

GENOTYPING STUDY ON HEPATITIS B VIRUS AMONG EGYPTIAN PATIENTS

By

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ABSTRACT

Background: Infection of hepatitis B virus (HBV) is foremost communal health disquiet around the world. In this sense, Egypt has a seroprevalence of HBV that is somewhere in the middle. There are ten genotypes of HBV (A–J), each with its own geographical spread. Africa is one of the supreme endemic areas, by five genotypes (A–E) were discovered for HBV. Genotype D is the most dominant in the Middle East. There is a scarcity of information about HBV genotyping in Egyptians.

Objective: The goal of this research was to find out the genotypes of HBV that were most prevalent among Egyptian patients.

Patients and methods: This study included 190 serum samples from patients at the Hospital of Al-Hussein at Al-Azhar University in Cairo, Egypt, whose hepatitis B surface antigen (HBsAg) test result was positive. Of the 190 serum samples, HBV DNA was detected in only one hundred (100) serum samples, and they were the only ones comprised in the research. Patients were sectioned into two collections: 40 from Upper Egypt governorates and 60 from Lower Egypt governorates. The INNO-LiPA technique, which is predicated on the notion of reverse hybridization, was used to determine HBV genotypes.

Results: Genotype C represented for 60% of all infections and was mostly distributed among patients in Lower Egypt's governorates, while genotype D represents for 40% of all infections and was mostly spread among patients in Upper Egypt's governorates.

Conclusion: These findings substantiated that the most dominant genotype in Lower Egypt governorates was genotype C, while the most dominant genotype in Upper Egypt governorates was genotype D.

INTRODUCTION

Above 350 million persons worldwide are diseased by the HBV, creating it a global health hazard [Sun et al., 2022]. Ten of HBV genotypes (designated from A to J) have been recognized [Kao, 2011]. Chronic carriers of HBV are found in from 5 to 10 % of infected adults besides

from 80 to 90 % of infected children [Razavi, 2020].

Geographically, variant HBV genotypes have diverse spreading. Genotype A prevails within North America and Northern Europe, while B and C genotypes prevail in East Asia. The D-genotype is the most popular in the

Mediterranean area. East, Central, and West Africa are the most common locations for genotype E [Athamneh *et al.*, 2021].

The F genotype is mainly found within South and Central America [Liu *et al.*, 2021]. The G-genotype has been discovered within France and United States [Locarnini *et al.*, 2021].

Central America has genotype H [Datfar *et al.*, 2021]. Genotype I was discovered within Vietnam and Laos, while genotype J was discovered in a Japan [Kao, 2011].

Five genotypes (A-E) of HBV have been found in Africa: Genotype A was discovered in Kenya [Okamoto *et al.*, 2019], genotype D was discovered in Tunisia [Kheirabad *et al.*, 2017], genotypes (A-D) have been found within South Africa [Toyé *et al.*, 2021], and genotype E was discovered in Nigeria [Fasola *et al.*, 2021].

Considering the significance of infection with HBV in this African region, little data on genotype distribution is available in Egypt [Duah *et al.*, 2018].

In Egypt, the endemicity of HBsAg is moderate from 2 to 8 %. Around from 2 to 3 million Egyptians have chronic infection of HBV [Elbahrawy *et al.*, 2021].

Functional and structure changes across genotypes can influence the course, severity, and possibility of problems. Furthermore, HBV genotypes may be linked to variances in antiviral medication responsiveness. According to certain research, in patients who have a chronic infection of HBV, variant genotypes respond differently to interferon [Zirabamuzale *et al.*, 2016].

Methods for determining HBV genotypes include the ligase chain reaction test [Gibriel *et al.*, 2017], colorimetric point mutation test [Khaled *et al.*, 2010], PCR using type-specific primers [Logoida *et al.*, 2021], the enzyme linked immunosorbent assay for genotype-specific epitopes [Okamoto *et al.*, 2019], direct sequencing [Locarnini *et al.*, 2021], line probe test [Rajput, 2020] and restriction fragment length polymorphism analysis [Logoida *et al.*, 2021].

The goal of this research was to find out the genotypes of HBV that were most prevalent among Egyptian patients via a line probe test (INNO-LiPA HBV Genotyping Assay).

PATIENTS AND METHODS

Ethical Committee:

The Ethics Committee of Al-Azhar University's Faculty of Medicine gave its approval to the study under the registration number [Mic.1Med.Research.Genotyping.HepatitisB.Inno/LIPA.Egy.Pts.000001]. Samples and patient data were obtained with permission from the University's Al-Hussein Hospital.

Inclusion criteria:

This study included 190 serum samples from Egyptian hepatitis patients who positive for HBsAg who were attending the Al-Hussein Hospital of Al-Azhar University. Only 100 serum samples positively identified for HBV DNA, and they were the only ones included in the research. A total of 100 HBV DNA positive patients, containing 26% females and 74% males, the average age were 42.4

years. Patients were divided into two main groups; Upper Egypt (Asyut, Beni Suef, Faiyum, Giza, Minya, Qena, and Sohag) and Lower Egypt (Alexandria, Beheira,

Cairo, Dakahlia, Gharbia, Kafr El Sheikh, Matruh, Monufia, and Qalyubia) patients. They were registered in this HBV genotypes research (Table 1).

Table (1): Demographic data of patients with positive PCR for HBV DNA

Characteristics	Values (%)
No of patients	100 (100%)
Gender	
Male	74 (74%)
Female	26 (26%)
Age in years, mean (range)	42.4 (24 – 61)
Region	
Lower Egypt	60 (60%)
Upper Egypt	40 (40%)

Exclusion criteria:

Patients who had the hepatitis C virus (HCV) or hepatocellular carcinoma (HCC) were not included in the study.

Serological assays:

To screen for HBsAg, HBc-IgM, and HCV-Ab, an Enzyme Linked Immunosorbent Assay (ELISA) approach was used [Rossi et al., 2015 and Ambachew et al., 2018].

Molecular assays:

Real time PCR (COBAS® AmpliPrep Instrument) was used to detect HBV DNA [Piratvisuth et al., 2013].

Biochemical assays:

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) [Thuy and Tseng, 2016], albumin (ALB), total bilirubin (TB) and direct bilirubin (DB) levels were determined using a commercial kit (Spinreact, spectrophotometer analyzer). Up to 42 IU/L, Up to 45 IU/L, 3.5 – 5.5 gm/dl, Up to 1.1 mg/dl, and Up to 0.25 mg/dl were the normal range values for ALT, AST, ALB, TB, and DB, respectively.

DNA Extraction:

Extraction of the DNA from serum samples was done by using the DNA extraction reagent kit QIAamp depending on the manufacturer's recommendations (QIAGEN GmbH, Hilden, Germany). In the LiPA techniques, the isolated DNA was employed for amplification. Following DNA extraction, LiPA analysis was completed in around 5 days. DNA extracts were kept at -20°C if they were not used right away.

INNO-LiPA detection:

The HBV polymerase gene domains B to C contain type-specific sequences that can be found using the INNO-LiPA HBV Genotyping assay to find out genotypes of HBV from A to H by a line probe assay. The HBV genotyping assay (LiPA) was not indicated for use as an HBV screening test or a diagnostic test to confirm HBV infection [Warkad et al., 2018]. HBV is a virus with partly double-stranded DNA that uses an RNA intermediary to replicate. In the first step, the viral DNA was extracted from the sample. In two rounds of PCR, the purified DNA was

amplified using biotinylated PCR primers [Nicolini *et al.*, 2019]. The primers on the outside amplified a part of the HBV polymerase gene (domain B and C) (first round of PCR) utilized in this test. The double strands of DNA helix were detached by heat (denaturation), letting the target to be exposed to the outside primers. These oligonucleotide primers complement the highly preserved flanking pieces of the target region. As a result, when the primers were cooled to a specific temperature (annealing at 45°C), they bound to their specific sequence. The annealed primers extended along the target template by the thermostable DNA polymerase at a higher temperature in the presence of dNTP's (extension). After one round of denaturation, annealing, and extension, an identical copy of the template was made. After 40 repetitions, the technique yielded a 409-bp multi-fold amplified target sequence. When the quantity of amplified product was inadequate, a nested (second round) PCR was used. The inkling of this amplification was the same of the first, with the exemption that the DNA changed by the amplified product of the first round PCR, and the outside primers changed by biotinylated nested primers. The denaturation, annealing, and extension processes were repeated 35 times,

providing a 342 bp amplified sequence. On the gel, a test was performed to find a nested or outer amplified production used. Afterward amplification, a specific oligonucleotide probes fixed as parallel lines on membrane-based strips was hybridized with biotinylated DNA, and then unhybridized DNA was washed away from the strips, and addition of streptavidin, to bind to any formerly created biotinylated hybrids, then incubation with a chromogen of BCIP/NBT to form a purple/brown precipitate. The INNO-LiPA strip for HBV genotyping consisted of a red marker line, two control lines, and 14 parallel probe lines (**figure 2**). The conjugate control line controls the color growth response. Also, the amplification control line detected the amplified HBV genomic material by universal HBV probes.

Statistical analysis:

The data were analyzed via SPSS 22.0. Quantitative data were presented as means, standard deviation, range and Pearson correlation. Qualitative data were presented as numbers and percentages and compared by Chi-square assessment, and P-values < 0.05 were deemed statistically significant.

RESULTS

Distribution of HBV genotypes in studied patients:

Only one hundred (100) serum samples confirmed positive for hepatitis B virus DNA out of one hundred and ninety (190), and these were the only ones included in the study. From these samples, 74% were male patients and 26% were female

patients, the genotype C represented [43/74 (58.1%)] in male patients, while genotype D represents [31/74 (41.9%)]. As for female patients Genotype C represented [17/26 (65.4%)], while genotype D represented [9/26 (34.6%)], (Table 2).

Table (2): HBV genotype distribution among infected patients (n=100)

Genotype	Male (%)	Female (%)	Total (%)
Genotype C	43 (58.1%)	17 (65.4%)	60 (60%)
Genotype D	31 (41.9%)	9 (34.6%)	40 (40%)
Total	74	26	100

Distribution of HBV genotypes in studied groups:

This study indicated that the genotype C constituted 60% of the overall infections, which spreaded mainly among patients in the governorates of Lower

Egypt. The genotype D constituted 40% of the total infections, which spreaded mainly among patients in the governorates of Upper Egypt. A, B, E, F, G and H genotypes for HBV were not detected in our research (Figures 1, 2, and 3).

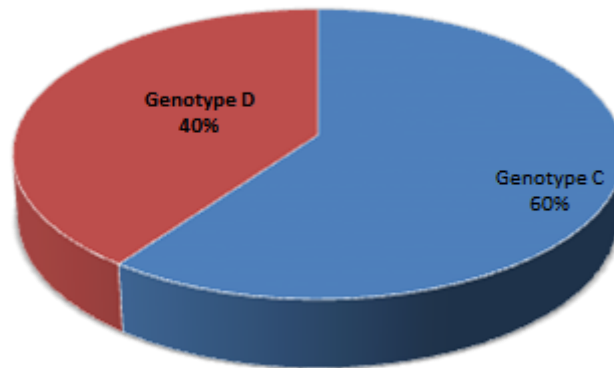


Figure (1): Distribution of HBV genotypes in the studied groups

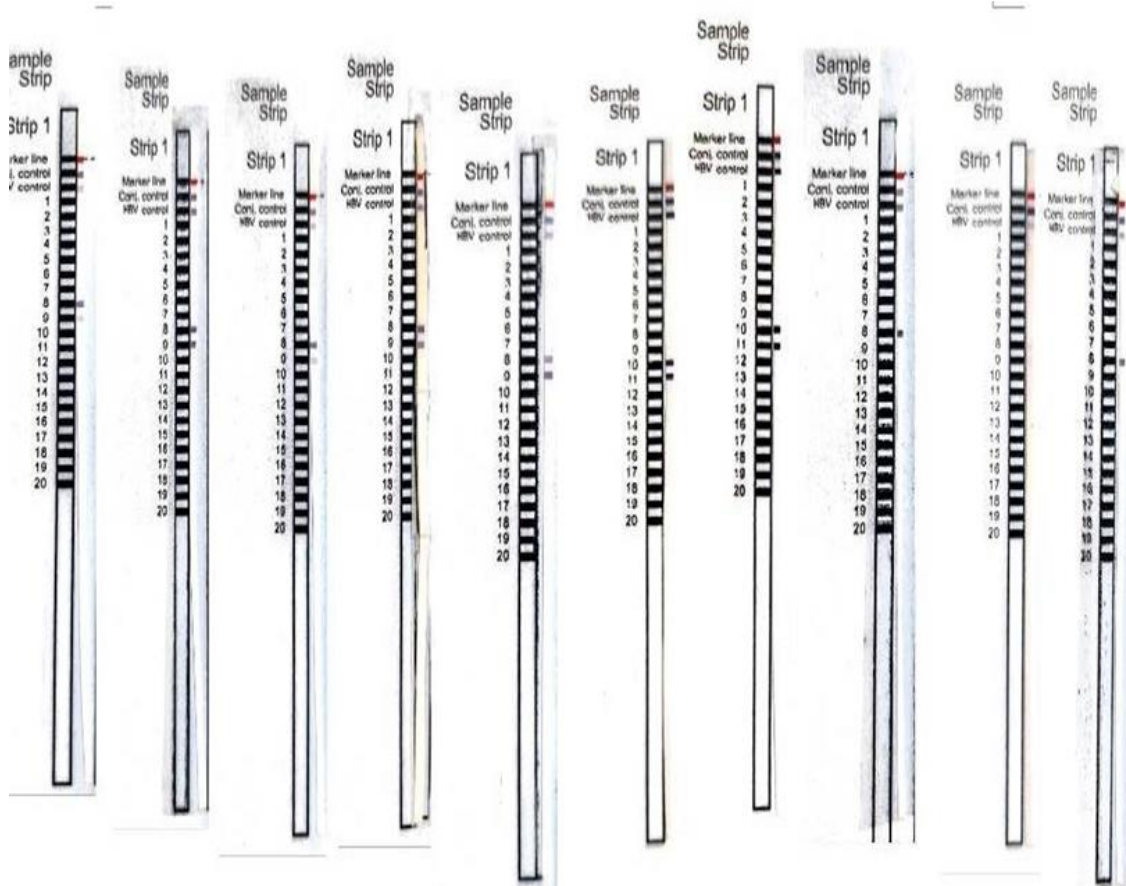


Figure (2): Bands representing HBV genotype-specific oligonucleotide probes in the study groups

Line	Genotype A				Genotype B			Genotype C			Genotype D			Genotype E			Genotype F		Genotype G		Genotype H	
1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3	x	x		x																		
4	x	x	x																	x		
5	x		x	x			x															
6							x		x													
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15																	x	x				x
16																				x	x	

Figure (3): Interpretation Chart for HBV Genotypes (Use the chart at all times for correct interpretation)

Distribution of HBV genotypes among the different governorates:

Out of 100 patients included in our study, 40 belonged to Upper Egypt governorates, where the genotype D was common among them, and they were distributed as follows [8/40 (20%)] from Asyut, [3/40 (7.5%)] from Beni Suef, [7/40 (17.5%)] from Faiyum, [10/40 (25%)] from Giza, [3/40 (7.5%)] from Minya, [5/40 (12.5%)] from Qena, and [4/40 (10%)] from Sohag. The other 60 patients belonged to Lower Egypt governorates, where the genotype C was common among them, and they were distributed as follows: [11/60 (18.33%)] from Alexandria, [22/60 (36.66%)] from Beheira, [11/60 (18.33%)] from Cairo, [1/60 (1.67%)] from Dakahlia, [5/60 (8.33%)] from Gharbia, [2/60 (3.33%)] from Kafr El Sheikh, [4/60 (6.67%)] from Matruh, [1/60 (1.67%)] from Monufia, and [3/60 (5.0%)] from Qalyubia, (Table 3 & Figure 4).

Table (3): Distribution of HBV genotypes among the different governorates

Location	Governorate	Genotype C (%)	Genotype D (%)
Upper Egypt	Assiute	0	8 (20%)
	Beni Suef	0	3 (7.5%)
	Faiyum	0	7 (17.5 %)
	Giza	0	10 (25%)
	Minya	0	3 (7.5%)
	Qena	0	5 (12.5%)
	Sohag	0	4 (10%)
Total		0	40
Lower Egypt	Alexandria	11 (18.33%)	0
	Beheira	22 (36.66%)	0
	Cairo	11 (18.33%)	0
	Dakahlia	1 (1.66%)	0
	Gharbia	5 (8.33%)	0
	Kafr El Sheikh	2 (3.33%)	0
	Matruh	4 (6.66%)	0
	Menoufia	1 (1.66%)	0
	Qalyubia	3 (5.0%)	0
Total		60	0

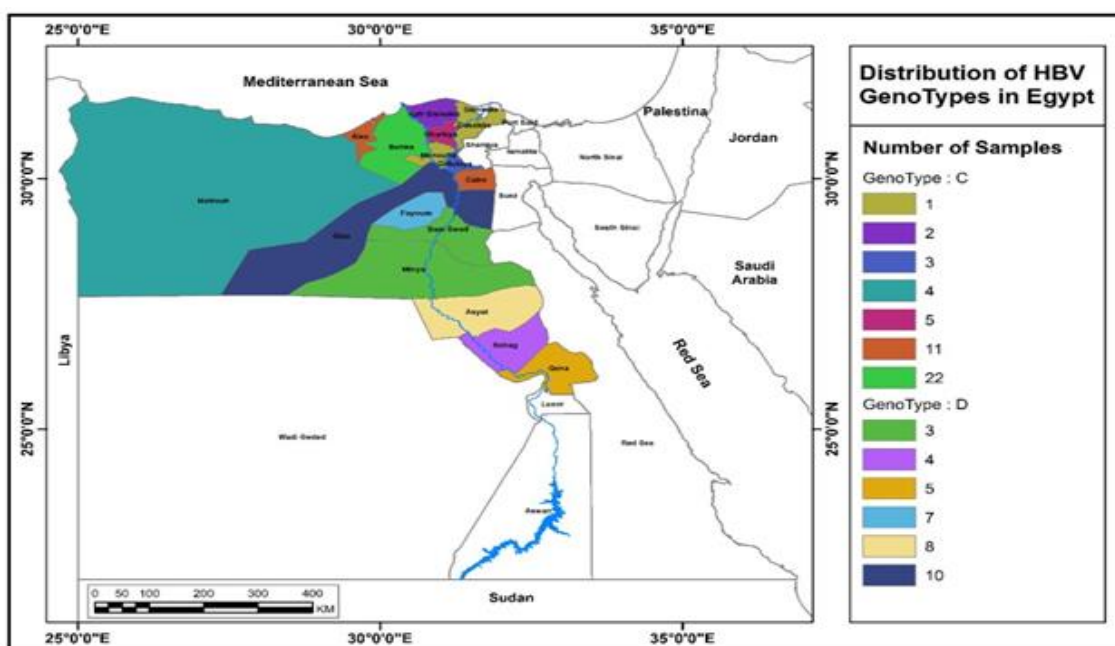


Figure (4): Distribution of HBV Genotypes in different governorates of Egypt

HBV-DNA Viral loads with different genotypes:

Based on the quantification of HBV DNA in the patients samples involved in our study, the viral load was allocated into three categories: high (> 100,000 IU/ml),

intermediate (10,000 - 100,000 IU/ml), and low (< 10,000 IU/ml). The viral load in all patients for each genotype; although genotype C patients have a high viral load, genotype D patients have a low to moderate viral load (Table 4).

Table (4): HBV-DNA Viral loads with different genotypes

Genotypes	HBV-DNA viral load by RT-PCR			Total
	Low Level (< 10,000 IU/ml)	Intermediate Level (10,000 – 100,000 IU/ml)	High Level (>100,000 IU/ml)	
Genotype C (%)	47 (55.29%)	7 (77.78%)	6 (100%)	60
Genotype D (%)	38 (44.71%)	2 (22.22%)	0	40
Total	85	9	6	100

Distribution of HBV genotypes with HBsAg, Anti-HBc, and types of infection:

Positive HBsAg was found in 100/100 (100%) of the patients. Positive results of Anti-HBc was found to be [12/100 (12%)] among HBsAg positive patients, while 88% of patients with HBsAg positivity also had negative anti-HBc results. The following associations were discovered

between liver diseases and the spreading of HBV genotypes: genotype C was found to be significantly more prevalent in patients with chronic infection and acute infection [52/88 (59.1%), 8/12 (66.7%)] than genotype D, which was found to be [36/88 (40.9%)] in chronic infection, and [4/12 (33.3%)] in acute infection (**Table 5**).

Table 5: HBV genotypes and the descriptive analysis for HBsAg, Anti-HBc, and the types of infection

Genotypes	HBs-Ag		Anti-HBc-IgM		Type of infection	
	Positive	Negative	Positive	Negative	Chronic	Acute
Genotype C	60 (60%)	0	8 (66.7%)	52 (59.1%)	52 (59.1%)	8 (66.7%)
Genotype D	40 (40%)	0	4 (33.3%)	36 (40.9%)	36 (40.9%)	4 (33.3%)
Total	100	0	12	88	88	12

Relationship between HBV DNA quantification by RT-PCR and other biochemical testing:

Our results indicated that there was no correlation between RT-PCR and ALT, AST, while a positive correlation was noticed between RT-PCR and total bilirubin and direct bilirubin. Negative correlation was noticed between RT-PCR and albumin in patients from Upper

Egypt. Moreover, negative correlation was noticed between RT-PCR and albumin in patients from Lower Egypt, while no correlation between RT-PCR and the other items were noticed in the same region (**Table 6, Figure 6 & 7**). However, there was non-significant differences between demographic and biochemical data in different genotypes (**Table 7**).

Table (6): Relationship between RT-PCR test for HBV DNA quantitation and the age of the patients in addition to the other biochemical assays

Variable	Upper Egypt			Lower Egypt		
	R	Slope	P-Value	R	Slope	P-Value
Age	0.2340	2.87E5	0.157	0.1830	9.05E3	0.155
ALT	0.0500	8.55E3	0.202	0.1840	9.75E	0.885
AST	-0.0010	-2.94E2	0.321	0.1470	1.11E3	0.919
Albumin	-0.5150	-2.36E7	0.016*	-0.5290	-7.06E	0.011*
Total Bilirubin	0.3440	7.96E6	0.000*	0.6920	3.16E5	0.279
Direct Bilirubin	0.3700	7.93E6	0.000*	0.7240	3.73E5	0.144

Note: r = Pearson Correlation, * Significance at P < 0.05.

Table (7): Differences in demographic and clinical data between patients infected with hepatitis B virus (HBV) genotype C and those infected with genotype D

Variable	Genotypes	Genotype C (n= 60)	Genotype D (n= 40)	P-Value
Mean age (range years)		40.5 (27 – 61)	45.2 (24 – 61)	0.140
Gender Male/Female		43/17	31/9	0.515
ALT (U/L)		72.3±10	56.3±11	0.340
AST (U/L)		56.2±7	47.1±9	0.452
Albumin (g/dl)		3.91±0.04	3.94±0.03	0.695
Total Bilirubin (mg/dl)		1.38±0.12	1.07±0.08	0.087
Direct Bilirubin (mg/dl)		0.64±0.11	0.39±0.08	0.120
PCR for HBV DNA (copies × 10 ³ /ml)		49.544±6.2	3.120±0.71	0.396

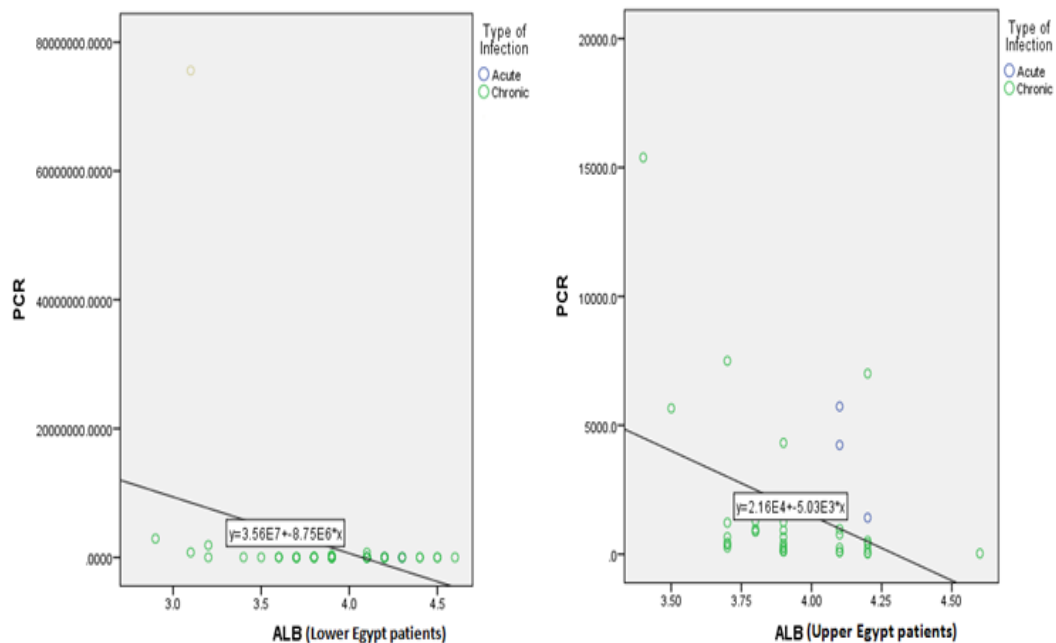


Figure (6): The negative correlation between RT-PCR and albumin in both Upper and Lower Egypt patients

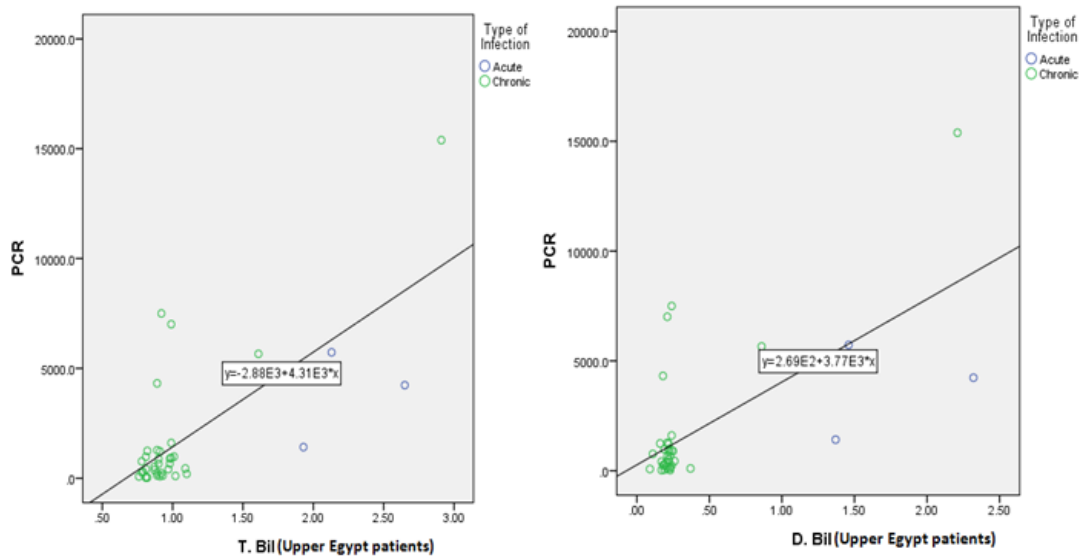


Figure (7): The positive correlation between RT-PCR and Total, Direct Bilirubin in Upper Egypt patients

DISCUSSION

HBV infection is a worldwide health tricky with a growing impact in developing countries. In Egypt, the prevalence of HBV infection decreased significantly since the institution of universal infantile HBV vaccination in 1992 [Boglione *et al.*, 2021, Quaye *et al.*, 2021 and Trimzi *et al.*, 2021]. Based on more than 8% variance in the whole genomic sequence of HBV, ten genotypes for HBV from A to J have been recognized according to virus sequence homogeneity, except for the genotypes I and J [Lazarevic *et al.*, 2012]. As a result, gathering more data on genotypes of HBV from throughout the world is critical in order to make an informed choice about their clinical value [Locarnini *et al.*, 2021].

The utmost important result in all findings was the prevalence of genotype C as the main HBV genotype in the Lower Egypt governorates, and genotype D as the main HBV genotype in the Upper

Egypt governorates. Other Egyptian studies have found similar results. By using Primer specific polymerase chain reaction to isolate HBV genotypes from 83 serum samples of Upper Egyptian carriers, Zaky *et al.* (2010) discovered that genotype D predominates in this region.

There were no previous studies concerned with the Lower Egypt governorates region. The presence of genotype C in Egypt was not a new finding, but it was compatible with a previous study, In Egyptian hemodialysis patients Esmail *et al.* (2016) discovered that the most common HBV genotypes were B (50 %), C (33 %), and D (17 %). However, the geographical dispersion of the patients was not taken into account.

The HBV C genotype was thought to be mainly found in Southeast Asia, but since Al-Azhar University and other Egyptian Universities attracted a large number of Asian students from these countries, those Asian students probably

transmitted the HBV C genotype to the Egyptians.

Also, previous studies did not take the patient's geographical location into account, and thus did not provide an explanation for the geographical distribution of HBV genotypes in Egypt.

In an Egyptian study, *Abdel-Rahman et al.* (2007) discovered the dissemination of HBV A-D genotypes in 70 patients of pediatric cancer (38 males and 32 females) were enrolled in the study at Cairo University's National Cancer Institute (NCI), but it was not considered a geographical distribution of.

In another Egyptian study, *Khaled et al.*, (2011) found that the genotype D was the most common in Egypt, which was found primarily in the patients with CAH and HCC, while mixed types D/F was found only in AH patients, where the study included 140 hepatitis patients, and the study also did not provide any details of the patients' geographical locations.

In our study, we considered the patient's geographic location and divided the genotypes discovered accordingly.

The relationship between the RT-PCR test for HBV DNA quantification and various clinical variables was evaluated in the statistical analysis. Our results presented that there was no correlation between RT-PCR and ALT, AST; this finding confirmed what study in chronic HBV patients, by *Biazar et al.*, (2015) who discovered a relation between the viral load of hepatitis B DNA in the liver and its histology. They found no association between liver viral load and AST, or vice versa.

Also, in our research work, a positive correlation was noticed between RT-PCR and total bilirubin and direct bilirubin, and a negative correlation was discovered between RT-PCR and albumin in patients from Upper Egypt. Moreover, negative correlation was discovered between RT-PCR and albumin in patients from Lower Egypt, while no correlation between RT-PCR and the other items were detected in the same region.

About 88% of the cases involved in the study were patients with chronic infection. Thus, over time, a reduction in the level of albumin and a rise in the level of bilirubin occur, as well as a balance in the level of liver enzymes, especially after taking medications that support liver cells.

However, there was non-significant variances between demographic and biochemical data in different genotypes. This finding was consistent with *Yuen et al.*, (2003), in their study; researchers found no statistically significant variances in demographic and clinical data between patients with HBV genotype B infections and those with genotype C infections.

Another study was conducted in China in 2005 where Zeng, et al. found that there were two basic genotypes (B, C) and upon statistical comparison between them, and claimed that there were no significant dissimilarities in ALT and total bilirubin levels between genotypes B and C. Based on serum ALT and bilirubin levels, there were no dissimilarities between the two genotypes in the severity of liver disease. Furthermore, Southern Ethiopian researchers genotyped and serovirologically characterized the HBV in blood, where *Ambachew et al.* (2018) detected that there were no significant

variations between genotypes in terms of donor age, gender, or biochemical assay findings in a study involving 103 HBsAg sero-positive samples, and he obtained two genotypes A and D.

CONCLUSION

These findings revealed that genotype C was the main genotype in Lower Egypt governorates, while genotype D was the main genotype in Upper Egypt governorates, with no statistically significant dissimilarities in demographic or biochemical data between genotypes.

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دراسة النمط الجيني لفيروس التهاب الكبد الوبائي (ب) بين المرضى المصريين

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خلفية البحث: عدوى فيروس التهاب الكبد (ب) هي مشكلة صحية عامة كسبب
لأمراض الكبد بما في ذلك سرطان الخلايا الكبدية وتليف الكبد. تم توثيق عشرة
أنماط جينية من (A-J) اعتمادًا على تجانس تسلسل الفيروس.

الهدف من البحث: أجريت هذه الدراسة للكشف عن الأنماط الجينية لفيروس
التهاب الكبد (ب) بين مرضى التهاب الكبد المصريين الذين كانوا يترددون على
مستشفى الحسين بجامعة الأزهر في الفترة من أكتوبر 2016 إلى أكتوبر 2017.

المرضي وطرق البحث: تم تسجيل مائة وتسعين (190) مصل مع مستضد التهاب
الكبد (ب) السطحي (HBsAg) في هذه الدراسة. من بين 190 عينة مصل، كانت
مائة (100) عينة مصل فقط موجبة للحمض النووي للفيروس (HBV-DNA)
وتم تضمين هذه فقط في الدراسة وكانت كالتالي: 74% ذكور و 26% إناث،
متوسط أعمارهم 42.4 سنة.

تم اختبار الأمصال لبحث وجود المستضد السطحي لفيروس التهاب الكبد
(ب) بواسطة تقنية ELISA، وتم تعريض الأمصال الموجبة للمستضد السطحي
لتفاعل البوليميراز المتسلسل (PCR). تم اختبار العدد المختار من الأمصال
الإيجابية للحمض النووي للفيروس للتمييز الجيني عن طريق مقايسة التتميط

الجيني HBV INNO-LiPA. تم تلخيص البيانات وعرضها وتحليلها باستخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS الإصدار 22).

من أصل 190 عينة مصل، فقط مائة (100) عينة مصل كانت موجبة للحمض النووي HBV وتم تضمين هذه فقط في الدراسة. من هذه العينات، كان 74% من المرضى الذكور و 26% من الإناث، ويمثل النمط الجيني C [43/74 (58.1%)] في المرضى الذكور، بينما يمثل النمط الجيني D [31/74 (9.41%)] [26/17 (65.4%)] بينما يمثل النمط الجيني D [9/26 (34.6%)] [9/26 (34.6%)].

النتائج: أظهرت هذه الدراسة أن النمط الوراثي C يشكل 60% من إجمالي الإصابات والتي تنتشر بشكل رئيسي بين مرضى محافظات الوجه البحري، ويشكل النمط الوراثي D 40% من إجمالي الإصابات والتي تنتشر بشكل رئيسي بين المرضى في محافظات صعيد مصر. لم يتم العثور على أنماط وراثية A أو B أو E أو F أو G في دراستنا.

الاستنتاج: كانت النتيجة الأكثر أهمية في نتائجنا هي هيمنة النمط الوراثي C باعتباره النمط الوراثي السائد في محافظات الوجه البحري، وهيمنة النمط الوراثي D باعتباره النمط الوراثي السائد في محافظات صعيد مصر.