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BIOINFORMATICS ANALYSES OF POLYMERASE (*pol*) GENE OF HBV GENOME OCCURRED IN BLOOD SAMPLES FROM DIFFERENT REGIONS OF KSA

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ABSTRACT

This study aimed to isolate of polymerase (*pol*) gene belonging to some hepatitis B virus (HBV) from some blood samples collected from different regions of KSA representing south, north, east, middle and west KSA. This was followed by determining the nucleotide sequences of this gene and carrying out some bioinformatics analyses on such genes. The presence of virus in some HBV-infected blood samples collected from different regions of KSA was serologically and molecularly confirmed by ELISA and Rt-PCR techniques. The nucleotide sequence of *pol* gene was determined and bioinformatically analyzed. Results showed that the nucleotide sequences of the partial sequences of the five HBV strains (LC152751.1, LC152752.1, LC152753.1, LC152757.1 and LC152759.1) were ranged from 600 to 899 nts and proved to be belonging to *pol* gene of HBV. Bioinformatics comparisons between the five HBV strains showed percent identities 92-98% and 80-95% at the levels of DNA and deduced amino acids, respectively, when compared to some overseas HBV strains recorded in GenBank. Types of domains (DNA_pol_viral_C and DNA pol viral-N) its accession (pfam00336 and pfam00242), super families (RT-like superfamily and cl02825) of the sequenced *pol* gene(s) were also addressed. Differences at the levels of DNA, encoding amino acids, *i.e.*, open reading frames and domains super families between the strains under investigation and those similar in GenBank were determined.

Keywords: Hepatitis, HBV, Polymerase (*pol*) gene, Bioinformatics.

INTRODUCTION

The relevance of infection caused by hepatitis viruses was related mainly to their wide geographic distribution and the large number of infected individuals in all parts of the world (Almeida et al., 2012; Lin and Kao, 2016 and Samal et al., 2017).

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are among the principal causes of severe liver disease (Akcam et al., 2009; Prasad et al., 2015 and Dai et al., 2015). Tadokoro et al. (2006) reported that HBV is a worldwide public health problem. HBV infection is transmitted sexually, parentally and prenatally from mother to child (Sharavanan et al., 2014). Therefore, Prasad et al. (2015) showed that health care workers are at high risk for transmission of hepatitis B.

There is limited data of epidemiology of Hepatitis B in community, more so in rural population (Prasad et al., 2015). Infection from Hepatitis B primarily results from peripartum vertical transmission and the risk increases in the presence of hepatitis Be antigen (Banks et al., 2016). Diagnostic assays allowing the quantification of HBV-DNA over a wide range of concentrations are important for monitoring patients during antiviral therapy (Zanella et al., 2002).

The epidemiological significance of HBV genotypes has been well established and becoming an essential concern day by day however, much little is known about the mixed infection with more than one HBV genotype and their clinical relevance (Alam et al., 2007). Using the emission transmission electron microscopy viral particles was measured at 42 nm in diameter (Howard 1986). It remains unclear whether HBV replicates in extra-hepatic tissues, and particularly in peripheral blood mononuclear cells, which may serve as a reservoir for the maintenance of infection (Mazet-Wagner et al., 2006). A serial invasive signal amplification react-ion assay (PCR-Invader assay) was developed for distinguishing the known eight genotypes (A to H) and four subgenotypes (Aa, Ae, Ba, Bj) of HBV. HBV, the hepadnavirus infecting humans, can be divided into the 7 genotypes A to G. By definition, genotypes differ by more than 8% at the nucleotide level. However, some genotypes differ by more than 14% from others (Schaefer et al., 2003).

The most prevalent genotype in the Mediterranean region is genotype D. Genotype E is observed in parts of East, Central, and West

Africa. Genotype F is found in South and Central America, genotype G in the USA and France, and genotype H is observed in Central America (Echevarrìa et al., 2005). This study was designed to analyze of the nucleotide sequence of polymerase (*pol*) gene of HBV amplified from the DNA-blood samples of different regions of Saudi Arabia, and this could be led to detecting the mutations at the level of DNA or its protein.

MATERIALS AND METHODS

Source of serum blood samples: Some HBV-infected serum blood samples were obtained from Central Laboratory [AJMS-01-2016 (Female-52y) and AJMS-02-2016 (Male-35y)] and King Abdul Aziz Hospital [AJMS-03-2016 (Male-41y)] at Taif; Asir Central Hospital [AJMS-04-2016 (Male-27y)] and General Abha Hospital [AJMS-05-2016 (Male-23y)] at Abha; General King Fahd Hospital [AJMS-06-2016 (Female-56y) and AJMS-07-2016 (Female -48y)] in Jeddah; Hospital in Hafr Al Batin [AJMS-08-2016 (Male-29y)] and Riyadh El Shamasy Hospital [AJMS-09-2016 (Female-46y) and AJMS-10-2016 (Male-34y)].

HBV serological testing: For detection of the surf-ace antigen of the hepatitis B in human serum or plasma by the enzyme immunoassay technique the Monolisa™ HBsAg ULTRA kit [BIO ELISA -BIO-RAD, la-Coquette, France] was used to assess the presence of HBsAg in collected blood samples.

PCR confirmation of HBV: To confirm the presence of HBV-DNA by PCR in the blood samples Cob-as *Taq*Screen MPX test was used among the COB-AS® TaqMan® Analyzer. Real time detection of PCR products was conducted using the following primer pair (Sense: 5' AGA CTC GTG GTG GAC TTC TCT 3' (5'252 position) and Antisense: 5' CAA AAG AAA ATT GGT AAC AGC GGT A 3' (5'794 position).

PCR program and amplification reaction: In a total volume of 25µL total which consists of Go*Taq* Green Master Mix, 2X (12.5µL); Sense Primer, 20µM (1.25µL); Antisense Primer, 20µM (1.25-µL); HBV-DNA Template 2.50µL; Nuclease-Free water 7.50 µL the PCR was conducted. The PCR program was started with one cycle of 95ºC for five minutes followed by 35 cycles each of 95ºC, 50ºC and 72ºC for minute for each. The final cycle (72ºC) was extended for 10 minutes.

Sequencing of PCR products: Using three primers: Sense: 5' GGA TGT GTC TGC GGC GTT T3' ; Sense: 5' AGA CTC GTG GTG GAC TTC TCT3' and Antisense: 5' CAA AAG AAA ATT GGT AAC AGC GGTA3' the PCR products of round 2 were gel purified and sequenced using automated DNA sequencing system (ABI 3100) and BigDye® Terminator v3.1 cycle sequencing Kit (Applied Bio-systems, Foster City, CA, USA).

Bioinformatics analysis: By using BLASTN 2.2.23 + software (http://www.ncbi.nlm. nih.gov/ blast/) DNA sequence was analyzed against compared to related HBV strains recorded in Genbank. The deduced amino acids *i.e*., open reading frame (ORF), types of domains and their super families were also analyzed using the same program. The sequence that showed the lowest E-value and maximum identity was taken as the genotype of the sample analyzed. On sequencing, the general primers of major surface antigen region (F1, F2, S2, S3 P3 and TPR1), and primers of each of PCR (PCR products 1, 2 and 3), sequencing (Sense 5'252, Sense 5'377 and Antisense 5'794) and mutagenic (M741VAY WF and L669 MAYWF) were detected in the obtained partial sequences of HBV strains.

RESULTS AND DISCUSSION

The HBV is a major health problem worldwide with more than 350 million people being chronic carriers (Aljarbou, 2013). HBV is one of the main etiological agents of acute and chronic liver disease that is still a major public health problem in the world (Amini-Bavil-Olyaee et al., 2005).

In this study, a number of ten blood samples infected with HBV were collected from Taif, Jeddah, Riaydh, Hafr Al Batin and Abha regions of KSA for bioinformatics studies on HBV genome. HBV is an enveloped DNA virus that belongs to the Hepadnaviridae family (NCBI taxonomy, ICTV, Viral Zone). It contains a small, partially ds, relaxed-circular (RC) DNA genome that replicates by reverse transcription to an RNA intermediate, the pgR-NA (Datta et al., 2006). Its length is comprised bet-ween 3182 and 3248 bp depending on genotypes (Jones and Jianming 2013).

The experimental results showed that the nucleotide sequences of the partial sequences of the five HBV strains belonging to the polymerase (*pol*) gene of HBV were recorded as 600 (AJMS-02-2016, LC152751.1), 898 (AJMS-03-2016, LC-1527-52.1), 855 (AJMS-04-2016, LC1527-53.1), 838 (AJ-MS-08-2016, LC152757.1) and 899 (AJMS-10-2016, LC152759.1) nts (Table 1). By comparison these nucleotides of the five strains with overseas HBV strains in Genbank, percent identities 98-99; 94-95; 92; 96 and 95-96 % were recorded, respectively.

Table 1: Characters of nucleotide sequences, ORFs and putative domains of the *pol* gene of HBV strains: LC152751.1, LC152752.1, LC152753.1, LC152757.1 and LC152759.1.

Levels	Characters	HBV Strains belonging to *pol* gene				
		LC152751.1	LC152752.1	LC152753.1	LC152757.1	LC152759.1
DNA	Length (nts)	600	898	855	838	899
	Identities (%)	99	94-95	92	94	97-98
Protein	ORF#-Frame	1-1	1-2	1-1	1-1	1-2
	ORF Length (nts)	600	798	855	837	897
	Eaa	200	266	284	279	299
	Stop codon	-	-	+	-	-
	Identities (%)	87-95	90-91	77-96	84-90	94
Domains	Name	DNA_pol_viral_C	DNA_pol_viral_N			
	Accession	pfam00336	pfam00242			
	Description	DNA_pol_viral_C terminal	DNA_pol_viral_N			
	Superfamily	RT-like superfamily	cl02825			

Eaa: Encoding amino acids. + : Present. - : Absent.

It is well known that HBV-polymerase region overlaps pre-S/S genes with high epitope density and plays an essential role in viral replication (Huang et al., 2013). The nucleotide sequences of open reading frames (ORFs) of the five HBV strains (LC152751.1, LC152752.1, LC152753.1, LC1527-57.1 and LC152759.1) were 600, 798, 855, 837 and 897 nts, respectively, and belonging to the *pol* gene of HBV (Table 2). These ORFs were deduced into 200, 266 (with stop codon), 285, 279, 299 amino acids, respectively. Identities of 90-91; 77-96; 84-90; 86 and 89-91%, respect-ively, were recorded bet-ween the ORFs of HBV strains compared to those similar HBV overseas strains recorded in GenBank. The intragenotypic divergence of the complete gen-ome sequence of Iranian strains was 1.8% and the intergenotypic in genotype D was 3.8% and with the other geno-types was 7.9-15.4%. The largest ORF in the HBV genome encodes for the hepatitis B poly-merase protein (HBp). The protein is 90 kDa in size and has RNA and DNA dependent polymerase activity (Toh *et al*., 1983). HBp plays a key role in HBV genome generation as well as pgRNA encapsidation. HBp is packaged together with pgRNA with in HBV nucleocapsids) (Mack *et al*., 1988).

Differences of 9 & 36; 57 & 27; 70 & 75; 51 & 44 and 30 & 24 were recorded between the partial sequences of HBV strains: (LC152751.1, LC1527-52.1, LC152753.1, LC152757.1 and LC-152759.1) *pol* gene & its ORFs, *i.e.*, deduced amino acids, respectively and the compared over-seas strains of HBV in GenBank. Phylogenetic tree of the HBV *P* gene, partial sequence, strains of this study compared the most related genes confirmed that the gene was belonging to the *pol* gene (Fig. 1). HBp has been divided into four characterized domains. Based on sequence homo-logies and studies on the mechanism of viral gen-ome replication, most parts of HBp are ind-ispensable (Lanford *et al*., 1999). The primase domain acts in priming (-) DNA strand synthesis and ends up covalently linked to the 5' end of the (-) DNA strand. The subsequent domain does not app-ear to have any enzymatic function but acts as a spacer between the first and third domains. The third domain gives HBp its name. It occupies app-roximately 40% of the protein and encodes for the RNA and DNA dependent polymerase activity.

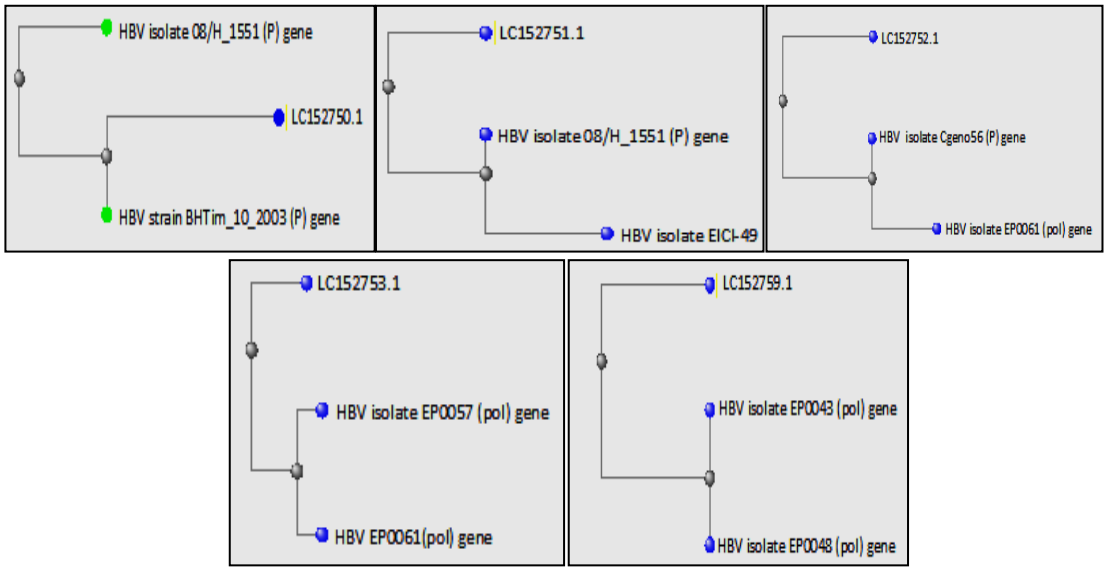


Figure 1. Phylogenetic tree of surface protein region genes of HBV strains and most related overseas strains in GenBank.

In the PCR-amplified sequences of HBV strains LC152751.1, LC152752.1, LC152753.1, LC152757.1 and LC152759.1, the general primers (Table 2) of surface antigen region genes were detected. Data show that the highest number of primers were recorded in the nucleotide sequences of LC152751.1 (Five primers) followed by LC152752.1 (Four primers). Three primers were detected in LC152757.1 and LC152759.1, while two primers were found in LC152753.1. The S2 primer was the most repeated one (four strains) followed by F1 & P3 primers (three strains), S3 & TPR1 primers (one strain). Both of M741VAYWF and L669MAYWF primers were only recorded in LC152751.1 (Fig. 2).

Table 2: Presences of general primers of the polymerase (*pol*) gene, and primers of each of PCR, sequencing and mutagenic recorded in the PCR-amplified sequences of HBV strains.

Primers names	LC152751.1	LC152752.1	LC152753.1	LC152757.1	LC152759.1
F1	-	+	-	+	+
F2	-	-	-	-	-
S2	-	+	+	+	+
S3	+		-	-	-
P3	-	+	-	+	+
TPR1	-		+	-	-
F58	+	+	-	-	-
R58	+	-	-	-	-
F252	-	-	-	-	-
M741VAYWF	+	-	-	-	-
L669MAYWF	+	-	-	-	-
Total	5	4	2	3	3

+: Present. -: Absent.

Only four partial sequences of HBV strains (LC152752.1, LC152753.1, LC152757.1 and LC-152759.1) showed putative conserved domains were belonging to DNA_pol_viral_N (pfam002-42) domain which described as DNA polymerase (viral) N-terminal domain of the superfamily cl02825 with Query E-value of 3.12e-89, 1.02e-96, 1.65e-89 and 2.89e-127, respectively. On the other hand, the putative conserved domains of the ORF number 1 of partial sequence of HBV strain LC152751.1 *pol* gene was DNA_pol_viral_C with accession number of pfam00336 described as DNA polymerase (viral) C-terminal domain, with Query interval and Query E-value of 175-200 and 1.92e-08, respectively, and belonging to the RT-like superfamily (Fig. 3).

When the amino acids between the putative conserved domains (Queries) of the four partial sequences of HBV strains: LC152752.1, LC1527-53.1, LC152757.1 and LC152759.1 which belonging to the DNA_pol_ viral_N (pfam00242) domain and the superfamily cl02825 were compared

with those in the same family differences of 2, 8, 12, and 12, were recorded, respectively (Figures 4 and 5).

The viral polymerase is composed of four domains, bearing three enzymatic activities: primase activity, reverse transcriptase & DNA-dependent DNA polymerase and RNase H: ribonuclease H activity. The *pol* gene contains 842 or 843 amino acids (aa) in most of genotypes, but it has 832 aa in genotype D and 845 aa in genotype A. The enzyme exhibits both a DNA polymerase and a reverse transcriptase activity and replicates the HBV genome from an encapsidated pregenomic RNA template (Nassal, 2008).

However, HBp also requires the presence of metal ions and the presence of the stem loop for polymerase/reverse transcriptase activity to occur (Bartenschlager and Schaller, 1992; Urban et al., 1998; Tavis et al., 1998). The fourth domain of HBp possesses its RNase H activity (Chang et al., 1990; Radziwill et al., 1990). This domain also plays a key role in HBV genome replication. In case of the putative conserved domains of the partial sequences of LC152751.1, multi domains: reverse transcriptases (RTs) from Rtvs (cd01645); DNA polymerase (vir-al) C-terminal domain (pfam00336) and reverse transcriptase (RNA-dependent DNA polymerase) (pfam00078) were obtained (Fig. 6).

TCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGATTATCAAGGTATGTT
GCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTATCAAACCTTCGGACGGAAATTGCACGTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGC
CCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTA
TTGGGGGCCAAGTCTGTACAGCATCGTGAGTCCCTGATTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAAAC
AAGAGATGGGGTTACTCTCTGAATTTTATGGGCTATGTCATTGGATGTTATGGGTCATTGCCACAAGAACACATCATACAAAAAATCAAAGAATGT
TTTAGAAAACTTCCTGTTAACAGG
Forward: 58-GGATGTGTCTGCGGCGTTT-38
L669MAYWF:(58-CAGCCCGTTTCTCATGGCTCAGTTTACTAGTGCC-38 **LC152751.1**
M741VAYWF:(58-GGCTTTCAGTTATGTGGATGATGTGGTATTGGG-38)
S3: (Forward, nt752-771): TATTGGGGGCCAAGTCTGTA
Reverse: 58-CAAAAGAAAATTGGTAACAGCGGTA-38

GTTAATGAAAAAAGGAGATTAAAATTAATTATGCCTGCTAGGTTCTATCCTAACCTTACCAAATATTTGCCCTTAGACAAAGGCATTAAACCGTAT
TATCCTGAACATGCAGTTAATCATTACTTTAAAACTAGGCATTATTTACATACGCTGTGAATGCTGGCCATTATATAAGAGAGAAACTACACGCAG
CGCCTCATCTTGTGGGTCACCATATTCTTGGGAACAAGAGCTACAGCATGGGAGGTTGGTCTTCCAAACCTCGATAAGGAAATTGGACGAGTCTTT
CTGTTCCCAATCGTGTGGGATTATTTCCAGATCACCAGTTGGACCGTGCGTTCGGAGCCAACTCAAACAATTCAGACAGGGACTTCAACTCCAACA
AGGATCACCGACCACAGGCAAATCAGGTAGGAGCGGGAGCTTTTGGGCCAGGGTTCACCCCACCACAGGGCGGTCTTGTGGGGTGGAGCCATTAGG
CTCAGGGCGTATTGACAACAGAGCCAGCCGAGCATCCTCGTGCCTCCGCCAATCGGCAGTGAGGAAGACAGCCTACTCCCATCTCTCCACCTCTAA
GAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCACCAAGCTATGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTG
GCTCCAGTTGCGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACCGAACATGGAGA
ACACAACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCCGGGTCTTTCTTGTTGACAAGAATCCTCACAATACCACAGAGTCTAGACTCGT
GGTGGACTTCTCTCAATTTTCTAGGGGGAGCACC
S2: (Forward, nt2816-2835) GTCACCATATTCTTGGGAAC
P3: (Forward, nt3193-3213) **LC152752.1**
CATCCTCAGGCCATGCAGTGG
F1: (sense, nt 56-76, CCTGCTGGTGGCTCCAGTTC
Forward: 58-AGACTCGTGGTGGACTTCTCT-38

CGCCCCGTCGCAGAAGATCTCAATCTCGGGAATCTCAATGTTAGTATCCCTTGGACTCAAAAGGTGGGAAACTTTACTGGGCTTTATTCTTCTACT
GTACCTGTCTTTAATCCCGAGTGGCAAACTCCCTCCTTTCCTCACATTCATTTACAGGAGGACATTATTAATAGATGTCAACAATATGTGGGCCCT
CTTACAGTTAATGAAAATAGGAGATTAAAATTAATTATGCCTGCTAGGTTCTATCCTAACCTTACCAAATATTTGCCCTTAGACAAAGGCATTAAA
CCGTATTATCCTGAACATGCAGTTAATCATAACTTCAAAACTAGGCATTATTTACATACTCTGTGGAAGGCTGGCATTCTATATAAGAGAGAGACT
ACACGCAGCGCCTCATTTTGTGGGTCACCATATTCTTGGGGAACAAGAGCTACAGCATGGAGAGGTTGGTCTTCCAAACCTCGACAAGGCATGGGG
ACGAATCTTTCTGTTCCCAATCCTCTGGGATTCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTC
AACCCCAAAAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCGGGAGCATCCACACCAAGGTTCACCCCTCTACAAGGCAGTCTTCTTGGAGGT
GGAGCCCGTCGGAGCTCCGGGCACATTGCACCACAGCTGCCGGCAGACCCCCCACCGGCGTCCACAAATCTTCCTGCTGGTAGATCGCCTACTCGA
ATATCTCCACCTGTTCCAAACACTGATCCTCACGCCACGCCATGCAACTCCACGACAGTCGACCCTGCTCTGCTAGAGCACAAGTGA
S2: (Forward, nt2816-2835) GTCACCATATTCTTGGGAAC **LC152753.1**
TPR1: Reverse, 50-TCGGGAAAGAATCCCAGAGGATTGG-30 at nt 2933-
2909

CCTTCGATAATGGCATTAAACCCTATTATCCTGAACATGCAGTTAATCATTACTTTAAATCTAGGCATTATTAACATACTCTGTGAATGCTGGCCA
TTATATAAGAGAGAAACTACACGCAGCGCCTCATCTTGTGGGTCACCATATTCTTGGGAACAAGAGCTACAGCATGGGAGGTTGGTCTTCCAAACC
TCGATAAGGAAATTGGACGAGTCTTTCTGTTCCCAATCGTGTGGGATTATTTCCAGATCACCAGTTGGACCGTGCGTTCGGAGCCAACTCAAACAA
TTCAGACAGGGACTTCAACTCCAACAAGGATCACCGACCACAGGCAAATCAGGTAGGAGCGGGAGCTTTTGGGCCAGGGTTCACCCCACCACAGGG
CGGTCTTGTGGGGTGGAGCCATTAGGCTCAGGGCGTATTGACAACAGAGCCAGCCGAGCATCCTCGTGCCTCCGCCAATCGGCAGTGAGGAAGACA
GCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACATTCCACCAAGCTATGCTAGATCCCAGAGT
GAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCGACTACTGCCTCACCCATATCGTCAATCTTCTCGAGGAC
TGGGGACCCTGCACCGAACATGGAGAACACAACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCCGGGTCTTTCTTGTTGACAAGAATCCT
CACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTACTAGGGGGAGCACCCACGTGTCCT
S2: (Forward, nt2816-2835) GTCACCATATTCTTGGGAAC **LC152757.1**
P3: (Forward, nt3193-3213)
CATCCTCAGGCCATGCAGTGG
F1: (sense, nt 56-76, CCTGCTGGTGGCTCCAGTTC)

CTATCCTAACCTTACTAAATATTTGCCCTTAGACAAAGGCATTAAACCGTATTATCCTGAACATGCAGTTAATCATTACTTCAAAACTAGGCATTA
TTTACATACTCTGTGGAAGGCTGGCATCCTATATAAGAGAGAAACTACACGCAGCGCCTCATTTTGTGGGTCACCATATTCTTGGGAACAAGAGCT

ACAGCATGGGAGGTTGGTCTTCCAAACCTCGAGAAGGAATCCGGACGAGTCTTTCTGTTCCCAATCCTCTGGGATTGTTTCCCGATCACCAGTTGG
ACCCTGCGTTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACTCCAACAAGGATCACCGACCAGAGGCAAATCAGGTAGGAGCGGGAGCAT
TCGGGCCAGGGTTCACCCCACCACACGGCGGTCTTGTGGGGTGGAGCCTTTAGGCTCAGGGCGTATTGACAACAGTGCCAGCCGCACCTCCTCCTG
CCTCCGCCAATCGGCAGTCAGGAAGACAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAACAT
TCCACCAAGCTATGCTAGATCCCAGAGTGAGGGGCCTATATTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCGACTACTGCCT
CACCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACCGAACATGGAGAACACAACATCAGGATTCCTAGGAGCCCTGCTCGTGTTACAGG
CGGGGTTTTACTTGTTGACAAGAATCCTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAGCACCCACGTGTC
CTGGCCAAATTTCGCAGTCCCCAACCTCCAATCAC
S2: (Forward, nt2816-2835) GTCACCATATTCTTGGGAAC **LC152759.1**
P3: (Forward, nt3193-3213)
CATCCTCAGGCCATGCAGTGG
F1: (sense, nt 56-76, CCTGCTGGTGGCTCCAGTTC

Figure 2. Primers of surface protein genes recorded in the partial sequences of HBV strains belonging to *pol* gene of HBV.

Mutations in the catalytic domain of the polymerase gene can affect the amino-acid sequence of the envelope protein (HBsAg) (Bartholomeusz et al., 1998). In particular, the genetic sequence for the neutralization domain of HBV known as a determinant, which is found within the HBsAg and located between amino acids 99 and 169, 5 actually overlaps the major catalytic regions of the viral polymerase protein from amino acid 454 to 524 and known as domains A and B. The amino acid sequence deduced from the overlapping polymerase gene showed a substitution of serine to threonine at position 413 in reverse transcriptase domain, due to the above T-A substitution. One can conclude that the presence of virus in some HBV-infected blood samples collected from different regions of KSA was serologically and molecularly confirmed by ELISA and Rt-PCR techniques. The nucleotide sequence of *pol* gene was determined and bioinformatically analyzed, and therefore, differences at the levels of DNA and encoding amino acids between the strains under investigation and those similar in GenBank and types of domains, its accession, and superfamily of the sequenced *pol* gene were reported.

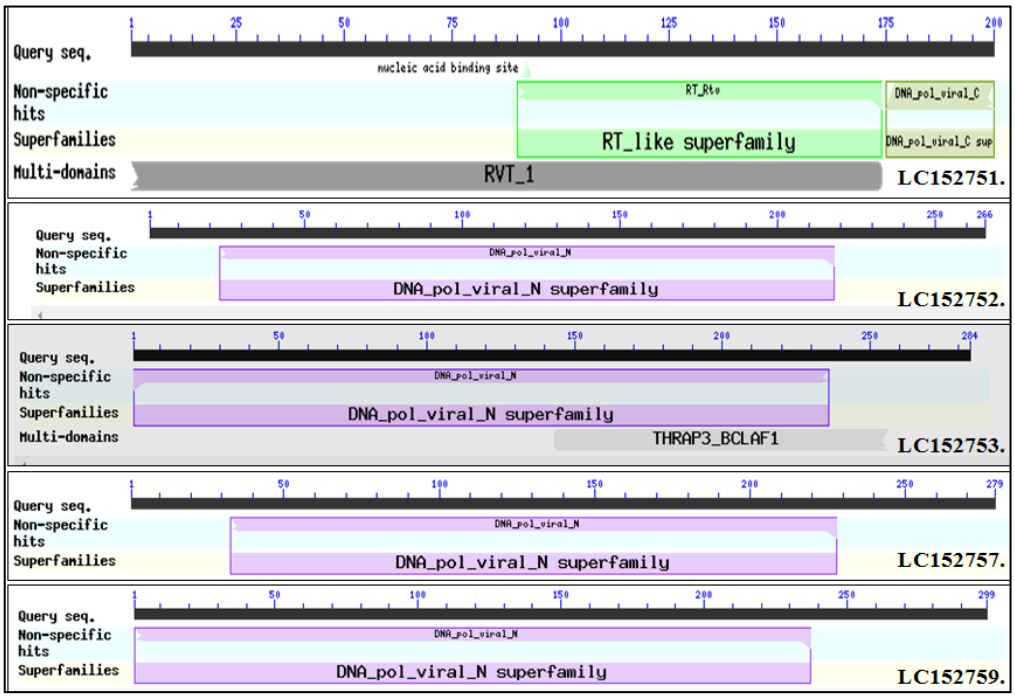


Figure 3. Putative conserved domains of the ORF number 1 of the partial sequences of HBV strains belonging to DNA_pol_viral_C and DNA_pol_viral_N domains.

gi 118896	1 .[161].LYLRKNQTTLTFKGKPYSWEHR.[2].VQHNG.[12].SSMVACSGHLLHNH.[25].RTGLCSYKQIQTDRL	256
LC152752.1	1 .[22].LYKRETTRSASSCGSPYSWEQE LQHGR.[11].ESFCSQSCGIISRS PVGPCVRSQLKQFRQ	89
gi 82008114	1 .[156].LYKRETTRSASFCGSPYSWEQE LQHGA EPVCQQSLGILPRA SVGSPVQSQLKQSRL	212
gi 81967260	1 .[156].LYKRESTRSASFCGSPYSWEQE LQHGS.[11].ESLCAQSTGILSRP SAGSSFQSKFQQSRL	223
gi 81934952	1 .[160].LYLRKNQTTLTFKGKPYSWGHR.[2].EQHNG.[12].SSMVASSGHILHKQ.[25].RTGGSVREKIQTNRL	255
gi 82005168	1 .[156].LYKRETTRSASFFGSPYSWEQE LHHGA.[12].ESFHQQSTRIFSRA PVGPCIQSKHQQSRL	224
gi 123844506	1 .[156].LYKRETSRSASFCGSPYTWEQD LQHGA.[10].EPFHQQSSRIPSRS PVGPSIQSKYQQSRL	222
gi 82001533	1 .[156].LYKRETTHSASFCGSPYSWEQE LQHGA ESFHQQSSGILSRP PVGSSLQSKHRKSRL	212
gi 81946769	1 .[158].LYKRETTRSASFCGSPYSWEQE LHHGR.[11].EPFCSQPSGILSRS SVGPCIRSQFKQSRL	225
gi 75554236	1 .[156].LYKRETTRSASFCGSPYSWEQE LQHGR.[11].ESFCSQSSGILSRA SVGPRDRSQHKQSRL	223

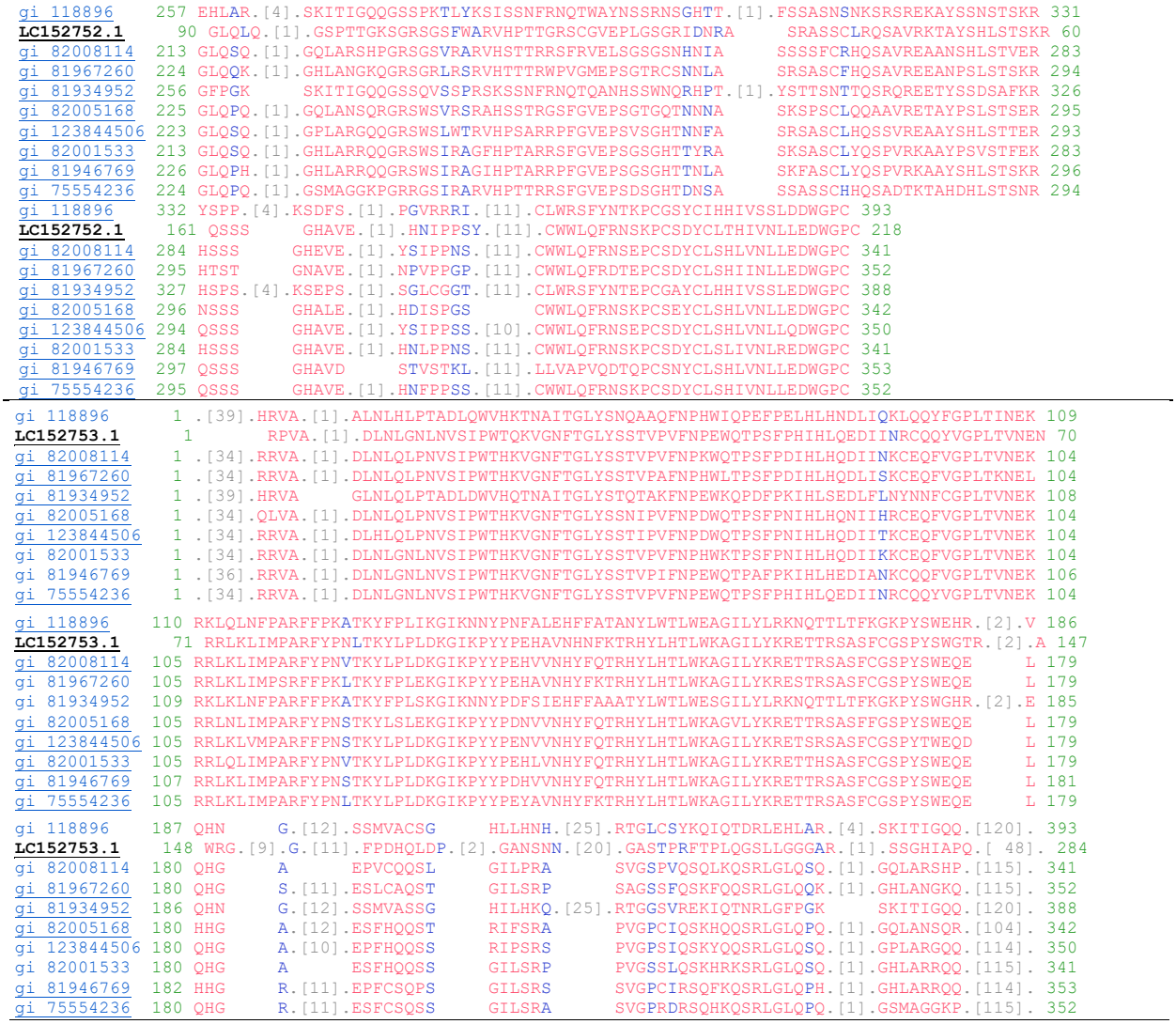


Figure 4. The superfamily (cl02825) of DNA_pol_viral_N domain of ORF number 1 of partial sequence of HBV strains LC152752.1 and LC152753.1 *pol* gene which belongs to the DNA polymerase (viral) N-terminal domain (pfam00242).

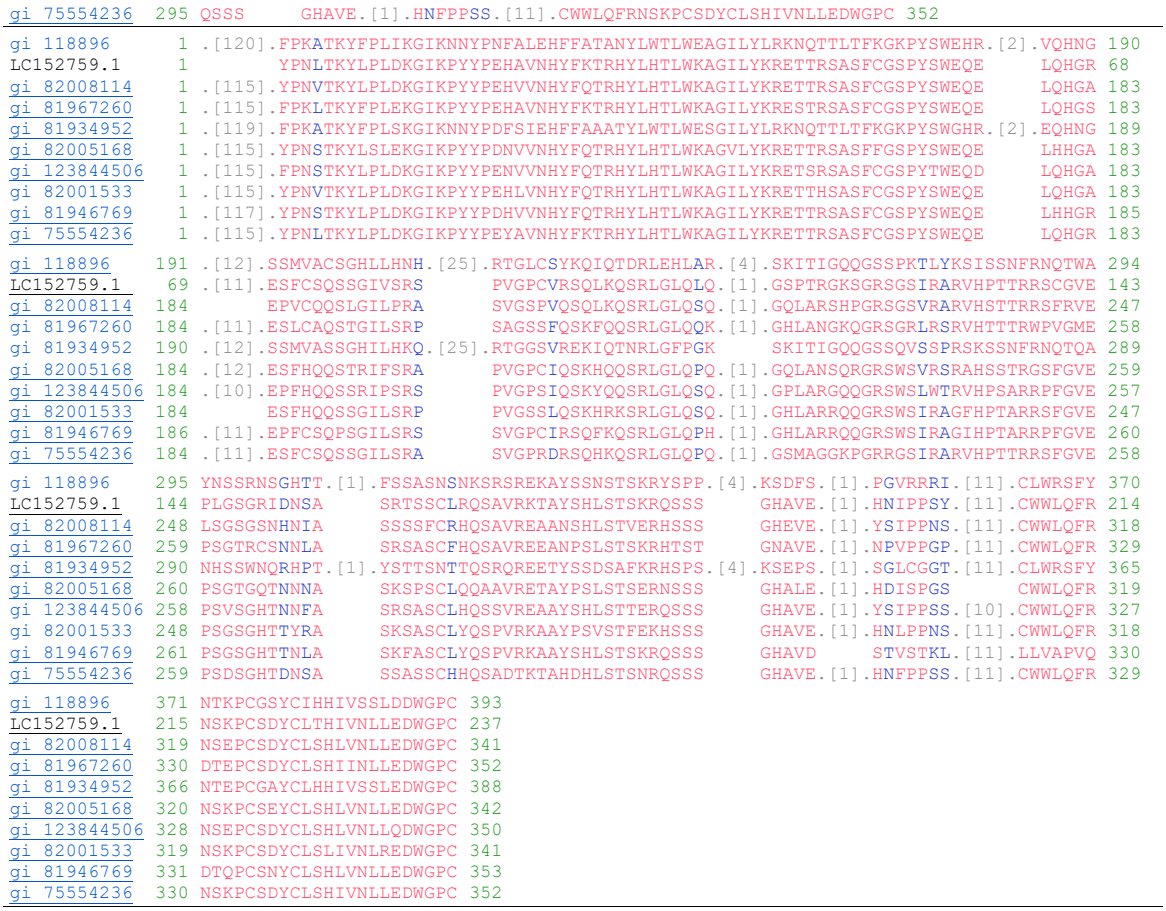
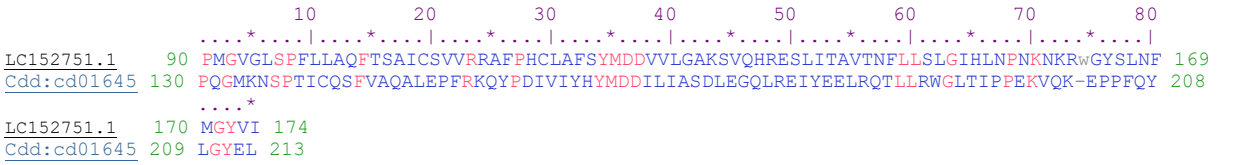


Figure 5. The superfamily (cl02825) of DNA_pol_viral_N domain of ORF number 1 of partial sequence of HBV strains LC152757.1 and LC152759.1 *pol* gene which belongs to the DNA polymerase (viral) N-terminal domain (pfam00242).

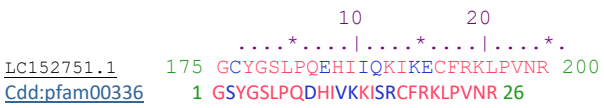
Name	Accession	Description	Interval	E-value
RT_Rtv	cd01645	RT_Rtv: Reverse transcriptases (RTs) from retroviruses (Rtvs). RTs catalyze the conversion of ...	90-174	1.77e-09

Pssm-ID: 238823 [Multi-domain] **Cd Length:** 213 **Bit Score:** 53.83 **E-value:** 1.77e-09



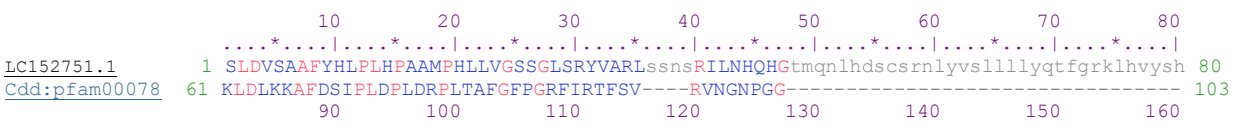
Name	Accession	Description	Interval	E-value
DNA_pol_viral_C	pfam00336	DNA polymerase (viral) C-terminal domain;	175-200	1.92e-08

Pssm-ID: 144068 **Cd Length:** 245 **Bit Score:** 51.46 **E-value:** 1.92e-08



Name	Accession	Description	Interval	E-value
RVT_1	pfam00078	Reverse transcriptase (RNA-dependent DNA polymerase); A reverse transcriptase gene is usually ...	1-174	4.15e-20

Pssm-ID: 249567 [Multi-domain] **Cd Length:** 196 **Bit Score:** 83.92 **E-value:** 4.15e-20



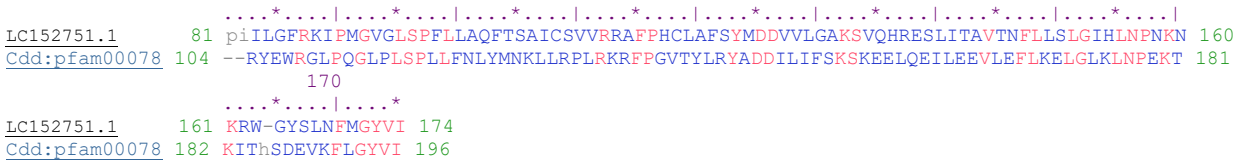


Figure 6. The superfamilies of DNA_pol_viral_N domains of ORF number 1 of partial sequence of HBV strain LC152751.1 *pol* gene which belongs to the DNA polymerase (viral) C-terminal domain.

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