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Intra-familial Transmission of Chronic Hepatitis B Infection: A Large Population-Based Cohort Study in Northern Iran

Aezam Katoonizadeh, MD[1#]; Nazgol Motamed-Gorji, MD[1#]; Maryam Sharafkhah, PhD[1]; Mohammadreza Ostovaneh, MD[1,2]; Saeed Esmaili, MD[1,3]; Layli Eslami, MD[1]; Abdolsamad Gharravi, MD[1]; Masoud Khoshnia, MD[1]; Amaneh Shayanrad, MSc[1]; Fatemeh Shakki Katouli, MD[4]; Abolfazl Shiravi Khuzani, MD[5]; Sedigheh Amini Kafi-Abad, MD[6]; Mahtab Maghsudlu, MD[6]; Jacob George, MD[3]; Hossein Poustchi, MD PhD[1*]; Reza Malekzadeh, MD[1*]

[1]Liver and Pancreatobiliary Diseases Research Center, Digestive Disease Research Institute, Shariati Hospital, Tehran university of Medical Sciences, Tehran, Iran

[2]Division of Gastroenterology and Hepatology, Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, USA

[3]Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Westmead, NSW, Australia

[4]Department of Radiology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

[5]Department of Radiology, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

[6]Iranian Blood Transfusion Organization Research Center, Department of Pathology, Tehran, Iran

Abstract

Aim: The aim of this study was to investigate the intra-familial transmission of chronic hepatitis B (CHB) in Golestan province, that has the highest prevalence of CHB in Iran.

Methods: The Golestan Cohort Study (GCS) is a population-based prospective study of 50045 individuals, 40 years or older, initially set-up to study upper GI cancers in Northern Iran. In 2008, a baseline measurement of hepatitis B surface antigen (HBsAg) on the stored serum of all GCS participants identified 3505 HBsAg+ individuals. In 2011, we assessed HBV serological markers in 2590 initially HBsAg+ individuals and their first-degree relatives including spouses (1454) and children (3934).

Results: The median (IQR) age of spouses and children were 52 (12) and 25 (12) years respectively. Out of 5388 family members, 2393 (44.5%) had no HBV markers, indicating susceptibility to infection. Of these, 378 (15.8%) were fully-vaccinated children with no apparent response to primary immunization. HBsAg was positive in 2.2% (n = 33) of spouses and 8.2% (n = 325) of children (overall rate of 6.6%). HBcAb was positive in 761 (52.3%) and 914 (23%) spouses and children, respectively. The rate of spontaneous loss of HBsAg (HBsAg-, HBsAb+ and HbcAb+) was 41.3% and 13.9% in spouses and children, respectively. A higher rate of HBsAg+ children (10.2%) was found in families in which the mother was positive for HBsAg compared with families where the father was positive for HBsAg (6.3%) ($P < 0.001$). When both parents were positive for HBsAg, the rate of HBsAg positivity was high (23.5%, $P < 0.001$). Despite high virus exposure rates between spouses (52.6 %), the prevalence of HBsAg positivity among them was very low (2.3 %).

Conclusion: Sexual and parent-to-child transmission are important routes of CHB spread in this population from northern Iran despite the fact that 24 years have passed since the beginning of hepatitis B vaccination in infants. Low percentage of HBsAg positivity in spouses is related to high HBsAg clearance rate among them.

Keywords: Chronic hepatitis B infection, Intra-familial transmission, Prevention, Risk factors

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Introduction

About 400 million people worldwide are infected with the hepatitis B virus (HBV). These individuals are at high risk for the subsequent development of cirrhosis and hepatocellular carcinoma (HCC), placing a substantial burden on health systems.[1,2] The primary routes of infection transmission are contact with blood and other body fluids of infected individuals. HBV can be transmitted both vertically (perinatal/during delivery from mother to child) and horizontally (sexual transmission between spouses/partners and non-sexual transmission between family members (child-to-child or household personal contact)) resulting in clustering of the infection in family units. In cases of vertically-transmitted infection, the likelihood of a neonate developing chronic hepatitis B (CHB) infection is as high as 90% for those born to HBsAg-positive mothers (with very high viral load). Most early childhood infections occur in households of families with CHB infection, probably via unapparent percutaneous or per-mucosal contact (e.g. bites, breaks in the skin, dermatologic lesions, and skin ulcers)

*Corresponding Authors: Hossein Poustchi, MD, PhD; Associate Professor, Liver and Pancreatobiliary Diseases Research Center, Digestive Disease Research Institute, Tehran university of Medical Sciences, Shariati Hospital, N. Kargar St., Tehran 14117, Iran. Tel: +98-21-82415141 Fax: +98-21- 8241 5400; Email: h.poustchi@gmail.com

Reza Malekzadeh, MD; Professor, Liver and Pancreatobiliary Diseases Research Center, Digestive Disease Research Institute, Tehran university of Medical Sciences, Shariati Hospital, N. Kargar St., Tehran 14117, Iran. Email: malek@tums.ac.ir

#Joint first authors.

*Joint corresponding authors.

Take-Home Message

It is crucial to conduct population-based studies to accurately determine the prevalence and patterns of intra-familial spread of CHB infection in the general population. This is an important subject for patients, their family members and health service providers. When patterns and routes of infection transmission and its associated risk factors among family members are clear, effective preventive and control measures could be developed.

with infectious body fluids. Sexual transmission has been estimated to account for 50% of new infections among adults in developed countries.[3]

The majority of studies dealing with intra-familial transmission of CHB infection are not population-based prospective cohort studies and often include small numbers of patients. On the other hand, asymptomatic individuals, who are unaware of their career status, are the largest reservoir of infection and therefore an important path of disease transmission.[4-8] Thus, it is crucial to conduct population based studies to accurately understand the prevalence and patterns of intra-familial spread of CHB in the general population.

Iran is a large country in which the prevalence of CHB in the general population varies from 1% to 9.7%, depending on the region studied.[9-13] We previously reported an overall prevalence of 7% and 6.6% for hepatitis B surface antigen (HBsAg) positivity in adults >45 years old from Golestan province and their first-degree family members (spouses and children), respectively.[12] The aforementioned high rates of HBsAg positivity demonstrate that despite Iran's expanded program of hepatitis B immunization of newborns (a course of 3 doses of HB vaccine before the age of 7 months since 1993), CHB remains a major health concern in Golestan. This prompted us to investigate the pattern of intra-familial spread of CHB infection and its associated risk factors. We expected that the insights gained from this study would provide valuable information to develop effective preventive and control measures, and therefore to restrict disease transmission.

Material and Methods

Study Area and Population

Golestan Province is located North-East of Iran. It has an area of 20 380 km² and a population of 1 710 000 residents. The population density for Golestan is 84 people per km².

The Golestan Cohort Study (GCS), which has been described before, is a prospective study of 50 045 adults (ages 40–75 years, 58% female) recruited between 2004 and 2008 designed to assess upper gastrointestinal cancers in Northern Iran. Cohort members comprise 74% Turkmen ethnicity and 80% live in rural areas. Eighty-eight percent were married, 83% were non-smokers, and 70% had no formal education.[14]

Measurement for HBsAg was performed on baseline stored serum samples of all GCS participants. Those who were HBsAg positive (n = 3,505; 7%) were enrolled in the Golestan Hepatitis B Cohort Study (HBCS).[12] In 2011, we assessed HBV serological markers in 2590 initially HBsAg+ individuals and their first-degree relatives including spouses (1454) and children (3934) who gave consent to participate in the study. All consenting subjects were enrolled in the Golestan HBCS and were interviewed to obtain their demographic characteristics, immunization history for hepatitis B, past medical history and exposure to risk factors of HBV infection. We checked HBV serological markers (HBsAg, HBsAb and HBcAb) in those initially HBsAg+ individuals and their first-degree relatives (spouses and children).

Serologic Testing

All serum samples were tested for HBsAg using the highly sensitive commercially available Enzygnost® HBsAg 6.0 kit (Siemens Healthcare Diagnostics Products, Marburg, Germany: Sensitivity of 100% and specificity of 99.89%) according to the manufacturer's instructions. Serum was also examined for HBsAb (DIA.PRO, Milano, Italy: Sensitivity of 100% and specificity of 98.8%) and HBcAb (DIA.PRO, Milano, Italy: Sensitivity of 94.9% and specificity of 99.5%) using commercially available enzyme-linked immunoassays according to the manufacturer's instructions in a central virology laboratory.

Statistical Analysis

Data are presented as mean and standard deviation (SD) or median and IQR or range or percentage (number). Statistical analysis was performed using STATA statistical software (Version 12, Stata Corporation, College Station, TX, USA). Differences in variables between groups were analyzed using the Mann-Whitney U-test, chi-square and Fisher exact tests as appropriate and P value < 0.05 was considered significant.

Results

Patient Characteristics

We completed blood samplings in 5488 children and spouses; referred to as the first-degree family members of initially HBsAg+ individuals of Golestan HBCS. Sera from 100 first-degree family members were not suitable for further analysis and excluded from the study. The remaining 5388 first-degree family members were left for final analysis. They included 3934 children and 1454 spouses who belonged to 1757 HBsAg positive index (initially HBsAg+) cases in the cohort. The number of first-degree family members screened was 1-16 persons per family. Selected baseline characteristics of first-degree family members are summarized in Table 1. The median (IQR) age of spouses was 52 (12) years (range: 25–85 years) of whom 451 (31%) were husbands. The median (IQR) age of the children was 25 (12) years (range: 8-60 years) and 37% (n = 1452) were male (Table 1).

Hepatitis B viral markers of the family members and their different combinations are presented in Table 2 and Table 3. Out of 5388 first-degree family members, 1291 (24%) had isolated HBsAb (without HBcAb, i.e. vaccinated) and 2393 (44.5%) had no HBV markers (HBsAg-, HBsAb-,

Table 1. Demographic Characteristics of 5388 First-Degree Family Members of Initially HBsAg Positive Individuals

Variables		Spouses			Children		
		Females (Wives) (n = 1003)	Males (Husbands) (n = 451)	Total Spouses (n = 1454)	Females (Daughters) (n = 2482)	Males (Sons) (n = 1452)	Total Children (n = 3934)
Age (year) Median (Q1, Q3)		50 (45–56.5)	57 (52–64)	52 (47, 59)	25 (20, 32)	26 (20, 33)	25 (20–32)
Age Distribution	<18, No. (%)	0	0	0	310 (12.5)	240 (16.5)	550 (14)
	18-29, No. (%)	5 (0.5)	0	5 (0.3)	1353 (54.5)	681 (46.9)	2034 (51.7)
	30-39, No. (%)	82 (8.2)	3 (0.7)	85 (5.9)	636 (25.6)	406 (28)	1042 (26.5)
	40-49, No. (%)	390 (38.9)	69 (15.3)	459 (31.6)	156 (6.3)	110 (7.6)	266 (6.8)
	50-59, No. (%)	366 (36.5)	202 (44.8)	568 (39.1)	25 (1)	13 (0.9)	38 (1)
	≥60, No. (%)	157 (15.6)	177 (39.2)	334 (23)	1 (0.04)	1 (0.1)	2 (0.05)
	Missing	3 (0.3)	0	3 (0.2)	1 (0.04)	1 (0.1)	2 (0.05)
Place of Residence	Urban, No. (%)	158(15.7)	69 (15)	227 (15.5)	345 (13.9)	151 (10.4)	496 (12.5)
	Rural, No. (%)	845 (84.3)	384 (85)	1229 (84.5)	2137 (86)	1301 (89.6)	3438 (87.5)

Table 2. Hepatitis B Markers of 5388 First-Degree Family Members of Initially HBsAg Positive Individuals

Variable	Spouse									Children									Total Population (n = 5388)		
	Wives (n = 1003)			Husbands (n = 451)			Spouse Total (n = 1454)			Female (n = 2482)			Male (n = 1452)			Total Children (n = 3934)					
	Pos[a]	Neg[b]	M[c]	Pos	Neg	M	Pos	Neg	M	Pos	Neg	M	Pos	Neg	M	Pos	Neg	M	Pos	Neg	M
HBsAg	14 (1.4)	989 (98.6)	0	19 (4.2)	432 (95.8)	0	33 (2.3)	1421 (97.7)	0	180 (7.2)	2302 (92.7)	0	145 (10)	1307 (90)	0	325 (8.3)	3609 (91.7)	0	358 (6.6)	5030 (93.3)	0
HBsAb	591 (59.1)	409 (40.9)	3	283 (62.7)	168 (37.2)	0	874 (60.2)	577 (39.8)	3	1003 (40.4)	1479 (59.6)	0	574 (39.6)	876 (60.4)	2	1577 (40.1)	2355 (59.9)	2	2451 (45.5)	2932 (54.5)	5
HBcAb	480 (48)	519 (51.9)	4	281 (62.9)	166 (37.1)	4	761 (52.6)	685 (43.4)	8	515 (20.7)	1967 (79.2)	0	399 (27.5)	1051 (72.5)	2	914 (23.2)	3018 (76.7)	2	1675 (31.1)	3703 (68.9)	10

[a] Pos, Positive; [b] Neg, Negative; [c] M, Missing.

Table 3. Different Categories of Hepatitis B Markers in First-Degree Family Members of HBsAg Positive Individuals

Categories	Spouse			Children			Total Population (n = 5373)*
	Female (Wives) (n = 996)	Male (Husbands) (n = 447)	Total Spouse (n = 1443)	Female (Daughters) (n = 2482)	Male (Sons) (n = 1448)	Total Children (n = 3930)	
HBsAg+ HBsAb- HbcAb-	0	0	0	10 (0.4%)	3 (0.2%)	13 (0.3%)	13 (0.2%)
HBsAg+ HBsAb- HbcAb+	12 (1.2%)	18 (4%)	30 (2%)	162 (6.5%)	137 (9.5%)	299 (7.6%)	329 (6.1%)
HBsAg+ HBsAb+ HbcAb+	2 (0.2%)	0	2 (0.1%)	7 (0.3%)	4 (0.3%)	11 (0.3%)	13 (0.2%)
HBsAg- HBsAb- HbcAb+	77 (7.7%)	55 (12.3%)	132 (9.1%)	36 (1.4%)	23 (1.6%)	59 (1.5%)	191 (3.5%)
HBsAg- HBsAb+ HbcAb+	388 (38.9%)	208 (46.5%)	596 (41.3%)	310 (12.5%)	235 (16.2%)	545 (13.9%)	1141 (21.2%)
HBsAg- HBsAb- HbcAb-	317 (31.8%)	93 (20.8%)	410 (28.4%)	1271 (51.2%)	712 (49.2%)	1983 (50.4%)	2393 (44.5%)
HBsAg- HBsAb+ HbcAb-	200 (20.1%)	73 (16.3%)	273 (18.9%)	685 (27.6%)	333 (23%)	1018 (25.9%)	1291 (24%)
HBsAg+ HBsAb+ HbcAb-	0	0	0	1	1	2	2

*Total missing: 15.

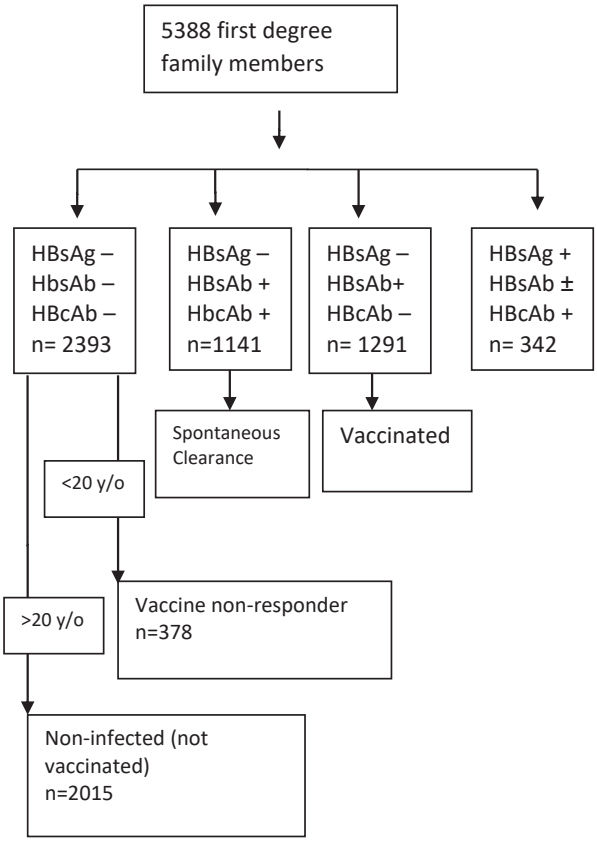

Figure 1. HBV Serological Markers in 5388 First-Degree Family Members.

HbcAb-), indicating their susceptibility to infection. Among the 2393 all marker negative individuals, 378 (15.8%) were previously vaccinated children, indicating no subsequent response to primary immunization or declined HBsAb titre (Table 3 and Figure 1).

Age Distribution of HBsAg and HBcAb Frequency

We further assessed HBV serological markers in the 5388 first-degree family members. The overall rate of seropositivity for HBsAg and HBcAb was 6.6% and 31.1%, respectively. The prevalence of HBcAb positivity in total population of the relatives (including spouses and children) were 35.7 % and 28.5% for males and females, respectively (P < 0.001). While investigating the age distribution of HBsAg frequency within the first-degree family members, the highest frequency of HBsAg seropositive cases was observed in the age group of 30–39 years olds (9.5%) in both males (12.2%) and females (7.9%). Regardless of gender, the youngest (<18 years old) and oldest (≥50 years old) age groups had the lowest frequencies for HBsAg positivity, suggesting the effect of vaccination in younger individuals and higher rate of HBsAg clearance in older people. In both genders, HBcAb seropositivity showed a statistically significant increasing trend with increasing age (Figure 2).

Patterns of Intra-familial Infection

According to the relationship of the initially HBsAg positive index case and first-degree family members, the family members included 1003 (18.6%) female spouses (wife), 451 (8.4%) male spouses (husband) and 3934 (73%) children. Overall, HBsAg was positive in 2.3% (n = 33) of the spouses (4.2% in husbands and 1.4% in wives, P = 0.02) and 8.3% (n = 325) of the children. HBsAg was positive in 10% of the male (n = 145) and 7.2% of the female children (n = 180) (P = 0.003). The rate of HBV-exposure (HBcAb positivity) was 48% (n = 480) in wives, 62.9% (n = 281) in husbands and 23.2% (n = 914) in children (Table 2). Despite high virus exposure rate among spouses, the rate of HBsAg positivity among them was very low (2.3%).

As presented in Figure 3, significantly higher rates of HBsAg positive (10.2%, n = 173) and HBcAb positive

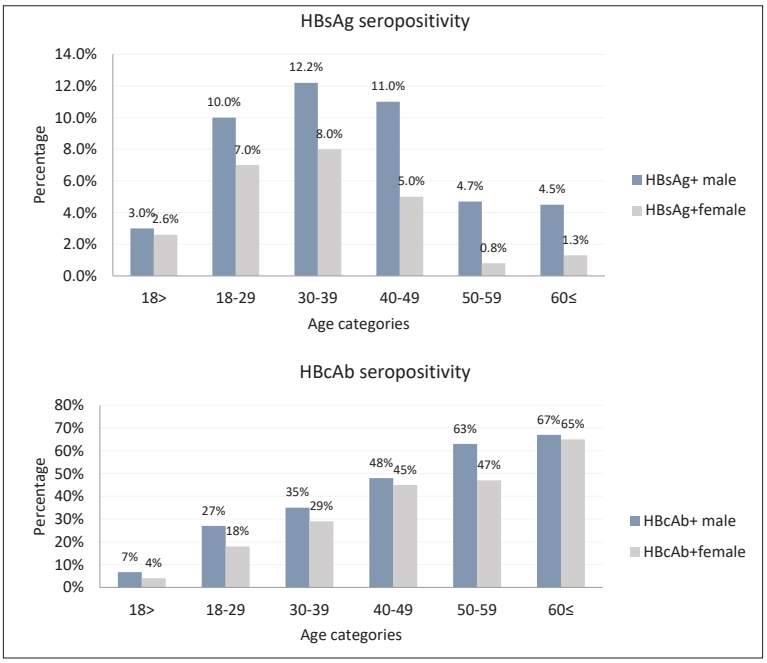

Figure 2. Frequency (%) and Age Distribution of HBsAg and HbcAb Seropositivity in First-Degree Family Members.

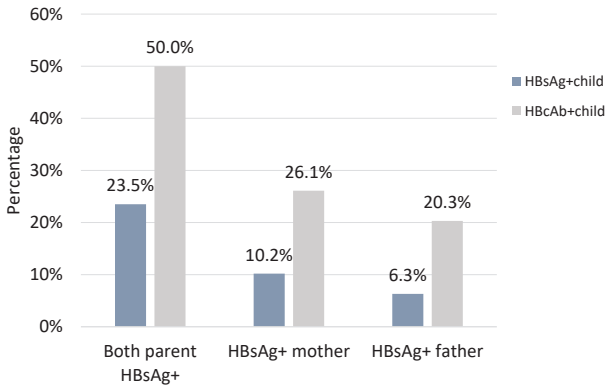

Figure 3. HBV Serological Status of the Offspring According to HBV Infected Parent(s).

(26.1%, n = 443) children were observed in families where only the mother was positive for HBsAg, compared to families in which only the father was positive for HBsAg (6.3%, n = 136) and HBcAb (20.3%, n = 437) ($P < 0.001$ for each). When both parents were positive for HBsAg, the rate of HBsAg and HBcAb positivity were as high as 23.5% (n = 16) and 50% (n = 34), respectively ($P < 0.001$ for each).

HBsAg Clearance

Among 5030 HBsAg negative first-degree family members, 22.7% (n = 1141) had antibodies against both HBs and HBc antigens suggesting spontaneous clearance of HBsAg. The mean (SD) age of HBsAg seroclearance was 43 (±14) years and 61.2% (n = 698) were female. Adjusting for the significant determinants of HBsAg clearance in bivariate analysis (BMI, age, gender, history of diabetes, and residence of a city) by logistic regression, we found gender and age as the only determinants of clearance. Females cleared HBsAg more frequently than males (odds ratio [OR]: 1.3; P=0.02). HBsAg seroclearance in family members also correlated with individual age with higher frequencies (95% and 83.3%) in the age groups of ≥ 50 (OR: 10.6, $P < 0.001$) and 40–49 (OR: 2.7, $P = 0.02$) years, respectively (Figure 4).

Discussion

To the best of our knowledge, this is the first large scale population based prospective cohort study to report CHB prevalence and patterns of intra-familial spread in northeastern Iran. This is in contrast to most studies dealing with intra-familial transmission of CHB infection in Iran that were case control, and usually only included smaller numbers of patients.[8-12]

Geographic variation in the prevalence of HBsAg positivity/CHB has been reported for decades. The prevalence of HBsAg positivity differs not only between countries but also regionally within countries. Accordingly, the frequency of HBsAg positivity in the general population depends on the region of the country from which the prevalence is reported. This is also the case for family members of infected individuals; the reported rate of HBsAg and HBcAb positivity in family members of infected individuals also depend on the target population in terms of country of origin, region of country, family member gender and age, and relationship to the index case.

In this study, we observed a gender- and age-dependent prevalence for HBsAg and HBcAb positivity in spouses and children of initially infected individuals. Our finding showed an overall prevalence of 6.6% (with a male prevalence predominance of 8.6%) and 31% (with a male predominance of 35.9%) for HBsAg and HBcAb positivity, respectively. These findings differ from reports on other parts of Iran.[4,7,15,16] Although our study is not directly comparable, the following rationales should be considered as reasons for the higher reported rates of HBsAg positivity in other studies: (1) Type of study (not being population-based), (2) the relationship of family members to the index case (parents, children, spouses and sibling of infected index cases) were not examined in detail, (3) Male gender proportion in different studies, (4) within country (regional) variability in frequency of HBsAg positivity with reference to life style and risk factors, and (5) Age and the vaccination status of the study population and (6) the number of individuals who consented to participate in the study.

Sexual transmission has been reported as a major route for HBV transmission. The current study did not find high rates of CHB infection among spouses, which is contradictory to the results of other national and foreign studies.[4,6,17] Some studies from Africa and Greenland underscore the role of horizontal spread of HBV.[18,19] Despite high virus exposure rates among spouses (52.6%), rate of HBsAg positivity among them was very low (2.3%); whereas, offspring of index cases had 23.2% exposure, with 8.3% of them being HBsAg positive. The findings of this cohort suggest that sexual transmission is a less significant route of CHB transmission compared with parent-to-child transmission. This can be explained by the fact that majority of our patients have HBe-Ag negative disease with low viral loads. Our results are in accordance with published data from high

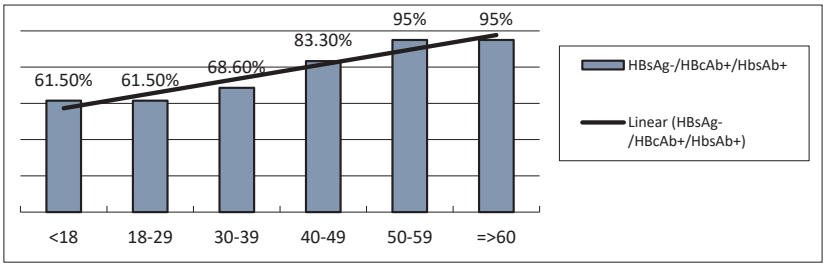

Figure 4. Frequencies of HBsAg Seroclearance in 1141 First-Degree Family Members of Initially HBsAg Positive Individuals.

endemic areas of HBV, in which it has been shown that older patients are more likely to have cleared HBsAg.[20,21]

In this study, we found a higher rate of HBsAg positivity in children whose mothers were HBsAg positive compared to those with positive fathers. This finding clearly underscores role of mothers in the transmission of HBV infection; a finding which has also been suggested in studies from regions with high endemicity in Iran,[22,23] as well as other countries such as Italy.[24] This finding emphasizes the importance of universal full schedule vaccination together with HBIG for infants born to HBsAg-positive mothers. When both parents were positive for HBsAg, the rate of HBsAg positivity was the highest. This finding accentuates the role of persistent close contact between parents and their children in the spread of HBV infection (in this area of Iran), and therefore stresses the necessity for proper HBV immunization in families with history of hepatitis B infection.

In summary, considering this population from northern Iran, sexual and parent to child transmission are important routes of CHB spread. Low percentage of HBsAg positivity in spouses was related to high HBsAg clearance rate among them. Further genotyping and phylogenetic analysis of HBV in infected families can help us to accurately determine different patterns of intra-familial transmission. It is also of utmost importance to recognize within and between country variability in the prevalence of intra-familial CHB infection and its associated risk factors. When pattern and routes of infection transmission and its associated risk factors among family members are better understood, effective preventive and control measures can be developed to limit disease transmission.

Authors' Contribution

Ak and NMG designed the study, analyzed the data and interpreted the results of the analysis and wrote the manuscript as co-first authors. MS participated in study design and did the statistical analysis. MO, SE, LE, AG, MK, FSK, ASK, SAKA, MM and AS were involved in acquisition of data. JG critically reviewed the manuscript for important intellectual content. HP supervised data collection and critically reviewed the manuscript for important intellectual content. RM critically reviewed the manuscript for important intellectual content and provided funding. All authors read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

The study protocol and the text of the consent was reviewed and approved by the ethics committee of the Digestive Disease Research Institute, Tehran University of Medical Sciences.

Informed Consent Statement

All subjects signed the consent form before being interviewed and prior to blood sample collection.

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Data Sharing Statement

No additional data are available.

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