



Persons with Intellectual Disability: A Potential Reservoir of Invasive Strains of Hepatitis B Virus

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Abstract

Background: A higher prevalence of hepatitis B virus (HBV) infection has been reported in persons with intellectual disability as well as the nurses working in closed institutions compared to the general population.

Objectives: In the present study, the serological and molecular markers of HBV infection in individuals with intellectual disability of closed institutions were investigated.

Methods: Blood samples were derived from 400 persons with intellectual disability living in six institutions in Tehran and tested for HBsAg and HBcAb. Nested PCR, direct sequencing, and phylogenetic analysis were performed to determine the HBV genotypes and mutational patterns of HBsAg. Also, HBsAb was tested for HBV DNA positive cases.

Results: Twenty-eight (7.0%) patients were positive for the HBsAg serological test. Furthermore, six HBV occult cases were identified. In total, out of 41 patients with HBV infection markers, 26 cases were positive for HBV DNA. Of these patients, 15 full-length HBsAg were successfully amplified and sequenced. All strains belonged to genotype D and subtypes ayw2 and ayw3. These 15 isolated strains carried several immune escape mutants in the S genes. Surprisingly, mutations related to antiviral resistance were detected in the overlapped pol genes of strains isolated from naïve-treatment patients.

Conclusions: The observed frequency of HBV infection in individuals with intellectual disability was higher than the reported estimation of HBV infection in Iranian blood donors and the general population. All HBV isolates from these patients represented a homogenous genotype and corresponded with other reported strains from Mediterranean countries. The high frequency of immune escape strains, despite vaccination and detection of identical mutational patterns in different genes, might indicate that persons with intellectual disability have shared vaccine-escape and drug-resistant HBV strains.

Keywords: Hepatitis B Virus, Intellectual Disability, Phylogenetic Analysis, Genotype, Hepatitis B Surface Antigen, Occult Hepatitis B

1. Background

Even half a century after the development of the hepatitis B virus (HBV) vaccine, HBV infection is still a serious public health problem. HBV is recognized as one of the seven known human oncogenic viruses and is responsible for one of the highest yearly mortality rates (1). A high genome diversity allows HBV to evade the host immune system, develop resistant mutations against antiviral therapy (at P gene) and vaccination, and generate diagnosis-escaping variants (at the S gene) (2). Of note, naturally occurring mutations associated with drug resistance have been detected in treatment-naïve individuals and can be

transmitted from person to person (3). These biological characteristics of HBV pose major challenges for the elimination of the virus. Therefore, HBV control is currently a priority to public health authorities. To this end, screening, vaccination, and monitoring of infected cases in high-risk groups is strongly recommended (4-6).

HBV is a blood borne virus and is transmitted through perinatal, sexual and parenteral/percutaneous routes. The high stability of HBV at room temperature (compared to other blood-borne viruses) (7), increases the chance of transmission through close contact in overcrowded places like prisons, refugee camps, and healthcare facilities (8-11).

Furthermore, the risk of transmitting HBV is further enhanced by poor sanitation and aggressive behaviour in institutions for people with intellectual disability (12). Besides patients, also treating nurses/physicians are at risk of getting infected infection; especially people taking care of aggressive patients (13).

In Iran, the implementation of the national vaccination against HBV has significantly decreased the HBV prevalence (14). Recent epidemiological studies among the Iranian general population and blood donors reported the circulation of HBV genotype D in chronic carriers (14). Despite a few studies performed in high-risk groups, there is a lack of data characterizing the HBV strains circulating in persons with intellectual disability in Iran.

2. Objectives

In this study we applied molecular analysis to determine the seroprevalence of HBV in individuals with intellectual disability. For the first time, this report will provide valuable information of HBV infection among this vulnerable population in Iran.

3. Methods

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences with approval number 25-1580-1393-122 and is in accordance with the Helsinki declaration of 1964. Individuals with intellectual disability from six different institutions in Tehran, Iran, were included in this study.

Inclusion criteria were defined based on census-based sampling. Informed consent was obtained from patient's parents or their guardian. Blood samples were taken after obtaining permission of the center director and based on the patient readiness for sampling. For instance, in some cases the patient mental state and aggressiveness could impede the sample collection. Individuals recruited in this study were semi-private and protected patients that suffered from mental and physical retardation. Institutionalized patients received treatment such as physiotherapy, occupational therapy and speech therapy for the intellectual disability patients. The patients had been admitted to the center by their families or without family supervision.

Blood samples were collected during 2011 - 13. Demographic data including age, sex, type of disability, period of visit to the centre, and HBV vaccination history were obtained through a questionnaire. HBV vaccination was reported by evaluating the patient files. If there was no record of HBV vaccination or incomplete vaccination, the

patient was reported as HBV vaccination naive. If no vaccine card was available, the patient was recorded as unavailable data for vaccination.

HBV serological markers, including HBsAg and HBcAb, were determined for all patients, while HBsAb was only checked for HBV DNA positive cases (bioELISA, Spain, Barcelona). Viral DNA was extracted from sera using the QIAmp® Viral DNA mini kit (QIAGEN, Hilden, Germany) in patients who were either HBsAg or HBcAb positive. To amplify the complete HBsAg gene, an in-house nested PCR technique was employed (15). Bi-directional sequencing was conducted using the ABI PRISM Big Dye® Terminator cycle sequencing reaction kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3130 automated sequencer. Primer-walking was performed and a consensus sequence was generated by assembling the S gene segments using the SeqMan II® software (DNASTar Inc., Madison, Wisconsin, USA). Sequences were submitted to NCBI GenBank under accession numbers KY225828-KY225842. Possible recombination events were investigated using the SimPlot software, version 3.5.1. A nucleotide BLAST and a web-based HBV genotyping program were used to determine HBV genotypes. To confirm the genotyping results, a phylogenetic analysis was performed. HBV S gene sequences were extracted from NCBI GenBank and aligned using ClustalW, version 1.83. A neighbour joining tree was constructed under the Kimura 2-parameter substitution model using MEGA software V6. The reliability of the pairwise comparison and the phylogenetic tree analysis were assessed by bootstrapping using 1000 replicates. Genotypes were determined by comparing the full-length HBsAg to the NCBI reference sequences representing all HBV genotypes and subgenotypes. HBV subtypes were assigned based on the amino acid sequences of the surface antigen region. Data analysis was performed using SPSS (Ver. 17.0, Chicago, IL) and R software (version 2.10.1). Categorical data were compared using the chi-square or Fisher exact test as appropriate. Numerical variables were analysed using the independent sample *t*-tests. Statistical significance was considered at two-sided $P < 0.05$.

4. Results

Samples were collected from 215 (53.8%) males and 185 (46.3%) females who were hospitalized for the duration of 1 to 30 years (mean \pm SD: 11.1 ± 4.9 years). The studied population included 293 (73%) persons with intellectual disability, 53 (13.0%) patients with Down's syndrome, 42 (10.5%) patients with cerebral palsy (CP), and 13 (3.2%) patients with autism. The mean \pm SD age of hospitalization was 4.53 ± 3.22 (0 - 13 years). HBV vaccination status was positive, negative, and unavailable in 194 (48.5%), 56 (14.0%), and 150

Table 1. Demographic, Serological, and Clinical Data of the Studied Population

Centers	Patients in Each Center, N	Age, y (Mean \pm SD)	Male, No. (%)	Female, No. (%)	HBsAg Positive, No. (%)	Anti-HBc Positive, No. (%)	Vaccination, No. (%)	Occult Infection, No. (%)
1	21	9.7 \pm 2.5	14 (67)	7 (33)	2 (9)	4 (19)	21(100)	2 (9.5)
2	51	11.9 \pm 2.9	23 (45)	28 (55)	6 (11)	7 (14)	37 (73)	0 (0)
3	99	11.3 \pm 2.8	65 (66)	32 (34)	2 (2)	7 (7)	74 (75)	1 (1)
4	64	4.0 \pm 2.3	25 (39)	39 (61)	8 (13)	8 (13)	48 (75)	1 (2)
5	144	12.6 \pm 3.4	78 (54)	66 (46)	6 (4)	10 (7)	13 (9)	1 (1)
6	23	20.4 \pm 5.6	10 (43)	13 (57)	4 (17)	5 (22)	1 (4)	1 (4)

Table 2. Association of Hepatitis B Serum Markers and HBV DNA with Patient's Demographic and Clinical Characteristic

Laboratory Findings	Age, y	P	Gender (M/F)	P	Years of Attendance	P	Clinical Manifestation [Down, CP, MR, Otism], No. (%)	P	History of Vaccination, No. (%)	P
HBsAg positive	11.18 \pm 6.39	0.96	12/16	0.28	5.32 \pm 4.17	0.18	4 (8), 7 (17), 20 (7), 1 (8)	0.17	20 (8)	0.6
HBsAg negative	11.14 \pm 4.83		199/173		4.48 \pm 3.13		49 (92), 35 (83), 276 (93), 12 (92)		230 (92)	
HBcAb positive	11.76 \pm 5.61	0.40	22/19	0.93	5.13 \pm 4.11	0.19	5 (9), 9 (21), 30 (10), 1 (8)	0.19	27 (11)	0.3
HBcAb negative	11.08 \pm 4.82		190/169		4.45 \pm 3.09		48 (91), 33 (79), 266 (90), 12 (92)		223 (89)	
HBV DNA +	12.56 \pm 6.03	0.13	12/14	0.41	5.89 \pm 4.68	0.13	3 (6), 6 (14), 16 (6), 1 (8)	0.17	18 (9)	0.1
HBV DNA -	11.07 \pm 4.80		204/170		4.44 \pm 3.11		50 (94), 36 (86), 276 (94), 12 (92)		232 (91)	

Abbreviations: CP, cerebral palsy; F, female; M, male; MR, mental retarded

Table 3. Demographic Data, HBV Serology and Molecular Markers of Patients Derived HBV Sequenced in This Study

Patient ID	Gender	Age, y	Mental Disease Type	Center	Period of Attendance, y	HBV Vaccination History	HBsAg	Anti-HBsAg; Titer, mIU/mL*	Anti-HBcAg	HBV Genotype	HBV Subtype	Immune Escape Mutations	YMDD Mutation
MR8	F	12	MR	1	2	Vaccinated	Pos	5	Pos	D1	ayw2	Pos	Pos
MR11	M	6	MR	1	1	Vaccinated	Neg	42	Pos	D1	ayw2	Neg	Neg
MR21	M	11	CP	1	2	Vaccinated	Neg	2	Pos	D1	ayw2	Pos	Neg
MR37	F	13	CP	2	3	Vaccinated	Pos	52	Pos	D1	ayw2	Pos	Pos
MR43	F	14	CP	2	11	Vaccinated	Pos	461	Pos	D1	ayw2	Neg	Neg
MR53	F	14	Down	2	11	Vaccinated	Pos	48	Pos	D1	ayw2	Neg	Neg
MR55	F	14	CP	2	10	Vaccinated	Pos	407	Pos	D1	ayw2	Pos	Neg
MR154	M	13	MR	3	2	Vaccinated	Neg	277	Pos	D1	ayw2	Neg	Neg
MR156	M	9	Au	3	3	Vaccinated	Pos	24	Pos	D1	ayw2	Neg	Neg
MR215	F	4	MR	4	1	Vaccinated	Pos	362	Pos	D1	ayw2	Pos	Pos
MR262	M	21	MR	6	13	No history	Neg	4	Pos	D1	ayw2	Pos	Pos
MR264	F	30	MR	6	13	No history	Pos	6	Pos	D1	ayw2	Neg	Neg
MR312	M	5	MR	5	0	No history	Pos	15	Pos	D1	ayw2	Pos	Pos
MR377	M	11	MR	5	5	No history	Pos	276	Pos	D1	ayw2	Pos	Pos
MR399	F	16	MR	5	10	No history	Pos	11	Pos	D1	ayw3	Pos	Neg

hypothesis in our study. In addition, the identical mutational pattern of the isolates and the identification of several occult cases (six out of 15) corroborate this idea. Likewise, the topology of the phylogenetic tree presents a signature of sharing a common ancestor of circulating strains among these patients. Particular behavioural characteristics of retarded patients such as increased incidences of bleeding, fissured or hypertrophied gums or lips, self-mutilation with bleeding wounds, excessive drooling or spitting, and scratches or biting may facilitate intra-

group viral transmission (12). In literature, transmission of HBV (wild or mutants strains) through close contacts has been documented. For instance, a study performed in the USA (28) reported the transmission of HBV and other blood borne viruses (e.g. HIV) among mentally ill residents at a long term care facility. Confirming horizontal transmission events requires meticulous phylogenetic analysis by using HBV full-length genomes (7). However, the low sample volume/availability and low viral loads restricted this analysis. HBV infection among different centers varied sig-

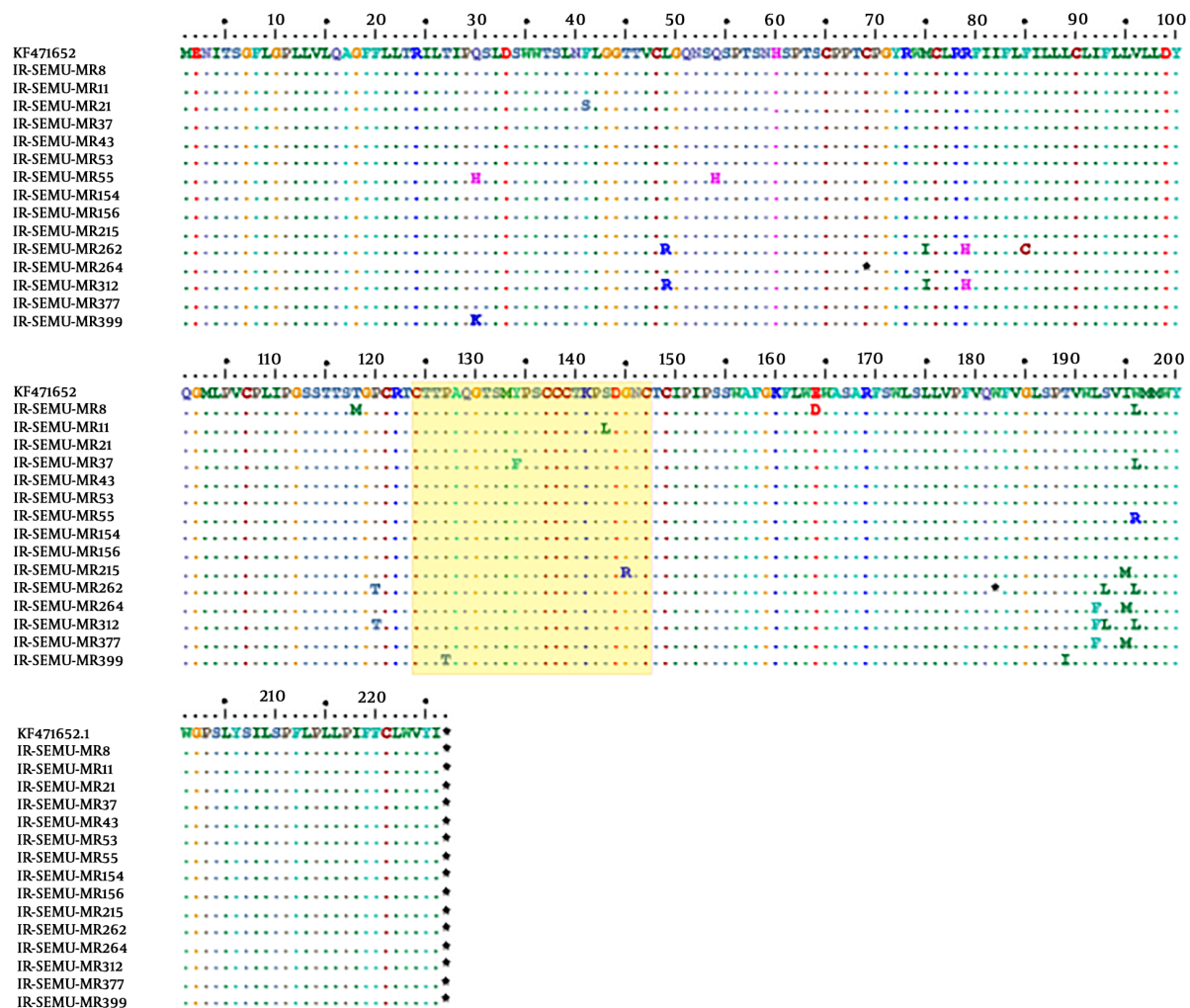


Figure 2. Distribution of amino acid mutations detected within the HBsAg among mentally retarded infected with HBV, the amino acid sequences represent positions at the 99-170 of the HBsAg is considered as Major Hydrophilic Region (MHR). The amino acid variations in the "a determinant" motif are framed in a yellow box.

nificantly ($P < 0.01$) between 2% - 17%. We could not find any possible cause for this marked difference based on our available data.

The detection of HBV genotype D is in line with previous epidemiological studies in Iran (29, 30) and the neighbouring countries (8, 31, 32). Although HBV D1 is the major subgenotype circulating in Iran (33), partial S gene sequences in the present study did not provide enough data for HBV subgenotyping (34). Previously, it has been shown that HBV subtypes ayw2 and ayw3, which were identified in this study, are associated with subgenotype D1 (35). In addition to the epidemiological role of HBV subtypes, it has been shown that 'ay' has less reactivity than 'ad' in ELISA assays and is accompanied by a lower vaccine response (36).

Single or combined mutations in HBsAg were detected in 73% of the isolates. These mutations can modulate the structure of the HBsAg molecule and help the virus to escape vaccine, immunotherapy, or diagnostic assays (37). In this study, the frequency of naturally occurring MHR mutations was 53.3%, indicating that a significant number of strains carry one or more mutations in this antigenic motif ($P < 0.05$). This rate is much higher than the numbers reported by previous studies involving blood donors (32.8%) (3) and the general Iranian population (17.2%) (38). Noteworthy, half of the mutations in MHR were located in the 'a determinant' region, which is also higher compared to previous studies from Iran (3).

In this study, codon 120 was most frequently mutated.

Table 4. Biochemical Characterization of Amino Acids Within the MHR Region and Their Clinical Importance in Wild Types and Mutants Variants

Position at the HBsAg	Wild-Type Amino Acid	Mutant Amino Acid	Position at "a Determinant"	Affect Serological Diagnosis	Vaccine Escape	Mutant Failure in HBIG Therapy	References
30	Glutamine	Histidine	No	ND	ND	ND	
41	Phenylalanine	Serine	No	Yes	ND	ND	(16)
54	Glutamine	Histidine	No	ND	ND	ND	
69	Cysteine	Stop codon	No	ND	ND	ND	(17, 18)
118	Threonine	Methionine	Yes	Yes	ND	ND	(19)
145	Glutamine	Arginine	Yes	Yes	Yes	Yes	(20)
164	Glutamate	Aspartate	Yes	ND	Yes	Yes	(21)
182	Tryptophan	Stop codon	No	ND	No	No	
195	Isoleucine	Methionine	No	ND	Yes	Yes	(21, 22)
196	Tryptophan	Leucine	No	No	Yes	No	(21, 23)
196	Tryptophan	Arginine	No	ND	ND	ND	

Abbreviation: ND, non-determined

This finding complies with previous studies from Iran (39), Morocco (40), and Serbia (41), where HBV genotype D is the predominant circulating strain. This mutation is mostly detected with HBs antibodies (42), and is responsible for most diagnostic and vaccine escape mutants in the HBV S gene (43). Moreover, we identified a variation in codon 145 (G145R) in one strain. Both mutations (P120T and G145R) are well known as vaccine, immunoglobulin escape causing mutations that can emerge under immunotherapy or antiviral therapies (44). Both can be transmitted vertically and horizontally.

Both upstream and downstream premature stop codons of the S gene can lead to truncated proteins and failure in the HBsAg diagnostic assays (45). Two strains with premature stop codons upstream (sC69*) and downstream (sW182*) were identified in the present study. These mutations were previously reported in Iranian blood donors and cirrhotic patients (17). Surprisingly, one of those strains carried P120T along with sW182* and was presented as HBsAg negative in the serological assay. However, there was no significant correlation between identified mutations in isolated strains from occult or overt cases.

The development of antiviral resistance mutations in HBV polymerase gene can have major clinical consequences. Furthermore, overlap of the polymerase and surface gene leads to variations that affect invasiveness, vaccine efficacy, and the intra-population transmission of the virus. The detection of antiviral resistance in treatment naïve patients that harbour viruses with these mutations increases the public health concern (3). In the present study, eight out of 15 strains developed single mutations as-

sociated with Lamivudine resistance (rtM204V/I). In seven strains these mutations were accompanied by rtL180M mutation, and in one strain together with rtL180M, rtV173L, and rtL80I. Surprisingly, none of our patients had a history of antiviral treatment. This rate (more than 50%) of antiviral resistance mutations in treatment naïve patients is much higher than previously reported (46, 47). This finding highlights the possibility of intra-group transmission of drug resistant variants. We hypothesize that some cases were born to HBsAg-positive mother(s) who were under antiviral therapy and had developed antiviral resistance before delivery. These resistant strains were vertically transmitted to the new-borns. Subsequently, close contact of children in healthcare institutions provided the setting for horizontal transmission. Although antiviral resistance mutations in treatment naïve patients have already been reported in Iran, the present study is the first report of horizontal transmission and with such a high rate. In line with our findings, several reports have demonstrated the transmission of HBV antiviral resistance mutations in high-risk cohorts (48, 49).

Of note, despite the importance of early and complete HBV vaccination of all health care workers, nurses are still at significant risk of developing needle stick injuries and mucocutaneous exposure. Therefore, a continuous training program to raise awareness of viral blood borne infection risks is a necessity among nurses. The knowledge about the nature of HBV disease, prevention, treatment and vaccine availability was unsatisfactory in different studies (50). This limited knowledge among health care workers may increase unsafe practices in the institutions (51).

One important finding in this study was that 81% of HBsAg positive patients were also positive for HBsAb. HBsAg coexistence with HBsAb has been reported previously among chronic hepatitis B patients with HBsAg mutants. However, in the present study 60% of patients, who were positive for HBsAg and HBsAb, were infected by immune escaped mutations. Researchers in 2006 and 2007 (52, 53) reported that in CHB patients, the coexistence of HBsAg and HBsAb was associated with an increase of “a” determinant variability (region between amino acid residues 99 and 169 which are involved in the binding of antibodies against HBsAg). Based on this observation they suggested a selection of HBV immune escape mutants during chronic carriage stage (52). The consequences of this selection process with regard to vaccine efficacy, diagnosis, and clinical evolution remain partially unknown. In our study, all centers directors mentioned that all institutionalized patients were regularly screened based on HBsAb titer. It can be concluded that if only HBsAb test is used for HBV screening, HBV positive cases can be missed in mentally retarded centers. Furthermore, we observed a high prevalence of mutations in “a determinant” region and immune escaped mutations in HBsAg and HBsAb positive patients.

In conclusion, residing and working in crowded places with the possibility of close contact has led to the spread of HBV and can be a potential reservoir for the dissemination of HBV. This epidemiology of HBV in institutions for persons with intellectual disability, calls for the need of comprehensive and national studies in similar establishments. These studies could have a key role in controlling HBV in the post-vaccination period.

Footnotes

Authors' Contribution: All authors have contribution to all steps of manuscript writing.

Conflict of Interests: Nothing to declare.

Ethical Approval: This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences with approval number 25-1580-1393-122 and is in accordance with the Helsinki declaration of 1964.

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Patient Consent: Informed consent was obtained from patient's parents or their guardian. Blood samples were

taken after obtaining permission of the center director and based on the patient readiness for sampling.

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