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Breakthrough infection of Hepatitis B Virus after vaccination

اختراق عدوى فيروس التهاب الكبد بي بعد التطعيم

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ABSTRACT

Background: Breakthrough infection are due to mutations in a determinant region of the HBsAg. These mutations allow the virus to break neutralizing antibodies from the administration of hepatitis B immunoglobulin and hepatitis B virus(HBV) vaccine. Despite of the progress made in vaccine and antiviral therapy development, HBV infection remains a major health care problem. The implementation of vaccination programs has led to an overall decrease in the prevalence of this disease worldwide but this may also have led to emergence of viral mutations that can evasion the protection of hepatitis B surface antibody. **Aim of the study**: To detect the frequency of HBsAg vaccine breakthrough among the patients with previous HBV infection. **Patients and Method**: A hospital control-based study included 69 persons. Twenty-seven with previous HBV infection patients ,23chronic hepatitis C patients and 19 apparently healthy persons as control. Patients and controls provided vaccination. None of the participant in either group had an active HB infection at time of enrollment. HBsAg, HBc Ab, HBeAg and HBs Ab were enrollment performed by micro particle immune assay. Polymerase real reaction (PCR) used to detected and quantify HBV DNA and identify HBsAg mutants. **Result**: HBV DNA was detected in 11(40.7%) in HBV patients with previous infection and after full dose of vaccination. HBsAg mutant s were detected in 9(33.3%) of them. In HCV patients with negative surface antigenemia HBV DNA was detected in 4(17.4%)

and HBsAg mutants were detected in 2(8.7%) of them. The frequency and levels of HBs Ab in HBV patients with previous infection and after full dose of vaccination were significantly decreased when compared to those with HCV infection and healthy control group after dose of vaccination. **Conclusion**: The presence of HBsAg variation in HBV after vaccination. HBc Ab and/or HBV DNA testing can help identify already infected patients and prevent the potential vaccine -induced immune pressure.

Keywords: Hepatitis B virus, vaccine breakthrough.

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ملخص البحث

تحدث العدوى الاختراقية نتيجة طفرات في منطقة محددة من المستضد السطحي لالتهاب الكبد الوبائي ب. تسمح هذه الطفرات للفيروس بكسر الأجسام المضادة المُحيِّدة الناتجة عن إعطاء الغلوبولين المناعي لالتهاب الكبد الوبائي ب ولقاح فيروس التهاب الكبد الوبائي ب . على الرغم من التقدم المحرز في تطوير اللقاحات والعلاجات المضادة للفيروسات، لا تزال عدوى التهاب الكبد الوبائي ب تُمثل مشكلة صحية رئيسية. وقد أدى تطبيق برامج التطعيم إلى انخفاض عام في انتشار هذا المرض عالمياً، ولكن ربما أدى ذلك أيضاً إلى ظهور طفرات فيروسية قد تفلت من حماية الأجسام المضادة السطحية لالتهاب الكبد الوبائي ب.

هدف الدراسة: الكشف عن معدل حدوث اختراق لقاح مستضد فيروس الكبد ب بين المرضى الذين يعانون من عدوى فيروس التهاب الكبد ب السابقة.

المرضى والطريقة: شملت دراسةٌ قائمةٌ على المقارنة في المستشفى 69 شخصاً. خمسة وعشرون منهم مصابون سابقاً بعدوى التهاب الكبد الفيروسي ب، و27 مصاباً بالتهاب الكبد الفيروسي ج المزمن، و19 شخصاً سليماً ظاهرياً كمجموعة ضابطة. تلقى المرضى والمجموعة الضابطة التطعيم. لم يُصَب أيٌّ من المشاركين في أيٍّ من المجموعتين بعدوى التهاب الكبد الفيروسي ب نشطة وقت التسجيل. أُجريت اختبارات علامات التهاب الكبد باستخدام اختبار مناعة الجسيمات الدقيقة. استُخدم تفاعل تفاعل متعدد السلاسل للكشف عن الحمض النووي لفيروس التهاب الكبد الفيروسي ب وتحديد كميته، وتحديد الطفرات.

النتيجة: تم الكشف عن الحمض النووي لفيروس التهاب الكبد ب لدى 11 مريضاً (40.7%) مصابين بعدوى سابقة وبعد تلقي الجرعة الكاملة من التطعيم. كما تم الكشف عن طفرات مستضدات فيروس الكبد ب لدى 9 مرضى (33.3%) مصابين. أما لدى مرضى التهاب الكبد ج ذوي المستضد السطحي السالب، فقد تم الكشف عن الحمض النووي لفيروس التهاب الكبد ب لدى 4 مرضى (17.4%) مصابين، بينما تم الكشف عن طفرات مستضدات فيروس الكبد ب لدى مريضين (8.7%) مصابين. وقد انخفض تواتر ومستويات الأجسام المضادة التهاب الكبد ب لدى مرضى التهاب الكبد ب المصابين بعدوى سابقة وبعد تلقي الجرعة الكاملة من التطعيم بشكل ملحوظ، مقارنةً بالمصابين بعدوى التهاب الكبد ج ومجموعة المراقبة الصحية بعد تلقي الجرعة.

الخلاصة: وجود تباين في مستضدات فيروس التهاب الكبد ب بعد التطعيم. يمكن أن يساعد فحص الأجسام المضادة لفيروس التهاب الكبد ب و/أو الحمض النووي لفيروس التهاب الكبد ب في تحديد المرضى المصابين بالفعل، ومنع الضغط المناعي المحتمل الناجم عن اللقاح.

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Introduction

Over the years, the pace of developing vaccines for HBV has never stopped. After more than 30 years of application, the HBV vaccine has reduced 80% of hepatocellular carcinoma. (1) However, vaccine escape variants occur under selective pressure induced by widespread vaccination and antiviral therapy, which results in fulminant infection and horizontal transmission. Several mechanisms have been studied to explain HBV vaccine change in determinant region of the HBsAg, including escape mutations in the major hydrophilic region, which leads to a decrease in the binding ability to neutralize antibodies. (2)

Hepatitis B (HBV) infection is a major public health problem and cause of chronic liver disease. In accordance with data provided by the World Health Organization (WHO), hepatitis B virus (HBV) chronically infects almost 240 million people worldwide despite the availability of the hepatitis B vaccine since 1982. (3)

Hepatitis B virus is very heterogeneous at the DNA, eight HBV genotypes from A to H have been classified on basis of genetic variability of 5 to 10% on over all genome. There are equally dominant genetic variant with geographic distribution, genotype D is prevalent in Mediterranean area. (4) The HBV genetic variability also concern the natural emergence of viral strain due to mutations that may occur in all portions of viral genome and may have relevant biological and clinical impact. (5)

During an active HBV infection, which can generate as many as 10^{11} viral particles per day, this high error rate can produce as many as 10^{7} base errors per day. (6) Due in part to the overlapping nature of the HBV protein reading frames, some of the mutants generated cannot survive or replicate as efficiently as the wild type. Nevertheless, an increase in HBsAg mutants has been associated with the selective pressure exerted by the available hepatitis B treatments (e.g., active immunization and passive immune prophylaxis. (7) HBV can evolve by the Major hydrophilic region (MHR) is the major region involved in this immune selection, and many strains with mutations in this region survive. These MHR mutations can also affect detection by commercial immunoassays because the MHR region which includes the “a” determinant contains the epitopes targeted by the antibodies used in many HBV immunodiagnostic tests. The most commonly reported mutation in HBsAg occurs at amino acid residue 145, where the wild-type glycine is replaced by an arginine (G145R). The presence of this G145R mutation has allowed some patient specimens to escape detection by certain commercial immunoassays. Occasionally, mutants carrying other amino acid changes within the “a” determinant have also been reported to evade detection by some commercial HBsAg immunoassays. (8)

Several studies shown that, HBV infection may occur in HBsAg-negative patients with or without serologic markers of previous infection (antibodies to HBsAg [anti-HBs] or to the hepatitis B core antigen (anti-HBc). The reasons for the lack of circulating HBsAg in such patients are unknown, but also have suggested that, the lack of HBsAg may be due to rearrangements in the HBV genome that

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interfere with gene expression or lead to the production of an antigenically modified S protein. (9)

Hepatitis B virus vaccination has been shown to be effective in preventing HBV infection. The protection is based on the induction of anti-HBs antibodies against a major cluster of antigenic epitopes of HBs Ag, defined as "a" determinant region of small HBsAg. (10)

Escape mutants of hepatitis B virus carry mutations in the major antigenic region of the hepatitis B surface antigen (HBsAg). (11) They are able to grow in the presence of antibodies against HBsAg (anti-HBs) and may escape detection by immune assays for HBsAg. Antibodies against HBV core antigen (anti-HBc) are considered to be a universal marker for active or resolved HBV infections, but they may appear late. (12) Highly sensitive detection of HBV DNA is the most universal reliable marker of infectivity. (13) Occult hepatitis B infection (OBI) refers to the presence of replication-competent hepatitis B virus (HBV) DNA in the liver, even when HBV DNA is not detectable in the blood using standard assays, (14) can be categorized as seropositive or seronegative. Seropositive OBI refers to individuals with detectable antibodies against the HBV core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs) in their serum, accounting for approximately 80 % of OBI cases. In contrast, seronegative OBI individuals lack all HBV serum markers (including anti-HBc and anti-HBs) but still harbor intrahepatic HBV DNA (and occasionally circulating HBV DNA). The duration of HBsAg positivity before its disappearance can vary significantly in seropositive OBI cases. (15)

Objective:

our aim was to determine the frequency of antigenic evasion (HBsAg) after of HBV vaccination.

Subjects and Methods

This study is hospital control-based study. It was performed on 69 adult persons, fifty-five males and fourteen females, their age ranged from 19 to 62 years with mean value ± SD (39.01 ± 7.6) years. The studied groups included (27 patients with previous HBV infection, 23 with HCV patient and 19 apparently healthy volunteers as controls). The patients and control groups were recruited from outpatient clinic at Al- Sadaqa Teaching Hospital -Aden-Yemen conducted from june 2023 to December 2024 after full dose of vaccination. All patients and control groups tested negative for the HBsAg when they were initially screened before being included in the study.

The immunization regimen consists of three doses of vaccine given according to the following schedule: First dose: 24 hours of elected date, second dose: 1 month after the dose 1, third dose: 6 months after the dose 1. The prophylactic doses were 1.0 ml (20 µg of Purified HBsAg) (rDNA) as active ingredient and 0.5 mg of Aluminum hydroxide gel (as Al) as adjuvant of EUVAX B and the therapeutic doses was 2 ml of EUVAX B (Store at 2-8°C.)

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A venous blood sample of 8 ml was collected from a fasting patient, divided into different tubes. 2ml were placed in an EDTA tube for complete blood. count in tube containing EDTA (ethylene diamine tetra-acetic acid) for complete blood count.1.8 ml in tube containing sodium citrate for prothrombin time testing including concentration and normalized ratio. The remaining 4 ml were collected in plain tubes. The samples were centrifuged within 30 minutes and the aliquoted and frozen at-70oC for further analysis.

The laboratory investigation focused on screening and diagnosing Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections. For HBV, serological markers like HBsAg, anti-HBs, anti-HBc (IgM and IgG), HBeAg, and anti-HBe were tested using commercially available immunoassays. Additionally, HBV DNA was detected using real-time PCR kits. HCV-Ab was also tested using similar enzyme immunoassays.

Data analysis:

Data was collected and analysis was done in line with objectives. Quantitative data were presented by using mean ± and standard deviation (SD). Qualitative data were presented by using proportion and percentages. Chi square was used as test of significance. P value less than 0.05 was taken as statistically significant.

Results In this study, significant differences were observed in HBsAb levels, HBV DNA levels, and the frequency of HBsAg mutants among control, HBV, and HCV patient groups. Specifically, the control group showed higher HBsAb levels and frequencies compared to both HBV and HCV patients, while HBV patients had significantly higher HBV DNA levels and HBsAg mutant frequencies compared to the other two groups. (Table 1)

In patients with HBV infection, those negative for hepatitis B surface antibody had a higher frequency of HBeAg and significantly higher HBV DNA levels compared to those with positive HBsAb. Furthermore, HBsAg mutants were found in 36.4% of HBsAb-negative patients, but not in those with positive HBsAb. (Table 2) In HBV patients, the presence of HBsAg mutants is associated with a significantly higher frequency of HBeAg, as well as increased levels and frequency of HBV DNA, compared to patients without these mutations. Specifically, the frequency of HBeAg was significantly higher in patients with HBsAg mutants ($p<0.05$). The frequency and level of HBV DNA were also significantly elevated in these patients, with p-values of <0.001 and <0.0001, respectively. Table (3)

In patients with HCV and positive HBsAb, HBeAg was detected in 36.4% (4 out of 11), while in those with negative HBsAb, it was detected in 8.3% (1 out of 12). While the difference in HBeAg detection rates was not statistically significant, HBV DNA levels were significantly lower in the HBsAb-negative group compared to the HBsAb-positive group. No significant difference in HBsAg mutant prevalence was observed between the two groups. (Table 4)

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In HCV patients, those with positive HBsAg mutants exhibited significantly higher frequencies and levels of HBeAg and HBV DNA compared to those without the mutants, with p-values of <0.05, <0.001respectively. However, no statistically significant difference was observed in the frequency or levels of HBsAb between the two groups. (Table 5)

Table (1) Prevalence of HBV markers and HBsAg evasion among studied patients

	Hepatitis B positive Group (n=27)	**HCV Group (n=23)**	**Control Group (n=19)**
HBsAg +	0	0	0
HBc IgM Ab+	0	0	0
HBc total Ab+	27(100%)	19(82.6%)	0
HBeAg +	6(22.2%)	3(13%)	0
HBeAb+	13(48.1. %)	7(30.4%)	0
HBs Ab+ Range Mean±SD IU/ml Median	5(18.5%)** 0-505 24.3±97.7 0.56**	11(47.8%)* 0-1000 267.9±419.5 10.7#**	14(73.7%) 0.9-1000 477.6±453.5 375
HBV DNA% Range Mean±SD IU/ml Median	11(40.7%) 0.142-2601x10^3 266.1x10^3± 808.3x$10^{3\#\#*}$ 0	4(17.4%) 0.66-0.21x10^3 23.6± 54.3* 0	0
HBsAg mutants	9(33.3%)#*	3(13%)	0

HBV and HCV group vs. control group, HBV group vs. HCV group and control group, * p <0.05, **p <0.01, *** p<0.001

Table (2) Prevalence of HBeAg, HBV DNA and HBsAg mutants in HBV patients group according to the presence of HbsAb

	HBV patients with HBs Ab positive (5)	**HBV patients with HBs Ab Negative (22)**
HBeAg +	0	5(22.7%)*
HBV DNA% Mean±SE IU/ml	1(20%) = 0.143 x10^3	9(40.9%) 316.8±762.3x10^{3***}
HBsAg mutants	0	8(36.4%)*

* HBV patients with HBsAb vs. HBV patients without HBsAb, * p <0.05, **p <0.01, *** p<0.001

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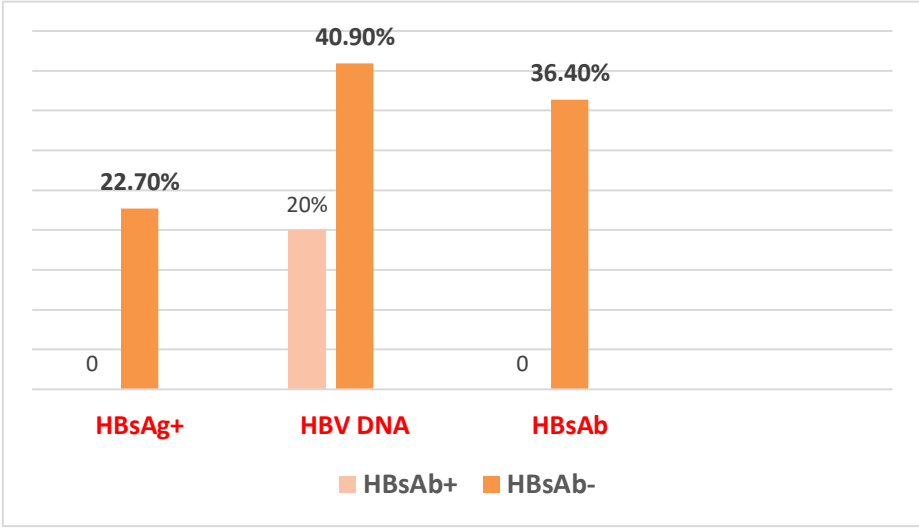

Table (3) Prevalence of HBeAg , HBsAb and HBV DNA in HBV patient group according to the presence of HBs Ag mutant

	HBV patients with HBsAg mutant (9)	**HBV patients without HBsAg mutant (18)**
HBeAg +	5(55.6%)*	0
HBs Ab+ Range Mean±SD IU/ml Median	0	4 (22.2%) 0.0-16.4 2.6±0.5.1 0.45
HBV DNA% Mean±SE IU/ml Median	9(100%)** 873±1098 x10^3 281.8 x10^3	2(11.1%)*** 0.96±0.281x10^3*** 0

* HBV patients with HBsAg mutant vs. HBV patients without HBsAg mutant* p <0.05, **p <0.01& *** p<0.001

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Table (4) Prevalence of HBe Ag, HBV DNA and HBsAg mutant in HCV patients group according to the presence of HBsAb

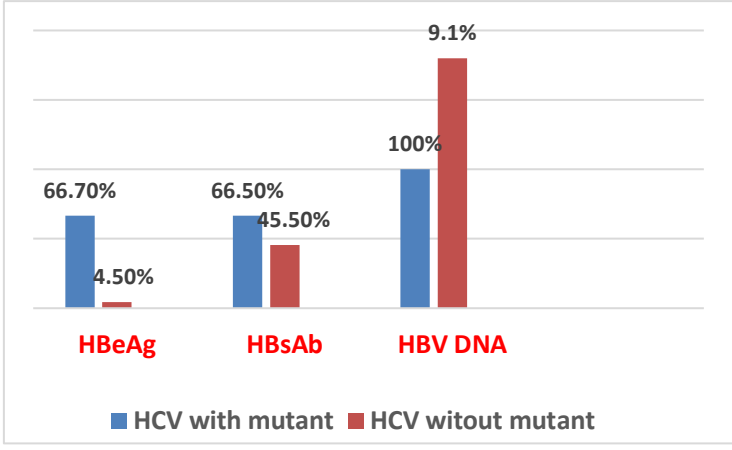
	HCV patients with HBs Ab positive (11)	HCV patients with HBs Ab Negative (12)
HBeAg +	4(36.4%)	1(8.3%)
HBV DNA% Mean±SE IU/ml	3(27.3 %) 0.152 x10^3	2(16.7%) 0.35±79.3x10^3*
HBsAg mutants	1(9.1%)	3(25%)

* HCV patients with HBsAb vs. HCV patients without HBsAb, * p <0.05, **p <0.01, *** p<0.001

Table (5) Prevalence of HBeAg , HBsAb and HBV DNA in HCV patient group according to the presence of HBs mutant

	HCV patients with (4HBsAg mutants)	HCV patients without (19 HBsAg mutants)
HBeAg +	2(66.7%)*	1(4.5%)
HBs Ab+ Range Mean±SD IU/ml Median	2 (66.6%) 0-1000 377.7±373.3 12.6	10(45.5%) 0.0-1000 281.3±429.9 6.9
HBV DNA% Mean±SE IU/ml Median	3(100%)** 0.88±0.22 x10^3 0.100 x10^3	2(9.1%) 0.15±0.51x10^3* 0

* HCV patients with HBsAg mutant vs. HCV patients without HBsAg mutant, * p <0.05, **p <0.01& *** p<0.001



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Discussion:

Various factors impact how adults respond to hepatitis B (HBV) vaccines, including unchangeable individual characteristics. Personalized vaccination strategies, taking these factors into account, can improve vaccine effectiveness, especially for high-risk individuals. Successful HBV vaccination programs have significantly lowered carrier rates and HBV-related complications, like liver cancer (HCC), in regions where adopted. (16) Our study reported that 73.3% of the healthy person had HBsAb after vaccination with mean value of 477.6±453.5 IU/ml. Over half (55.8%) in similar study done by Albash et al., strongly believe that vaccinations keep individuals healthy with the present of HBsAb in average production 479.7 ±21.5 IU/ml. (17)

In another study indicated that among fully vaccinated individuals, HBsAb was detected in about 69% of males and 81% of females, but these individuals exhibited a poor antibody level response. Several studies suggest these individuals might benefit from a single booster dose of the vaccine and no further retesting is needed. (18)

Our work revealed that, HBsAg mutant was detected in 9(33.3%)of patients with previous HBV infection after full dose of vaccination. This finding is in agreement with Iranian study who reported the occurrence of vaccine-associated HBsAg mutations8(20.8%) which principally have been identified in the `M124T' determinant and attributed these mutants to possibly vaccine pressure. They related that to the sequence variation in antigenic regions is one of the most powerful viral strategies for escaping recognition by both the B and T cell-mediated immune system of the host and facilitates viral persistence. (19) While Hepatitis B vaccination programs have successfully reduced overall chronic Hepatitis B infection, they have also led to an increase in HBV variants with mutations in the surface antigen protein, particularly in regions like Italy, Thailand, and Taiwan. These mutations, especially those affecting the "a" determinant, can alter the antigenicity of the surface protein, potentially making it less susceptible to antibody neutralization. (20) Mutations in the "a" determinant of the Hepatitis B virus (HBV) surface antigen (HBsAg), arising from selection or natural variation, can significantly impact immunity and protection against HBV infection. These changes can lead to altered HBsAg antigenicity, causing issues like false-negative HBsAg test results, evasion of immunoglobulin therapy, and vaccine-induced immunity. reported by Lazarevic, Kay&Zoulim verified that Antibodies found in vaccinated people and those used in immunoassays for HBsAg, are directed against this region; in particular, to a cluster of B-cell epitopes called the ‘‘a’’ determinant, which comprises two loops of amino acids 124-147.(21) As a consequence of this arrangement, the polymerase gene, which encodes the reverse transcriptase enzyme targeted by antiviral drugs, shares a portion of its sequence with the surface gene. This overlapping region includes the "neutralization domain" of the surface antigen, which is the target of antibodies produced by the hepatitis B vaccine. Consequently, mutations in the polymerase gene induced by

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antiviral therapy can potentially affect the surface antigen, impacting the effectiveness of the vaccine. (22)

The Present study showed that HBV DNA was detected in 11 cases (40.7%) of HBV group after full dose of vaccination. HBs antigen mutant was detected in 9(33.3%) of them and occult hepatitis B was detected in other three patients. Our result aligns with the statement by Candotti& Allain that were they confirms a shift in focus within Hepatitis B Virus (HBV) research, acknowledging that alongside traditional HBV infection, there's growing attention to "occult" HBV infection and HBsAg mutants. These alternative forms of HBV infection, characterized by the absence of detectable HBsAg but with the presence of viral DNA, and the emergence of mutants that evade detection by standard assays, are gaining increasing attention. This shift is particularly relevant as these forms of infection can lead to disease despite "protective" anti-HBs antibody titers post-vaccination. (23) Pue et al, also mentioned that in most adolescents and adults with acute HBV infection lose HBsAg and develop both anti-HBc and anti-HBs, HBV DNA remains present in small amounts, both in the circulation and within the liver , viral DNA also remains present after resolution of chronic HBV. The infecting strains of HBV in such cases often have mutants that impair HBV replication, leading to low concentrations of HBV DNA in the circulation. They also reported that in most cases, the infection remains quiescent, but when the individual's immune system becomes suppressed (e.g., with chemotherapy or with use of immunosuppressant drugs), viral replication may increase. When the immune status of the person recovers, recurrence of hepatic injury (often termed "reactivation") may develop and, in some cases, will progress to acute liver failure. (24) Moreover, Hemert et al, Occult HBV infections are defined as the presence of HBV DNA and the absence of HBV surface antigen (HBsAg encoded by the S gene) in plasma or serum of HBV-infected patients. This infection may persist in individuals for years without emerging symptoms of overt HBV infection. (25) Emara also reported that in occult hepatitis B virus (HBV) infection, where HBV DNA is present in the liver or serum of individuals testing negative for HBsAg, a variety of mutations have been observed in HBV sequences from serum samples. These mutations, including point mutations, deletions, and splicing variations, are often found in the S gene and regions regulating its expression, as well as in the core and polymerase genes. However, it remains unclear whether these mutations are a cause or a consequence of occult HBV infection, meaning whether they lead to the occult state or arise as a result of it. (26)

In current study found that 55.6% of vaccinated patients with prior HBV infection tested positive for HBeAg, and this frequency was significantly higher in patients with HBsAg mutants compared to those without such mutations. This suggests that the presence of HBsAg mutations may be associated with increased viral replication and infectivity, as indicated by HBeAg positivity. In agreement with our study, the findings from the Bonino et al, research, specifically the coexistence of wild-type and mutant HBV with HBeAg in HBsAg-negative patients, are noteworthy. The mutant virus exhibited mutations in both preS2 (ATG to ATA) and

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S genes (15-nucleotide repeat insertion in the 'a' determinant), according to a medical information site. This suggests a complex interplay between viral replication and immune response in these patients. (27) Further studies have indicated that HBeAg positivity in the context of occult HBV infection can be due to either superinfection in a previously immune individual or reactivation of a latent infection. The mutated HBV strain, with reduced replication capacity, results in low-level viral replication and a mild form of occult HBV infection. (28)

In our study we demonstrated that, HBsAg mutant was significantly increased in HBV patients group with negative HBsAb (18 patients) when compared to those with positive HBsAb (9 patients), this finding is in agree with existing research of Leong et al, who also identified certain mutations in the Hepatitis B virus's surface antigen (HBsAg) can render them ineffective against natural or vaccine-induced anti-HBs antibodies. These mutations, often found within the "a" determinant of HBsAg, can alter the antigen's conformation, preventing the antibodies from binding and neutralizing the virus, leading to potential breakthrough infections. (29) In other words, even successful vaccination does not protect against this HBV mutation. If HBsAg remains undetectable despite indications of an active HBV infection, a mutation of HBsAg should be considered. Even a successful hepatitis B vaccination may not protect against certain HBV mutations. If HBsAg, the hepatitis B surface antigen, is undetectable despite other signs of active HBV infection (like detectable HBV DNA), it suggests a possible mutation in the HBsAg gene, which could render the vaccine ineffective against that specific viral strain according to the Hepatitis B Foundation. These mutations are often referred to as immune escape mutations. (30)

In the current study HBsAb was detected in HCV patients group 11(47.8%) were found to have detectable HBsAb, with a median value of 10.7 IU/ml. This was significantly lower than in healthy controls, according to a study published by Virus Medical Journal, this finding aligns with previous research by Hassnine et al., which showed a higher rate of non-response to hepatitis B vaccination in HCV patients compared to healthy individuals. Consequently, the study suggests that individuals with HCV and HBsAb titers below 10 IU/ml should receive a booster dose of hepatitis B vaccine to ensure adequate protection. (31) Lower immune response in HCV patients after full dose of vaccination when compared to healthy control and attributed these findings to immune compromise in individuals with chronic HCV infection reported by Park. (32)

In this work, HBV DNA has been detected in the blood of 4(17.4%) HCV patients group after a full vaccination dose. This is in agreement with study by Fernandez-Rodriguez et al. found that a significant portion (23%) of hepatitis C (HCV) positive individuals also had occult hepatitis B virus (HBV) infection, characterized by low HBV viral load (102-104 copies/mL). Furthermore, the study indicated that the presence of occult HBV in these patients was associated with higher HCV RNA levels, increased liver inflammation, and more advanced fibrosis, compared to those without detectable HBV DNA.(33) In addition, Kao et al. reported the prevalence of occult HBV infection in patients with chronic HCV infection, did

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not parallel the severity of liver disease (14.5% in patients with chronic hepatitis, 8% in patients with liver cirrhosis, and 22% in patients with hepatocellular carcinoma). Moreover Fernandez-Rodriguez et al. detecting occult HBV infection by using PCR in plasma, peripheral blood mononuclear cells (PBMCs) and liver compartments in (41.6%) patients with biopsy proven chronic hepatitis C and found no association between occult HBV infection and the degree of liver necro-inflammation and fibrosis. (34)

The current study showed that 3(13%) of HCV patients group after full dose of vaccination had HBsAg mutants. This finding supported by Yang et al., that (HBsAg) mutations can occur in patients infected with (HCV), even when HBsAg is not detected by standard tests. These mutations, particularly the Thr118-Ala118 change, can impact the structural integrity of the "a" determinant within the HBsAg, potentially affecting its antigenicity and leading to false negative results in diagnostic assays. (35) Moreover Lok et al. reported focus more on occult HBV infection (OBI) in vaccinated persons and is a growing apprehension regarding the inability of HBV vaccines to ensure complete immunity. The occurrence of OBI in HCV patients is a condition where HBV DNA is present in the liver despite a negative HBsAg test, indicating active viral replication without detectable surface antigen. A subset of these cases, termed "false" OBI, exhibits higher levels of HBV DNA but still lacks detectable HBsAg, often due to mutations in the HBsAg gene that interfere with antibody binding in standard assays. (36)

Conclusion: In some HBV and HCV patients, HBsAg mutants can emerge after hepatitis B vaccination, leading to negative surface antigenemia. Sensitive HBV DNA testing is vital for detecting HBV infection, particularly in cases with mutated HBsAg or occult HBV. Prophylactic vaccination can reduce immune response in patients with previous HBV or HCV infection, potentially leading to HBsAg mutation development. Therefore, HBcAb and/or HBV DNA testing should be considered before vaccination to minimize the risk of vaccine-induced pressure and subsequent HBsAg mutations.

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العام	2021	2022	2023	2024	2025
معامل أرسيف	0.2692	0.1954	0.3759	0.3068	0.5978
معامل التأثير العربي	1.60	1.73	1.25	1.55	1.59