccine and missing dose of HB vaccine may be also responsible for this variation in results. In comparing accelerated and standard schedules of vaccination, 58.3% and 60% of our studied health care workers retained their protective anti-HBs level with respectively.There was no significant difference between two schedules of vaccination in maintaining protective anti-HBs titre 6 -10 years after vaccination. This was in agreement with **Scheiermann et al., (1990),** who noticed that 98% of those who follow standard vaccination schedule & 94% of those who follow accelerated vaccination schedule retained anti-HBs ≥10mIU/ml 4 years after last dose **(24)**.

Some studies assessed immune memory in those with low or undetectable levels of circulating anti-HBs by looking at the anamnestic response to revaccination. Collectively, both the ELISpot and ICS assays are capable of reproducibly measuring both quantitative and qualitative parameters of antigen-specific T cell responses. Additionally, these assays can be performed with cryopreserved PBMC samples, thus permitting batch testing as well as retrospective immunogenicity studies **(25).** The limitations with ICS assay may place with the background responses detected and requirement of expensive instrumentation and trained personnel for sample analysis **(26).** The ELISpot assay which is the ‘‘ex vivo’’ assay **(27)** with the lowest limit of detection of IFN-gamma producing T-cells, allowing the reliable detection of as few as 10 specific T-cells per 1 million PBMC **(28,29)** and direct visualization of each IFN-gamma-secreting cell **(30)**, is becoming the assay of choice for evaluation cell mediated immune responses induced by vaccines or natural infections **(31)**. In this study we found that approximately 73% of the participants displayed HBsAg specific IFN-gamma producing cells. Comparison between this result and antibody response demonstrated that 90% of participants who displayed protective anti-HBs titre were also positive for HBsAg specific IFN-gamma producing cells & 65% of participants who displayed unprotected anti-HBs titre were positive for HBsAg specific IFN-gamma producing cells. This means that most of vaccinees with unprotected anti-HBs titre still have their primed immune cells to HBsAg.Our findings strengthen **Bauer and Jilg (2002)**, who showed that HBsAg-specific memory T cells could be detected by ELISpot assay and were enhanced by revaccination in all subjects who had responded to

HB vaccination 4–8 years earlier and subsequently lost their anti-HBs **(23)**.The results of our study were comparable to **Chinchai et al.(2009)** in Thailand who found that 50% of the participants displayed HBsAg specific IFN-gamma producing cells **(10)**. Furthermore 48.6% of participants who displayed protective anti-HBs titre were also positive for HBsAg specific IFN-gamma producing cells & 54.5% of participants who displayed unprotected anti-HBs titre were positive for HBs Ag specific IFN producing cells. Their study populations were 18 to 20 years old participants who had received the complete course of recombinant HB vaccine. These differences in the percentage of participants with protective Anti-HBs titre and positive for HBsAg specific IFN-gamma producing cells may be explained by difference in schedules of vaccination and, time since vaccination during testing. Moreover, time of incubation with HBsAg in this study was 16 hours which wasn't enough for HBsAg processing and presentation.Furthermore cellular immune response is rather complicated, as it is influenced by various factors such as the presentation of antigen by antigen presenting cells, HLA of the individual and regulatory T cells.

The present study showed that, the most prevalent HLA-DRB1 alleles in those with anti-HBs ≥10 mIU/ml were HLA-DRB1*15. These alleles were significantly higher (P<0.05) in those with anti-HBs ≥10 mIU/ml than those with anti-HBs <10 mIU/ml. Also HLA-DRB1*15 association with HB vaccine responder was demonstrated in these studies **(33-36)**.However, these findings were not in agreement with those of **Lin et al.(2008),** who noticed that long-term immunological memory to HB vaccination were closely linked with the presence DRB1*08 **(8).** the differences may be related to the great variability of the frequency of HLA alleles in different populations and HLA genotyping methods.

Moreover we demonstrated that in those with anti-HBs <10 mIU/ml, the most prevalent HLA-DRB1 alleles were HLA-DRB1*13 and HLA-DRB1*03 but it was not statistically significant. This may due to the small sample size. Similar results were obtained by **Peces et al.(1997), Caillat-Zucman et al.(1998) & Amirzargar et al.(2008),** who demonstrated that the lack of antibody response to HBsAg is significantly associated DRB1*03 **(33,34,36)**.On the contrary to this study,several authors found that the lack of antibody response to HBsAg is significantly associated DRB1*07 **(35,37-38)**. Also **Höhler et al. (1998)** demonstrated that DRB1*13 was associated with increased antibody response to HBsAg **(35)**. The controversy among the above mentioned results could be attributed to different genetic background among different ethnic groups. As allelic differences between MHC class II molecules are clustered around the antigen binding groove the most obvious explanation for the observed HLA–class II associations would be variable efficiency in the antigen presentation between DRB1* antigens **(39).**

The studies on association between HLA-DRB1 alleles and cell mediated immunity to HBV are very limited and there are only studies on cell mediated immunity to rubella. The immune response to rubella and other viruses involves processing involves processing of viral peptides and their presentation to both CD8+ cytotoxic T lymphocytes (CTL) restricted by HLA class I alleles and CD4+ T cells restricted by HLA class II alleles **(40).**

We noticed non-significant differences (P>0.05) in HLA-DRB1 alleles types between those with ELISpot negative & those with ELISpot positive. Similar findings were obtained by **Ovsyannikova et al., (2009)** who studied the association between HLA alleles and rubella-specific IFN-gamma (Th1) and interleukin-10 (IL-10) (Th2) cytokine responses among 106 healthy children (ages 14 to 17 years) previously immunized with two doses of rubella vaccine, they found non statistically significant association between IFN-gamma levels and HLA-DRB1 (*P* values of 0.42) **(40)**. This results may be due to small sample size and also may be due to other factors that affect IFN-gamma response as IFN-gamma gene polymorphism.

In conclusion, T cell memory to HBsAg can be demonstrated by ELISpot IFN-gamma many years after HB vaccination, even in the majority of persons with serum anti-HBs <10 IU/L. One third of the studied adolescent who was vaccinated in infancy had unprotected anti-HBs titre & non detectable HBsAg-specific memory T cells and this puts them at risk of HB infection. Standard & accelerated schedules of vaccination of adults are equal in maintaining protective anti-HBs titre & HBsAg-specific memory T cells for 6 -10 years after vaccination. Finally, we suggested that HLA-DRB1*15 influence long term humoral memory to HB vaccine. Additional long term studies should be conducted to explore vaccine memory & the need of booster dose in different schedules of vaccination. Further prospective studies should be performed in infants using vaccination schedules that start at birth to accurately clarify the effet of first dose timing on immune memory.

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استمرارية الأستجابة المناعيه النوعيه بعد التطعيم ضد الألتهاب الكبدي (ب) في مصر: العلاقه مع أليلات إتش.إل.إيه- دي.أر.بي.١ والجدول الزمني للتطعيم

ملخص الدراسه:

يعد الألتهاب الكبدي (ب) من المشاكل الصحيه الرئيسيه في العالم. كما يعد التطعيم بلقاح الألتهاب الكبدي (ب) من أكثر الوسائل الفعاله في منع انتقال فيروس الألتهاب الكبدي (ب).

الهدف من الدراسه:

تقييم استمرارية كلا من المناعة الخلطية و الخلوية ضد فيروس الالتهاب الكبدي (ب) بعد التطعيم ضد الألتهاب الكبدي(ب) ، و دراسة بعض العوامل المؤثره علي استمرار الاستجابه المناعيه بعد التطعيم ضد الألتهاب الكبدي (ب). والتي تشمل علي: مستضدات الكرات البيضاء البشريه دي.أر.بي.١ و الجدول الزمني للتطعيم

طرق الدراسه:

اشتملت الدراسه ثلاث مجموعات بجداول تطعيم مختلفه للقاح الألتهاب الكبدي (ب) بفتره تتراوح من ٦-١٦ سنه منذ تلقي التطعيم. وتم تقييم ذاكرتهم المناعيه بقياس كلا من مستوي الأجسام المضادة للمستضد السطحي للألتهاب الكبدي (ب) لتقييم المناعة الخلطية ، و فحص "بقعة المناعه المرتبطة بالإنزيم لتقييم المناعة الخلوية لأكتشاف الخلايا الخاصه بفيروس الألتهاب الكبدي (ب) المنتجه للأنترفيرون جاما.

نتائج الدراسه:

٦٥% من المشاركين الذين أظهروا مستويات غير واقيه من الأجسام المضادة لمستضد السطحي للألتهاب الكبدي (ب) كانوا ايجابيين للخلايا الخاصه بفيروس الألتهاب الكبدي (ب) المنتجه للأنترفيرون جاما ، و ٩٠% من المشاركين الذين أظهروا مستويات واقيه من الأجسام المضادة لمستضد السطحي للألتهاب الكبدي (ب) كانوا ايجابيين للخلايا الخاصه بفيروس الألتهاب الكبدي (ب) المنتجه للأنترفيرون جاما. و كان الأليل ١٥ لمستضدات الكرات البيضاء البشريه دي.أر.بي.١هو الأكثر انتشارا بين اليلات مستضدات الكرات البيضاء البشريه دي.أر.بي.١في المشاركين الذين كان مستوي الأجسام المضادة لمستضد السطحي للألتهاب الكبدي (ب) بتركيز مساوي أو أكبر من ١٠ وحدة ملي دوليه.

المستخلص من الدراسه:

لاتزال الخلايا المناعيه الذاكره لمستضد السطحي للألتهاب الكبدي (ب) موجوده لسنوات حتي بعد اختفاء المستويات الواقيه من الأجسام المضادة لمستضد السطحي للألتهاب الكبدي (ب). كما توجد علاقه بين الأليل ١٥ لمستضدات الكرات البيضاء البشريه دي.أر.بي.١، و المناعة الخلطية طويلة الأجل للقاح الألتهاب الكبدي (ب).