Microbes and Infectious Diseases 2022; 3(4): 878-889

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Intense intra-familial transmission of HBV in a rural area in Egypt is a probable cause of non-response to vaccination: A cross-sectional-seroprevalence-community-study

Nader Nemr [1], Rania Kishk [2], Mohamed Mandour [3], Mostafa Ragheb [1], Hebatalla Mohamed Aly [4], Eman Fahmy [5], Mohamed Eida [1], Nageh Louis [1]

1- *Endemic and Infectious Diseases Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*
2- *Microbiology and Immunology Dpartment, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*
3- *Clinical Pathology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*
4- *Department of Occupational and Environmental Medicine, Faculty of Medicine, Suez Canal University, Ismailia , Egypt.*
5- *Internal Medicine Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*

ARTICLE INFO

Article history:
Received 17 July 2022
Received in revised form 4 September 2022
Accepted 6 September 2022

Keywords:
HBV
Intrafamilial transmission
OBV
Vaccination
Egypt

ABSTRACT

Background: Worldwide, the prevalence of hepatitis B virus (HBV) infection is decreasing particularly in the vaccinated population. However, there are foci of increased transmission particularly in localized communities and within families. **Objective:** This study aimed at identifying HBV infection status among family members (FMs) of a cohort of HBsAg positive index cases (ICs) living in a village near Ismailia City, North-East Egypt. **Design and participants:** The study targeted ICs with chronic hepatitis B and their FMs. All were inquired for socio-demographic data, previous vaccination, kinship, and risk factors. All were tested for hepatitis markers and in HBcAb positive sera, HBV DNA and ALT were added. **Results:** The study included 96 participants including 14 ICs, 51 (53.1%) were females and 73 (76%) self-reported receiving hepatitis B vaccine after birth. Among 82 FMs, HBcAb was found in 49 (59.76%) of whom overt and occult HBV were diagnosed in 24 (49%) and 18 (36.7%). HBs Ag and HBcAb were more frequent in unvaccinated compared to vaccinated FMs; being 60.9% vs. 32.9% for HBsAg (p=0.017) and 91.3% vs. 57.5% for HBcAb (p=0.003). Among the FMs, active HBV were more related to male than female ICs (54.9% vs. 45.2%, p=0.73) while among children, it is more related to females more than male ICs (38.9% vs. 25%, p=0.33). In all HBsAg positive participants, HBeAg was negative and HBV DNA load was higher among female than male ICs (median 3500 vs. 2594.5 IU/mL, p=0.82). **Conclusion:** The study shows a high rate of HBV transmission among FMs of HBsAg carriers living in a remote area in North-East Egypt. Both overt and occult HBV infections were frequent despite previous vaccination.

Background

Worldwide, an estimated 257 million individuals are affected by chronic hepatitis due to infection by hepatitis B virus (HBV). Unfortunately, about one-tenth of individuals with chronic hepatitis B (CHB) are aware of their infection and only 1 in 6 is treated [1]. This shortage in diagnosis and

DOI: 10.21608/MID.2022.150592.1349
* *Corresponding author:* Nageh Louis
E-mail address: NagehLouis@med.suez.edu.eg

treatment facilities could be responsible for the continuous infection particularly where surveillance and immunization are not available.

According to the prevalence of HBsAg seropositivity, regions throughout the world are described as areas of high (> 8%), intermediate (2–8%) or low (< 2%) endemicity [2-5]. Three decades ago, all types of endemicity were reported in Egypt depending on geographical differences, population density, and diversity in socioeconomic status. Nowadays, the country witnessed much progress due to the implementation of hepatitis B vaccination, periodic surveillance of hepatitis viruses B and C throughout the country and provision of antiviral drugs to infected individuals. In 2017, a nationwide survey reported a lower prevalence of HBV fluctuating from low to intermediate endemicity [6].

The principal global mode of HBV transmission is vertical (mother-to-child) transmission, particularly in high endemic areas [7, 8]. Other forms of transmission include sexual, parenteral, as well as horizontal transmission due to close contact within the same family [9]. Despite the value of hepatitis B vaccination in decreasing the prevalence of HBV infection in many country reports, foci of increased intensity of infection have been reported in localized communities and within families of infected patients [10-12]. The intra-familial spread could happen via long contact of susceptible household contacts with the body fluids of the infected person [13].

Ismailia Governorate lies in the middle of Suez Canal of Egypt. It includes urban areas and other rural and Bedouin communities around. Three major hospitals and a network of primary health care (PHC) centers provide different levels of health care for the citizens in this area. All benefit from services of the PHC facilities including universal hepatitis B vaccination to all newborns. However, familial clusters of patients with CHB are occasionally encountered during our clinical practice in the Suez Canal University Hospital (SCUH). All were blood-related or living in a closed community in distant areas [14]. This observation motivated us to study the prevalence, pattern, risk factors, and impact of vaccination on HBV transmission among family members of HBsAg positive index cases from one village in a remote area in Ismailia.

Patients and methods

Sample type was non-randomized convenience sample. This cross-sectional study included two groups, the first was the index cases who were HBsAg positive patients, ≥18 years, of both sexes, diagnosed with chronic hepatitis B for at least 6 months and living in one village. The second group included all the available family members living in the same house of the index cases regardless of their age.

Sample size

All index cases and available family members we could meet (convenience sampling).

The study excluded HBsAg positive individuals with HCV co-infection or with other types of liver diseases as metabolic, drug-related, or autoimmune. If two or more family member were diagnosed at the outpatient clinic, the one who was first diagnosed is considered an index case. All individuals from both groups were inquired about their socio-demographic data (age, sex, residence, kinship to the index case), history of exposure to the main risk factor of viral hepatitis, history of acute hepatitis or jaundice, therapy by antiviral drugs, history of vaccination, and immunization with hepatitis B immune globulin (HBIG). All were tested for HBV serological markers. In HBcAb positive individuals, HBV-DNA was quantified by real-time PCR, and serum levels of liver transaminases; alanine aminotransferase (ALT) were determined.

Screening for HBV infection

Testing for HBsAg, HBsAb, and HBcAb was done using the following commercial enzyme immunoassay (ELISA) kits [Murex HBsAg Version 3, ETI-AB-AUK-3 (anti-HBs), and Murex anti-HBc (total), Diasorin, Italy] respectively. Sera positive for HBsAg samples were further tested for HBeAg/HBeAb (Monolisa HBe Ag-Ab PLUS, Biorad) as well as liver transaminases ALT (Cobas, Roche). All serologic assays were carried out according to the manufacturer's instructions. An anti-HBs titer's concentration ≥ 10 mIU/ml was considered protective [15, 16].

Hepatitis B virus DNA quantification

The viral load was quantified by real-time polymerase chain reaction (PCR). Viral DNA was isolated from 200 µl of serum samples positive for HBsAg using the QIAamp DNA MiniKit (QIAGEN, Hilden, Germany), and re-suspended in 100 µl of a storage buffer provided by the kit manufacturer. HBV-DNA was quantified by real-

time PCR using 7500 Real-Time PCR machine to determine the viral load as described previously [17].

Outcomes of the study

Interpretation of hepatitis markers was according to the CDC criteria [15, 16]. A person was considered to have an infection if he/she is positive for HBsAg and HBcAb, resolving infection if HBcAb was only positive, while positive HBsAb was considered a marker of immunity due to vaccination if HBcAb was negative or as a sequence of the previous infection if HBcAb was positive. A susceptible person is considered if the three markers are negative. The infection status of HBV DNA/HBcAb positive individuals is defined as overt when HBsAg is positive and as occult when HBsAg is negative. Hepatitis is described if ALT exceeds the reference value (>40 IU/L). Vaccination's non-response is considered if no sufficient antibody is produced (<10 IU/mL) after the three doses or if there is evidence of HBV breakthrough infection after mounting sufficient antibodies. In this case, there should be viremia in a previously vaccinated individual with coexistent HBsAb and HBcAb.

Ethical aspect

The study was approved by the committee of research ethics, Faculty of Medicine, Suez Canal University (Ethical approval number #4242). Informed signed consent was obtained from each participant (as for children, the signed consent of their father or mother was obtained).

Statistical analysis

The collected data were managed by the SPSS-version 20 program of statistical analysis. Continuous data were described as range, mean and standard deviation, and qualitative data were summarized by frequencies and percentages. In analytic data, the Chi-square test was used to detect the difference between qualitative data, while the Student t-test was used to detect the difference between continuous data with normal distribution and Mann Whitney U test for non-parametric data. A *p*-value <0.05 was considered statistically significant.

Results

Descriptive results

This study included 96 individuals; 14 ICs and 82 FMs. Their mean age was 17.8 ± 13.1 years. Males represented 46.88% (45/96) and 73 (76%) recalled immunization by hepatitis B vaccine after birth.

Of 14 ICs, 12 were parents (6 fathers and 6 mothers), one son and one daughter. Inquiry of the main risk factors for HBV infection revealed previous surgery in 75 (78.1%), dental procedures in 55 (57.3. While blood transfusion was recalled in one (1%) individual being an index case, and none reported intravenous drug use. Interpretation of hepatitis B markers revealed chronic hepatitis B in 38/96 (39.58%), infection related immunity in 16 (16.67%), vaccine related immunity in 29 (30.2%), isolated HBcAb in 9 (9.38%) and negative markers in 4 (4.16%) susceptible individuals **(Table 1).**

In the studied 14 families, the number of relatives living in the same house ranged from 2-11 (mean: 5.9±2.2). Of all, evidence of HBV infection was found in 12 families, overt in two, occult in 4 and both types in 6. Serology revealed positive HBcAb, HBsAg and HBsAb in 63 (65.6%), 38 (39.58%) and 50 (52.1%) respectively **(Table 2).**

HBsAb positive individuals included 5 (5.2%) OVBI (with coexistent HBsAb), 16 (16.6%) positive HBcAb (infection-related immunity) and 29 (30.2%) HBcAb negative (vaccine-related immunity). HBV DNA was positive in 55 of 63 (87.3%) HBcAb positive individuals, with a viral load range of 134-135,000 IU/mL (median=524.5). HBV DNA was positive in 37 of 38 (97. %) HBsAg positive/HBcAb positive individuals (overt HBV), 18 of 49 (28.6%) HBsAb negative/HBcAb positive individuals (occult HBV). The latter group included 8 of 9 (88.9%) with isolated HBcAb and 10 of 16 (62.5%) sero-reactive to HBcAb/HBsAb. In sera of all HBcAb positive participants, HBeAb was negative and HBeAb was positive.

In 38 HBsAg positive chronic hepatitis B, 24 reported previous vaccination (61.2%) and 14 were not vaccinated (36.8%). Among 18 OBI cases, 15 reported vaccinated (83.3%) and 3 were not vaccinated (16.7%). In 73 individuals who reported vaccination, OVBI and OBI represent 32.9% and 20.5% respectively.

Comparison between index and family members

Self-reporting vaccination was significantly more frequent in FMs (82.9%) than ICs (35.7%), p=0.0006. Previous exposure to dental and surgical procedures were recalled by all ICs compared to 50% and 74.4% of FMs (p<0.001 and 0.035 respectively). HBsAb was significantly more frequent in FMs than ICs (56.1% vs. 28.57%, *p*=0.004), By definition, all ICs had OVBI type of infection, while in 49 HBcAb positive FMs, overt

and OBI were found in 49% and 36.7% (representing 29.3% and 22% of all the 82 contacts). Meanwhile, both patterns of infection were more frequent among female (25/44 or 56.8%) than male FMs (17/38 or 44.7%), p=0.38. Interpretation of hepatitis B markers revealed all individuals with isolated HBcAb, with vaccine-related immunity and infection-related immunity were FMs **(Table 2).** Coexistent HBsAb with OVBI was present in 5, 4 ICs, and one FM. In the 5 index cases who received vaccination, HBsAb was positive in only one who had a titer of 45 IU/ml. Among the other 9 index cases who were not vaccinated, HBsAb was positive in 3 with a titer of 10 each. In such 4 index cases, the combination of sero-positivity to HBsAg, HBcAb and HBsAb could signify the possibility of vaccine escape mutant infection.

The characteristics of such 5 individuals are shown in **table (4).**

Evidence of hepatitis in the two types of infection

In this study, the mean ALT was significantly higher in OVBI than OBI (42.1±11.0 vs. 24.7±8.9 IU/L, p<0.001). ALT was elevated in 20 of 38 (52.6%) with OVBI and 1 of 18 (5.6%) with OBI, p=0.008.

Hepatitis B virus infection relation to the sex of index cases, age, and kinship of family members

In this study, Of 14 ICs, 12 were parents (6 fathers and 6 mothers), one son and one daughter. Of 82 FMs, 31 (37.8%) were belonging to female ICs and 51 (62.2%) belonging to male ICs.

Among 82 FMs, HBcAb was positive in 49 (59.76%), of whom HBV DNA was positive in 42 (85.7%) including 24 (57.1%) HBsAg positive (overt HBV) and 18 (42.9%) HBsAg negative (occult HBV). Both types of infection were more frequent among FMs of males (28/51 or 54.9%) than female ICs (14/31 or 45.2%), p=0.73.

The female index cases were associated with evidence of HBV infection in 7 offspring, one husband, one grandparent and 5 siblings. While in families of the 6 male ICs, HBV infection was shown in 6 offspring, one wife, 3 grandparents, and 18 siblings. The prevalence of infection among offspring was higher in female ICs (7/18 or 38.9%) than male ones (6/24 or 25%), (p=0.33). In this study, the prevalence of HBcAb, overt and occult HBV infection increased with age to have a peek at the age interval of 21-30 years and decreased thereafter **(Figure 1).** HBV infection (overt and occult) was present in 66.67% of grandparents and parents each, 74.2% of siblings, and 31% of offspring. However, according to kinship, the prevalence of OBI was reflected by age. It was 9.5% of offspring (mean age 5.9), 32.26% of siblings (mean age 22.3), 33.3% of parents (mean age 32.67), and 50% of grandparents (mean age 44.8). The mean age of individuals with OBI was higher compared to overt HBV; being 24.26±10.9 vs. 22.45±14.3 years (p=0.58).

HBsAb in overt and occult HBV

In this study, the median HBsAb was 10 IU/mL with a range of 2-460 IU/L. Levels of HBsAb were significantly higher in OBI than OVBI (median of 10 and 7 respectively, 0=0.0028).

HBV DNA according to the type of HBV infection

The HBV DNA levels were significantly higher in individuals with OVBI than OBI (median=860 and 303 IU/ml respectively, p<0.001). In OBI, the median HBV DNA was lower in HBcAb positive with coexistent HBsAb compared to individuals with lone HBcAb (median of 344 vs. 289 respectively, p>0.05) **(Table 5)**.The median HBV DNA load was higher among female than male index cases (median 3500 vs. 2594.5 IU/mL) with no significant difference (p=0.82).

Vaccination, immunization, and medications

In this study, history of vaccination following birth was given by 73 (76%) including 5 of 14 ICs and 68 of 82 FMs, some of them had documents of vaccination from Primary Health Care units and Directorate of Health Affairs while the history of vaccination in others relied largely on self-reporting by the participants or their sponsors **(Table 1).** Of all, participants reporting vaccinated had significantly lower frequency of HBsAg and HBcAb compared to non-vaccinated (32.9%, 57.5% vs. 60.9%, and 91.3% respectively). However, the prevalence of overt and OBI showed no significant differences between both vaccinated and non-vaccinated FMs; being 19/68 (27.9%) and 15/68 (22%) in vaccinated and 5/14 (35.7%) and 3/14 (21.4%) in non-vaccinated respectively; p=0.5382 and 0.5164 respectively. Furthermore, among 42 vaccinated offspring, both overt and OBIs were diagnosed in 9 (21.4%) and 4 (9.5%) respectively.

It is worth noting that none of the participants were using antiviral drugs before the study. Furthermore, none of the HBsAg positive mothers recalled the use of HBIG to their newborns after birth.

Table 1. Sociodemographic characteristics and risk factors in index cases and their family members.

	Total population (n=96)	Index cases (n=14)	Family members (n=82)
*Age: Range (median) Mean±SD	0.5-50 (16) 17.8 ±13.1	22-40 (27.5) 29.78±5.82	0.5-50 (12) 15.87±13.19
**Male female	45/96 (46.88%) 51/96 (53.1%)	7/14 (50%) 7/14 (50%)	38/82 (46.3%) 44/82 53.7%)
^Reporting Vaccination	73/96 (76.0%)	5/14 (35.7%)	68/82 (82.9%)
$Dental procedures	55/96 (57.3%)	14/14 (100.0%)	41/82 (50.0%)
#Surgery	75/96 (78.1%)	14/14 (100.0%)	61/82(74.4%)
Blood transfusion	1/96 (1.0%)	1/14 (7.1%)	0/82 (0.0%)
Infection related immunity	16 (16.67%)	-----	16 (19.5%)
Vaccine related immunity	29 (30.2%)		29 (35.37%)
@Chronic hepatitis B	38 (39.58%)	14 (100%)	24 (29.27%)
Lone HBcAb	9 (9.38%)	------	9 (10.98%)
Susceptible	4 (4.16%)	------	4 (4.88%)

* $p<0.001$ ** $p>0.05$ ^ $p=0.0006$ $ $p=<0.001$ # $p=0.035$ @ $p<0.001$

Table 2. Laboratory findings of the studied population.

		Total population (n-96)	Index cases (n=14)	Family members (n=82)
HBsAg positive chronic hepatitis (overt HBV)		38/96 (39.58%)	14/14 (100%)	24 (29.27%)
HBcAb positive		63/96 (65.6%)	14/14 (100%)	49 (59.76%)
HBsAb positive		50/96 (52.1%)	*4/14 (28.57%)	46 (56.1%)
HBsAg negative chronic hepatitis (occult HBV)		18/63 (28.6%)	----------	18/49 (36.7%)
HBV DNA	positive	55/63 (87.3%)	13/14 (92.8%)	42/49 (85.7%)
	median	524.5	2450	442.5
	range	134-135000	234-135000	134-6500
ALT	mean range	34.5±14.16 7-66	40.14±10.66 23-66	32.77±14.73 7-66
	elevated	21/63 (33.3%)	7/14 (50%)	14/49 (28.57)

* HBsAb coexistent with HBsAg and HBcAbsero-reactivity

Table 3. Comparison between individuals reporting vaccinated and non-vaccinated.

	Self-reported vaccination (n=73)	**Unvaccinated (n=23)**	***p* value**
Age in years (mean ± SD)	12.05±8.3	36.1±7.3	<0.001
HBsAg positive	24 (32.9%)	14 (60.9%)	0.017
HBcAb positive	42 (57.5%)	21 (91.3%)	0.003
HBsAb concentration (median)	20 (0-460)	8 (0-230)	0.049
HBsAb positive	42 (57.5%)	8 (34.8%)	0.057
Index cases	5 (6.84%)	9 (39.1%)	0.0013
Family members	68 (93.16%)	14 (60.9%)	
Significance of hepatitis markers			
Susceptible	3 (4.1%)	1 (4.3%)	>0.05
Immune due to natural infection	12 (16.4%)	4 (17.4%)	>0.05
Immune due to vaccination	28 (38.4%)	1 (4.3%)	0.0014
HBsAg positive chronic Hepatitis B	24 (32.9%)	14 (60.9%)	0.017
Lone HBcAb	6 (8.2%)	3 (13.0%)	>0.05
***HBV DNA Viral Load Median**	450	727	0.065
Range	134-65000	234-135000	

- In 37 vaccinated and 18 unvaccinated

Table 4. Characteristics of five overt hepatitis B infection cases with coexistent HBsAb.

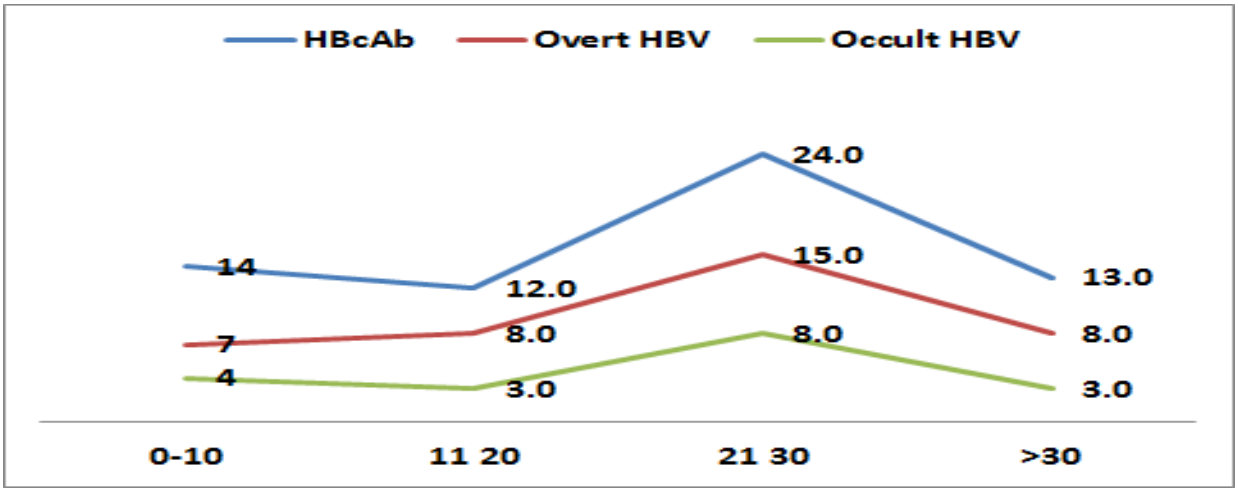
case	**Age (year)**	**Sex**	**HBsAb conc***	**HBV DNA**	**ALT**	**Vaccination history**	**Index/family member**
1	25	M	45	Negative	43	Yes	IC
2	32	M	10	5400	24	No	IC
3	38	F	10	3500	23	No	IC
4	30	F	10	234	32	No	IC
5	9	F	10	760	43	Yes	FM

*HBsAb concentration in IU/mL

Table 5. Comparison between vaccinated and unvaccinated subjects regarding HBV DNA viral load.

	Range	**Median**	***p*-value**
Vaccinated (n=37)	134-65000	450	0.065
Non-vaccinated (n=18)	234-135000	727	

Figure 1. Prevalence of HBcAb, overt and occult HBV infections in the studied family members according to age groups.



Discussion

In Egypt, hepatitis B vaccination has been introduced in late 1992 for universal immunization of all newborns. Till 2011, the doses of the vaccine were given at 2, 4, and 6 months with the other vaccines. Thereafter, the schedule includes the birth dose in addition to 2, 4 and 6 month doses. Despite the high vaccination coverage in infants (97%), immunization of the adults is limited to risky individuals including health care workers and relatives of HBsAg carriers [18,19]. The overall response to vaccination is good and the prevalence of HBsAg progressively decreases [19,20]. However, breakthrough HBV infection in vaccinated individuals has been reported in Egypt. Hepatitis B vaccine non-response was documented in family members of HBsAg positive index cases, among pregnant women screened during antenatal care, and in a series of children born of HBsAg positive mothers [21, 22].

This work aimed at highlighting the status of HBV infection among family members of HBsAg positive index cases. What makes this study unique is the extremely high prevalence of HBcAb and HBsAg (59.8% and 29.3%) compared to other studies in Egypt. According to a representative population-based study, the prevalence of HBcAb and HBsAg varies from 6.2-11.7% and 0.5-1.9% respectively. In Ismailia Governorate, where the target families were studied, the prevalence of HBcAb and HBsAg was 10.4% and 0.8% [19]. The rate of seropositivity for HBsAg in this study was more than 12.1% of 330 FMs of 55 HBsAg positive ICs in Suez Governorate in Egypt and higher than 23.3%, 20.7% and 19.8% among similar clusters in Iran, Croatia and Brazil [11, 14,23, 24].

The second alarming finding is the high exposure to HBV infection among individuals who claimed vaccination after birth (41/73 or 57.5%). The explanation is difficult to discuss particularly in the absence of documented evidence of compliance to the three doses. The socio-demographic characteristics of the studied families might play role; being blood-related from one tribal origin, living in a small village, and residing in houses that were very close. The spouses usually share manual work in agriculture and their socioeconomic status is low. This study included 96 individuals; 14 ICs and 82 FMs. Of 14 ICs, 12 were parents (6 fathers and 6 mothers), one son and one daughter. Inquiry of the main risk factors for HBV infection revealed previous surgery in 75 (78.1%), dental procedures in 55 (57.3). While blood transfusion was recalled in one (1%) individual being an index case, and none reported intravenous drug use. The high frequency of exposure to surgery and dental procedures could carry the risk of viral hepatitis as well. It is also difficult to discuss the timing of infection in individuals who reported previous vaccination. Due to the local infection pressure, HBV infection probably occurred during birth or during the subsequent first months taking into consideration missing the birth dose of the vaccine in most of FMs and missing hepatitis B immune globulin in all [25]. Even with the availability these two post-exposure protective measures, transmission of HBV late in pregnancy or during birth has been reported in up to

15% in newborns of mothers with high viral load [26, 27].

In this study, 5 participants showed co-existent HBsAg/HBsAb seroreactivity with viremia. This breakthrough infection could be due to surface escape mutant while the possibility of vaccine escape mutant could not be excluded in 2 of 5 who reported previous vaccination [21-30]. However, to verify this hypothesis, a molecular sequencing study is recommended.

In this study, OVBI and OBI were evident in 24 (32.9%) and 15 (20.5%) of 73 vaccinated participants of different ages. Furthermore, both infections were diagnosed in 9 (21.4%) and 4 (9.5%) of 42 children respectively, most of them did not receive a birth dose of the vaccine and none had hepatitis B immune globulin. All four cases of OBI aged 3-7 years. On the other hand, among non-familial cluster studies, the relation of vaccination to the type of HBV infection is different from shown in our study. In Taiwan, among children and adolescents, vaccination was associated with a higher rate of OBI and a lower rate of OVBI infection [31]. **Lu and colleagues** emphasized the effectiveness of the dual immunization, by hepatitis B vaccine and immune globulin, in prevention of OVBI, but not OBI, in babies born to HBsAg positive mothers. The authors added that OBI among such infants was transient as loss of viremia occurred in most of the children during 36-month-follow up [32]. Among fully immunized infants born to HBsAg mothers, OBI could signify a stage towards resolving infection [33-35].

In the current study, the prevalence of HBsAg among vaccinated FMs (32.9%) was higher than other cohorts in Egypt including; 10.6% of vaccinated FMs of HBsAg positive ICs in Suez City, Egypt, 4.9% among vaccinated pregnant, and 1.55% of vaccinated infants and young children born of HBsAg positive mothers [14,21,22]. These examples of HBV infection highlight the critical significance of vaccinating some risky individuals such as close contacts of HBsAg positive individuals, newborns of HBsAg positive mothers that were immunized only by the birth dose of the vaccine without immune globulin, or vaccinated children born to mothers with unknown infection status.

In many familial cluster studies, the significant role of female ICs in the transmission of HBV to their close contacts and offspring has been reported [14,36]. However, the pattern of transmission, in the current study, is quite different. The frequency of HBV infection among FMs of all ages was more related to male than female ICs (54.9% vs. 45.2% respectively). Meanwhile, exposure of the offspring to HBV infection (past or current) was more frequently related to female than male ICs (38.9% and 25% respectively). These findings emphasize the probable role of mother-to-child transmission during the peri-natal and early childhood periods and the role of the father ICs in horizontal transmission to their offspring, siblings and parents. This pattern of transmission is an important route of infection encountered in low socio-economic families [34, 35].

In this study, all the four OBI cases among the offspring were related to HBsAg positive parents, three mothers, and one father. However, the possibility of vertical transmission from HBsAg negative parents to their vaccinated children was reported by **Ghaziasadi and colleagues** in Iran. Among parents of 49 OBI children, OBI was diagnosed in 17 (34.7%); 6 mothers and 11 fathers [35]. HBV infection among the vaccines could be due to non-compliance or missing one or more doses of hepatitis B vaccine.

It has been reported that sero-reactivity to HBeAg and high viral load during pregnancy are well-known risk factors for perinatal mother-to-child transmission in 90% [36]. However, in this study, all the HBsAg carriers were HBeAg negative/HBeAb positive and had low to moderate viral load. Therefore, the probability of perinatal transmission might be low as 10-20% [37].

In the current study, the HBV DNA levels were higher in individuals with overt than occult HBV infection (median of 860 and 303 IU/ml respectively, $p<0.001$) and in female index cases than males (median: 3500 vs. 2594.5 IU/mL respectively).The coexistence of anti-HBs with HBV DNA could signify recovery from past infection (or resolving infection). Contrary to this, the association of isolated HBcAb with HBV DNA may represent a variant of chronic HBV infection with undetectable levels of HBsAg [33]. Many studies reported low infectivity from OBI cases probably due to the low viremia particularly in anti-HBs positive individuals [37-39].

The current study revealed that most of HBV in FMs was an overt type that increases the risk transmission to their nearby community.

Despite this innocent nature of OBI, the risk of transmission to others, progression to cirrhosis and HCC has been described. Patients with cryptogenic liver disease and OBI should be closely monitored for such outcomes [40, 41]. Meanwhile, reactivation of OBI could occur following treatment with immunosuppressive drugs or chemotherapy. However, this possibility is infrequent compared to reactivation of inactive HBsAg positive infection (2.7% vs. 48%) [38-41]. In both scenarios, patients on immunosuppressive or immune-modulatory drugs should be strictly followed for viral replication and treated with antiviral drugs if a viral breakthrough is diagnosed [42, 43]. The other sequel of OBI is the possibility of transmitting infection via blood transfusion in low-resource countries. Testing sera of HBsAg negative donors for HBcAg and nucleic acid amplification for HBV DNA in HBcAb negative sera extremely minimize the risk of blood transmitted infection [44- 46].

Study limitations

The source of HBV transmission within the family members could not be exactly determined and needs further sequencing studies. Meanwhile, the history of vaccination given in this study relied largely on self-reporting by the participants or their sponsors.

Finally, as this study is mainly a seroprevalence one, we did not inquire the detailed risks and behavior that is usually associated with viral hepatitis to avoid recall bias.

In conclusion, this seroprevalence study represents an exceptionally high transmission of HBV infection among family members of HBsAg positive individuals living in a remote area, most of them reported immunization by hepatitis B vaccine at birth.

Nowadays, further steps are taken by the ministry of health and population to minimize transmission of HBV within families; including premarital screening and screening of pregnant women during antenatal care.

Conflict of interest

The authors declare no conflict of interest.

Funding:

The study was funded by the contributing authors.

Author contributions

NN, RK, MR and MM conceived the study. RK, MM, EF and NL collected data and performed experiments. NN, RA, MR,MM, HA and NL analyzed the data. NN, RK, MM, MR and EF wrote the manuscript. The final manuscript was read and approved by all authors.

Abbreviations

HBV: Hepatitis B virus, **HBsAg**: Hepatitis B surface antigen, **HBcAb:** Hepatitis B core antibody, **HBsAb**: Hepatitis B surface antibody, **HBeAb:** Hepatitis B e antibody, **OBI**: occult HBV infection, **OvBI:** overt HBV infection. ICs: index cases, FMs: family members.

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