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Original Research Article

Herpes Simplex in Neonatal Sepsis: Prospective Study in Egyptian Neonates

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ABSTRACT

Introduction: Herpes simplex infections in neonates have been described to be associated with significant morbidity and mortality**.** The aim of the present study was to assess the presence of herpes simplex 1 and 2 associated with neonatal sepsis in Mansoura University Children hospital, Egypt.
Methodology: The study was carried out in neonatal intensive care unit Mansoura University Children hospital, Egypt. All neonates with clinical diagnosis suggesting sepsis were included in the study. Blood samples were obtained for serological study of specific IgM for herpes simplex1&2 (HSV1&&2) and for multiplex polymerase chain reaction (PCR) for HSV1/2.
Results and conclusion: The study included 80 neonates affected with sepsis. They were 38 males and 42 females with mean age ±SD 38.6± 2.6 days.
Sepsis was associated mainly with low birth weight (33.4%) and congenital anomalies (26.3%). Neonates with positive virological markers for HSV1/2 either IgM or/and positive PCR was 40% with positive PCR in 29 neonates (36.2%) and positive IgM in 22 neonates (27.5%).
Comparative study between neonates affected by HSV1/2 and those non affected with HSV1/2 reveled non statistical significant difference in age (P=0.9), clinical diagnosis associated with sepsis (P=0.2). However, there was statistically significant association between fatal outcomes of sepsis and HSV1/2 affection (P=0.02).
In comparison of the finding by PCR and serology, 21 neonates were positive by PCR with negative serology and 14 neonates were positive for HSV1/2 by serology alone.
Herpes simplex types as identified by PCR was herpes 2 in 22 samples and 7 Herpes simplex type 1.
The present study highlights that HSV viral infections are common among neonates presented with sepsis. The presence of HSV1/2 should be thought carefully in neonates presented with sepsis. Polymerase chain reaction associated with specific IgM measurement is useful for diagnosis of such condition. Prevention should be considered by careful antenatal screening for maternal diagnosis of HSV1/2.

Keywords: HSV1/2, neonates, sepsis, PCR, IgM

INTRODUCTION

Herpes simplex 1&2 (HSV1, HSV2) are two viruses of the human herpes family. They are enveloped viruses with icosahedral nucleocapsid composed of 162 capsomers around double strand DNA core. [1]

Herpes simplex virus infection can be associated with significant morbidity and mortality in neonates and infants.

Transmission might occur in one of three stages intrauterine, perinatal or postnatal, with incidence rates around 5 %, 85 % and 10 %, respectively. [2] The incidence of neonatal HSV infections in different parts of the world is 1.7–3/100000. [3] There are few studies about the prevalence of HSV in pregnant women in developing countries, [4-8] but very few studies have reported the neonatal HSV infection in such countries. [9,10]

Clinical manifestations in the first three weeks of life in neonates vary from localized skin/eye/mouth disease to disseminated disease. It is estimated that one third of infected neonates lead to encephalitis and one fourth develop disseminated disease. [11,12] HSV infection is rarely considered in different clinical situations in neonates and antiviral therapy is advised only if there is a either a maternal history of HSV or ulcerative lesions.

The outcomes of neonates with HSV disease is improved when advances in the diagnostics tools are available to clinicians and the most valuable tool is the application of PCR to diagnose suspected neonatal HSV disease even with absence of vesicular skin manifestations. [13]

To our best of knowledge, there are no reports about the presence of herpes simplex in neonatal infections in Egypt.

The aim of the present study was to assess the presence of herpes simplex1 and 2 associated with neonatal sepsis in Mansoura University Children hospital, Egypt.

MATERIALS AND METHODS

The study was carried out in Mansoura University Children hospital, Egypt in the period from March 2013 till July 2014. Neonates admitted to the neonatal intensive care unit during this period complaining of sepsis were eligible for study. Clinical sepsis was defined as the presence of three or more of the following categories of clinical signs derived from a validated sepsis score: (a) temperature instability (hypothermia, hyperthermia); (b) respiratory (grunting, intercostal retractions, apnea, tachypnea, cyanosis); (c) cardiovascular (bradycardia, tachycardia, poor perfusion, hypotension);(d) neurologic (hypotonia, lethargy, seizures); (e) gastro intestinal (feeding intolerance, abdominal distension). [14] Informed written consent was obtained from the parents of each infant.

HSV2 IgG & IgM

Serum samples from each neonate were analyzed for qualitative specific IgM for HSV1/2 (ELISA-Equipar Via G, Ferrari, Saronno, Italy). Further aliquots of serum were stored at -70^{0}C to be analyzed for HSV1/2 by PCR

Multiplex Nested PCR for HSV-1/HSV-2

DNA was extracted with the commercially available Qiagen kit (GmbH, Hilden). Primers were designed to bracket a well-conserved region in the DNA polymerase gene. [15] Primer pair HSVP1 (5′-GTGGTGGACTTTGCCAGCCTGTAC CC-′3) and HSV-P2 (5′-TAAACAT GGAGTCCGTGTCGCCGTAGATGA-′3) were used to amplify HSV-1 and HSV-2.11 *Taq* (0.25 µL) and extracted DNA (10 µL) were added to each premixed supplied tube. Negative control was analyzed by adding water instead of DNA. The following program was used for the thermal cycle: 1 cycle at 94^{0}C for 2 minutes, 35 cycles (94^{0}C for 30 seconds, 56^{0}C for 30 seconds, 72^{0}C for 30 seconds), and 1 cycle at 72^{0}C for 5 minutes.

Following, the amplicon (137 bp for HSV-1 and 100 bp for HSV-2) was resolved on a 1.5% agarose gel and visualized using ethidium bromide (0.5 µg/mL) under ultraviolet illumination.

Statistical Analysis

The results were analyzed by SPSS 16. Values were represented as means± SD,

median (range), or the number of subjects and proportions. One-way analysis of variance test and independent samples Student *t* test were used for group comparisons of normally distributed variables, and the Kruskal-Wallis test and Mann-Whitney *U* test were used for comparisons of variables with skewed distribution. The chi-square test was used to compare proportions. The patient was considered to have HSV1/2 if either IgM and/or PCR were positive.

RESULTS

The study included 80 neonates affected with sepsis. They were 38 males and 42 females with mean age ±SD 38.6± 2.6 days.

Sepsis was associated mainly with low birth weight (33.4%) and congenital anomalies (26.3%). Neonates with positive virological markers for HSV1/2 either IgM or/and positive PCR was 40% with positive PCR in 29 neonates (36.2%) and positive IgM in 22 neonates (27.5%), table 1

Table 1. Demographical, clinical and laboratory finding in neonates with sepsis

Parameter	Data
Age (days) Mean± SD	3.8±2.5 P=0.1
Sex Male -No.(%) Female-No.(%)	 38 (47.5%) 42 (52.5%)
Clinical Diagnosis associated with sepsis Congenital anomalies-No.(%) Low birth weight-No.(%) Pneumonia-No.(%) Respiratory distress syndrome-No.(%) Preterm-No.(%)	 21(26.3%) 27(33.4%) 13(16.3%) 11 (13.8%) 8 (10%)
Outcome of the neonates Died-No.(%)	 10 (12.5%) P=0.03
Total Positive for Herpes simplex-No.(%) Positive Herpes IgM-No.(%) Positive PCR for HSV1/2-No.(%)	32 (40%) 22 (27.5%) 29 (36.2%)

Comparative study between neonates affected by HSV1/2 and those non affected with HSV1/2 reveled non statistical significant difference in age (P=0.9), clinical diagnosis associated with sepsis (P=0.2). However, there was statistically significant association between fatal outcomes of sepsis and HSV1/2 affection (P=0.02), table 2.

Table 2. Demographical, clinical and laboratory finding in neonates with sepsis associated with HSV1/2 compared with neonates negative for HSV1/2

Parameter	Neonates with no HSV infection (n=48)	Neonates with HSV1/2 Infections (n=32)	P
Age Days Mean ±SD	38. ±5 2.2	38± 2.1	P=0.9
Congenital anomalies-No.(%)	12 (25%)	9 (28.1%)	P=0.2
Low birth weight-No.(%)	20 (41.7%)	7 (21.8%)	-
Pneumonia-No.(%)	6 (12.5%)	7 (21.8%)	-
Preterm-No.(%)	4 (8.3%)	4 (12.5%)	-
Respiratory distress syndrome-No.(%)	6 (12.5%)	5 (15.6%)	-
Fatality-No. (%)	3 (6.3%)	7 (21.9%)	P=0.02

In comparison of the finding by PCR and serology, 21 neonates were positive by PCR with negative serology and 14 neonates were positive for HSV1/2 by serology alone, table 3.

Table 3. Comparison of detection of HSV1/2 by IgM and PCR

Herpes IgM	PCR		Total
	Negative	Positive	
Negative	37	21	58
Positive	14	8	22

P= 0.6

Herpes simplex types as identified by PCR was herpes 2 in 22 samples and 7 Herpes simplex type 1.

DISCUSSION

Neonatal infections caused by maternal HSV transmission are common including disseminated HSV infection causing severe multiorgan dysfunction and has a high mortality rate if left untreated. [16]

This is the first report about the rate of herpes simplex virus among high risk neonates and infants with sepsis from Egypt. This study had demonstrated high proportion of HSV1/2 infection (30%) among septic neonates compare to low prevalence of HSV in other studies in developed countries. The estimate rate of HSV 1/2 prevalence ranges widely from 1/3200 to 1/20000 of life births. [9,17-20] This can be explained by the higher prevalence of HSV1/2 reported among pregnant women in developing countries. For example, in sub-Saharan Africa, high HSV1/2 rates (30 - 80 %) were reported in women. In South America, the prevalence of HSV2 ranges from 20 to 40 %. Lower prevalence (10 - 30 %) has been reported in the general population in Asian countries. [21] HSV infection of the newborn can be acquired by various routes either during pregnancy, at time of birth or after birth. The mother is the most common source of infection. [22] In 85–90% of neonatal HSV infections, HSV is acquired at the time of birth and 5–10% is caused by early postnatal viral acquisition. [23]

The HSV1/2 DNA was common in the present study with negative IgM finding as 21 neonates have positive PCR results and negative serology. This finding may indicates late maternal infection with HSV1/2 during pregnancy. When primary HSV infection occurs during late pregnancy, there is no time to develop antibodies needed to suppress viral replication before labor. So, the infection with herpes can be detected only by finding viral DNA.

In contrary, neonates with positive IgM alone for HSV1/2 may indicates early maternal infection with herpes simples during pregnancy leading to formation of serological response to the virus.

The mortality rate among the affected neonates was 21.9%.The perinatal mortality was reported to be high up to 50% in previous study. [10] The infection prognosis in neonates depend on many factors such as the type of herpes simplex virus as HSV-2 carries a graver prognosis than that caused by HSV-1, [6,23] the time of infection the type of maternal infection whether primary or recurrent and the immunological response of the neonates. Both primary and recurrent maternal infection can result in congenital disease, even if the risk after recurrent infection is small.

Intrauterine viral transmission is more likely to occur during the first 20 weeks of gestation leading to abortion, stillbirth and congenital abnormalities in infants who survive. [8]

The common findings in the present study was low birth weight and congenital anomalies, however there was insignificant difference in neonates with and those without HSV. This finding can be explained by the fact that sepsis in general is common in those neonates.

HSV infection should also be considered in any infant who presents during the first month of life with symptoms like poor feeding, irritability lethargy or convulsions. [24]

The clinical presentation of infants with neonatal HSV infection depends mainly on the site of the infection and the extent of viral replication. [22] Congenital intrauterine infection which usually manifested during the first 48 hours is characterized by skin lesions such as vesicles. [8] Even in early herpes infections, over 20% of infants with disseminated infection do not develop skin vesicles during the course of their illness. [22]

The neonates included in the present study were older than 48 hours, with mean age± SD 38.6± 2.5 days. On line with our finding, it was reported previously that symptoms may occasionally be present at birth, but occur in 60% later than 5 days

after birth and sometimes are present after 4–6 weeks of life. [25,26] In previous studies in US about the age of neonatal herpes, it was reported that age of ≤ 21 days at onset of symptoms comprised 90% of all infected infants with HSV admitted to a children's hospitals. [27,28]

The relatively older age of neonates in the present study can explain absence of any skin manifestations associated with herpes simplex.

In our study, no history was reported of symptoms in mothers suggesting of HSV infections during delivery. In previous study also mothers had no herpes simplex virus lesions at delivery. [29] In another studies, maternal HSV infections were reported to be 11 % [30] up to 60. [31]

An important factor for delayed HSV treatment appears to be lack of available data of this disease amongst the clinicians. [32] Appropriate diagnosis requires accurate laboratory diagnosis. PCR assay for HSV is an accurate tool for diagnosis of HSV within 24 hours. [33]

The American Academy of Pediatrics Committee on Infectious Diseases suggests that HSV infection should be evaluated in neonates with suggestive symptoms like fever, irritability or lethargy and the presence of convulsions. [34] Moreover, HSV infection should be considered in differential diagnosis of sepsis even though in mothers without any history of HSV infection. In these neonates, proper laboratory samples should be taken and intravenous acyclovir administration (60 mg/kg/day) should be started immediately. [32]

In our study the majority of HSV DNA belongs to type 2 22 (75.9%) type 2. Genital infection is typically caused by HSV type 2 although the ratio of infections caused by HSV type 1 is increasing. [35,36] HSV-1 as a genital infection has a higher risk of transmission to the neonate than HSV-2. [37]

The present study highlights that HSV viral infections are common among neonates presented with sepsis. The presence of HSV1/2 should be thought carefully in neonates presented with sepsis. Polymerase chain reaction associated with specific IgM measurement is useful for diagnosis of such condition. Prevention should be considered by careful antenatal screening for maternal diagnosis of HSV1/2.

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