The Prevalence of Combined Three Markers of Hepatitis B in Indonesia: Further Analysis of National Basic Health Research, 2013

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Abstract. Hepatitis B is still a health problem in the world and Indonesia. A combination of three markers, HBsAg, anti-HBc, and anti-HBs, provides further information on the hepatitis B active infection and immunity status. Therefore, the objective of this study is to identify the prevalence of combined three markers of hepatitis B in the Indonesian population. Serum was collected from respondents aged ≥ 1 year in biomedical testing which was part of the National Basic Health Research conducted by the Ministry of Health in 2013. Up to 37,076 samples were examined for HBsAg, anti-HBc, and anti-HBs using a Chemiluminescent Immunoassay method. Data were then analyzed to calculate the prevalence of the combined three markers of hepatitis B.The result shows that more than half samples were negative (54.23%) and 0.88% were positive for those three markers. The prevalence of only HBsAg positive was found in 1.65% of samples, with positive anti-HBc (3.86%) and anti-HBs (0.40%). Around 22.16% of samples had antibodies both anti-HBc and anti-HBs, 4.52% (only anti-HBc), and 12.29% (only anti-HBs).In summary, most Indonesian populations were still prone to catching hepatitis B and hence need wider access to hepatitis vaccination. The rest had at least a history of hepatitis infection that either might have resolved or still lasted to a chronic state and past vaccination. A small proportion of cases might be suggestive of mutant hepatitis B virus. Therefore, thorough surveillance via genomic sequencing can be performed to identify genotypes of hepatitis virus circulating in our population and help us develop more proper vaccines or targeted therapy for such variants.

Keywords: Hepatitis B · HBsAg · anti-HBc · anti-HBs · Indonesia

1 Introduction

Infection of Hepatitis B becomes a health problem in the world and also in Indonesia. Globally, The World Health Organization (WHO) estimates that 296 million people experienced to chronically hepatitis B in 2019, and emerged 1.5 million new cases every year. In the same year, hepatitis B infection also caused 820.000 mortality, mostly due to cirrhosis or liver carcinoma. In highly endemic areas, hepatitis B is usually transmitted

I. Nurlaila et al. (Eds.): ICHR 2022, AHSR 56, pp. 220–227, 2023.
https://doi.org/10.2991/978-94-6463-112-8_22

by perinatal (from the infected mother to her baby) or horizontal transmission (from infected people to others), particularly in children under 5 years old. However, sharing the use of needles, tattoos, piercing, and exposure to infected blood or body fluids are other sources of hepatitis B transmission. It is targeted that the global elimination of hepatitis B would be achieved by 2030 [1].

Manifestation of hepatitis B infection is varied, from asymptomatic carrier to chronic and severe symptoms, therefore, a laboratory confirmation for prompt diagnosis is essential. Serological markers used for hepatitis B diagnosis include HBsAg (hepatitis B surface antigen), anti-HBs (antibody against hepatitis B surface antigen), anti-HBc (antibody against hepatitis B core antigen), HBeAg (hepatitis B protein antigen which indicated active replication)), total anti-HBe, and anti-HBe IgM /IgG (antibody against HBeAg). By using these hepatitis B serologic markers we will get information related to the patient's infection status, the natural course of chronic hepatitis, the phase of clinical infection, and the impact of antiviral treatment [2]. In 2013, the Ministry of Health conducted basic health research in all provinces in Indonesia. The objective of that research was to collect the community-based data used to evaluate the health status on a district, province, and national level. Part of the research was biomedical-based research accompanied by serum collection and serologic testing of immunization-preventable diseases, including hepatitis B. Specifically for biomedical-based research, the data generated was only representative of the national scale [3]. This research aimed to obtain the prevalence data of combined three serological markers of hepatitis B using biomedical-based research specimens conducted in 2013. It is hoped that the result provides further information on the hepatitis B active infection and immunity status of the Indonesian people to hepatitis B.

2 Materials and Methods

2.1 Materials

This research used stored biological material in the form of serum collected by the Ministry of Health through Basic Health Research in 2013. The study design was cross-sectional and carried out in 33 provinces in Indonesia. The serum came from selected respondents aged > 1 year old. A sampling of the census block, households, and respondents was determined by the Central Bureau of Statistics (BPS), then updated by enumerators. Research ethics approval was obtained from the ethics committee of the National Institute of Health Research and Development no: LB.02.01/5.2/KE.246/2014 dated 13 June 2014. In addition to serum specimens, three serological markers of hepatitis B kits (HBsAg, anti-HBs, and anti-HBc), chemiluminescence immunoassay automatic analyzers, and a set of micropipettes were required to perform the tests.

2.2 Laboratory Examination

The examination of Hepatitis B serology was carried out in 2014 at the National Institute of Health Research and Development, using the direct sandwich chemiluminescence immunoassay (CLIA) method. Serological tests included HBsAg, anti-HBc, and anti-HBs markers, with the catalog number of each marker being HBsAg (Liaison XL Murex

HBsAg Quant, cat 310250); anti-HBc (Liaison anti-HBc, cat 310130) and anti-HBs (Liaison anti-HBs, cat 311220) [4].

The principle of the CLIA method is the reaction between hepatitis B antigen/antibody with conjugates and magnetic particles, then with the addition of a starter reagent, a chemiluminescence reaction will be formed and read by the CLIA machine automatically [4]. Before being tested, the samples should be prepared and diluted, the control reagent and the CLIA machine also be calibrated according to the manual instructions. The sample was examined singly, but if the titer value showed equivocal results, the sample should be re-examined in duplicate. The cut-off value of each examination is as follow; HBsAg (reactive if titer ≥ 0,05 IU/m), anti-HBs (positive if titer B ≥ 11 mIU/ml), and anti-HBc (positif if titer < 1,0 IU/ml). All data then be entered and linked with other variables and analyzed to calculate the prevalence of three hepatitis B markers (HBsAg, anti-HBs, and anti-HBc) simultaneously using the SPSS program. The limitation of the research was not all required variables were available in the main research, therefore, data analysis was adjusted to the existing and appropriate variables.

3 Results

The serological examination of hepatitis B was carried out on the serum of respondents who came from 33 provinces in Indonesia and aged > 1 year. A total of 37,238 sera were successfully examined with the characteristics of the respondents as shown in Table 1. More than half of the respondents came from rural areas (55.3%) and were female (55.9%). The number of adult respondents (aged 15 years and over) who were examined was five times more than children (85.6%).

The results of the analysis of three serologic markers of hepatitis B, namely HBsAg, anti-HBc, and anti-HBs, are shown in Table 2. The total serum for further analysis was 37,076 because 162 sera showed equivocal values (borderline or cannot be determined yet as either positive or negative), so they were not included in the final analysis. The

Table 1. Characteristics of respondents who were examined with hepatitis B serological markers

Variables	n	Proportion
Area		
Urban	16,634	44.7
Rural	20,604	55.3
Sex		
Male	16,411	44.1
Female	20,827	55.9
Age group		
1–14 years old	5,378	14.4
≥ 15 years old	31,860	85.6
Total	37,238	100

Table 2. The prevalence of combined HBsAg, anti-HBc, and anti-HBs and their interpretation

Serologic markers of Hepatitis B			n	Proportion	Interpretation
HBsAg	Anti- HBc	Anti-HBs			
+	+	+	325	0.88	Chronic infection (considered a healing infection)
+	+	-	1,432	3.86	Acute/ chronic infection, ongoing depend on Ig M /IgG
+	-	+	150	0.40	Possible HBsAg mutant
+	-	-	612	1.65	Chronic infection (isolated HBsAg)
-	+	+	8,217	22.16	Past infection, cleared, immunity achieved
-	-	+	4,557	12.29	immune due to vaccination
-	+	-	1,677	4.52	Past natural infection, cleared, anti-HBs have waned over time
-	-	-	20,106	54.23	Never expose, Susceptible to infection
Total			37,076	100	

examination showing equivocal values should be repeated, however, this could not be performed due to limited serum aliquot.

Based on the assessment of the three hepatitis B markers simultaneously, nine combinations marker were obtained. Most respondents (54.23%) had never been infected with hepatitis B and had no hepatitis B antibodies, which is indicated by negative results for all three markers. Respondents with this condition are considered susceptible to hepatitis B infection. The next highest prevalence were respondents with positive anti-HBc and anti-HBs (22.16%). In this group, respondents had experienced hepatitis B infection but had recovered and developed immunity. Vaccination-induced immunity was found in 12.29% of respondents. Chronic/acute hepatitis B infection with positive HBsAg and anti-HBc but negative anti-HBs was found in 3.86% of respondents. A small prevalence of less than 2% was found in respondents who were HBsAg positive only (1.65%) or plus anti-HBc and anti-HBs positive (0.88%) or plus anti-HBs positive (0.40%). Respondents in the first two groups were likely to undergo chronic hepatitis B, while the latter are likely to be infected with mutant hepatitis B virus.

4 Discussion

This is one of the largest hepatitis B studies, involving more than 37,000 respondents in both urban and rural areas of Indonesia. However, because the sampling design used is only representative of the national scale, so the data cannot be translated by the province. In addition, because the available variables are not intended for certain diseases, consequently not all the necessary data are available, for example, the immunization

status of the respondents who were only asked to children under five years old, so this variable cannot be used for analyzing all age groups.

Hepatitis B virus is the main cause of chronic liver disease which can be transmitted through injection, sexual intercourse, or vertically from mother to baby. Based on the serological results indicated that most respondents have never been infected with the hepatitis B virus, nevertheless, they also have no antibodies, meaning that they are quite susceptible to hepatitis B infection later in life. If we highlight the prevalence of HBsAg alone, this value (7%) belongs to the moderate to almost high endemicity. Endemicity status based on the prevalence of HBsAg can be categorized into three groups, namely high (above 8%), moderate (2–7%), and (low 0.5–2%) (5).

History of hepatitis B infection was detected almost quarter respondents, indicated by positive anti-HBc and/or anti-HBs, although the HBsAg was no longer detectable. In this case, the infection might be healed and the person has sufficient protective immunity. However, in certain cases, the presentation of anti-HBs sometimes wanes or even disappears until it's not detected anymore [2]. Anti-HBs is known as neutralizing antibody, at a certain level can protect the people from the hepatitis B infection in long term. The protective level of anti-HBs is more than 10 mIU/mL, develop if someone has a vaccination of hepatitis B or have been infected with the hepatitis B virus before, the last case is characterized by developing anti-HBc as well [2]. Anti-HBc is persistent and detectable in the long term. Detection of anti-HBc can be divided into anti-HBc IgM indicates an acute infection and anti-HBc IgG indicates chronic infection. In this study, the anti-HBc marker examined was total anti-HBc, so it could not explain whether the infection was acute or chronic, however, it was sufficient to provide information on the infection status.

Although the immunization status of respondents is not known with certainty, the impact of vaccination does not appear to be optimal, since only 12% of respondents have positive anti-HBs. Low immunization coverage and inadequate antibody levels will encourage hepatitis B infection to continue. In addition, the presence of a mutated virus can cause the inability of antibodies to recognize and overcome infection [6]. Anti-HBs will persist for several years (even for life) but the titer can decrease and the humoral response goes away. In Indonesia, the immunization program for hepatitis B has been launched in 1997, and since 2018 the immunization of hepatitis B was given five times; when a baby is born, 2–3-4 months of age (pentavalent vaccine of DTP-HB-Hib), and at 18 months of age (given together with measles/Measles-Rubella vaccine) (7,8), with the hope to provide long-term protective immunity. However, there is a small portion of respondents that show positive anti-HBs and also HBsAg, it may be that the existing antibody is unable to neutralize the viral antigen so that the patient still carries the virus (2), or the patient is infected with hepatitis B virus that undergoes mutation (9).

The serological markers sometimes do not give enough information related to the patient's clinical condition, so additional tests are often needed to confirm the diagnosis or treatment, for example, molecular examination. Molecular detection of hepatitis B can be applied to determine the viral load, which indicates the replication activity. This marker is usually detected in the early stages of infection and reaches a peak 3 months after exposure, then the infection can develop into chronic or heal (2).

The main strategies to control hepatitis B other than immunization for susceptible populations are treatment of chronic hepatitis B infection and interruption of transmission routes. It starts with treating pregnant women with hepatitis B to prevent perinatal infection [10]. Knowing that more than half the Indonesian population is still vulnerable to hepatitis B, implementing universal vaccination not only in children but also in adults is very encouraged. Aside from mandatory immunization in young infants, a booster vaccination should be made available to all who need it the most at a low cost [11]. After learning from experience during the COVID-19 pandemic, the need to develop a database registry for other infectious diseases like hepatitis B is getting bigger. This will help our healthcare system not only evaluate its program but also address the situation more effectively by using local approaches [12, 13].

Considering the limitation of serological marker detection, genomic surveillance should be performed for cases of occult hepatitis B infection (OBI), particularly ones caused by mutant variants [14, 15]. Whole-genome sequencing (WGS) technology can monitor closely the circulating hepatitis B viruses, their behavior as well as their genotypes. Moreover, further necessary steps can be taken based on this valuable information, such as giving specific clinical treatment when one becomes infected with a certain variant. Identification of new mutation position also provides a solid basis when we would like to design new vaccines and targeted therapy which can be potentially effective against this mutated virus [16, 17].

Most Indonesian populations were still prone to catching hepatitis B and therefore this group needs wider access to hepatitis vaccination. The rest had at least a history of hepatitis infection that either might have been resolved or continued to a chronic state while some others had past vaccination. There is still an indication of occult hepatitis B infection (OBI) although the proportion was relatively small. Hence, thorough surveillance via genomic sequencing can be performed to identify genotypes of hepatitis virus circulating in our population and help us develop better strategies to tackle this problem. These may include but are not limited to adjustment of clinical treatment, proper vaccine design, or targeted therapy for such variants.

Acknowledgements. We would like to thank the Ministry of Health for conducting basic health research regularly so that the data can be used for publication, evaluation, and policy input for future programs.

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