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Seroprevalence of hepatitis B virus infection in pre-mass vaccination era among children residing in a rural area of Bangladesh

Masuda Mohsena[1], Amal K Mitra[2], MA Sayeed[1*], Akhter Banu[3], J Ashraful Haq[4*]

[1]*Department of Community Medicine, Ibrahim Medical College, Segunbagicha, Dhaka, Bangladesh;*
[2]*Department of Epidemiology and Biostatistics, School of Public Health, Jackson State University, Jackson, Mississippi, USA;*
[3]*Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh;*
[4]*Department of Microbiology, Ibrahim Medical College, Segunbagicha, Dhaka, Bangladesh*.

Abstract

***Background and objectives*:** There are few details available regarding the prevalence of hepatitis B virus (HBV) infection in the general Bangladeshi population. There is a dearth of data on prevalence of HBV infection in children and adolescents who were born before the hepatitis B vaccine was introduced in the Expanded Program on Immunization (EPI). The objective of the current study was to use archived data to describe the seroepidemiology of HBV infection (HBsAg and anti-HBc Antibody) among school children in a particular rural area of Bangladesh. Also, the study correlated serum vitamin A level with the HBV infection status among a subset of children.

***Materials and method*:** The study analyzed the archived data of a study conducted in 2003 and 2004. The samples were collected from 1995 children, aged 5 to 15 years, from a purposively selected rural area located about 100 km north-east of capital Dhaka. HBsAg (HBV surface antigen) and anti-HBc antibody were determined by ELISA method. Vitamin A (retinol) in blood was assayed by HPLC technique. The prevalence rates of HBsAg and anti-HBc antibody was determined by simple percentages. All associations between different characteristics were tested by Chi square test.

***Results*:** Of the total 1995 children, 988 (49.5%) and 1007 (50.5%) were male and female respectively. Among them, 23 (1.2%) were HBsAg positive or HBV carriers and 79 (8.1%) were anti-HBc antibody positive. Neither HBsAg nor anti-HBc antibody positivity rate showed any difference in male and female children. There was also no significant difference of HBsAg positivity rate amongst children of different age groups; whereas, anti-HBc antibody positivity rate increased significantly ($p < 0.005$) with increase of age. Serum vitamin A was estimated in a subset of children. The mean serum vitamin A concentration was found significantly ($p<0.05$) lower among HBsAg positive children compared to their age and sex matched healthy control group.

***Conclusion*:** This study has demonstrated that rural children are in risk of exposure to HBV infection. Increasing HBV seropositivity with age emphasizes the need for devising prevention strategies and to create awareness among the rural children. Further studies are necessary to find out the hitherto undetected sources namely occult hepatitis B cases and the ways of spread of HBV in the community.

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***Correspondence**: *M Abu Sayeed, Department of Community Medicine, Ibrahim Medical College, 1/A, Ibrahim Sarani, Segunbagicha, Dhaka 1000, Bangladesh; Email: sayeed1950@gmail.com*; J. Ashraful Haq, *Department of Microbiology, Ibrahim Medical College, Segunbagicha, Dhaka, Bangladesh*; Email: jahaq54@yahoo.com

Introduction

Hepatitis B virus (HBV) infection is a global health problem; it is estimated that two billion people worldwide are infected with HBV and that more than 350 million people have chronic hepatitis B infection [1].

There are few details available about the prevalence of HBV infection in Bangladeshi general population, and so far no national-level research has been carried out on seroepidemiology of HBV. HBsAg (Hepatitis B surface antigen) seroprevalence in Bangladeshi adults was found to be 8.6%, 6.4%, 5.5%, and 6.5%, respectively, in studies done in 1991, 1997, 2008, and 2011[2]. Bangladesh is not an exception to the global lack of data on HBV infection in children and adolescents.

In Bangladesh, the Expanded Program on Immunization (EPI) schedule included the hepatitis B vaccine in a phased manner between 2003 and 2005 [3]. Very little information regarding HBV infection is available among children and adolescents who were born before the program's launch. In terms of hepatitis B care, le *et al* [4] referred them as the "missing generation" and voiced concern about the fact that they are in the age bracket with a tendency of risky behavior, increasing their risk of HBV exposure. Therefore, it is useful to study the prevailing situation against which this vaccination program was implemented.

It has long been established that vitamin A has a protective role against a number of infectious diseases. This vitamin has essential roles in immunity, cellular differentiation, maintaining epithelial surfaces, growth, reproduction and vision [5]. In children, severity of measles, pneumonia, and diarrhea have all been linked to vitamin A deficiency [6–8]. Vitamin A storage and metabolism takes place in liver. Hepatitis B virus causes inflammation and damages the liver over time, so there may be a relationship between HBV biomarkers and vitamins A concentrations in humans [9].

The objective of the current study was to use archived data to describe the seroepidemiology of HBV infection (HBsAg and anti-HBc Antibody) among school children in a particular rural area of Bangladesh. These findings may be used to establish baseline estimates of pediatric chronic HBV infection and determine whether Bangladesh is on track to meet regional and global targets for the eradication of HBV. Also, the study correlated serum vitamin A level with the HBVinfection status among a subset of children.

Material and methods

The study analyzed the archived data of a study conducted in 2003 and 2004 to find out the prevalence of HBV carrier and exposure rate to the virus amongst rural children. At that time, HBV vaccination was not included in EPI schedule in that area. Information on all data, test methods and results were retrieved from the stored data sheets. A structured data sheet was used to record the age, gender, comorbid condition and test results. Approval of the Institutional Review Board was obtained for analysis of the archived data.

The study was conducted at a purposively selected rural area located about 100 km north-east of capital Dhaka. The area covered 19 villages with a population of 16,400 above one year age. Apparently healthy children between 5 to 15 years of age were selected randomly from the entire population. Each participant and their guardians were informed about the objectives and the procedural details of the investigation. Informed consent and assent were obtained from the participants and the guardians, respectively. Participants were informed about the results of the tests and advised accordingly.

At the time of enrollment in the study 2 ml of venous blood was collected aseptically from all participants. A second sample of blood (2ml) was collected 12 months after the first sample from those who were HBsAg positive. Immediately, serum was separated from collected blood samples and transported to laboratory by maintaining a cold chain for detection of HBsAg and anti-HBc antibody and estimation of serum vitamin A concentration. Serum vitamin A was measured among HBsAg positive cases and in age and sex matched healthy HBsAg negative children (controls).

HBsAg and anti-HBc antibody were determined by ELISA method. Any child who had HBsAg in serum for more than 6 months with no clinical symptoms was considered as HBV carrier [10]. Any child who

was positive for anti-HBc antibody but HBsAg negative was considered exposed to hepatitis B virus [11]. Vitamin A (retinol) in blood was assayed by HPLC technique [12]. The HPLC was carried out using Shimadzu HPLC system (Tokyo, Japan).

All data were analyzed by using SPSS (version 22.0). The prevalence rates of HBsAg and anti-HBc antibody was determined by simple percentages. All associations between different characteristics were tested by Chi square test.

Result

A total of 1995 children aged 5 to 15 years were included in the analysis. Of the total, 988 (49.5%) and 1007 (50.5%) were male and female children, respectively. Mean age of the study population was 9.62 ± 3.19 years. Detail age groups and gender distribution of the study population is shown in Table-1. Out of 1995 enrolled children, 23 (1.2%) were HBsAg positive or HBV carriers (Table-2). The rate of positivity of HBsAg in male and female children was not significantly different (1.4% vs 0.9%; $p < 0.05$). There was also no significant difference of HBsAg positivity rate amongst children of different age groups.

Table-3 shows the exposure rate to HBV among the study children. Out of 1995 children, anti-HBc antibody was determined in 973 children of which 485 and 488 were male and female respectively. Overall anti-HBc antibody was positive in 79 (8.1%) children, of which 40 (8.2%) and 39 (8%) were male and female, respectively ($p > 0.05$). Among the children, anti-HBc antibody positivity rate increased significantly ($p <0.05$) with increase of age. Age group 11-12 and 13-15 years had significantly ($p <0.05$) higher anti-HBc antibody positivity rate compared to age 5-6 and 7-10 years age groups (13.7% and 9.5% vs. 5.8% and 4.7%).

Serum vitamin A was estimated in 18 HBsAg positive children and in 118 age and sex matched HBsAg negative apparently healthy children (Table-4). The mean serum vitamin A concentration was significantly ($p < 0.05$) less in HBsAg positive children (20.43 ± 2.15 μg/dl) compared to healthy children (24.89 ± 0.68 μg/dl).

Table-1: *Age and gender distribution of the study population (n=1995)*

Age groups (years)	Male n (%)	Female n (%)	Total n (%)
5- 6	305 (50.7)	297 (49.3)	602 (30.2)
7-10	260 (51.3)	247 (48.7)	507 (25.4)
11-12	195 (48.8)	205 (51.2)	400 (20.1)
13-15	228 (46.9)	258 (53.1)	486 (24.4)
Total	988 (49.5)	1007 (50.5)	1995

Table-2: *Distribution of HBsAg positive (HBV carrier) cases according to the gender and age groups (n=1995)*

Age groups (years)	Male		Female		Total Number	HBsAg +ve n (%)
	Number	HBsAg +ve n (%)	Number	HBsAg +ve n (%)		
5- 6	305	4 (1.3)	297	2 (0.7)	602	6 (1)
7-10	260	4 (1.5)	247	1 (0.4)	507	5 (1)
11-12	195	4 (2.1)	205	1 (0.5)	400	5 (1.3)
13-15	228	2 (0.9)	258	5 (1.9)	486	7 (1.4)
Total	988	14 (1.4)	1007	9 (0.9)	1995	23 (1.2)*
		[CI=0.008, 0.023]		[CI=0.003, 0.015]		[CI=0.007, 0.016]

*Note: * In all 23 cases, HBsAg remained positive 12 months after detection in follow up test;*
Male vs. female: p = ns; CI = 95% confidence interval; HBsAg: Hepatitis B virus surface antigen.

Table-3: *Anti-HBc antibody positivity rate according to the gender and age groups (N=973)*

Age groups (years)	Male		Female		Total	Anti-HBc +ve n (%) [CI]	p value
	Number	Anti-HBc +ve n (%)	Number	Anti-HBc +ve n (%)			
5- 6	155	9 (5.8)	153	9 (5.9)	308	18 (5.8) [0.037 , 0.09]	
7-10	106	7 (6.6)	107	3 (2.8)	213	10 (4.7) [0.026 , 0.084]	p <0.005*
11-12	104	12 (11.5)	86	14 (16.3)	190	26 (13.7) [0.095 , 0.19]	
13-15	120	12 (10)	142	13 (9.2)	262	25 (9.5) [0.065 , 0.14]	
Total	**485**	40 (8.2) [0.061, 0.11]	**488**	39 (8) [0.059, 0.11]	**973**	**79** (8.1) [0.065 , 0.1]	p = ns M vs. F

*Note: *4x4 chi-square test, χ^2 = 11.7645; CI: confidence interval; ns = not significant*

Table-4: *Comparison of vitamin A levels of HBsAg positive (HBV carrier) and negative children*

HBsAg status	Number	Vitamin A (μg/dl) Mean ± SE	p value*
HBsAg positive	18	20.43 ± 2.15	p < 0.05
HBsAg negative	118	24.89 ± 0.68	

*Note: *p value calculated by unpaired t test*

Discussion

The HBsAg prevalence among pre-mass vaccination era children, aged 5-15 years was found to be 1.2%, which was lower than the findings from previous small-scale studies in Bangladesh conducted before the introduction of hepatitis B vaccine. The majority of earlier investigations, however, involved either high-risk populations or hospital patients, or participants from particular urban regions. Children under the age of 10 were found to have a 5.4% HBsAg prevalence in a study conducted in 1997–1998 among participants in a hospital's outpatient department for pre-vaccination HBsAg screening [13]. In another study, conducted in 2005–2006 among under-five children in an impoverished area of Dhaka, known for its high burden of infectious diseases, HBsAg prevalence was found to be 12.5% [14]. However, a different study carried out in 1995 in Dhaka among school children aged 6 to 15 found a lower rate (0.8%) [15]. Because of the difference in sample selection methodology, these findings might not be comparable to the current study findings.

Since the discovery of HBsAg, it has been known that males typically have a higher prevalence of HBV than females [16]. This finding is supported by almost all reports on the prevalence of HBV in Bangladesh that included gender-specific data, and in the majority of cases, the differences were statistically significant [17]. On the other hand, the current study revealed no discernible variation in anti-HBc antibody prevalence across the gender categories.

According to the results of the age-group analysis, children between the ages of 11 and 15 had a significantly higher anti-HBc antibody prevalence rate than those between 5 and 10 years old. From the data available, it was not possible to draw any firm conclusions about the reason of this finding. In Bangladesh, vertical transmission is one of the most common ways for HBV to spread, but present study was unable to assess this since it excluded children under the age of 5. Additionally, serological markers of other household members

were not evaluated, and therefore it was not possible to estimate the risk of HBV transmission at the household level. As indicated earlier, le *et al*. [4] stated that teenagers and young adults tend to engage in risky behavior, which may increase their risk of being exposed to HBV. However, more thorough studies are required to fully comprehend the dynamics of HBV infection transmission and risk factors in Bangladeshi children.

Vitamin A level was measured in a subset of sample and it was found that HBV seronegative children had significantly higher levels of vitamin A than seropositive children. For a long time, vitamin A has been referred to as "the anti-infective vitamin" [18], but scientific interest in vitamin A as "anti-infective" therapy has declined due to recent developments in antibiotics. Recent clinical trials and systematic reviews, however, demonstrate that regular vitamin A administration can reduce mortality and morbidity of HIV-infected children [19]. According to Sinopoli *et al.* [20], vitamin A supplemented individuals had a better prognosis and outcomes in several diseases like clearing up of HPV lesions or fewer complications from the measles. A previous study demonstrated that having a positive HBsAg test result was a strong independent predictor of low vitamin A concentration and that those who were HBsAg- positive were almost 6 times as likely to have low vitamin A concentration as those who were HBsAg-negative [9].

Recent studies have demonstrated that vitamin A is more effective to enhance recovery from infection than to prevent infection in first place [21]. It would have been preferable if the current study had done a follow-up to evaluate the HBV markers after supplementing vitamin A in the seropositive subjects.

In conclusion, this study has demonstrated that rural children are in risk of exposure to HBV infection. Increasing HBV seropositivity with age emphasizes the need for devising prevention strategies and to create awareness among the rural children. Further studies are necessary to find out the hitherto undetected sources namely occult hepatitis B cases and the ways of spread of HBV in the community.

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Conflict of interest

The authors declared no conflict of interest.

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Reference

1. Hou J, Liu Z, Gu F. Epidemiology and prevention of hepatitis B virus infection. *Int J Med Sci.* 2005; **2**(1): 50-57. doi: 10.7150/ijms.2.50.
2. Banik S, Datta A, Ghosh A, Ghosh KY, Debi H. The prevalence of hepatitis B virus infection in Bangladesh: a systematic review and meta-analysis. *Epidemiol Infect*. 2022 Feb 14; **150**: e47. doi: 10.1017/S0950268822000061.
3. Paul RC, Rahman M, Wiesen E, Patel M, Banik KC, Sharif AR, et al. Hepatitis B surface antigen seroprevalence among prevaccine and vaccine era childhood in Bangladesh. *Am J Trop Med Hyg.* 2018 Sep; **99**(3): 764-771. doi: 10.4269/ajtmh.17-0721.
4. Ie SI, Turyadi, Sidarta E, Sadhewa A, Purnomo GA, Soedarmono YS, et al. High prevalence of hepatitis B virus infection in young adults in Ternate, Eastern Indonesia. *Am J Trop Med Hyg*. 2015 Dec; **93**(6): 1349-1355. doi: 10.4269/ajtmh.15-0331.
5. Semba RD, Vitamin A, immunity and infection. *Clin Infect Dis*. 1994; **19**(3): 489-499.
6. Markowitz L, Nzilambi N, Driskell WJ, Sension MG, Rovira EZ, Nieburg P, et al. Vitamin A levels and morality among hospitalized measles patients, Kinshasa. *Zaire J Trop Pediatr*. 1989; **35**(3): 109-112. doi: 10.1093/tropej/35.3.109.
7. Sommer A, Katz J, Tarwotjo I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. *Am J Clin Nutr*. 1984; **40**(5): 1090-1095. doi: 10.1093/ajcn/40.5.1090.
8. Semba RD. Vitamin A and immunity to viral, bacterial and protozoan infections. *Proc Nutr*

Soc. 1999; **58**(3): 719-727.
doi: 10.1017/s0029665199000944

9. Obuseh FA, Jolly PE, Jiang Y, Shuaib FM, Waterbor J, Ellis WO, et al. Allflatoxin B1 albumin adducts in plasma and aflatoxin M1 in urine are associated with plasma concentrations of vitamins A and E. *Int J Vitam Nutr Res*. 2010 Dec; **80**(6): 355-368. doi: 10.1024/0300-9831/a000021.
10. Hoofnagle JH, Shafritz DA, Popper H. Chronic type B hepatitis and the healthy HBsAg carrier state. *Hepatology*. 1987; **7**(4): 758-763. doi: 10.1002/hep.1840070424
11. Centers for Disease Control and Prevention. Viral hepatitis: interpretation of hepatitis B serologic test results. USA: Centers for Disease Control and Prevention; Jan 2023. USA.
12. Driskell W, Neess JW, Bryant CC, Bashor MM. Measurement of vitamin A and vitamin E in human serum by HPLC. *J Chromatogr*. 1982; **231**(2): 439-444. doi: 10.1016/s0378-4347(00)81869-1.
13. Zaki H, Darmstadt GL, Baten A, Ahsan CR, Saha SK. Seroepidemiology of hepatitis B and delta virus infections in Bangladesh. *J Trop Pediatr.* 2003; **49**(6): 371-374. doi: 10.1093/tropej/49.6.371.
14. Ashraf H, Alam NH, Rothermundt C, Brooks A, Bardhan P, Hossain L, et al. Prevalence and risk factors of hepatitis B and C virus infections in an impoverished urban community in Dhaka, Bangladesh. *BMC Infect Dis*. 2010 Jul; **10**: 208. doi:10.1186/1471-2334-10-208.
15. Laskar MS, Harada N, Khan F. Prevalence of hepatitis B surface antigen (HBsAg) in Viqarunnessa noon girls' school children in Dhaka, Bangladesh. *Cent Eur J Public Health*. 1997; **5**(4): 202-204.
16. Blumberg BS. Sex differences in response to hepatitis B virus. I. History. *Arthritis Rheum.* 1979 Nov; **22**(11): 1261-1266. doi:10.1002/art.1780221114.
17. Uz-Zaman MH, Rahman A, Yasmin M. Epidemiology of Hepatitis B Virus Infection in Bangladesh: Prevalence among General Population, Risk Groups and Genotype Distribution. *Genes (Basel).* 2018; **9**(11): 541. doi: 10.3390/genes9110541.
18. Semba RD, Vitamin A as "Anti-Infective" Therapy, 1920–1940. *J Nutr*. 1999; **129** (4): 783–791. doi: 10.1093/jn/129.4.783.
19. Semba RD, Ndugwa C, Perry RT, Clark TD, Jackson JB, Melikian G, et al. Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus-infected children in Uganda: A controlled clinical trial. *Nutrition.* 2005; **21**(1):25-31. doi: 10.1016/j.nut.2004.10.004. Erratum in: Nutrition. 2005 Feb; **21**(2): 287.
20. Sinopoli A, Caminada S, Isonne C, Santoro MM, Baccolini V. What Are the Effects of Vitamin A oral supplementation in the prevention and management of viral infections? A systematic review of randomized clinical trials. *Nutrients.* 2022; **14**(19): 4081. doi: 10.3390/nu14194081.
21. Villamor E, Fawzi WW. Effects of vitamin a supplementation on immune responses and correlation with clinical outcomes. *Clin Microbiol Rev*. 2005; **18**(3): 446-464. doi: 10.1128/CMR.18.3.446-464.2005.

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