ORIGINAL ARTICLE

Seroprevalence of Neonatal Herpes Simplex Virus Infection at A Tertiary Teaching Hospital in Malaysia

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ABSTRACT

INTRODUCTION: Neonatal herpes simplex virus (HSV) infection is generally infrequent, despite being a life-threatening illness. Knowledge of its prevalence is limited in Malaysia since most cases are asymptomatic infections and only limited routine neonatal HSV screening is conducted. This study therefore provides a comprehensive investigation of the seroprevalence of HSV-1 and HSV-2 in neonates. **MATERIALS AND METHODS:** Serological screening for HSV-1 IgG and HSV-2 IgG antibody tests using the Electrochemiluminescence assay was performed on serum samples of 215 neonates delivered from January until December 2022 at Hospital Universiti Sains Malaysia. **RESULTS:** Of the neonates, 54.4% were found to be HSV-1 positive, while 4.2% were HSV-2 positive. All the HSV-2 neonates were co-infected with HSV-1. Newborns aged 0–10 days were the most infected group by HSV-1 (92.3%) and HSV-2 (55.6%). The most reported clinical presentation was small gestational age (SGA) (60%). Microcephaly and macrocephaly were observed in one neonate each. The clinical presentations of reactive HSV-1 and HSV-2 cases revealed the presence of fever with rash in both cases. **CONCLUSION:** The high seroprevalence of HSV-1 is alarming. It is hoped that these data will support the advocacy of screening women for HSV before or during pregnancy as a precautionary approach to reducing the risk of vertical transmission.

Keywords
Neonatal HSV infection, HSV-1, HSV-2, Seroprevalence.

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INTRODUCTION

Herpes simplex virus (HSV) infection poses significant effects on the community. The virus has the capacity to induce a latent scenario within the sensory ganglia, persisting for the span of human longevity, and is capable of causing recurrent reactivation. In several instances, individuals infected with the disease may not display any noticeable symptoms, but those who do exhibit symptoms may present with painful blisters or sores in the afflicted region. HSV can be classified into two types: HSV-1 and HSV-2. These types differ in terms of their clinical characteristics, severity, genetic profile, primary mode of transmission, predominantly afflicted body site, seroprevalence, and reactivation rate.[1–5]

Both HSV-1 and HSV-2 demonstrate a high level of infectiousness in pregnant women, and it can be transmitted from the mother to the newborn through placenta or, more commonly, after vaginal delivery. This transmission leads to neonatal herpes, which causes higher rates of mortality.[6,7] Neonatal HSV infection is an infrequent occurrence but has serious effects for the neonate and is a potentially lethal illness.[6,8,9] The rate of neonatal HSV infection is determined by factors such as seroprevalence, birth rates, and infections during pregnancy. It is estimated that there are approximately 10 cases of neonatal HSV per 100,000 live births, with a yearly average of 14,000 cases.[10–12]

Neonatal HSV disease can show up in three different ways: i) as a localized infection affecting the mucous membranes, skin, or eyes; ii) as an infection mainly affecting the central nervous system; or iii) as a disseminated disease that may or may not involve the CNS.[3,13] The clinical presentations of this condition may include characteristic manifestations such as herpetic skin and mucosal lesions, as well as seizures. However, it is

important to note that symptoms can also be non-specific, encompassing fever, decreased fluid intake, lethargy, or even the absence of symptoms.[14,15]

Numerous diagnostic tests, including culture, molecular, and serological tests exist for detecting HSV infection, yet only a subset have been deemed useful and validated for use in newborns.[16] Serological diagnosis stands out as one of the most convenient and reliable tests available. At present, the direct fluorescent antibody assay (DFA) is a widely employed method in clinical virology laboratories for the direct detection of HSV in clinical specimens.[16] Moreover, serological tests are helpful given that antibodies specific to glycoproteins G-1 and G-2 can distinguish between HSV-1 and HSV-2, respectively.[3] It is worth noting, however, that negative serological testing in newborns does not entirely rule out HSV disease. Moreover, the presence of IgG in the neonate could be indicative of active or passive immunity from the mother.[16]

The available data on neonatal HSV infections in Malaysia is limited due to the fact that most cases are asymptomatic, and the absence of routine screening for HSV in neonates is not consistently implemented. Thus, the current study aims to fill this research gap by conducting a comprehensive investigation into the seroprevalence of HSV-1 and HSV-2 in suspected neonates. This study not only underlines the importance of conducting screenings for neonates with suspected HSV infection, but it also highlights the importance of screenings for pregnant women to minimise the risk of congenital transmission.

MATERIALS AND METHODS

Study Population

The present study consists of a total sample size of 215 neonates with clinically suspected cases of congenital HSV infections. The neonates were delivered at Hospital Universiti Sains Malaysia (HUSM) throughout the timeframe of January 2022 to December 2022. The neonates were divided into three groups; 0-10 days, 11-20 days and 21-28 days to see the frequency of seroprevalence and their correlation with clinical presentations.

Sampling Method and Laboratory Interpretation

Serum samples were obtained from the neonates and subjected to serological screening for HSV-1 IgG and HSV-2 IgG tests using an Electrochemiluminescence assay (ECLIA) (Roche, Germany) following the instructions given by the manufacturer. The outcomes of the samples were categorized as either 'reactive' or 'non-reactive' and were presented in the form of a cut-off index (signal sample/cutoff). Samples that have a cutoff index value of less than 0.6 in the Elecsys HSV-1 IgG and HSV-2 IgG assays are classified as 'non-reactive' and were interpreted as 'negative'. Samples that have a cutoff index falling within the range of greater than 0.6 and less than 1.0 are designated as 'Gray-zone'. In such cases, an additional sample is necessary to perform further testing. Samples exhibiting a cutoff index greater than 1.0 are classified as 'reactive'.

Selection Criteria

Newborns over 28 days of age were excluded. A total of 215 cases were analysed to investigate the serological profiles and then compared with the clinical data obtained from neonatal records.

Statistical Analysis

The data were gathered and examined using the statistical software SPSS, version 27.0. Descriptive statistics were used to analyse the data with the objective of determining the incidence of congenital HSV-1 and HSV-2 infections among newborns, as well as their relation to various clinical presentations, by using two types of statistical tests, namely, the Pearson chi-square test and Fisher's exact test. $p<0.05$ was considered significant.

RESULTS

Characteristics of the Specimen and Patients

In terms of the gender distribution, out of a total of 215 serum samples collected from neonates, 52% were female.

Regarding the age distribution, a significant proportion of individuals (89.3%) fell into the age range of 0–10 days.

Serological Detection of HSV-1 and HSV-2

The outcomes of the serological investigation revealed that 54.4% (n=117) of participants were reactive for HSV-1 antibodies, while 4.2% (n=9) for HSV-2 antibodies. All HSV-2 reactive samples were also found reactive to HSV-1, suggesting the presence of co-infection. In addition, it was observed that HSV-1 and HSV-2 reactivity were predominant among neonates aged 0–10 days, accounting for 92.3% (p=0.163) and 55.6% (p=0.002), respectively. Furthermore, the majority of newborns who were reactive for HSV-1 (59%, p=0.027) were females. Similarly, higher proportion of female neonates 55.6% (p=1.000) were reactive for HSV-2 (Table I).

Table I: Demographic data regarding reactive HSV-1 and HSV-2

Variables	Total (n)	HSV-1 reactive [n=117] n (%)	p value	HSV-2 reactive [n=9] n (%)	p value
Age (days)					
0-10	192	108 (92.3)	0.163[a]	5 (55.6)	0.002[b]
11-20	11	3 (2.6)		0 (0.0)	
21-28	12	6 (5.1)		4 (44.4)	
Gender			0.027[a]		1.000[b]
Female	112 (52%)	69 (59.0)		5 (55.6)	
Male	103 (48%)	48 (41.0)		4 (44.4)	

Note: [a] Pearson chi-square test, [b] Fisher's exact test

Clinical Presentations of Congenital HSV-1 and HSV-2 Neonatal Infections

The neonates suspected of having congenital HSV-1 and HSV-2 infections showed a predominance of certain clinical symptoms. The most frequently observed symptom was small for gestational age (SGA), which occurred in 60.0% (129/215) of cases. This was followed by neonatal jaundice (NNJ), which was present in 6.0% of cases. Additionally, sepsis and presumed sepsis were reported in 2.8% (6/215) of cases each (Table II). The clinical presentation of HSV-1 reactive neonates revealed that two neonates had fever with rash (2/2, p=0.502), whereas one presented with microcephaly, and another, with macrocephaly. Among neonates infected with HSV-1, a total of 72 out of 129 cases were found to have SGA, representing 55% of the sample. The results also indicated that two HSV-2 reactive neonates had fever with rash, with a statistically significant p-value of 0.002 (2 out of 2 cases). On the other hand, SGA was reported in 2.3% (3 out of 129 cases) of HSV-2 reactive neonates, with a p-value of 0.161, which was not statistically significant. These findings are summarized in Table II.

Table II: Clinical presentations of the neonates in relation to HSV status

Clinical symptoms n (%)	HSV-1 Non-reactive n (%)	Reactive n (%)	p-value	HSV-2 Non-reactive n (%)	Reactive n (%)	p-value
SGA 129 (60.0)	57(44.2)	72 (55.8)	0.615[a]	126 (97.7)	3 (2.3)	0.161[b]
Sepsis 6 (2.8)	5 (83.3)	1 (16.7)	0.095[b]	6 (100)	0 (0.0)	1.000[b]
Presumed Sepsis 6 (2.8)	4 (66.7)	2 (33.3)	0.415[b]	6 (100)	0 (0.0)	1.000[b]
NNJ 13 (6.0)	9 (69.2)	4 (30.8)	0.077[a]	13 (100)	0 (0.0)	1.000[b]
Premature 3 (1.4)	2 (66.7)	1 (33.3)	0.593[b]	3 (100)	0 (0.0)	1.000[b]
Fever with rash 2 (0.9)	0 (0.0)	2 (100)	0.502[b]	0 (0.0)	2 (100)	0.002[b]
IUGR 2 (0.9)	1 (50.0)	1 (50.0)	1.000[b]	2 (100)	0 (0.0)	1.000[b]
Microcephaly 1 (0.5)	0 (0.0)	1 (100	1.000[b]	1 (100)	0 (0.0)	1.000[b]
Macrocephaly 1 (0.5)	0 (0.0)	1 (100)	1.000[b]	1 (100)	0 (0.0)	1.000[b]

SGA: Small for gestational age, NNJ: Neonatal jaundice, IUGR: Intrauterine growth restriction
Note: [a] Pearson chi-square test, [b] Fisher's exact test

DISCUSSION

Neonatal herpes is an infrequent condition that impacts newborns, although it is classified as one of the most severe diseases acquired during pregnancy. Hence, it is imperative to include neonatal HSV infection in the list of potential diagnoses for every neonate presenting with an acute illness.[17,18] Performing seroprevalence studies on HSV-1 and HSV-2 is crucial to enhancing our understanding of neonatal herpes. This study aimed to analyse the seroprevalence of HSV-1 and HSV-2 in neonates, considering that there is limited knowledge on this issue, especially in the study area.

The present study revealed that 54% of neonates had HSV-1 IgG reactivity, whereas a mere 4.2% showed HSV-2 IgG reactivity and were co-infected with HSV-1. The significant variations in the seroprevalence rates of HSV-1 and HSV-2 have been based on factors such as age, gender, ethnicity, and geographical location. In several regions across the globe, the predominant cause of HSV infections is attributed to HSV-1.[19,20] The global prevalence of HSV-1 and HSV-2 infections is estimated at 67% and 11%, respectively.[21] According to a European study, the seroprevalence of HSV-1 in various countries was found to range between 52% and 84%. Additionally,

the seroprevalence of HSV-2 was between 4% and 24%.[22] In Asia, the seroprevalence of HSV-1 continues to be elevated, with a seropositivity rate of 50% among children and 75% among adults.[23] Meanwhile, the seroprevalence of HSV-2 is approximately 12% among the general population of this geographical area.[24] Various studies in Southeast Asia have shown the prevalence of HSV-1 in different populations. In Indonesia, the seroprevalence rate is 72.7%. In Thailand, it ranges from 61.1% to 92.9%, while in the Philippines, it is 82.5%. Rates ranging from 49% to 78% have also been reported in Singapore.[23] As for HSV-2, a combined seroprevalence of 21.2% was documented among different populations in Southeast Asia.[24]

The high prevalence of HSV-1 IgG indicates a high efficiency of transmission of HSV-1 from mother to infant, probably from primary infection or reactivation of genital HSV-1.[25,26] HSV-1 has emerged as the most prevalent viral agent associated with genital herpes, accounting for an important percentage (60%–80%) of genital herpetic infections in specific groups of young females [27,28]. It has the potential to cause severe newborn disease and can potentially result in an infection that is transmitted across the placenta.[29,30] The chance of newborn infection during delivery appears to be greater when HSV-1 is present in genital secretions than HSV-2.[25]

In this current study, a total of nine neonates (4.2%) who showed reactivity in HSV-2 IgG were also found to have reactivity in HSV-1 IgG. This finding suggests the presence of a co-infection involving both HSV-1 and HSV-2. The findings of our study are in line with Sauerbrei et al.'s investigation into the seroprevalence of HSV-1 and HSV-2 in Thuringia, Germany, between 1999 and 2006. Sauerbrei et al. observed that out of a total of 191 individuals who tested positive for HSV-2, 147 individuals (77.0%) were found to be co-infected with HSV-1.[1] Studies showed that the most common method used to differentiate between HSV-1 and HSV-2 infections in serological diagnostics is immunoassays or immunoblots that test for HSV type-specific IgG using type-specific glycoprotein G-1 (gG-1) from HSV-1 and gG-2 from HSV-2.[3] Recently, molecular techniques have become a feasible alternative to serological methods, providing several advantages, such as faster data collection, suitability for different clinical samples, and increased levels of sensitivity and specificity.[31]

Clinical presentation alone is not sufficient for a proper diagnosis due to the overlapping symptoms with various other illnesses.[32] In this current study, fever with rash was found in both reactive HSV-1 and HSV-2. Various viral and non-infectious disorders can lead to children presenting symptoms of fever with rash.[33] In the statistical analysis, none of the symptoms were related to HSV-1 or HSV-2 infection, except for fever with rash in HSV-2 infection. Therefore, it is recommended that future studies evaluate with a large sample size in order to further investigate the significance of the association.

Information regarding neonatal HSV in Malaysia is inadequate. A study was undertaken in 1976 by Tan et al.'s "TORCHES" program, which focused on congenital disorders, specifically in women of childbearing age. Using complement-fixing antibodies approach, up to 79% HSV infection was found in Malaysia.[34] Between 1961-1979, Tan and Stern conducted another serological study on the prevalence of CMV and HSV infections in Peninsular Malaysia. The study, conducted on a diverse group of people (aged 0-55 years) from different parts of Peninsular Malaysia, found that 954 of the 1554 people (61.4%) had HSV antibodies. These antibodies were also detected using complement-fixing antibodies.[35] Balasubramaniam et al. led a group of researchers in 1994. A total of 1688 infants, aged 0–4 months, presenting with congenital anomalies, underwent screening to detect the presence of congenital CMV infection and the rest of the TORCHES. The study, which also utilised the complement fixation test, indicated a 0% incidence of congenital HSV infection among infants in Malaysia.[36] In another study carried out by Hooi between January 1990 and December 1999, individuals presenting with a preliminary clinical diagnosis of mucocutaneous HSV infections in the oral and genital areas were examined. Out of the 504 specimens analysed, 18.0% showed positive results by direct immunofluorescence (IF) testing, while 55.0% tested positive through virus isolation.[37] However, the seroprevalence of HSV-1 and HSV-2 in newborns in Malaysia remains poorly understood due to

limited diagnostic virology facilities and a shortage of published studies. Consequently, the significance of these infections acquired during pregnancy, which can result in severe diseases in newborns, has not been adequately understood. Our study addresses this gap by reporting a substantial seroprevalence of HSV-1 and HSV-2 in newborns, highlighting the importance of screening pregnant women and suspected cases of neonates.

This study has certain limitations. Only serological method was used. The use of more advanced methodologies, such as polymerase chain reaction (PCR), in addition to serology, will help provide more robust result. Additionally, the duration of the data collection was limited. Also, a mother serum test was not conducted in order to define if the observed case of genital HSV was a result of primary infection or reactivation.

CONCLUSION

This study provides the first serological investigation that includes both HSV-1 IgG and HSV-2 IgG based approaches, as much as our current understanding allows. The ECLIA method revealed a high prevalence of HSV-1 IgG antibodies (54.4%) in neonates. Up to 4.2% of HSV-2 IgG antibodies were also observed in the neonates tested. It is suggested that future studies be conducted using techniques such as PCR to improve our understanding of active infections in neonates or passive immunity from the mother. Furthermore, the present study reveals the need for laboratory confirmation of HSV infection as clinical presentation alone is not sufficient for a proper diagnosis, especially due to the overlapping symptoms with other illnesses. It is hoped that these data will support the advocacy of screening of women before or during pregnancy as a preventive measure to reduce the risk of vertical transmission.

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