

*Full Length Research Paper*

# Novel mutations of hepatitis B virus surface antigen genotype D among chronic Egyptian patients

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**Hepatitis B virus (HBV) is a major health problem in Egypt. Mutation studies concerning molecular identification of HBV genotypes/sub genotype are still limited. 100 HBV isolates obtained from Egyptian HBV chronic patients were sequenced to identify the full-length sequence of HBV surface antigen (HBsAg) gene. Obtained sequences of HBsAg isolates were submitted to Gene Bank database under accession numbers HM014049 and HM014050. Phylogenetic analysis performed between them and other HBV genotypes (A-I) verified that our isolates belong to genotype D and more specifically to sub genotype D1. Their amino acid alignments illustrated several missense mutations at HBsAg gene compared to other previously identified Egyptian isolates. These results are important for the identification of HBsAg antigenicity. In addition, if these recent results are compared with the results of other researchers working all over the world it could help for explaining the increased risk of hepatocarcinoma (HCC) in Egypt.**

**Key words:** Hepatitis B surface antigen, Egyptian HBV genotype, HBV surface antigen mutations, HBV genotype D mutations.

## INTRODUCTION

Many countries in the world suffer a great financial load from the high percentage of chronic Hepatitis B virus (HBV), which is considered as public series health problem. HBV infection can cause acute and chronic liver diseases, ranging from liver cirrhosis to hepatocellular carcinoma (HCC) (Okamoto et al., 1988; Norder et al., 1994). Human HBV is high compact circular double stranded DNA containing four overlapping open reading frames (ORFs) encoding the large S, core, polymerase, and x protein (Stuyver et al., 2000). Presently, eight genotypes of HBV (A-H), have been identified based on sequence divergence of greater than 8% over the entire HBV genome (Arauz-Ruiz et al., 1997; Tong, et al., 2010; Chu et al., 2003; Miyakawa and Mizokami, 2003). Genotypes are further categorized into subgenotypes

based on nucleotide divergence between 4 and 8% (Schaefer, 2005). Some of the genotypes could map to a special geographic distribution. Genotype A is prevalent in sub-Saharan Africa, North America and Europe (Kimbi et al., 2004), B and C in Asia (Michitaka et al., 2006), E in Africa, and F and H in Central and South America (Sanchez et al., 2002; Kato et al., 2004). Genotypes D and G, on the other hand, seems to be scattered worldwide (Stuyver et al., 2000; Kato et al., 2001; Sanchez et al., 2002; Vieth et al., 2002; Chu et al., 2003; Bahri et al., 2006). In addition to these acknowledged genotypes, there are putative genotypes that could not be classified into the previous groups. Such variants include genotype I (Santos et al., 2010) and J (Tatematsu et al., 2009). Prior to the definition of genotypes, HBV strains were classified into nine serotypes, according to the different serological analysis results of surface antigen (Norder et al., 1992).

Genotyping of HBV is important to clarify the route and pathogenesis of the virus. In particular, the examination

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**Table 1.** Primers for HBsAg full length amplification and their positions.

Primer	Primer sequence	Primer length	Nucleotide position
F1	GGGTCACCATATTCTTGGG	19	2859-2878
R1	CCCACCTTAGAGTCCAAGG	19	873-892
F2	GAACAAGAGCTACCGCATGGG	21	2877-2898
R2	CAAGAGACAAAAGAAAATTGG	21	810-789

of sequence diversity among different isolates may differ in their patterns of serologic reactivity, pathogenicity, virulence, and response to therapy. This will help in determining the clinical severity and development of chronic infection (Arauz-Ruiz et al., 1997). HBV genotype C is associated with more severe liver diseases than genotype B (Kramvis et al., 2008; Sugauchi et al., 2004; Banerjee et al., 2006; Huy et al., 2006; Sakamoto et al., 2006). Patients infected with genotype D appear to have a higher incidence of HCC (Chan and Sung, 2006), a higher risk for HBV recurrence, and a higher mortality rate after liver transplantation than patients with genotype A (Buti et al., 2002). In addition, patients with HBV genotypes C and D have a lower response rate to treatment with IFN compared to those with genotypes A and B (Zöllner et al., 2001). Genotype may also influence the emergence of lamivudine resistance mutations, which appear to be more strongly associated with genotype A than genotype D (Wen, 2004).

HBV genotype D is widespread and was segregated into either four subgenotypes (Norder et al., 2004), five sub genotypes (Tallo et al., 2008) or eight subgenotypes (Chekaraou et al., 2010). Geographical distribution of these sub genotypes are so far less defined than those for other genotypes (Tallo et al., 2004). In Egypt, although HBV sequences among HBV carriers have not been evaluated sufficiently (Saudy et al., 2003), most serological studies reported that HBV genotype D is the predominant HBV genotype (37.1%) followed by genotype B that constituted 25.7% and mixed D/B infections in 15.7% of patients. HBV genotypes A and C infections were the less observed constituting 10% and 8.6% respectively of the total infections. Children with pediatric malignancies have a relatively high prevalence of mixed A/D genotype infections (Zekri et al., 2007). The association of HBV and liver cancer is well documented. The burden of HCC has increased in Egypt with a doubling in the incidence rate in the past 10 years (Lehman and Wilson, 2009).

HBsAg are able to induce protection against HBV infection and therefore can be used as vaccines. The three regions of complete HBsAg (large S, pre S2, and pre S1) share the C-terminal 226 amino acid residues (Szmuness et al., 1981; Carman, 1997). The role of HBsAg is related directly to B-cell epitopes where it is the major target of neutralizing antibodies to HBsAg. Mutant HBsAg with amino acid substitutions such is known to affect the binding of specific anti-HB antibodies and their

detection by conventional diagnostic assays (Tian et al., 2007). There is a noticed relation between low antigenicity of HBV (lead to HBV reinfection) and increased incidence of HCC in Egyptian HBV chronic patients. We aimed to analyze the complete sequence of HBsAg gene (pre S1, pre S2 and S regions) isolated from Egyptian patients chronically infected with HBV, where HBsAg molecular genotypes/sub genotypes studies in Egypt are still limited. Results of the present sequences gave us an insight about missense mutations compared with previous published Egyptian isolates.

## MATERIALS AND METHODS

### Sample collection

Hundred HBV-positive sera samples were collected from Egyptian patients with HBV infection from different hospitals of Cairo. All patients were positive for HBsAg. The collected sera were divided into aliquots and stored at -70°C until used for DNA extraction, amplification and sequencing steps.

### HBV surface antigen primer design

With the aid of specific software for primer design (Primer 3 software), candidate regions were selected for primer design. We designed four primers for the sequence of HBsAg gene as described in Table 1. A complete genome sequence for all HBV genotypes (A-I) available in Gene Bank were downloaded and aligned. Using Primer 3, primers were selected to match all expected HBV genotypes. Primer length and sequence, GC content, and Tm were considered.

### HBV surface antigen gene amplification

HBV DNA were extracted from 100 patients' sera by using QIAamp DNA blood Mini kit (Qiagen) following the manufacturer's instructions. The complete HBsAg ORF was chosen for amplification. All PCR contamination precautions were observed and negative controls using included sera from subjects with no HBV markers were included. The amplification were carried out in a 100 ul reaction mixture consisting of 10 ul of 10 x PCR buffer, 1 ul of 50 mM MgCl<sub>2</sub>, 200 mM deoxynucleoside triphosphates (dNTPs), 1 ul of 50 pmol of both F1 and R1 primers (Table 1) and 1 U of Amplitaq DNA polymerase. The first round of PCR were covered pre S1, pre S2 and S regions were amplified using the selected primers F1 and R1. The cycling conditions were performed with an initial 5 min of preheating at 95°C, followed by 35 cycles of denaturing for 30 s at 95°C, annealing for 30 s at 49°C, and an elongation for 1 min at 72°C, with a final extension period for 10 min at 72°C. Nested PCR were performed using the F2 and R2 primers (Table 1) and PCR products were obtained from the first round

**Table 2.** Nucleotide differences between HBsAg genes of HM014049 and HM014050 after alignment represented by their nucleotide positions.

Present study isolates accession number	Nucleotide position									
	Pre S1					PreS2		S		
	25	36	264	286	346	385	733	755	889	955
HM014049	A	O	G	T	C	A	G	A	G	C
HM014050	O	A	T	C	T	G	C	C	A	A

amplification as templates. The second round of amplification was performed with an initial 5 min preheating at 95°C, followed by 35 cycles 95°C of denaturing for 30 s at 95°C, annealing for 30 s at 55°C, and an elongation for 1 min at 72°C, with a final extension period for 10 min at 72°C. Approximate concentration and length of either HBV DNA or PCR products were determined after each extraction and amplification steps by electrophoresis in a 2% agarose gel stained with Ethidium bromide and visualized under ultra-violet light (Stuyver et al., 2000).

### Sequencing of PCR products

PCR fragments were purified using the QIAquick Gel Extraction Kit. (Qiagen) following the manufacture's instruction. Purified PCR products were sequenced directly using Big Dye terminator cycle sequencing reaction kit version 3.1 (Applied Biosystem, CA). ABI PRISM 3100 Genetic automated analyzer at VACSERA Research Center was used for electrophoresis and data collections (McElhinney et al., 2011).

### Data analysis of sequences and phylogenetic

All forward and reverse sequences were assembled using SeqMan software (Lasergene, DNASTar) and consensus sequences were obtained. Multiple sequence alignment of assembled sequences and other previously published HBsAg sequences were performed using Mega 5.05 software (Wen, 2004). Sequence identity and divergence were calculated based on the number of nucleotide changes per total number of nucleotides analyzed (1170 bp). Phylogenetic tree analysis was performed by using MEGA 5.05 software depending upon the Tamura-Nei model of evolutionary distance, and the topology was evaluated by bootstrap analysis (1,000 replicates) using the neighbor joining method (Tallo et al., 2008).

### Analysis of amino acid HBV surface antigen

Protein coding of pre S1, Pre S2, and S regions were translated into amino acid sequences using the Megaline software (Lasergene, DNASTar). Translations were performed using the standard and universal genetic codes, and were compared to surface antigen of some previously isolated HBV strains which were isolated from Egypt. The accession numbers of the previous Egyptian HBV isolates were AB104709, AB104711, and AB104712, that retrieved from Gene Bank.

## RESULTS

### Primer selection and HBsAg amplification

Two sets of full-length surface antigen primers were

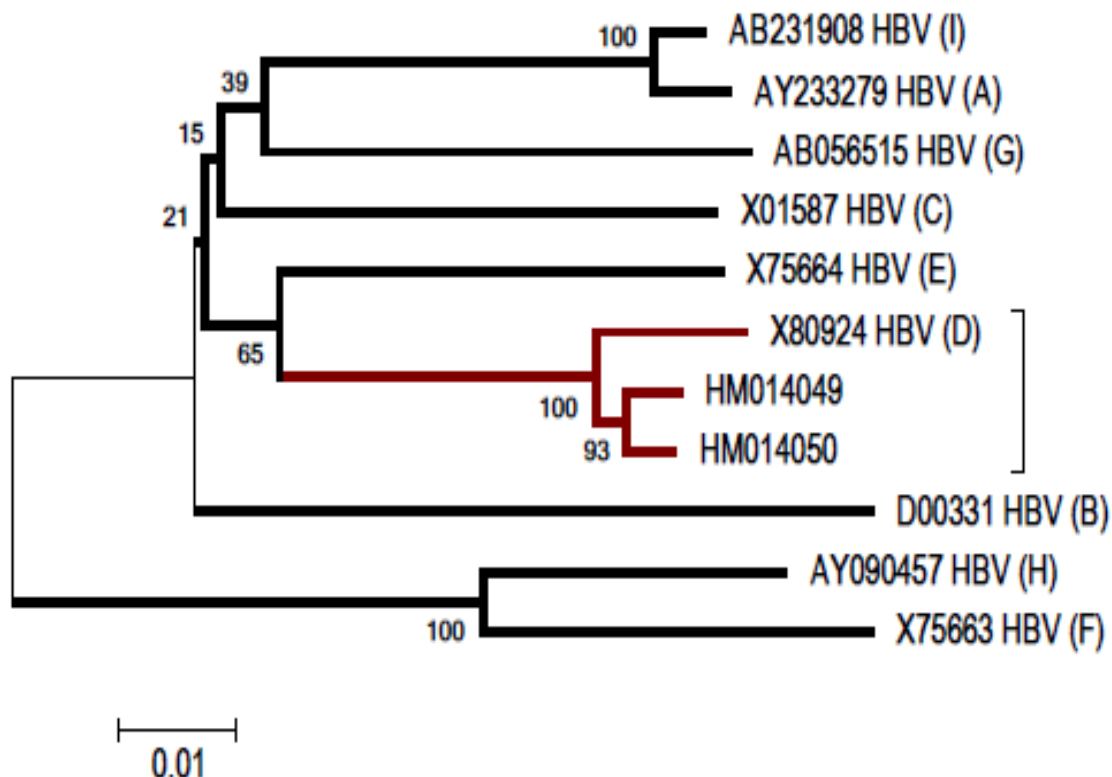
designed with the aid of Primer 3 program. The selected primers were aligned with HBV genotypes A (AY233279), B (D00331), C (X01587), D (X80924), E (X75664), F (X75663), G (AB056515), H (AY090457) and I (AB231908) retrieved from Gene Bank database to detect the position of the primers and the expected product lengths for each product. Candidate regions were defined as sites within the desire location that had >17+ bases from 3'end. When primers were tested for its ability to amplify human DNA in Silico PCR, they gave negative results. HBsAg fragments were detected and the PCR reaction products were found to be located at the expected size approximately 1170 bp.

### Analysis of nucleotide sequence of full-length surface antigen of HBV

Genomic heterogeneity analysis of the present HBV isolates were performed, where HBsAg genes were directly amplified, sequenced and aligned to establish the homology among the sequenced fragments. All the positive PCR reaction products were purified and two novel characteristic HBsAg nucleotide sequences mutations were observed among the sequenced isolates and they were submitted to NCBI Gene Bank database under accession numbers HM014049 and HM014050. Alignment between HM014049 and HM014050 sequences showed some nucleotide differences that were verified according to their positions (Table 2).

Phylogenetic tree was generated to analyze the nucleotide heterogeneity of HM014049 and HM014050 isolates against different HBV genotypes (A to I). The tree showed nine distinct clusters corresponding to the HBV genotypes where HM014049 and HM014050 isolates seems to be more related to genotype D with nucleotide identity of 98% (Figure 1). Distance value equal 0.0195 and this result was verified while the distance between HM014049 and HM014050 recorded 0.009, which was closed to intra-genotype distance (Table 3).

In order to extend the study to genotype D subgenotype, another phylogenetic tree was constructed based on the HBsAg entire nucleotide sequence of 90 isolates of different subgenotype of HBV genotype D (including the two HBsAg sequence obtained in the present study). Accession number and the country of



**Figure 1.** Phylogenetic tree constructed by neighbor-joining method (NJ), based on full length of HBsAg gene of the obtained strains HM014049, HM 014050 (red color branch) compared to HBsAg reference sequences retrieved from Gene bank database. Bootstrap values indicate the major nodes as a percentage of the data obtained from 1000 resamplings.

**Table 3.** Pair wise distances between entire nucleotide sequence of HBsAg gene of genotypes A-I, HM014050 and HM014049 generated by MEGA5.05 software. Values represent the mean distances within each genotype.

HBV genotype	HBV (A)	HBV (B)	HBV (D)	HBV (C)	HBV (E)	HBV (F)	HBV (G)	HBV (H)	HBV (I)	Hm014050
HBV(A)										
HBV (B)	0.096									
HBV (D)	0.096	0.101								
HBV (C)	0.085	0.103	0.092							
HBV (E)	0.095	0.107	0.078	0.082						
HBV (F)	0.138	0.148	0.133	0.131	0.135					
HBV (G)	0.083	0.112	0.090	0.089	0.085	0.132				
HBV (H)	0.128	0.138	0.129	0.129	0.127	0.059	0.127			
HBV (I)	0.010	0.095	0.095	0.082	0.093	0.138	0.077	0.126		
HM014050	0.093	0.092	0.019	0.090	0.069	0.131	0.081	0.123	0.090	
HM014049	0.090	0.092	0.020	0.088	0.072	0.133	0.081	0.127	0.089	0.009

The red color represent the closest distance which related to HBV genotype D while the highest distance was represented by green that related to genotype G. The pink represents the distance between the two obtained isolates. The calculation is based on the Maximum composite likely hood distance analysis.

origin denote references sequences. The 90 isolates were grouped into five clusters D1, D2, D3, D4, and D5.

The results show that our obtained strains belong to subgenotype D1. Although isolates of the present study

were clustered in different clades compared with the other Egyptian isolates, they were still closely related (Figure 2).

### Amino acid comparison of different Egyptian isolates

In the present study, the lengths of pre S1, pre S2 and S (ORFs) of HBV isolates were as expected for genotype D, where pre S1 regions contains 108 amino acids (324 bp), pre S2 region 55 amino acids (165 bp) and S region 226 amino acids (678 bp). The common 11 amino acids deletion in the pre S1 region categorization genotype D was observed. Both HM014049 and HM014050 showed nucleotide sequence mutations, which lead to amino acids substitution, compared to previously identified Egyptian HBV strains (AB104709, AB104711, and AB104712). Regarding the pre S1 regions, both HM014049 and HM014050 agreed in one amino acid substitution of Ala22Pro due to change in the nucleotide sequence G65C. In addition, HM014050 showed more unique five amino acid substitutions where amino acids frame shift consisting of four amino acids Asn9Ile, Pro10Leu, leu11Trp, Gly12Glu were observed respectively due to the deletion of A25 and insertion of A36 while Arg88Leu was recorded to be the result of nucleotide mutation G264T (Table 4). In accordance to pre S2 region, both HM014049 and HM014050 showed compatibility of one nucleotide change (C385T). In addition, one more nucleotide change was demonstrated separately for each of the present strains (G346A in HM014049 strain and C391T in HM014050 strain). No pre S2 nucleotide mutations of both HM014049 and HM014050 were associated with any amino acid substitutions (silent mutation) (Table 4). Both of the present isolates reported missense mutations associated to S gene region, where HM014049 isolate showed two amino acid substitution of Ile244Met and Leu252Ile due to nucleotide mutations of C733G and C755A respectively; HM014050 isolate showed amino acid substitution of Met296Ile corresponding to nucleotide mutation of G889A. Otherwise, HM014050 demonstrated one silent amino acid mutation due to change of C955A (Table 4).

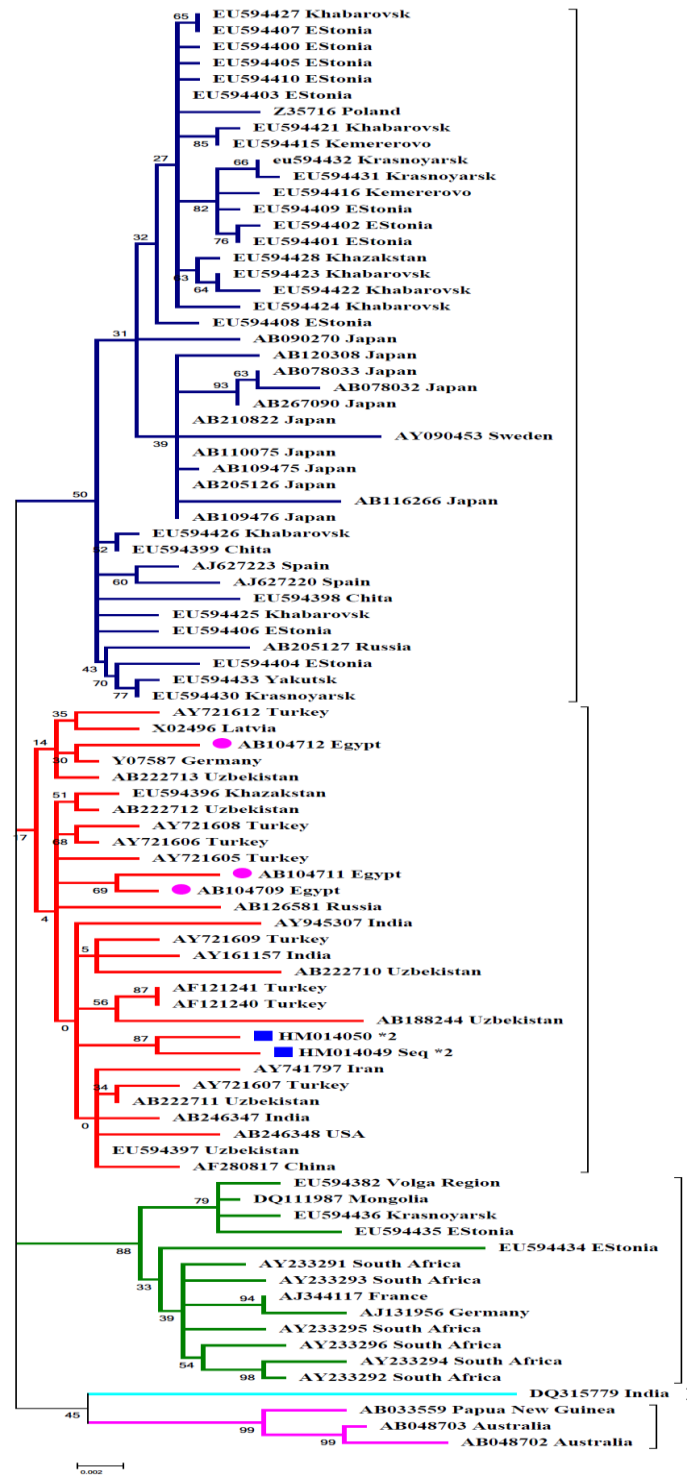
Total amino acid composition of HM014049 and HM014050 were evaluated; it showed slight differences compared to other Egyptian isolates, AB104709, AB104711, and AB104712. No differences were noticed on amino acids composition level of Trp, Gly, Lys, Glu, and Cys among all the Egyptian isolates HBsAg gene including HM014049 and HM014050 (Figure 3). An increase was recorded in amino acids composition levels of Asp, Gly, His, Ile, Thr, Met, and Phe (Figure 4), but decreases were noticed in Try, Val, Ser, Arg, Pro, Asn, Leu, and Ala amino acids level on both or either HM014049 and HM014050 isolates compared to other previous Egyptian strains AB104709, AB104711 and

AB104712 (Figure 5).

### DISCUSSION

HBV is a typical example of a virus that attracts attention with its different nine immunological genotypes, showing special geographic distribution around the world (Kao et al., 2003; Liu et al., 2009). In the context of the findings described in this manuscript, the amplification and sequencing of full-length gene of HBsAg from chronic Egyptian patients lead to isolation of two HBV strains (HM014049, HM014050). Alignments between HM014049 and HM014050 isolates showed slight differences in nucleotide sequences. Phylogenetic tree using complete sequence genome of HBsAg gene showed nine distinct clusters of HBV genotypes from A to I. The present isolates HM014049, HM014050 clustered to genotype D and were established by 0.0195 distance values. The distance between HM014049 and HM014050 was 0.009, which was considered as intra-genotype distance value. Many previous studies that confirmed these results, postulated that genotype D appears to be predominating in the Mediterranean basin and the Middle East countries (Saady et al., 2003; Norder et al., 2004). Also, other serological studies reported that HBV genotype D includes the highest percentage of HBV infection in Egypt followed by genotype B, while the lowest percentage of infections were recorded with mixed D/B genotypes. HBV genotypes A and C infections were less observed (Zekri et al., 2007). Previous study reported that subgenotype of Egyptian HBV strains belonging to D1 compared to D2, D3, D4, and D5 (Tallo et al., 2008). This is confirmed by the present results of pair wise sequence and phylogenetic analysis between HM014049, HM014050, and the 88 isolates of different D subgenotypes retrieved from Gene bank database. The present HBV isolates seems to be more related to others derived from different world wide regions including Turkey, India, Uzbekistan, Iran, China and USA.

The development of DNA sequencing methodologies facilitated the elucidation of amino acid mutation in HBsAg because of nucleotide mutations. Both HM014049 and HM014050 showed nucleotide sequence mutation, which leads to amino acids substitution, compared with previously identified HBV strains infecting Egyptian (AB104709, AB104711, and AB104712). Regarding the pre S1 regions, both HM014049 and HM014050 shared one amino acid substitution with Ala22Pro due to change in nucleotide sequence G65C. Moreover, HM014050 showed more unique five amino acids substitutions where, deletion of A25 and insertion of A36 were an indication of the frame shift of four amino acids Asn9Ile, Pro10Leu, leu11Trp, Gly12Glu respectively, and G265T mutation led to substitution of Arg88Leu. Three silent mutations were reported at the pre S2 region of both HM014049 and HM014050 although three different

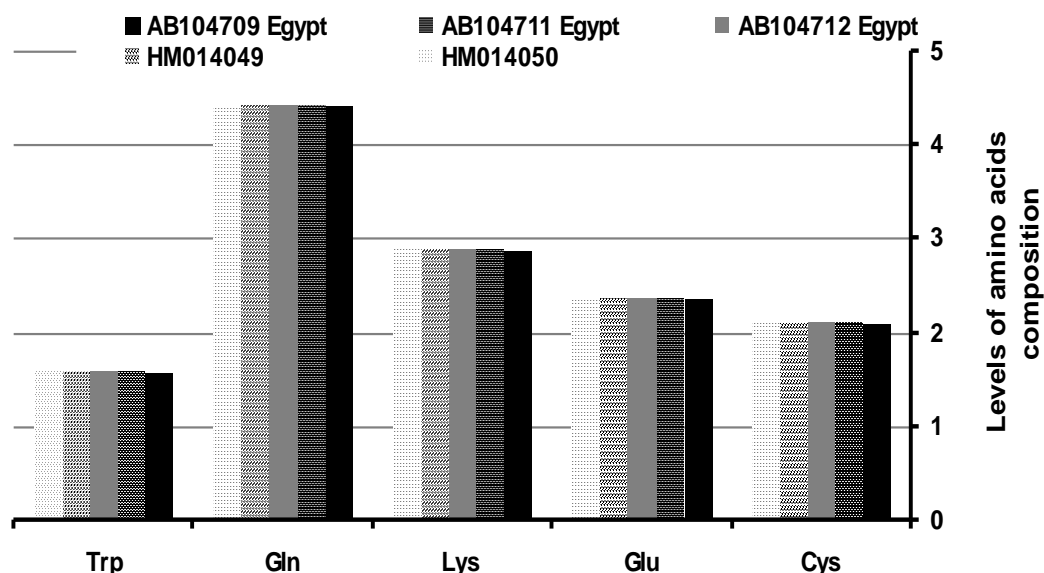


**Figure 2.** Phylogenetic tree constructed by neighbor-joining method (NJ), based on complete HBsAg gene for D subgenotype. The sequences include all available reference sequences, and the representative 88 isolates of subgenotype (D) derived from different regions of the world. All sequences of all reference isolates were retrieved from Gene Bank; blue (D2), green branches (D3), pink branches (D4) and red branches (D1). ■ Represents previous HBV isolates infected Egyptian patients; ● represent the present isolates represented. Bootstrap values indicate the major nodes as a percentage of the data obtained from 1000 resamplings.

**Table 4.** Amino acid residues in HBsAg gene (Pre S1, Pre S2, S) derived from three references sequences, of three Egyptian isolates (AB104709, AB 104711, and AB104712) and two isolates obtained from the present study (HM014049, HM014050).

HBsAg regions	Nucleotide mutation						Amino acid substitution					
	Nucleotide position	AB104 709	AB10 4711	AB104 712	HM014 049	HM014 050	Amino acid position	AB104 709	AB10 4711	AB104 712	HM014 049	HM01 4050
Pre S1	25	A	A	A	A	-	9	Asn	Asn	Asn	Asn	Ile
							10	Pro	Pro	Pro	Pro	Leu
	36	-	-	-	-	A	11	Leu	Leu	Leu	Leu	Try
							12	Gly	Gly	Gly	Gly	Glu
	65	G	G	G	C	C	22	Ala	Ala	Ala	Pro	Pro
	126	A	A	A	G	G	43	Ala	Ala	Thr	Ala	Ala
	255	T	T	T	C	C	86	Thr	Thr	Thr	Thr	Thr
264	G	G	G	G	T	88	Arg	Arg	Arg	Arg	Leu	
286	C	C	C	T	C	96	Leu	Leu	Leu	Leu	Leu	
Pre S2	346	G	G	G	A	G	116	Phe	Phe	Phe	Phe	Phe
	385	C	C	C	T	T	129	Tyr	Tyr	Tyr	Tyr	Tyr
	391	C	C	C	C	T	131	Pro	Pro	Pro	Pro	Pro
S	733	C	C	C	G	C	244	Ile	Ile	Ile	Met	Ile
	755	C	C	C	A	C	252	Leu	Leu	Leu	Ile	Leu
	889	G	G	G	G	A	296	Met	Met	Met	Met	Ile
	955	C	C	C	C	A	318	Ser	Ser	Ser	Ser	Ser

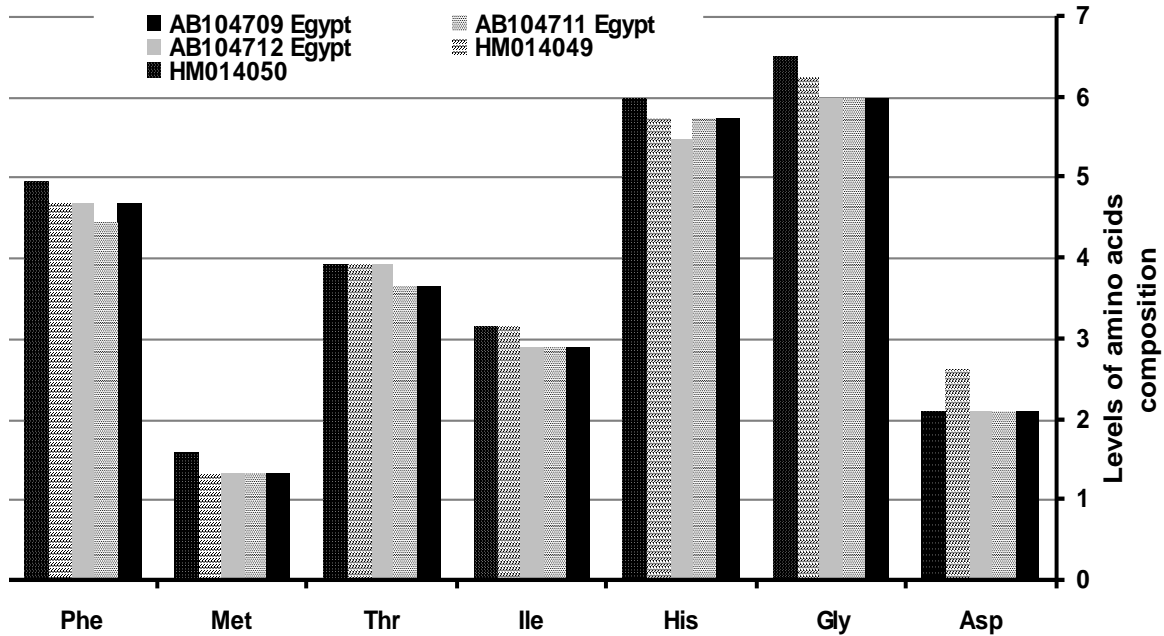
The amino acid sequences were derived from the nucleotide sequences. Subgenotypes- specific substitution are colored with red.



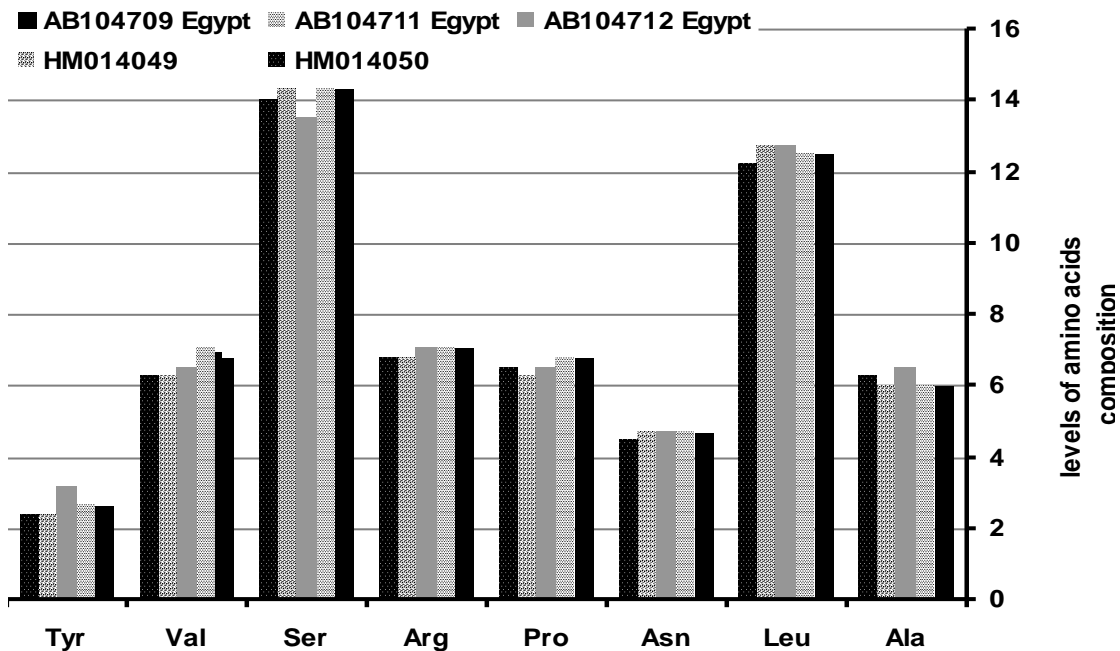
**Figure 3.** Level of Trp, Gln, Lys, Glu, and Cys. HBsAg amino acids showed no differences among HM014049, HM014050 and the previous Egyptian strains. All isolates are represented by their Gene bank accession numbers.

nucleotide mutations were verified. The association between HBV mutations and hepatocarcinogenesis

remains controversial because of conflicting data in the literature (Liu et al., 2009). An increase risk of HCC are



**Figure 4.** Increase in the level of Phe, Met, Thr, Ile, His, Gly, and Asp amino acids composition of HBsAg gene of either or both HM014049 and HM014050 compared to previous strains. All isolates are represented by their Gene bank accession numbers.



**Figure 5.** Decreasing level of Trp, Val, Ser, Arg, Pro, Asn, Leu, and Ala amino acids composition of HBsAg gene of either or both HM014049 and HM014050 compared to previous strains. All isolates are represented by their Gene bank accession numbers.

associated with specific HBV mutations in the Pre S and BCP regions, irrespective of HBV genotypes (Kao et al., 2003; Liu et al., 2006; Lin et al., 2007). The relation

between pre S deletions and hepatocarcinogenesis could be described that pre S deletions decrease the expression of middle and small surface proteins of HBV,



resulting in intracellular accumulation of HBV surface proteins and viral particles, which may contribute to hepatocarcinogenesis by inducing DNA damage (Chen et al., 2006, 2007; Kay and Zoulim, 2007).

In the present study, S gene of HM014049 showed two amino acids substitutions Ile244Met and Leu252Ile, which were directly related to nucleotide mutations C733G, and C755A. HM014050 showed only one amino acid substitution of Met296Ile corresponding to nucleotide mutation of G889A. Mutations can lead to the creation of escape mutants, which can alter group-specific antigenicity (Kohno et al., 1996; Miyake et al., 1996; Kfoury Baz et al., 2011). It can disrupt the antigenicity of HBsAg by modifying amino acids directly involved in expression of the antigen (Rodriguez-Frias et al., 1999). Alterations of the structure of HBsAg can disrupt the binding ability of polyclonal antibodies to it, because they contain several epitopes for T or B cells. S mutants emerge during chronic HBV infections, often in patients treated with interferon, and may represent the way by which the virus overcomes host immune responses (Roznovsky et al., 2000; Seddigh-Tonekaboni et al., 2001; Wakil et al., 2002). Some mutations involve amino acid insertions into the determinant (Hou et al., 2001; Weinberger et al., 2000) or the creation of nonsense mutations (Rodriguez-Frias et al., 1999; Thuyle et al., 2005). Existence of HBV quasi-species (Schätz et al., 1997) has facilitated the development of mutants with specific ability to escape antibody detection and antibody neutralization. These mutants may lead to re infection, because it replicates through an RNA intermediate synthesized by reverse transcriptase of viral genomes (Kreutz, 2002; Ohishi et al., 2004) and quasi-species are generated (Torresi, 2002; Liu et al., 2002; Hsu et al., 2004). This results in the production of viral mutants during naturally occurring infections (Chong-Jin et al., 1999). In this study we investigated (for the first time) HBsAg genes isolated from HBV chronic Egyptian patients. The sequence results obtained from isolates showed two different sequences (accession numbers: HM014049, Hm014050). Analysis of sequence results showed several missense mutations at the HBsAg gene compared to other previous isolates retrieved from gene Bank database. These HBV isolates clustered with other isolates derived from Turkey, India, Uzbekistan, Iran, China, and USA. The obtained results could be combined with the results of other researchers working all over the world to explain either the increasing incidence of HCC in Egypt (Zekri, et al., 2007) or the disruption of the antigenicity of HBsAg (Shaaban et al., 2007).

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