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Epidemiological study of the dominant genes of hepatitis B virus in Salah Al-Din Governorate, Iraq·

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Abstract

The genotype of patients with viral hepatitis B in Salah al-Din Governorate was determined through the use of Real Time PCR technology. Real Time PCR technology is the most precise method for detecting DNA and determining the genotyping of infected individuals· The study demonstrated a significant discrepancy in the genotyping of patients and that the genotype distribution in the governorate was as follows: The total number of patients in the Governorate was divided as follows: genotype A accounted for 69.0%, genotype B for 38.0%, genotype C for 35.0%, and genotype D for 61.0%.

Mix genotypes were also observed in the patients, indicating that an individual possessed more than one genotype. In 6% of cases, the combination of all four genotypes (A+B+C+D) was identified, while in 10% of cases, the three-genotype combinations A+B+C, A+B+D, and A+C+D were identified. In terms of the frequency of two-genotype combinations, A+B was present in 10%, A+D in 19% (the highest among all combinations), A+C in 3%, B+C in 4%, B+D in 2%, and C+D in 3%.

Introduction

The seroprevalence of HBV in the Mediterranean region ranges from intermediate (≥2%) to high prevalence (≥7%) (Tarky et al., 2013). The treatment response of various viruses is influenced by the nucleotide diversity of the HBV genome. identified eight genotypes (A to H) of the hepatitis B virus, each of which has a unique geographic distribution that is employed to track the virus's evolution and transmission· These genotypes are distinguished by differences in more than 8% of the virus's entire genomic sequence (Guirgis et a.,2010). These genotypes exhibit

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variations due to a variety of mutations, such as primary mutations and deletion mutations, which influence the severity of the disease and the probability of complications. These genotypes do not predict advanced liver disease; however, they support the identification of the most effective treatment and the response to it. The genotypes are differentiated by the geographic distribution variations of the viruses and the nucleotide sequence divergence of more than 4% or 7-5%-8% in the complete viral genome (Kramvis et al., 2008). Genotype A is predominantly found in Southeast Africa, Northwest Africa, North America, and Northern Europe. It has a global distribution (Liu et al., 2021). Genotype A of Hepatitis B virus (HBV) is the most prevalent genotype worldwide and is presently divided into six subgenotypes (A1-A6). The distribution of these subgenotypes is as follows: Enotype B of Hepatitis B virus (HBV) is more prevalent in South Africa and significantly higher in East Africa than in West Africa. Genotype B is associated with a lower tendency for chronicity and a better response to interferon-based treatments· Additionally, it is associated with an increased risk of liver cirrhosis and the development of hepatocellular carcinoma (Paul et al., 2023; Lin and Kao, 2015; Sunbul, 2014). A1 is prevalent in South Africa, A2 in Europe, A3 in Cameroon, and A5 in Haiti (Cai et al., 2016). East Asia and Australia are the regions where Hepatitis B virus (HBV) genotype C is more prevalent (Guirgis et al., 2010).The Hepatitis B virus (HBV) genotype D is the most prevalent in Europe, Mediterranean countries, India, and Russia (Kafeero et al., 2023). Additionally, genotype D is prevalent in a variety of regions, such as Myanmar, where it was identified as the second most prevalent genotype at 32%, following genotype C, which has a prevalence of 66.7% (Kyaw et al., 2020). Genotype D is prevalent in the region, with countries such as the United Arab Emirates, Saudi Arabia, Iran, and Iraq (Janahi et al.,

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2019). Recent studies have concentrated on the distribution of hepatitis B virus (HBV) genotypes in Iraq, and the results indicate that genotype D is prevalent among HBV patients in various regions of the country, particularly in the Kurdistan region. Genotype D is the most common genotype in various areas, particularly in Duhok, where it was found in 99.2% of chronic HBV carriers studied (Abdulla and Goreal, 2016). In Sulaymaniyah, a recent study discovered that all tested patients were infected with genotype D, a finding that is in stark contrast to previous results that reported mixed infections involving multiple genotypes (A, B, C, D) in the region (Abdulqadir et al., 2023). Genotype E is the most prevalent genotype in Africa, and it is estimated to affect approximately 20% of chronic HBV carriers worldwide. Nevertheless, genotype E has not been the subject of as much research as other genotypes (Toyé et al., 2023; Ingasia et al., 2021). Genotype F is the most diverse of the Hepatitis B virus (HBV) genotypes and is subdivided into six subgenotypes, ranging from F1 to F6. It is primarily distributed in Central and South America and is considered the most prevalent genotype in the majority of these countries (Liu et al., 2021).

France, Germany, and the Americas are the locations where genotype G is prevalent· The evolutionary geography of genotype G indicates that it may have descended from a common ancestor with other genotypes; however, its evolutionary history remains poorly comprehended (Kafeero et al·, 2023; Araujo, 2022). Genotype H is a genotype that is relatively uncommon and is predominantly found in Mexico and Central America· It has a low global prevalence in comparison to other Hepatitis B genotypes (Kafeero et al., 2023; Panduro et al., 2023).

Materials and Methods

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Study Population

Patients from Salah al-Din Governorate were the subjects of the present investigation. A survey of individuals with Hepatitis B virus infection was conducted in the districts of Samarra, Tikrit, Al-Alam, Al-Dour, Al-Ishaqi, and Balad as part of the study. A total of 130 patients, both male and female, were gathered, with an age range of 18 to 70 years. This comprised 100 samples from individuals who were experiencing symptoms of the virus and 30 control samples.

Sample collection

Following the sterilization of the venipuncture site with 70% ethanol, a 5 cc plastic cannula was used to obtain a venous blood sample· The blood was then collected in gel containers and let to coagulate at ambient temperature. The materials were then centrifuged at 3000 rpm for 5 minutes. The serum was transferred to fresh, uncomplicated containers with an automated pipette. A fast test was used to quantify HBsAg· The samples were then frozen and kept at -28°C. To enable subsequent ELISA and RT-PCR experiments, the samples were frozen and let to reach room temperature before testing·

Hepatitis B Virus Genotyping

DNA was extracted from blood samples with the Preto Mini gDNA Kit, and its concentration was quantified with a NanoDrop spectrophotometer (Thermo Scientific). RT-PCR reactions were performed using the Perfectstar Green qPCR Super Mix Kit, obtained from TRANS (China). The primers used were those indicated in Table 1. RT-PCR testing was used to determine the genotypes.

Table 1. Primers sequences use in this study and size product for each HBV genotype:

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No.	Genotypes	DNA Sequence	Size (pb)	Position
1.	A	AAA CTA CTG TTG TTA GAC GAC GAC ACC CTG GAT TGT TTG AAT TGG CTC CG	644	2334 - 2360 2955 - 2977
2.	B	CAA AAC TCT TCA AGA TCC CAG AGT CA ACA AGT TGG TGA GTG ACT GGA GAT TT	331	16 - 41 321 - 346
3.	C	CTC CCA TCT CTC CAC CTC TAA GAG ACA GT CAG GGG TCC TAG GAA TCC TGA TGT TG	242	3164 - 3192 165 - 190
4.	D	CAG ACG CCA ACA AGG TAG GAG CT GAG TGT TTC TCA AAG GTC GAG ACA GA	189	2972 - 2994 3135 - 3160

Detection of deoxyribonucleic acid using real time PCR technology

Preparation of Primers:

- Add 250 microliters of nuclease-free water to dilute the material.
- Take 10 microliters of the previous mixture and add 90 microliters of nuclease-free water to achieve a concentration of 10 picomolar.
- Add 0·5 microliters of the forward primer.
- Add 0·5 microliters of the reverse primer.

Real-Time PCR Additions:

- Add 10 microliters of Master Mix.
- Add 3 microliters of nuclease-free water.
- Add 6 microliters of the extracted sample.
- Add 0·5 microliters of the forward primer.

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- Add 0·5 microliters of the reverse primer.

Results and Discusstion

Using Real-Time PCR, we investigated the presence of four genotypes A, B, C, and D in the most prevalent blood serum samples within our study· 100 samples from infected individuals were tested. The results indicated that the primer detection rate for genotype A was 69.0% (69/100), for genotype B it was 38.0% (38/100), for genotype C it was 35·0% (35/100), and for genotype D it was 61.0% (61/100). The present investigation suggests that genotypes A and D are the most prevalent types in comparison to the other genotypes. This indicates a substantial difference at a probability level of 0.05. This is illustrated in Table 2.

Table 2· Positive relative and negative relative for the genotypes·

	A	%	B	%	C	%	D	%
Positive test	69	69·0 %	38	38·0 %	35	35·0 %	61	61·0 %
Negative test	31	31·0 %	62	62·0 %	65	65·0 %	39	39·0 %
Total	100	100·0 %	100	100·0 %	100	100·0 %	100	100·0 %
P-Value	<05·0							

These findings correspond with the research conducted on 87 patients in Iraq by (Shakir, 2007). The research revealed that genotype D was the predominant variant, present in 93.1% (81/87) of the samples, while genotype C of the Hepatitis B virus was detected in 3.45% (3/87) of the

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examined specimens· (Idrees et al.,2011) demonstrated that genotype A was the most prevalent. Genotype A has the greatest occurrence rate of 72.8% in East Africa. (Croagh et al.,2015; Shrestha et al.,2007) found that genotype D constituted the predominant Hepatitis B virus infection in Nepal, including 69.0% of patients, while genotype A represented 22.0% of cases·(Liu et al.,2006) identified genotype A in two patients, representing 0.6% of the total sample. Genotype B was identified in 256 instances, accounting for 78.8% of the total cases. Genotype C was identified in 10 instances, representing 3.1% of the total population. (Bello et al.,2023) identified genotype C as the major Hepatitis B viral genotype throughout Asia, with an overall prevalence of 30.9%. (Velkov et al.,2018) determined that 96% of worldwide chronic Hepatitis B virus infections are associated with specific genotypes, with genotype B having a prevalence of 17.8% and genotype D a prevalence of 15.4%. Genotype C is the prevalent variation, comprising 26% of the population. Genotype D ranks second, constituting 22%, followed by genotype A at 17% and genotype B at 14%. In contrast, the study conducted in Brazil by (Reuter et al.,2022) showed that genotype A was the most prevalent, representing 65.3%, whilst genotype D included 32.7%. (Janahi et al.,2019) conducted a study in Bahrain that analyzed the distribution of genotypes across the general population, indicating that genotype D was the most prevalent at 63.6%, followed by genotype A at 18.2% and genotype B at 9.1%. Another study by (Scotto et al.,2010) identified the distribution of Hepatitis B virus (HBV) genotypes among migrants to Italy from Eastern Europe, Sub-Saharan Africa, East Asia, Central Africa, and the Middle East: Genotype D was found in 18.1% of the population, genotype B in 15.3%, genotype C in 13.2%, and genotype A in 4.9%. Genotype D was identified in 33·.3% of patients, while genotype A was identified in 17.4% of patients in previous research. The research

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revealed that Vietnam had the largest incidence of genotype B for Hepatitis B virus, Lebanon had the greatest prevalence of genotype C, and Jordan had the highest prevalence of genotype D. The predominant genotype of Hepatitis B virus globally is genotype D, with a cumulative prevalence rate of 72·8% (Sant'Anna et al., 2023). Additionally, a distinct analysis shown that genotype D accounted for more than 95% of Hepatitis B infections in Italy (Miyakawa et al., 2003). However, migration may influence the distribution of Hepatitis B virus genotypes, perhaps bringing genotypes beyond genotype D (Scotto et al., 2010). Genotype D is the predominant type among blood donors in Mosul, as noted by (Khalid, 2014). Furthermore, it has been identified as the most prevalent genotype in the Arab region, including Saudi Arabia (Abdo et al., 2006), the United Arab Emirates (Alfaresi et al., 2010), Yemen (Sallam and William, 2004), Lebanon (El Chaar et al., 2012), Jordan (Masaadeh et al., 2008), Egypt (Zekri et al., 2007), Libya (Salem et al., 2012), Morocco (Kitab et al., 2011), and Syria (Antaki et al., 2010).

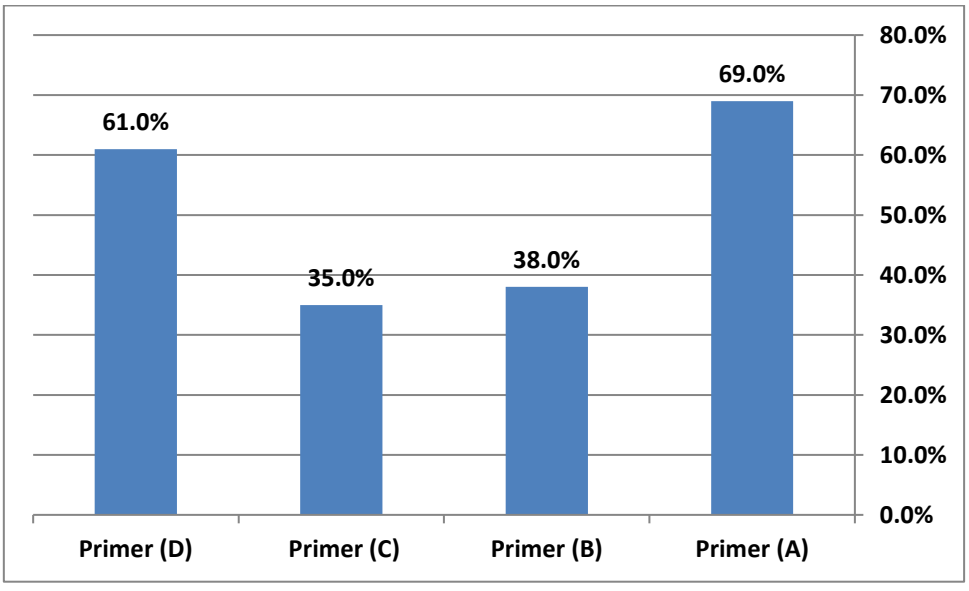

Figure 1:the results of the genotypes of hepatitis B virus.

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The patients exhibited a mixture of genotypes, as indicated by the results. The four genotype combinations (A+B+C+D) were present in 6.0% of cases, while the three-genotype combinations (A+B+C, A+B+D, and A+C+D) were present in 10.0%, 6.0%, and 8.0% of cases, respectively. In terms of the frequency of two-genotype combinations, A+B was present in 10%, A+D in 19% (the highest among all combinations), A+C in 3%, B+C in 4%, B+D in 2%, and C+D in 3%· Table 3

Table 3: Numbers and percentages of mix genetic patterns among the patients.

Mix Genotyps	No·	%
A+B+C+D	6	%0·6
A+B+C	10	%0·10
A+B+D	6	%0·6
A+C+D	8	%0·8
A+B	10	%0·10
A+D	19	%0·19
A+C	3	%0·3
B+C	4	%0·4
B+D	2	%0·2
C+D	3	%0·3

The study conducted in Iraq (Al-Suraifi et al., 2016) for the age group 1-91 years found that the most common genetic pattern was A+B+C+D+E, occurring in 77.7% of cases, followed by A+B+D+G at 16.66%, A+B+C at 2.77%, A+B+E at 1.38%, and A+D+E at 1.38%.

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Another study in Iraq (Rashid and Salih, 2015) documented all four positive HBV-DNA samples with mix genetic infections. Of these, 75% (3 out of 4) carried four different genetic patterns, while 25% (1 out of 4) carried three different genetic patterns. Another study (Liu et al., 2006) found that the mix genotype pattern A and B was present in 18 patients (5.5%), B and C in 30 patients (9.2%), B and D in 1 patient (0.3%), A and C in 1 patient (0.3%), and the combined genotypes A, B, and C in 3 patients (0.9%). In another study (Hamida et al., 2021), specific mix genotype patterns were identified, with C+D occurring at 10.0%, A+C+D at 0.8%, and A+D at 4.9%. Figure 2.

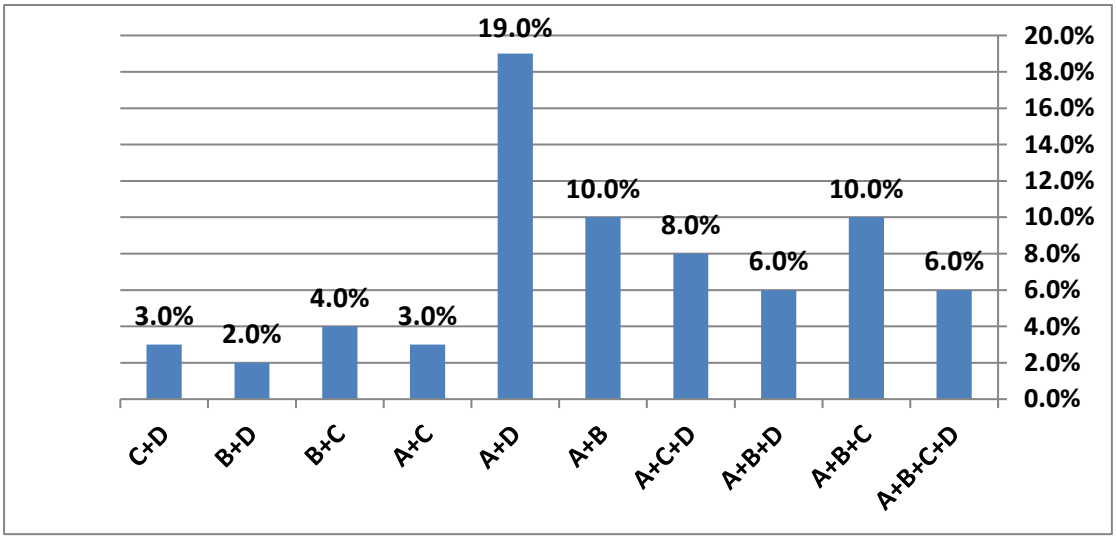

Figure 2: shows the percentages of mix genetic patterns.

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