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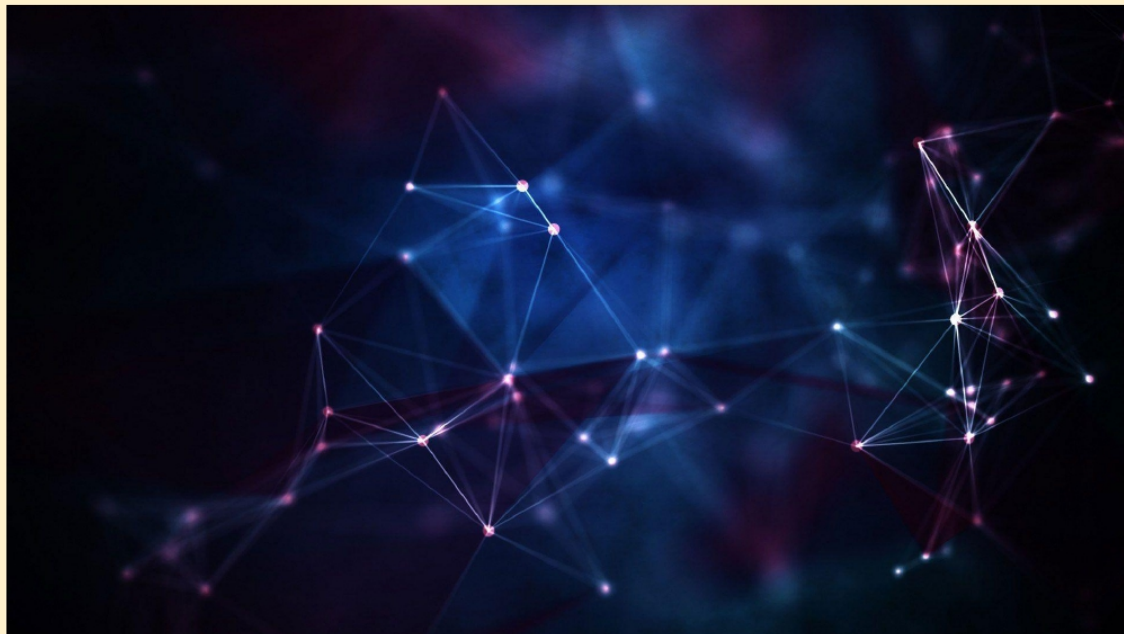
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Factors Associated with Anti-HBs Seroprotection Among Children Aged 10-18 Years Old in Bangkalan Islamic Boarding School, Indonesia

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Abstract: - Hepatitis B (HB) is a liver inflammation due to Hepatitis B virus (HBV) infection. Currently, HB immunization is included in the national immunization program and significantly decreases the incidence of HB. However, previous evidence reported that formed anti-HBs are low or even absent among individuals with complete HB immunization. This study aims to analyse the factors associated with long-term seroprotection against HBV infection among children aged 10-18 years in Bangkalan Islamic boarding school, Indonesia. This study was conducted at the Bangkalan Islamic Boarding School, Madura, Indonesia in January 2022. A total of 100 healthy children aged 10-18 years old (45% boys) were included. Baseline characteristics such as age, sex, nutritional status, immunization status, and anti-HBs levels were collected. Thirteen participants had seropositive anti-HBs, while 87 participants did not. Age and immunization status was associated with seropositive anti-HBs with a p-value of 0,013 and 0,034, respectively. On the contrary, sex and nutritional status based on BMI did not associate with anti-HBs status. Additional boosters' immunization for adolescents in Indonesia could be considered to prevent HBV infection and chronic HB. Further studies are needed to explore the protection duration of complete HB immunization.

Key Words: — *Hepatitis B, Immunization, Anti-HBs, Antibody, Risk Factors.*

I. INTRODUCTION

Hepatitis B (HB) is inflammation in the liver due to Hepatitis B virus (HBV) infection. In some cases, the disease progresses into chronic HB and endangers public health (Kementerian Kesehatan Republik Indonesia, 2012). The HBV infection is responsible for 4 million new HB cases and a million deaths due to chronic HB complications such as steatohepatitis, cirrhosis, and hepatocellular carcinoma (HCC). The HCC is ranked as the fifth cause of death in cancer worldwide and is responsible for 620 thousand deaths related to HBV infection (World Health Organization, 2021).

Hepatitis B is a preventable disease. Currently, HB immunization is included in the national immunization program and demonstrated a significant effect in suppressing the incidence of HB in Indonesia (Kementerian Kesehatan Republik Indonesia, 2014). However, evidence regarding the life-long protection of primary HB immunization against HBV infection is scarce. The antibody of HB surface (anti-HBs) is an objective indicator of an individual's protection against HBV infection. A level of 10 mIU/mL of anti-HBs is categorized as seropositive or seroprotective. In some cases, the formed anti-HBs were low or even absent. Ironically, anti-HBs is not routinely examined in Indonesia.

A study in Hongkong reported that after three dosages of HB immunization, only 90% of participants were protected for 30 years with declining anti-HBs titer (Das et al., 2019). In contrast, a study in South Korea showed that the level of anti-HBs was reduced by 40% in 10 years after vaccination. Factors such as age, sex, obesity, smoking, and alcohol consumption were associated with participants' immunogenicity against HBV infection, resulting in anti-HBs production failure (Kim et al., 2003).

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Islamic boarding school is a special environment and associated with a higher risk of HBV infection. Evidence regarding anti-HBs seroprotection status among children and adolescents in Islamic boarding schools in Indonesia is limited. Therefore, the current study aims to analyze the factors associated with long-term seroprotection against HBV infection among children aged 10-18 years in Bangkalan Islamic boarding school, Indonesia.

II. METHODS

This study was a cross-sectional study that explored factors associated with anti-HBs seroprotection among children aged 10-18 years old in an Islamic boarding school in Bangkalan, Indonesia. The study was conducted in January 2022 at Ar Rohmani Al Ishaqy Islamic boarding school, Bangkalan, Indonesia. This study method had been approved by the Ethics Committee of Dr. Soetomo General Academic Hospital Surabaya and granted an ethical clearance (No. 233/EC/KEPK/FKUA/2022).

The participants of this study were students at respective Islamic boarding school who fulfilled inclusion criteria which were: children aged 10-18 years old, classified as healthy individuals based on anamnesis and physical examination, and the parents signed the information for consent and informed consent form. While children who were not healthy or under emergency conditions, history of blood transfusion, history of intravenous drugs abuse, and refused to participate were excluded from the study. The minimal sample size was calculated based on the Lemeshow formula. Based on our calculation, the minimal sample size was 80 participants. After adjustment for dropout (20%) the final minimum size was 96 participants.

The data regarding age, sex, nutritional status, and immunization status were collected through medical records. While anti-HBs titer was tested by ELISA method with 10 mIU/mL as the cut-off value. Baseline characteristics including age, sex, nutritional status, immunization status, and anti-HBs status were presented in a dedicated table.

The association between the anti-HBs titer and other factors was analysed with the Chi-square test or Fischer exact probability test. All analysis was performed using IBM SPSS Statistics. A p-value of 0.05 or below after adjusting age and sex was considered statistically significant.

III. RESULTS

3.1 Baseline Characteristics

A total of 100 students from Ar Rohmani Al Ishaqy Islamic boarding school were included in this study. The majority of the participants were female (55%) and aged 10-15 years old (62%). Seventy-three children had normal BMI, while 17 and 10 children were classified as overweight and obese, accordingly (Table 1.).

Table.1. Baseline Characteristics

Characteristics	Total (%) n=100
Age	
10-15 years old	62
>15-18 years old	38
Sex	
Boys	45
Girls	55
Nutritional Status (BMI)	
Normal (<85%)	73
Overweight (85-95%)	17
Obese (>95%)	10
Immunization Status	
Complete	76
Incomplete	24
Anti-HBs Status	
Seropositive	13
Seronegative	87

3.2 The Seroprotection of Anti-HBs

All participants were tested for anti-HBs using the ELISA method. An anti-HBs of 10 mIU/mL or higher was defined as seropositive or have protection against HBV (seroprotection), while anti-HBs below 10 mIU/mL was defined as seronegative. Most participants were seronegative (87%), while only 13% were seropositive.

3.3 Factors Associated with Anti-HBs Status among Children Aged 10-18 Years Old

A Chi-square test was performed to evaluate factors associated with the seroprotection status of anti-HBs. Four following factors were evaluated: age, sex, nutritional status, and immunization status. All factors were presented as categorical data and underwent statistical analysis.

Most of the seropositive children were aged >15-18 years old (69,2%). Based on the Chi-square test, age is significantly associated with anti-HBs status Children aged 10-15 years old

had higher seropositive ($p=0,013$).

Even though the majority of participants were girls, the boys were more likely to have protection against HBV infection (53,8%). Yet, this difference was not statistically significant ($p=0,492$).

According to BMI classification, 11 out of 13 seropositive children had normal BMI, followed by one child with overweight and obese, respectively. While among seronegative participants, 62 children had normal BMI. The difference between groups was not statistically significant ($p=0,572$).

Seventy-six participants received complete immunization. All seropositive children had received complete HB immunization, while 63 out of 87 seronegative children had complete HB immunization. This difference was statistically significant with a p-value of 0,034, yet the odds ratio was not able to calculate since all seropositive children had received HB immunization. All analysed factors are presented in Table 2.

Table.2. Factors Associated with Anti-HBs Status

Characteristics	Sero+ (%) n=13	Sero- (%) n=87	P value
Age			
10-15 years old	4 (31)	58 (66)	0,013
>15-18 years old	9 (69)	29 (33)	
Sex			
Boys	7 (54)	38 (44)	0,492
Girls	6 (46)	49 (56)	
BMI			
Normal	11 (85)	62 (71)	0,572
Overweight	1 (7,5)	16 (19)	
Obese	1 (7,5)	9 (10)	
Immunization Status			
Complete	13 (100)	63 (72)	0,034
Incomplete	0 (0)	24 (28)	

IV. DISCUSSION

4.1 Baseline Characteristics

The World Health Organization (WHO) recommends HB immunization be included in every national immunization program worldwide. Indonesia had included HB immunization in the national immunization program since April 1997. Physiologically speaking, inoculation of HB immunization would produce anti-HBs, which is an objective

indicator of an individual's protection against HBV infection (Astuti & Kusumawati, 2014). Conventionally, 10 mIU/mL is the minimum concentration of anti-HBs that protects individuals from HBV infection reflected by HBsAg testing (Bonanni & Bonaccorsi, 2001; Westmoreland et al., 1990). Therefore, this study also used an anti-HBs cut-off value of 10 mIU/mL. The immunization experts from WHO and the international hepatitis organization reported that the second inoculation of HB immunization has a higher titer of anti-HBs compared to the first inoculation (80-95% vs 16-40%). While third inoculation or booster increases the anti-HBs titer to 98-100%.

Ironically, there were numerous cases in which anti-HBs are not formed in children with complete HB immunization. In this study, anti-HBs of all participants (45 boys and 55 girls) were tested. The seropositive participants were only 13%, while the remaining demonstrated no formed anti-HBs. All seropositive children and 72.4% of seronegative had received complete HB immunization.

A previous study showed that anti-HBs lasts for more than 5 years. Subsequently, this protection during the first 5 years of life is considered adequate to suppress chronic infection of HBV, despite no booster being administered (Pracoyo & Wibowo, 2016).

Several factors had been associated with declining anti-HBs titer. Age, sex, obesity, smoking, and alcohol consumption are significantly related to lower immunogenicity against HB immunization and failure of anti-HBs production (Kim et al., 2003). Another study reported that age during vaccination, vaccination dosage, and genetic aspects play a crucial role in maintaining anti-HBs concentration in serum. The higher immune response to HB vaccination is associated with longer protection of anti-HBs (Stefanati et al., 2019).

4.2 Age and Anti-HBs Status

The current study evaluated anti-HBs seroprotection status after serial vaccination during the first year of life. Evidence showed that antibody titer will decline over time. The peak of antibody titer usually occurs after the first month of the last complete vaccination, followed by a steep reduction in the few months and stagnant in the following years (Haber & Schillie, 2021).

Based on our sample, the last HB vaccination was given around 10-18 years ago. Despite titer reduction over time, anti-HBs might be detected since the immunological memory lasts for more than 20 years after vaccination. In addition,

healthy individuals with a declining antibody are still protected from HBV infection (West & Calandra, 1996).

Seropositive participants were older compared to seronegative participants and statistically significant. Our result was contrary to a study among 1200 children aged 3-10 years in South Korea which stated that anti-HBs are reduced following their age (Kwon & Jeong, 2019). Another study among children aged 15-20 years reported that older age of vaccination is associated with a seropositive status of anti-HBs (Stefanati et al., 2019). Subsequently, children who received HB vaccination during the infancy period had lower anti-HBs concentration compared to those who were vaccinated during the adolescent period. However, the immunological memory still existed for 11-23 years (Bini et al., 2018).

4.3 Sex and Anti-HBs Status

Sex was not related to the seropositive status of anti-HBs ($p=0,492$). Similar to an earlier study among 621 participants which reported that seropositive status was not associated with sex. The seropositive males were 74 out of 244 male participants (30,3%), while seropositive females were 98 out of 377 female participants (25,9%) (Stefanati et al., 2019). In addition, seropositive anti-HBs was more likely in the males compared to females (53,8% vs 46,2%).

Demographical data from Asian and non-Asian countries showed that males had a higher immunological response to the HB vaccine compared to females (Giefing-Kröll et al., 2015). This difference was more prominent when the route of HB vaccine administration was intracutaneous (Cardell et al., 2008). Yet, the difference in seronegative was not statistically significant.

Epidemiological and clinical reports showed the opposite result. Chronic HBV infection was more prevalent among males compared to females. Moreover, complications related to hepatitis such as fibrosis, cirrhosis, and HCC were more prevalent in males (Grebely & Dore, 2014). A cohort study suggested that the androgen pathway upregulates HBV transcription through direct activation of the sensitive-androgen element in the viral multiplication process. This mechanism might explain why HBV titer and risk of HCC were higher among males. Females also have a better humoral and cellular immune response to vaccination compared to males in all age groups (Fish, 2008).

Our study showed that anti-HBs titer among females was higher compared to males. The mechanism behind this

phenomenon might be related due to higher immunological genes in X chromosomes than in Y chromosomes. Estrogen activates the monocytes to produce IL-10 and induces secretion of IgG and IgM via B cells. While testosterone alters IgG and IgM production and dampens monocytes to produce IL-6 (Kanda & Tamaki, 1999). Another hypothesis is related to virus clearance during early infection. Host factors such as IL-28 and viral genotype 1a in X chromosomes are associated with spontaneous viral clearance in acute HBV infection (Lebray et al., 2004).

4.4 Nutritional Status and Anti-HBs Status

A study in 2013 evaluated the role of BMI on post-HB vaccine progressivity. The study grouped the BMI based on WHO classification which were underweight ($<18,5 \text{ kg/m}^2$), normal ($18,5-24,9 \text{ kg/m}^2$), overweight ($25-29,9 \text{ kg/m}^2$), and obese ($\geq 30 \text{ kg/m}^2$). The BMI was not related to progressivity, yet obese participants were more likely to be seronegative. The BMI was not significantly different between the seropositive and seronegative group. Normal BMI in seropositive was 84,6%, while in seronegative was 84,9% (Young et al., 2013).

The same study also reported an association between BMI and vaccine-specific immunoglobulins with 10 mIU/mL as the cut-off value ($p=0,009$). Obese participants had a higher risk of seronegative or non-responder to the second dose of the rHBV vaccine (Adjusted Odds Ratio 8,75; $p=0,043$). (Young et al., 2013). This result indicated that immunological response after HB vaccination was lower among obese participants compared to normal participants (Young et al., 2013).

Correlation between obesity and immunological response to the vaccine was firstly observed in 1985 among health workers who received the HB vaccine. After 25 years, clinical and laboratory data were published during the influenza A/pH1N1 pandemic. According to the data, obese individuals had a higher risk of influenza and its complication. Moreover, the antibody protection after tetanus and rabies vaccine was lower in obese individuals. This phenomenon indicates that immunological response is associated with BMI, which seroconversion among obese individuals is not optimal (Painter et al., 2015).

A meta-analysis of 16 studies concluded that obesity is associated with seronegative of anti-HBs after HB vaccination (adjusted OR: 2,46, 95%CI: 1,50-4,03). (Fan et al., 2016). The majority of the vaccine were administered during the first month of life and obesity had not occurred. Some vaccines,

HB and influenza, need a booster dosage during pre-school age and had been recommended for obese population annually, yet the immunological response was unknown. Decreased immunological response to the vaccine is not a solely an individual problem, but also public health concern (Tagliabue et al., 2016).

4.5 Immunization Status and Anti-HBs Status

Currently, a complete HB immunization consists of a serial dosage of vaccine which consist of the first dose right after delivery or before the first 24 hours; 2, 3, and 4 months combined with DTwP or DTap; and 18 months booster. This study only considered complete immunization as HB vaccine during the age of 2,3, and 4 months. Previously, a vaccination at the age of 18 months was not regulated. This additional dose is expected to provide protection among school-age children and adolescents since the anti-HBs titer is low if the HB vaccination was administered during the infancy period (Lee et al., 2006).

Formation of anti-HBs after HB vaccination indicates individuals' immunological response and protection against HBV infection (Astuti & Kusumawati, 2014). Some cases reported that anti-HBs is not formed in healthy children after vaccination. Subsequently, the protection duration of HB vaccination is still controversial.

In our study, 76 children had complete immunization and 13 of them were seropositive. However, another 63 children had no formation of anti-HBs. This proportion was significantly different ($p=0,034$). Our result was similar to an earlier study in Italy. Administration of HB vaccine during infancy showed a lower long-term titer of anti-HBs compared to those who received HB vaccine during adolescent period (12,2% vs 51,1%, $p < 0.001$). However, this study did not distinguish seroconversion between children with basic immunization and those who received basic and booster.

Another study in 2018 enrolled 2203 children and young adults aged 11-23 years old. This study compared the immunological response of basic and boosters of the HB vaccine. As predicted, those who received basic and boosters vaccine showed higher immunological response compared to those who only received basic HB vaccine ($p < 0,001$) (Bini et al., 2018).

4.6 Strength and Limitation of the Study

The result of this study is applicable for clinical and public health considerations. However, limitations of our

study were small number of the overweight-obese children, no data regarding types of HB vaccine administered, no data regarding age during first vaccination, and recall bias of immunization status.

V. CONCLUSION

Age and HB immunization status were associated with anti-HBs seroprotection among children aged 10-18 years old. Sex and nutritional status were not associated with anti-HBs seroprotection. Booster of HB immunization could be considered among children and adolescents in Indonesia. In addition, further studies are required to confirm our findings.

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Conflicts of Interest: The authors declare no conflict of interest

Ethical Clearance: This research was approved by the Ethics Committee of Dr. Soetomo General Academic Hospital Surabaya and granted an ethical clearance (No. 233/EC/KEPK/FKUA/2022).

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