ORIGINAL ARTICLE

Hepatitis B virus infection: Epidemiology and seroprevalence rate amongst Negrito tribe in Malaysia

Noraziah Sahlan, MPath[1], Fadzilah Mohd Nor, MPath[1,3], Azdayanti Muslim, MMedSc[1,2], Syahrul Azlin Shaari, MPath[1], Thuhairah Hasrah Abdul Rahman, MPath[1], Hoh Boon Peng, PhD[4]

[1]Department of Microbiology and Parasitology, Faculty of Medicine, Universiti Teknologi MARA, Sg. Buloh Campus, Sungai Buloh, Selangor, Malaysia, [2]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [3]Integrative Pharmacogenomics Institute (i-PROMISE, Universiti Teknologi MARA, Puncak Alam, Shah Alam, Malaysia, [4]Centre of Excellence in Research, Value Innovation and Entrepreneurship (CERVIE), UCSI University

ABSTRACT

Introduction: Prevalence of Hepatitis B virus (HBV) infection among the non-indigenous people in Malaysia has been well established and range between 3% and 5%. However, data from the indigenous (*Orang Asli*) people is still lacking. The Negrito population is the most remotely located Orang Asli tribe with limited access to health care facilities. This study was undertaken to determine the epidemiology and seroprevalence of HBV infection among the Negrito.

Methods: Surveys were conducted in five Negrito settlements in Kelantan and Perak states in Malaysia. A total of 150 participants were recruited. Clinical history was taken and physical examination was performed. Five millilitres of whole blood were collected and tested for hepatitis B surface antigen (HBsAg) using electrochemiluminescence immunoassay.

Results: Participants were mainly from the *Bateq* (49.3%) and Mendriq (29.4%) sub-tribes. Overall, 13 subjects (8.7 %); nine males and four females were HBsAg positive. Nine of the HBsAg positive subjects were ≥35 years old. All of them had history of home deliver without evidence of antenatal record. Six (46%) of the HBsAg positive subjects had tattoo and body piercing in the past.

Conclusion: The prevalence of HBV infection rate amongst the Negrito tribe is almost three-fold compared to the national rates. The reason for this finding remains unclear. Tattooing, body piercing and vertical transmission could be the main possible routes of transmission of HBV among the Negrito population in Malaysia.

KEY WORDS:
Hepatitis B, Orang Asli, Negrito, prevalence, Malaysia

INTRODUCTION

Hepatitis B virus (HBV) is one of the major aetiological agents that may cause acute or chronic hepatitis. Its infection results into well-known and established long-term sequelae such as cirrhosis and hepatocellular carcinoma (HCC). The HBV infection is transmitted haematogenously and sexually via blood, semen and vaginal secretions. Parenteral or mucosal exposures pose potential risk of acquiring the infection. Perinatal infection is vast in highly endemic region, whereas horizontal transmission is the major route in intermediate endemic region.[1] Approximately 25% of those acquired with HBV infection prenatally, and 15% of adults infected with HBV developed cirrhosis or HCC respectively.[2]

The disease burden of HBV is substantial worldwide. Globally, chronic HBV infection affects 350 to 400 million people,[3] with disease prevalence varying between geographic regions. According to the WHO, the Africans and the Western Pacific regions have the highest of HBV prevalence, while the America regions has the lowest prevalence rate of 6.1% and 0.7% respectively. To date, the South East Asia region was estimated to have approximately 2% of HBV infection prevalence rate.[4]

The HBV seroprevalence rate for Malaysia ranges from 3% to 5%, that categorised Malaysia as an intermediate endemic country for HBV.[5,6] The neighbouring countries including Thailand, Indonesia and Philippines are endemic countries with prevalence rates of >8%, 2-5% and 5-16% respectively.[5] Such discrepancy is most probably related to the differences in the mode of transmission, including iatrogenic transmission, and the age of the patients at the time of infection.

Malaysia is a multiracial country with three major ethnics: Malays, Chinese and Indians. The indigenous people of Malaysia make up the minority of the population. It has been shown that the prevalence of HBV infection is the highest among the Chinese (36%), followed by Malays (26%) and Indians (15%).[7] The major route of transmission among the general population in Malaysia is via vertical transmission. Horizontal transmission also plays important role especially among adults at risk such as people who inject drug, HIV positive persons and sexual workers.

Literature search revealed that the data on prevalence of HBV among Malaysia indigenous people, the *Orang Asli* is currently scarce. Thus, the true HBV burden in Malaysia is not accurately reflected. The true picture of Hepatitis B infection burden is important as it helps to gauge the health cost and social impact on Malaysia's population as a whole.

This article was accepted: 1 June 2019
Corresponding Author: Noraziah Sahlan
Email: aziah94@salam.uitm.edu.my

Determining the prevalence and epidemiology of Hepatitis B among the *Orang Asli* also helps to identify Hepatitis B potential. As Hepatitis B is a chronic disease, identifying the risk population is beneficial in term of planning of disease prevention and long-term management.

There are three main tribes of *Orang Asli* in Peninsular Malaysia, which includes Senoi, Proto-Malay (aboriginal Malay) and Semang (Negrito), and they represent 0.6% of the Malaysian population.[7] Among the Orang Asli tribes, the Negrito have the least population compared to the other two tribes.[8] They are known to be the earliest inhabitants that arrived in Peninsular Malaysia 25,000 years ago. Some of them still practice semi nomadic life up to this day, maintaining their hunting-gathering lifestyle. They are also the most geographically remote tribe compared to the others, and therefore most unaffected by urbanisation and modernisation.

As highlighted in previous publication,[9] poor access to health service is of the barrier to health promotion among *Orang Asli*. The Negrito, being the smallest population with the most remote geographically location with limited access to health care, they are very much affected by this. Thus, we believe they are at higher risk for Hepatitis B infection.

The main objective of this study was to provide preliminary resourceful data on seroprevalence and epidemiology of HBV infection among the Negrito in Malaysia.

MATERIALS AND METHODS

Ethical Approval

The protocol of the study was reviewed, and institutional ethics approval was granted by approved by the of Universiti Teknologi MARA (UiTM) [600-RMI (5/1/6)]. Permission to conduct the study among the *Orang Asli* population was obtained from the Department of *Orang Asli* Development (JAKOA), Gua Musang, Kelantan, Malaysia. A preliminary visit was made to the selected villages with JAKOA officers and headmen of the sub-tribes (*Tok Batin*). Explanation regarding the study and usage of the collected data for future interventions were explained to the prospective subjects. Those subjects who agreed to be enrolled in the study either duly signed or placed their thumb-print on a written consent form. Interview was performed in Malay and witnessed by the accompanying JAKOA officer.

Study population and study areas

In brief, the Negritos are the smallest tribe among the three *Orang Asli* tribes encompassing 2.6% (~3600) of total Orang Asli population in Malaysia. There are further divided into six subtribes; the *Bateq, Jahai, Lanoh, Mendriq, Kensiu* and *Kintak*. Geographically, the Negrito settlements are isolated and scattered but mainly distributed in highland areas in the northern parts of Peninsular Malaysia. Most of the Negritos in this study were hunter-gatherers, and their villages are either in the secondary forest, near rivers or require specific transportation facilities such as boats or four-wheel drives. There were from six to 25 houses in all studied settlements with five to 10 family members per house.

Our survey was conducted in five different Negrito villages from April 2014 to May 2016. The villages were: Kg. Sungai Aring (4º 56.45′ 114″ N, 102º 20′ 38″ E) and Kg. Sg Dedari (4º 38′ 25″ N, 102º 40′ 78″ East) which were mainly habited by the Bateq sub-tribe. While Kg. Kuala Lah (5º 12′ 279″ N, 101º 97′ 30″ E) was the main settlement of the Mendriq sub-tribe. The main settlement for Jahai and Lanoh sub-tribe were in Perak; RPS Banun (5º 55′ 82″ N, 101º 41′ 46″ E) and Kg Air Bah (5º 22′ 25″ N, 101º 60′ 27″ E) respectively (Figure 1).

Demographic data and Physical Examination

All subjects were interviewed using a questionnaire that consisted of demographic details such as age, gender, sub-tribes, educational level, socioeconomic and marital status. Emphasis on clinical symptoms and signs related to HBV infection such as jaundice, abdominal pain, hepatomegaly, and ascites, along with cutaneous manifestation of liver disease, for example spider naevi, telangiectasia, palmar erythema and finger clubbing were included during the clinical history and physical examination. All finding was then recorded.

Blood collection

Five millilitres of blood were collected from each subject using serum separator tubes and were centrifuged for 15 minutes at 2500rpm. All sera were transferred into biohazard plastic vial and transported with dry ice to the Microbiology laboratory, Centre for Pathology and Research Laboratories, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Malaysia. These sera were then subjected for Hepatitis B surface antigen (HBsAg) evaluation. In the event that they were not tested immediately, sera were stored at -40°C in aliquots.

Automated immunoassay test

Hepatitis B surface antigen qualitative assay was carried out according to standard operating procedures using the E411 analyser (Roche Diagnostics, Mannheim, Germany) to determine the HBsAg based on the principle of electrochemiluminescence immunoassay (ECLIA). Hepatitis B surface antigen qualitative test calibration and quality control were performed using calibrators and controls (negative and positive) prior to running every batch of tests. All calibration, controls, and tests were performed and interpreted in accordance to the manufacturers' recommendations.

Statistical analysis

Analysis was performed using SPSS version 20 (SPSS, Chicago, IL, USA) and for descriptive analysis, rate (percentage) and median (interquartile range) was used to describe the characteristics of the studied population. The association between height, weight and Body Mass Index with the gender and age groups were analysed using Mann Whitney U-Test. A two -way contingency table analysis between two categorical variables were conducted by using a Pearson Chi-squares test ($\chi 2$) and Cramer's V. The level of statistical significance was established at $p<0.05$.

Table I: Overall characteristics of our studied Negrito population (n=150)

Variables	Frequency (%)	Median (IQR)
Age (years)	-	36.0 (13.0)
Age group		
< 35 years	72 (48.0)	
≥ 35 years	78 (52.0)	
Gender		
Male	70 (46.7)	
Female	80 (53.3)	
Subtribes		
Bateq	74 (49.3)	
Mendriq	44 (29.4)	
Jahai	24 (16.0)	
Lanoh	8 (5.3)	
Anthropometric measurements		
Weight, kg		51.1 (11.1)
Height, cm		157.6 (8.0)
BMI, kg/m^2		20.4 (4.7)
Nutritional status		
Underweight (BMI <18.5)	38 (25.3)	
Normal	77 (51.4)	
Overweight (BMI ≥25)	35 (23.3)	

IQR interquartile range.

Table II: HBsAg positivity according to gender and age groups

Variables	No. examined	No. positive HBsAg N (%)	χ2 (df)	Cramer's V	P value
Gender					
Male	70	9 (12.9)	2.91 (1)	0.139	0.088
Female	80	4 (5.0)			
Total	150	13 (8.7)	-	-	-
Age groups					
<35 years	72	4 (5.6)	1.69 (1)	0.106	0.193
≥35 years	78	9 (11.5)			
Total	150	13 (8.7)	-	-	-

χ^2 : Pearson Chi- Square
P value < 0.05 = statistically significant

RESULTS

Demographic profiles and physical examination

A total of 150 Negritos participated in this study, representing of nearly 5% of Malaysia's total Negrito population (n=3600). The subjects were mainly from Bateq sub-tribe; 74 (49.3%), followed by *Mendriq*; 44 (29.3%), *Jahai*; 24 (16%) and *Lanoh*; 8 (5.3%). The number of males was almost equal to the number of female subjects with the ratio of 7:8. The age of the studied population ranged from 18 to 79 years, with the median age of 36 years. For anthropometric measurement, overall median body weight, height and BMI in adult Negritos were 51.1 (IQR: 11.1), 157.6cm (IQR: 8.0) and 20.4kg/m^2 (IQR: 4.7), respectively.

Further Mann Whitney U Test analysis indicated that by gender, the median body height among Negritos was significantly higher in male (Z statistic= -5.703, p<0.001) compared to the females. However, no significant different was found in the mean body weight (Z statistic= -0.829, p=0.407) in male versus female. Moreover, the median BMI was significantly different between gender female Negritos (median: 21.2kg/m^2) as compared to male (20.06kg/m^2) (Z statistic= -2.509, p=0.012). Similarly, stratification by age groups showed no significant different was discovered in term of median body weight and BMI except in median body height which was significantly higher in younger group <35 years oldy/o compared to≥35 years old (Z statistic= -2.12, p=0.034).

Meanwhile, the overall prevalence of underweight and overweight in our studied population was 25.3% and 23.3%, respectively. By gender, our results revealed that the prevalence of overweight was very statistically higher (χ^2=13.4, df=1, p <0.001) in female adult (45.9%) as compared to male (13.7%). However, there was no difference in underweight status among gender. General characteristics of sample population according to the Negrito subtribes are shown in Table I.

Prevalence of Hepatitis B infection and possible risk factors

Overall, of the 150 participants, 13 individuals were found to be HBsAg positive with the prevalence rate of 8.7%. A two-way contingency table analysis was conducted to evaluate whether different gender and age groups were associated with the HBsAg positivity. The percentage of HBsAg positive was found to be higher in males, nine (12.9%) and in those aged

≥35 years (11.5%). When compared to females and those aged <35years respectively, these results were not statistically significant (Table II).

None of the subjects had a history of receiving blood or blood products previously. The clinical findings of all HBsAg positive subjects were unremarkable. There was neither hepatomegaly nor peripheral stigmata of chronic liver disease documented, and none of them complained of having symptoms suggested compromised liver function during the interview. During our interview session, all of the positive participants were born at home, through spontaneous vaginal delivery unattended by any medical personnel. None of the mothers attended antenatal care clinic, however they did receive a few visit from assistant nurses from the District Health care clinic. In addition to that, about 46% of the positive subjects (n=6) had history of tattoo or body-piercing.

DISCUSSION

Numerous studies have been conducted in Malaysia to ascertain the prevalence and epidemiology of HBV infection among the non-indigenous population. Findings of our study showed that the prevalence rate of Hepatitis B Virus (HBV) infections among Negrito population in Malaysia was 8.7%. Our data showed higher prevalence rate as compared to current Malaysia's national rate of HBV prevalence which is around 3-5%.

According to WHO Global hepatitis report 2017, the indigenous people have a high prevalence of HBV infection worldwide. In Taiwan, the prevalence of HBV among the non-indigenous population was estimated around 17%,[10] while the prevalence among their indigenous population was substantially higher and ranges from 50 to 95%.[11] Similarly, in Australia, the prevalence among the indigenous population was four times higher than the non-indigenous population.[12] The primitive tribes of India have prevalence rate of HBV infection that ranges from 21.2% to 65%, which were significantly much higher than their national rate of 3%.[13] Our results showed that the prevalence rate of HBV infection among Negritos were 8.7%, and this was almost three times higher than Malaysia's national rate. This finding is in keeping with previous reported studies on indigenous population elsewhere.

It is widely known that most indigenous population usually live in relative isolation from other communities with limited accessibility to health care, and Malaysia is not an exception. The health services provision for the indigenous population in this country has always been challenging due to the geographical, political, and socio-economic issues.[14,15] Many studies showed that the health status of *Orang Asli* remains poor and in dire need of attention.[14-16] In the setting where the provision of health care is limited, the main groups that would be most affected are women and children, hence in the perspective of HBV infection women may disregard antenatal care opportunities that usually includes HBV screening, vaccination, diagnosis and treatment, while most children would miss their vaccination.

Antenatal care plays an important role in ensuring quality maternal health care. As per outlined by the Perinatal Care Manual by the Ministry of Heath Malaysia, it is strongly recommended that all pregnant women attend first antenatal check-up during their first trimester. During this antenatal check-up, among other assessment, HBsAg positive pregnant women will be identified and will be managed to reduce risk of vertical transmission. New-borns to HBsAg positive women will be given Hepatitis B immune globulin and first dose of Hepatitis B vaccination. As all HBsAg positive subjects were delivered at home without proper antenatal and delivery records, questions remain whether they received this standard care. Thus, it is possible that vertical transmission is a potential transmission risk factor for the studied population.

The rate of HBsAg positivity was higher among those ≥35 years of age compared to among those <35 years. This finding is comparable with studies done on other aboriginal tribes worldwide that found the peak was among those ≥35 years of age.[17-20] A possible explanation for this finding would be the possible increase of high-risk activity within the stated age group. Based on the conducted interview, 46% of the HBsAg positive subjects had a history of tattooing and body piercing. There were no record or evidence that showed the subjects observed hygienic tattooing and body piercing practices. However, tattooing and body piercing is well documented to carry significant risk of HBV infection.[21-24] Some publications suggested that risk of tattooing depends on the background prevalence of the studied population.[25-30] Our studied population was considered to have a high circumstantial prevalence therefore in agreement with the previous reports, we believe that tattooing and body piercing practices are probable risk factors for HBV transmission in this study.

In Malaysia, one of the initiatives to curb HBV infection is to implement WHO's expanded programme on immunization (EPI). In this initiative, HBV vaccination is included in the national childhood vaccination programme with the first dose given after birth follow with second and third dose given at one and six months of age respectively. The implementation of EPI started in 1989 and since then Malaysia has significantly reduced the national rate of HBV infection.[8] Our findings showed higher prevalence among those aged ≥35 years old. This cohort of people were born after the implementation of EPI and thus their immunisation level would be low. Among those who was born after the implementation of EPI, the success of EPI programme may not necessarily have reached the *Orang Asli* population. It is possible that our HBsAg positive subjects did not receive HBV vaccination, as all of them were delivered at home unattended by a healthcare professional. A previous study reported that 85.2% of *Orang Asli* children were not vaccinated or partially vaccinated,[31] with only 15% of population is vaccinated.

In our study, we found that HBV infection mainly involved the older age group. We believe this is because these subjects were the recipient of inadequate antenatal care and low HBV vaccination coverage and immunisation level. Another possibility is the increase of high-risk activity when they reach adulthood.

This study has certain limitations that might affect the interpretation of the results including language barrier during the interview session, as not all *Orang Asli* were fluent in the Malay language although the sub-tribe leader (*Tok Batin*) and JAKOA officer were present to facilitate the process. This could result in loss of information during the translation. Furthermore, important data such as year of birth or actual age of the subject might not be accurate, as all the subjects were delivered at home and capturing such details are not common practice among them. In addition to that, some of them were unable to recall their own age due to failure of presenting their national identification card. Moreover, the data in relation to risk factors were reliable only to the extent that the subjects were willing to provide the factual information, which create another limitation. In addition to it, questions regarding sexual orientation and injectable drug use may not have been answered truthfully, as the sociocultural characteristics of the subjects may have had effect on their answers. Therefore, to prove risk factors for HBV infection in this population would be statistically challenging.

Other relevant limitation in this study was in relation to the HBsAg testing whereby repeated HBsAg testing was not performed. Ideally repeat HBsAg test should be performed at least six months after the initial HBsAg test, along with other HBV serological markers (Hepatitis B surface antibody, Hepatitis B 'e' antigen, Hepatitis 'e' antibody, Hepatitis B core antibody) and liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase). However, all the mentioned tests were not carried out in the studied population as subject tracing was difficult due to their nomadic lifestyle and inability to access the settlements due to flood disaster.

It is noteworthy that majority of the data presented in this study was from Negrito sub-tribes (*Bateq, Mendriq, Jahai, Lanoh*), without including data from the remaining two subtribes (*Kensiu, Kintaq*). However, we believed that, the studied population satisfactorily represent Negrito population as the total population of Kensiu and Kintaq represents only 10% of the 3600 Negritos living in Malaysia.

In conclusion, this study provides preliminary data on epidemiology and seroprevalence rate of HBV infection among Negritos in Malaysia. The reason for a higher prevalence among the Negrito compared to national rate remains unclear. However, in this study we identified possible transmission risk factors for HBV infection among the Negrito. A comprehensive study which includes all tribes of Orang Asli with appropriate protocols should be conducted to elicit the comprehensive epidemiology of HBV infection among the Orang Asli in Malaysia.

CONFLICT OF INTERESTS

The authors declare that they have no conflicting interests.

FINANCIAL SUPPORT

This research was funded by the Research Acculturation Grant Scheme. RAGS/1/2014/SKK04/UITM//2 Ministry of Education Malaysia.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Ministry of Education Malaysia for funding this research through the Research Acculturation Grant Scheme. RAGS/1/2014/SKK04/UITM//2. We also gratefully acknowledged the Department of Orang Asli Development (JAKOA), Ministry of Rural and Regional Development, Malaysia for granting us permission to conduct this research. We also extend our gratitude to Microbiology Laboratory, Centre for Pathology and Research Laboratories, Universiti Teknologi MARA for their technical support. Lastly, we thank all the participants for their cooperation and commitment.

REFERENCES

1. Koziel MJ, Thio CL. Hepatitis B Virus and Hepatitis Delta virus. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7th ed. New York: Churchill Livingston; 2000.
2. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. Clin Microbiol Rev 1999; 12(12): 351-66.
3. Nebbia G, Peppa D, Maini MK. Hepatitis B infection: current concepts and future challenges. QJM 2012; 105(2): 109-13.
4. World Health Organization. Global hepatitis report 2017 [cited May 2017] Available from http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/.
5. Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 2000; 15(12): 1356-61.
6. Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. Vaccine 2000; 18: S20-2.
7. Raihan R. Hepatitis in Malaysia: Past, Present and Future. Eurosian J Hepato-Gastroenterol 2016; 6(1): 52-5.
8. Masron T, Masami F, Ismail N. Orang Asli in Peninsular Malaysia: Population, Spatial Distribution and Socio-Economic Condition. Journal of Ritsumeikan Social Sciences and Humanities 2013; 6: 75-115.
9. Aniza I, Norhayati M. Barriers to health promotion for indigenous communities: lessons for Malaysia. Malaysian Journal of Public Health Medicine 2016; 16(1): 6-14.
10. Chen CH, Yang PM, Huang GT, Lee HS, Sung JL, Sheu JC. Estimation of seroprevalence of hepatitis B virus and hepatitis C virus in Taiwan from a large-scale survey of free hepatitis screening participants. J Formos Med Assoc 2007; 106(2): 148-55.
11. Lin HH, Li YH, Yu JH, Wang YW, Lua AC, Huang LC et al. Ethnic and geographic variations in the prevalence of hepatitis A, B and C among aboriginal villages in Hualien, Taiwan. Infection 2000; 106(4): 205-8.
12. Graham S, Guy RJ, Cowie B, Wand HC, Donovan B, Akre SP et al. Chronic hepatitis B prevalence among Aboriginal and Torres Strait Islander Australians since universal vaccination: A systematic review and meta-analysis. BMC Infect Dis 2013; 13: 403.
13. Puri P. Tackling the Hepatitis B Disease Burden in India. J Clin Exp Hepatol 2014; 4: 312-9.
14. Center for orang Asli concerns. Health Care For The Orang Asli: Consequences of Paternalism and colonialism. 2007 [cited may 2017] Available from https://www.coac.org.my/main.php?section=articles&article_id=13
15. Phua KL. The Health of Malaysia's "Orang Asli" Peoples: a review of the scientific evidence on nutritional outcome, parasite infestations, and discussion on implications for clinical practice. Malaysian Journal of Public Health Medicine 2015; 15 (1): 83-90.
16. Cheng YX, Chong CP, Kiew CF, Bahari MB. An assessment of health and social-economic status among Lanoh ethnic sub-group of Orang Asli (indigenous peoples) in Air Bah I village, State of Perak, Malaysia. Journal of Applied Pharmaceutical Science 2014; 4(10): 32-7.
17. Wood N, Warlow M, Quinn H, Selvey C, Lum G, McIntyre P et al. Establishment of a surveillance system (utilising midwifes data collection systems) for monitoring the impact of hepatitis B vaccination on the population prevalence of chronic hepatitis B virus infection in Australia. Aust N Z J Public Health 2008; 32(3): 272-5.
18. Nguyen VT, McLaws ML, Dore GJ. Highly endemic hepatitis B infection in rural Vietnam. J Gastroenterol Hepatol 2007; 22(12): 2093-100.
19. Luo ZB, Xie YR, Deng M, Zhou X, Ruan B. Prevalence of hepatitis B in the southeast of China: a population-based study with a large sample size. Eur J Gastroenterol Hepatol 2011; 23(8): 695-700.
20. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012; 30(12): 2212-9.

21. Jafari S, Buxton JA, Afshar K, Copes R, Baharlou S. Tattooing and risk of hepatitis B: a systematic review and meta-analysis. Can J Public Health 2012; 103: 207-12.
22. Yang S, Wang D, Zhang Y, Yu C, Ren J, Xu K et al. Transmission of hepatitis B and C virus infection through body piercing: a systematic review and meta-analysis. Medicine 2015; 94: e1893.
23. Mele A, Corona R, Tosti ME, Palumbo F, Moiraghi A, Novaco F et al. Beauty treatments and risk of parenterally trans-mitted hepatitis: results from the hepatitis surveillance system in Italy. Scand J Infect Dis 1995; 27(5): 441-4.
24. Mariano A, Mele A, Tosti ME, Parlato A, Gallo G, Ragni P et al. Role of beauty treatment in the spread of parenterally transmitted hepatitis viruses in Italy. J Med Virol 2004; 74(2): 216-20.
25. Zhang Y, Fang W, Fan L, Gao X, Guo Y, Huang W et al. Hepatitis B surface antigen prevalence among 12 393 rural women of childbearing age in Hainan Province, China: a cross-sectional study. Virol J 2013; 10: 25.
26. Zenebe Y, Mulu W, Yimer M, Abera B. Sero-prevalence and risk factors of hepatitis B virus and human immunodeficiency virus infection among pregnant women in Bahir Dar city, Northwest Ethiopia: a cross sectional study. BMC Infect Dis 2014; 14: 118.
27. Urbanus AT, Van den Hoek A, Boonstra A, Van Houdt R, de Bruijn LJ, Heijman T et al. People with multiple tattoos and/or piercings are not at increased risk for HBV or HCV in the Netherlands. PLoS One 2011; 6: e24736.
28. Samuel MC, Doherty PM, Bulterys M, Jenison SA. Association between heroin use, needle sharing and tattoos received in prison with hepatitis B and C positivity among street-recruited injecting drug users in New Mexico, USA. Epidemiol Infect 2001; 127(3): 475-84.
29. Ko YC, Lan SJ, Chang PY. An increased risk of hepatitis B virus infection from tattooing in Taiwan. Gaoxiong Yi Xue Ke Xue Za Zhi 1990; 6(5): 237-43.
30. Jafari S, Buxton JA, Afshar K, Copes R, Baharlou S. Tattooing and risk of hepatitis B: A systematic review and meta-analysis. Can J Public Health 2012; 103(3): 207-12.
31. Singh A. Mortality, morbidity & malnutrition in Orang Asli children. 2008 [cited May 2017]. Available from http://hoag.moh.gov.my/images /pdf_folder/simposium/tujuh.pdf.