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# Detection of Cytomegalovirus and Herpes Virus Infection in High Risk Newborns

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## Abstract

ELISA was used to detect specific viral antibodies (IgG and IgM) for both cytomegalovirus (CMV) and herpes simplex virus II (HSV-2). The study included 77 newborns classified as 33 preterms, 32 infants of diabetic mothers (DMs) and 12 controls. The mean of CMV IgG and IgM were higher in preterm newborns than in IDMs and both were higher than in control. As regards HSV-2, the mean of IgG was equal in both preterm newborns and IDMs, but higher than in controls. For IgM the mean in IDMs was higher than in preterm newborns and both were higher than the controls. Thirty three per cent (33%) of preterm newborns were CMV IgG positive and 21.2% were CMV IgM positive. As regards HSV-2, 24.2% of preterm newborns were IgG positive and 9.1% were IgM positive. Both results are statistically highly significant. With maternal infection during pregnancy, the risk for the baby to acquire congenital CMV infection is 63.71%, while it is 37.6%, in cases of HSV-2 infection.

## Introduction

IT is increasingly apparent that certain viruses have predilection for the fetus and may cause abortion, stilbirth, intrauterine infection, congenital malformations, acute disease during the neonatal period, or chronic infection with subtle manifestations that may be recognized only after a prolonged period. It is important to recognize these viral infections in the neonatal period not only to diagnose the acute infection, but also to anticipate the potential implication for the subsequent growth and development of the infant [1].

As stated by Alford [2] "neonatal diagnosis of infections acquired in utero, natally or postnatally, are inherently difficult". Twenty years later, this statement is still true. Physicians faced with a newborn infant with signs and symptoms of perinatal infection must consider a multitude of diseases and may need to embark on a complex differential diagnosis [3].

To identify intrauterine infections the determination of specific IgM in cord blood or early neonatal sera is more important than the quantitative elevation of IgM [4].

The aim of this study is to find out the incidence of CMV and HSV-2 infections in high risk newborns, namely prematures and infants of diabetic mothers (IDMs). The prognosis of these diseases has improved since the introduction of antiviral agents such as acyclovir, gancyclovir and vidarabine. Therefore simple and reliable markers of CMV and HSV are urgently needed to screen large numbers of babies and to give treatment as early as possible after birth.

## Patients and Methods

The work was carried out in the Neonatal Intensive Care Unit, Kasr-El-Ainy Hospital. A total number of 77 neonates were enrolled in the study. Sixty five neonates (preterm and IDMs) were selected showing some of the clinical findings suggesting congenital infection (icterus, hepatosplenomegaly, skin rash, congenital anomalies etc.). Patients were classified in the following groups:

Group I: Included 33 preterm neonates, 17 males and 16 females, having a gestational age ranging from 30-36 weeks and their birth weights ranged from 1300-2700 gms. Twenty six were born by uncomplicated vaginal delivery, while seven were born by cesarean section.

Group II: Included 32 neonates born to diabetic mothers, 16 males and 16 females with their gestational age ranging from 34-41 weeks and their birth weights ranging from 2250-5200 gms. Eleven were delivered vaginally, while 21 were born by C.S. Among these neonates 8 were born prematurely.

Control group: Twelve full term healthy neonates were selected as control. They were 7 males and 5 females, having birthweights ranging from 2650-3700 gms. Nine were delivered vaginally, while 3 were born by C.S.

The study entailed a full clinical examination for each neonate. Gestational age was estimated using the Dubowitz Scoring System [5]. Venous samples were taken for estimation of the following viral antibodies by quantitative methods using ELISA Technique:

1- Cytomegalovirus IgG [6].
2- Cytomegalovius IgM [7].
3- Herpes Simplex Vius II IgG [8].
4- Herpes Simplex Virus II IgM [9].

Kits were supplied by Diamedix Corporation.

This procedure is a rapid and easy method allowing screening of a large number of babies as early as pobsible.

## Results

Cytomegaloviral antibodies were positive in 7 cases, while HSV-2 antibodies were positive in 3 cases of the preterm group. The infants of diabetic mothers showed one case positive for CMV and 2 cases positive for HSV-2.

The levels of viral antibodies (CMV, HSV-2 IgG and IgM) in the two studied groups are shown in tables I and II. Fig. I shows the mean distribution of viral antibodies among preterm neonates. IDMs and controls.

*Among preterns newborns:*

33.3% of cases were CMV IgG positive and 21.2% were CMV IgM positive. This is statistically highly significant compared to negative cases ($p$ = 0.0211 and <0.001 respectively). This means that with maternal infection during pregnancy the risk for the fetus to acquire congenital CMV infection is 63.7%.

24.2% of preterm cases were HSV-2

IgG positive and 9.1% were IgM positive. This is also highly significant compared to negative cases ($p = 0.002$ and $< 0.0001$ respectively). This means that with maternal infection during pregnancy the risk for the fetus to acquire congenital HSV-2 infection is 37.6% (Table III).

*Among infants of diabetic mothers:*

6.3% of cases were CMV IgG positive while 3.1% were IgM positive. The risk to acquire congenital infection is 49.2%. As regards HSV-2 28.1% were IgG positive and 6.3% IgM positive. The risk for fetal infection in this group is 22.4% (Table IV).

Table (I): Viral Antibodies Preterms.

| Case No. | CMV | | | | HSV-2 | | | |
|---|---|---|---|---|---|---|---|---|
| | IgG | | IgM | | IgG | | IgM | |
| 1* | 187 | +ve | 152 | +ve | 3 | -ve | 163 | +ve |
| 2** | 6 | -ve | 29 | -ve | 49 | +ve | | |
| 3 | 4 | -ve | 2 | -ve | 12 | -ve | | |
| 4* | 240 | +ve | 91 | +ve | 17 | -ve | | |
| 5 | 239 | +ve | 20 | -ve | 15 | -ve | | |
| 6 | 2 | -ve | 3 | -ve | 4 | -ve | | |
| 7 | 5 | -ve | 13 | -ve | 10 | -ve | | |
| 8 | 6 | -ve | 35 | -ve | 17 | -ve | | |
| 9 | 11 | -ve | 10 | -ve | 16 | -ve | | |
| 10 | 9 | -ve | 16 | -ve | 17 | -ve | | |
| 11 | 93 | +ve | 12 | -ve | 11 | -ve | | |
| 12 | 210 | +ve | 17 | -ve | 13 | -ve | | |
| 13 | 12 | -ve | 29 | -ve | 4 | -ve | | |
| 14 | 5 | -ve | 28 | -ve | 3 | -ve | | |
| 15* | 163 | +ve | 154 | +ve | 13 | -ve | | |
| 16 | 103 | +ve | 10 | -ve | 33 | +ve | 26 | -ve |
| 17* | 57 | +ve | 145 | +ve | 12 | -ve | | |
| 18* | 184 | +ve | 57 | +ve | 14 | -ve | | |
| 19 | 15 | -ve | 16 | -ve | 12 | -ve | | |
| 20 | 13 | -ve | 12 | -ve | 16 | -ve | | |
| 21* | 155 | +ve | 61 | +ve | 11 | -ve | | |
| 22 | 14 | -ve | 2 | -ve | 4 | -ve | | |
| 23** | 11 | -ve | 17 | -ve | 22 | +ve | 100 | +ve |
| 24 | 9 | -ve | 12 | -ve | 25 | +ve | 40 | -ve |
| 25 | 10 | -ve | 12 | -ve | 13 | -ve | | |
| 26* | 240 | +ve | 118 | +ve | 0 | -ve | | |
| 27 | 7 | -ve | 17 | -ve | 17 | -ve | | |
| 28 | 13 | -ve | 24 | -ve | 25 | +ve | 35 | -ve |
| 29 | 6 | -ve | 26 | -ve | 41 | +ve | 22 | -ve |
| 30 | 10 | -ve | 12 | -ve | 9 | -ve | | |
| 31 | 7 | -ve | 14 | -ve | 16 | -ve | | |
| 32 | 10 | -ve | 18 | -ve | 38 | +ve | 121 | +ve |
| 33 | 10 | -ve | 19 | -ve | 24 | +ve | 25 | -ve |

N.B. * = +ve cases of CMV. ** = +ev cases of HSV-2

Ranges:

CMV
IgG > 23 EU/ml = +ve.
< 18 EU/ml = -ve.
IgM > 40 EU/ml = +ve.
< 30 EU/ml = -ve.

HSV-2
IgG ≥ 20 = +ve.
< 20 = -ve.
IgM > 60 = +ve.
< 60 = -ve

Table (II): Viral Antibodies among IDMs.

| Case No. | CMV | | | | HSV-2 | | | |
|---|---|---|---|---|---|---|---|---|
| | IgG | | IgM | | IgG | | IgM | |
| 1 | 5 | -ve | 6 | -ve | 13 | -ve | | |
| 2 | 10 | -ve | 12 | -ve | 12 | -ve | | |
| 3 | 228 | +ve | 15 | -ve | 14 | -ve | | |
| 4 | 14 | -ve | 14 | -ve | 1 | -ve | | |
| 5 | 13 | -ve | 6 | -ve | 11 | -ve | | |
| 6 | 6 | -ve | 16 | -ve | 12 | -ve | | |
| 7 | 12 | -ve | 26 | -ve | 9 | -ve | | |
| 8 | 11 | -ve | 11 | -ve | 37 | +ve | 24 | -ve |
| 9 | 3 | -ve | 18 | -ve | 3 | -ve | | |
| 10 | 6 | -ve | 7 | -ve | 10 | -ve | | |
| 11 | 4 | -ve | 16 | -ve | 12 | -ve | | |
| 12 | 9 | -ve | 22 | -ve | 24 | +ve | 35 | -ve |
| 13 | 5 | -ve | 13 | -ve | 3 | -ve | | |
| 14 | 6 | -ve | 27 | -ve | 7 | -ve | | |
| 15 | 7 | -ve | 21 | -ve | 1 | -ve | | |
| 16 | 10 | -ve | 15 | -ve | 4 | -ve | | |
| 17 | 6 | -ve | 15 | -ve | 20 | +ve | 15 | -ve |
| 18 | 11 | -ve | 12 | -ve | 19 | -ve | | |
| 19 | 12 | -ve | 9 | -ve | 12 | -ve | | |
| 20 | 11 | -ve | 20 | -ve | 15 | -ve | | |
| 21* | 184 | +ve | 162 | +ve | 55 | +ve | 30 | -ve |
| 22 | 12 | -ve | 22 | -ve | 18 | -ve | | |
| 23 | 9 | -ve | 22 | -ve | 17 | -ve | | |
| 24 | 8 | -ve | 18 | -ve | 18 | -ve | | |
| 25 | 6 | -ve | 13 | -ve | 25 | +ve | 32 | -ve |
| 26 | 7 | -ve | 17 | -ve | 19 | -ve | | |
| 27** | 6 | -ve | 13 | -ve | 23 | +ve | 93 | +ve |
| 28 | 5 | -ve | 12 | -ve | 39 | +ve | 40 | -ve |
| 29 | 11 | -ve | 7 | -ve | 22 | +ve | 20 | -ve |
| 30** | 13 | -ve | 16 | -ve | 26 | +ve | 182 | +ve |
| 31 | 16 | -ve | 10 | -ve | 6 | -ve | | |
| 32 | 15 | -ve | 13 | -ve | 3 | -ve | | |

N.B.
* = -ve cases of CMV
** = +ve cases of HSV-2
Ranges:

| CMV | GSV-2 |
|---|---|
| IgG > 23 EU/ml = +ve. | IgG ≥ 20 +ve. |
| < 18 EU/ml = -ve. | < 20 = -ve |
| IgM > 40 EU/ml = +ve. | IgM > 60 = +ve. |
| < 30 EU/ml = -ve. | < 60 = -ve. |

Table (III): Percentage Distribution of Viral Antibodes among Preterms.

| Parameter (Total no = 33) | Present No. | Present % | Absent No. | Absent % | *t* test | *p* | Sig. |
|---|---|---|---|---|---|---|---|
| *HSV:* | | | | | | | |
| IgG Eu/ml | 8 | 24.2 | 25 | 75.8 | -3.45 | 0.0002 | High. Sig. |
| IgM Eu/ml | 3 | 9.1 | 30 | 90.9 | -8.18 | < 0.0001 | High. Sig. |
| *CMV:* | | | | | | | |
| IgG Eu/ml | 11 | 33.3 | 22 | 66.7 | -2.03 | 0.0211 | High. Sig. |
| IgM Eu/ml | 7 | 21.2 | 26 | 78.8 | -4.05 | < 0.0001 | High. Sig. |

Table (IV): Percentage Distrubution of Viral Antibodies among IDMS.

| Parameter (Total no = 33) | Present No | Present % | Absent No | Absent % | *t* test | *p* | Sig. |
|---|---|---|---|---|---|---|---|
| *HSV:* | | | | | | | |
| IgG Eu/ml | 9 | 28.1 | 23 | 71.9 | -2.75 | < 0.002 | High. Sig. |
| IgM Eu/ml | 2 | 6.3 | 30 | 93.7 | -10.4 | 0.0001 | High. Sig. |
| *CMV:* | | | | | | | |
| IgG Eu/ml | 2 | 6.3 | 30 | 93.7 | -10.4 | 0.0001 | High. Sig. |
| IgM Eu/ml | 1 | 3.1 | 31 | 96.9 | -15.24 | 0.0001 | High. Sig. |

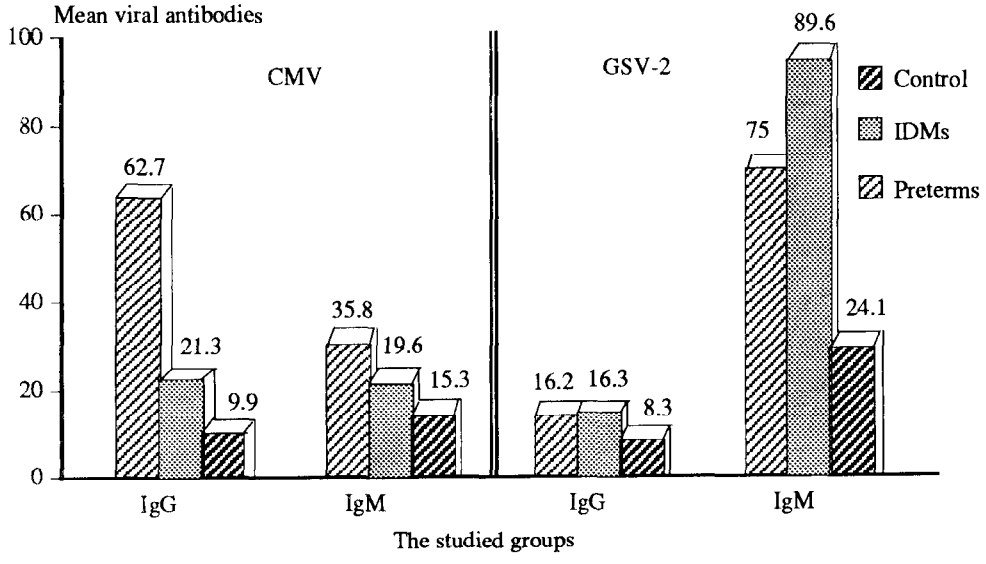


Fig. (1): The mean distribution of viral antibodies among the studied groups.

## Discussion

In 1974, Nahmias coined the acronym TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes) to call attention to a group of pathogens for the fetus and newborn infant, all sharing similar features. A more comprehensive acronym "CROTCHS" was suggested by Alpert and Plotkin [11]. Fetal and neonatal infections can occur at different times during pregnancy, from conception to birth. The damage done by the organism depends largely on the gestational age of the fetus at the time of infection. Viruses make up the largest number of infectious agents that produce significant pathology in the fetus [12].

This study has focused only on two viruses causing congenital infections, namely CMV and HSV-2. Specific viral antibodies for both CMV and HSV-2 (IgG and IgM) were determined. The prevalence of infection among pregnant mothers was reflected by positive IgG, while the incidence of congenital infections was reflected by positive IgM, as it does not cross the placenta and so its presence is entirely of fetal origin and is a marker of congenital infection.

In our study the incidence of congenital CMV infection was much higher in preterms (21.2%) than in IDMs (3.1%). A lower rate of 3-4% in preterms and 1-2% in all newborns was found by Hanshaw and Dudgeon [13] using urine culture method for CMV. The prevalence of maternal infection during pregnancy with CMV was 33.3% in mothers of preterm newborns and 6.3% in diabetic mothers. A level of 56% was found by Peckham et al. [14] and higher levels of 88% and 83.5% were reported by Hossein et al. [15] and Bashir [16] respectively. The rate of transplacental spread of CMV to the fetus was 63.7% for preterm and 49.2% for IDMs. The higher rate in preterms favours the idea that congenital CMV infection leads to preterm delivery. A rate of 25% was found by Griffiths et al. [17]. Kumar et al [18] reported 50% rate of transplacental spread.

The incidence of congenital HSV-2 infection was also higher in preterms (9.1%) than in IDMs (6.3%). Hossein et al. [15] failed to detect any cases of congenital HSV-2 infection inspite of the high prevalence of maternal infection among their studied groups. In our study the prevalence of maternal infection with HSV-2 was higher in diabetic mothers than in preterms, being 28.1% and 24.2% respectively. This could be explained by the fact that the diabetic mothers are more prone to acquire infections. Secondary to their diabetic state . Proben et al. [19] found that 86% of pregnant women had serological evidence of HSV-2 infection.

The rate of transplacentel spread of HSV -2 infection was 37.6% for preterms and 22.4% for IDMs. Inspite of the higher prevalence of maternal infection in diabetic mothers, yet the incidence of congenital HSV-2 infection was higher in preterms than in IDMs and this strengthens the view that congenital HSV-2 infection leads to preterm delivery. Brown et al. [20] reported a rate of 20% to be increased to 40% if the infection was primary.

The death rate among preterm congenitally infected with CMV was 57.1% and 33.3% among those infected with HSV-2 Hutto et al. [21] reported a rate of 15% among all newborns infected with HSV-2. Our rate is higher probably because our study comprised mainly preterm newborns, who represent a high risk group.

The possibility of false negative and

false positive serologic results is present, therefore the "gold standard" for confirming the diagnosis is to supplement serology with virus isolation. It is hoped that over the next decade, the development of safe and efficient vaccines, as well as better understanding of the factors associated with transmission of viruses from mother to infant, will allow ultimate prevention of congenital infections.

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