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COMPARATIVE EPIDEMIOLOGY OF INFECTION WITH HUMAN CYTOMEGALOVIRUS IN CAIRO AND SOUTH LONDON

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ABSTRACT

Human cytomegalovirus (HCMV) infection induces HCMV IgM antibodies in acute and HCMV IgG antibodies in convalescent phases while HCMV IgG antibodies last for years. Testing for HCMV IgG antibodies as an age-related pattern of infection was studied in Cairo and South London showed that HCMV infection was acquired earlier in life in Cairo than in London. At large 95.6% of 720 sera from the general population in Cairo were seropositive by the age of 5 years compared with 33.3% of 313 sera from British subjects in London. HCMV IgM antibodies were detected only in a small proportion of elderly individuals in both cities. Testing of urine specimens by virus culture showed that congenital infection with HCMV was more common in Cairo (1.28%), compared with (0.0%) in London. Urine samples from 8.6% of Egyptian children aged 2 weeks to 5 years were positive for HCMV by culture, compared with 3.6% aged 6-10 years and 5.8% aged 11-15 years. In children from London, HCMV was isolated from the urines of 11% aged 2 weeks to 5 years, 5.2% of those aged 6-10 years but from none aged 11-15 years.

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INTRODUCTION

HCMV infection occurs world-wide and, once it is acquired, persists in the host probably for life either in states of latency or low level replication, with sporadic episodes of reactivation. Reactivation is detected with greater frequency in immunocompromised subjects, especially kidney and bone marrow transplant **(Griscelli *et al.*, 2001; Kamar *et al.*, 2008)** recipients and infection with human immunodeficiency virus (HIV) **(Kim *et al.*, 2006)**. That reactivation occurs in immunocompetent individuals is witnessed by the birth of significant numbers of congenitally-infected babies, mostly in the developing world, whilst almost everyone acquires HCMV and develops a detectable immune response within the first year of life. Molecular analysis has shown that some individuals harbor more than one strain of HCMV which suggests that exogenous reinfection, despite a specific immune response, is a feature of the natural history of HCMV **(Yamamoto *et al.*, 2010; Novak *et al.*, 2008).**

Although considerable information exists on the prevalence of HCMV infection, with age, in different populations, as determined by serology, somewhat less is known about the prevalence of active infection in the general population, as shown by virus excretion, especially in developing countries. In the latter congenital HCMV affects up to 2% of births compared to 0.5% in developed countries; however, an incidence of 1.4% has been reported in one location in West Africa **(Boppana *et al.*, 2001; Schopfer *et al.*, 1978).**

Like congenital infection, perinatal and postnatal infection results in excretion of HCMV in infants' urine, persisting for several years until such time as the immune response matures. Cell mediated immune lymphoproliferative responses to HCMV are weaker in HCMV antibody-positive adults from Arabic and West African countries than adults native to Britain (**Omisakin *et al.*, 1996; Bello and Whittle, 1991; Booth, *et al.*, 1987).** Whether this is reflected in a higher prevalence of virus excretion is not known. In order to provide a direct comparison of the epidemiology of HCMV infection in developed and developing societies we have undertaken a series of investigations, in a single laboratory, on specimen materials collected in Cairo, Egypt, and in a cosmopolitan population in South London, England.

MATERIALS & METHODS

Subjects and specimens:

Serum specimens were collected from 720 Egyptians: three hundred and ninety eight apparently healthy subjects whose age ranged from 16 years to 75 years; ninety sera from neonates plus children (age range from few days old up to 15 years), and one hundred and sixty six sera were obtained from pregnant females in labor. Mostly the sera were obtained from the mothers of the babies whose urines were examined for evidence of congenital CMV infection. In addition sixty six sera were obtained from hospitalized patients suffering from bilharzial complications whose ages were between 15 and 73 years old. One hundred and thirteen sera from British neonates and children and two hundred sera from British adults were kindly given by Virus Laboratory at St. George's Hospital Medical School, London.

Urine specimens from Egyptian and British newborn babies.

Urine specimens from Egyptian and British older children and adolescents.

Tests for antibody to HCMV:

Sera were tested for CMV IgG by an in-house indirect enzyme-immunoassay (EIA) **(Booth *et al.*, 1982)** and for CMV IgM by both in-house indirect EIA **(Kangro *et al.*, 1986)** and commercial capture EIA (CMV-IgM-ELA-Medac, Hamburg).

Detection of HCMV in urine samples:

Urine specimens were inoculated into duplicate tube cultures of primary human embryonic lung (HEL) fibroblasts which were examined microscopically every other day for evidence of cytopathology, for five weeks before being regarded as negative, with refeeding with fresh medium every 3-4 days. Confirmation of cytopathology as being due to CMV was by immunofluorescent staining with CMV monoclonal antibody (MAB) to a CMV early protein (HCMV-3; Cogent Diagnostics Ltd, Edinburgh).

Urine specimens were also tested for CMV by a rapid culture method in monolayers of HEL fibroblasts growing in 24-well culture plates **(Steel *et al.*, 1988)**, which were washed thoroughly with fresh medium after allowing the

inoculums to adsorb for 2 hours at 37°C then refed and returned to the incubator for two days. The culture medium was discarded and the cell sheets were fixed in methanol, and then were stained for 1 hour at 37°C with HCMV-3 monoclonal as above. After thorough washing in phosphate-buffered saline (pbs), the cell sheets were reacted 1 hour at 37°C with rabbit anti-mouse $F(ab')_2$; (Tago, Burlingame, Ca., USA) labeled with alkaline phosphatase (AP) followed by incubation with a solution of AP substrate Fast Red TR (Sigma). CMV-infected cells were identified by their prominent, red cytoplasmic staining when the culture plates were examined under an inverted microscope at 100x magnification.

RESULTS

Seroepidemiology:
Of the serum samples that were tested for HCMV IgG, overall 97.1% of those from Egypt and 50.1% from Britain were positive. Among the Egyptians, 92% were already positive for HCMV IgG by 5 years of age and 100% by age 50 years (**Table 1**) among the British, 29-37% were positive in the age groups less than 15 years, rising to 71% in those over 45 years (**Table 2**).

Tests for CMV IgM were positive in the serum from a British subject aged 76 years and in three Egyptians over the age of 40, namely a 43 year-old woman, a 52 year-old woman and a 71 year-old man. All were in good health. Sera from 66 bilharzial patients positive for HCMV IgG were all negative on testing for HCMV IgM. Interestingly the mean level of HCMV IgG was higher in bilharzial patients than for healthy Egyptians of the same age (**Figure 1**).

Intrauterine CMV infection:
Urine specimens from 178 Egyptian babies less than 2 weeks old yielded HCMV isolates in 2 cases (1.3%) (**Table 3**); 176 urines from babies less than 2 weeks old in South London were negative (0%) (**Table 4**). Both CMV and adenovirus were isolated from a urine specimen belonging to an Egyptian 6-day old healthy baby boy.

Perinatal and postnatal infection:
Urines from 8.6% of Cairene children aged 2 weeks to 5 years were positive for HCMV by culture, compared with 3.6% aged 6-10 years and 5.8% aged 11-15 years (**Table 3**). In children from South London, HCMV was isolated from the urines of 11% aged 2 weeks to 5 years, from 5.2% of those aged 6-10 years but from none aged 11-15 years (**Table 4**).

Table 1. Detection of CMV-IgG and IgM in sera from Egyptians of different age groups.

Age range	Number of sera tested	No of CMV-IgG positive sera (%)	No of CMV-IgM positive sera (%)
1 day - 6 months	8	8 (11)	-
7 months - 1 year	7	5 (71.4)	-
13 months - 5 years	46	44 (95.6)	-
6 years – 10 years	13	12 (92.3)	-
11 years – 15 years	16	16 (100)	-
16 years – 20 years	82	79 (96.3)	-
21 years – 30 years	123	119 (96.7)	-
31 years – 40 years	78	77 (98.7)	-
41 years – 50 years	42	41 (97.6)	1 (2.3)
Over 50 years	73	73 (100)	2 (2.7)
Total	**488**	**474 (97.1)**	**3 (0.6)**

Table 2. Detection of CMV-IgG and IgM in sera from Londoners (UK) of different age groups.

Age range	Number of sera tested	No of CMV-IgG positive sera (%)	No of CMV-IgM positive sera (%)
1 day - 6 months	32	13 (40.6)	0
7 months - 1 year	13	6 (46.1)	0
13 months - 5 years	33	11(33.3)	0
6 years – 15 years	35	10(28.6)	0
16 years – 24 years	41	21 (51.2)	0
25years – 35 years	125	72 (57.6)	0
Over 35 years	34	24(70.6)	1(2.9)
Total	**313**	**157 (50.1)**	**1 (0.3)**

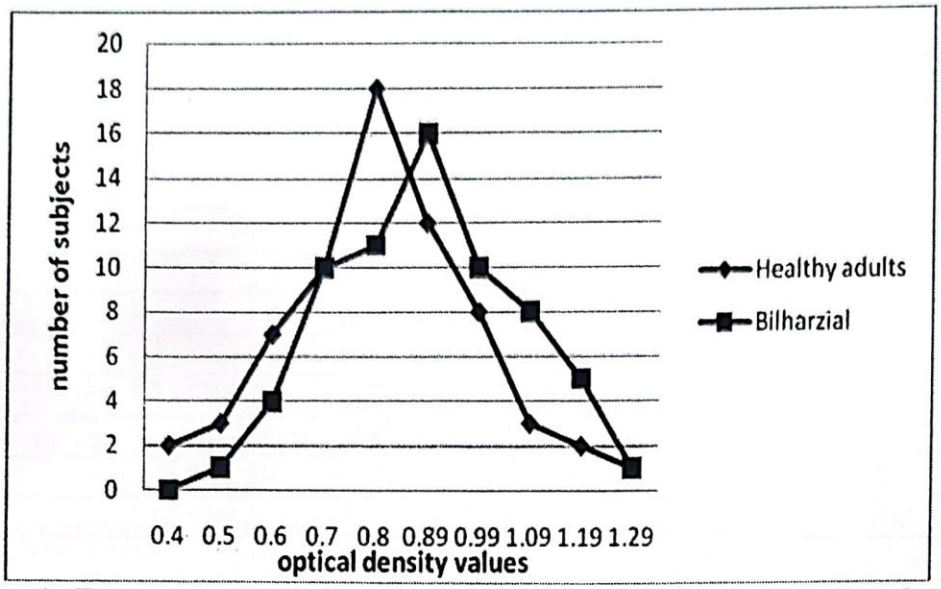

Figure 1. Frequency distribution for CMV-IgG antibodies in Egyptian healthy adults and bilharzial patients; detected by ELISA test and reported as optical density values.

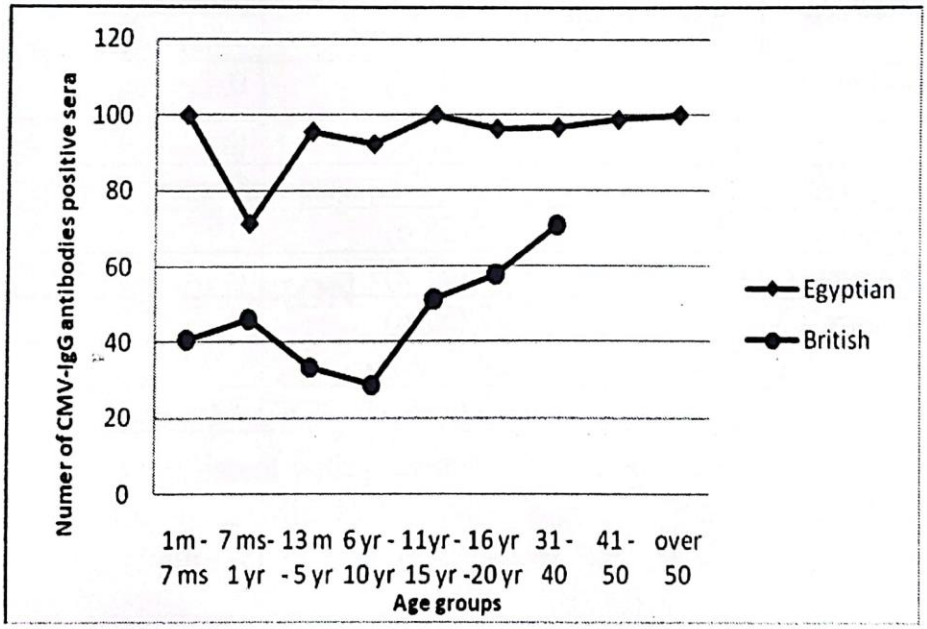

Figure 2. Comparison of prevalence of CMV-IgG antibodies detected by Elisa test among the studied Egyptian and British subjects.

Table 3. CMV isolation from Egyptian children and neonates urines.

Age range	No. of urines inoculated into HEL culture	No. of CMV positives (%)
Less than 2 weeks	178	2 (1.28)
2 weeks – 5 years	23	2 (8.6)
6 years – 10 years	55	2 (3.6)
11 years – 15 years	17	1 (5.8)

* HEL: Human embryonic lung fibroblast

Table 4. CMV isolation from British children and neonates urines.

Age range	No. of urines inoculated into HEL culture	No. of positives (%)
Less than 2 weeks	176	None (0)
2 weeks – 5 years	451	50 (11)
6 years – 10 years	211	11 (5.2)
11 years – 15 years	21	0 (0)

* HEL: Human embryonic lung fibroblast

DISCUSSION

The high prevalence of HCMV IgG from an early age in the population of Cairo is consistent with previous findings for other Middle Eastern countries (figure 2) **(Amer *et al.*, 1989; Peckham, 1989; Daboubi and Al-Zaben, 2000; Abou-El-Yazed *et al.*, 2008)**. Likewise, the pattern for South London confirms previous reports for this and other similar locations **(Stern and Elek, 1975)**. An unexpected finding was the detection of HCMV IgM particularly in older adults in Cairo. In London one HCMV- IgM case detected may have been the result of a primary infection in someone who had escaped contact exposure

with HCMV in infancy, the more so because 29.4% of subjects over 35years old were HCMV IgG-negative. However this was unlikely to have been the explanation in the subjects from Cairo especially two of these were mothers who had children. It may be that they had experienced recent household contact reinfection or had reactivated their latent HCMV. In bilharzial patients, the failure to detect HCMV IgM, suggests that antivirus cell mediated immunity (CMI) is not depressed to an extent as to favour reactivation of latent HCMV. Others have also reported detecting HCMV IgM in the elderly **(McVoy and Adler, 1989; Weymouth *et al.*, 1990)**; its presence was attributed to virus reactivation due to declining antiviral immunocompetence with increasing age **(Saltzman and Peterson, 1987)**.

The incidence of congenital CMV infection in Egypt (1.28%) compares well with the data that are available in reports from Egypt (5.71%) **(Morgan *et al.*, 2003)**; low socioeconomic population in London (1.4%) **(Stagno *et al.*, 1986);** and in Chilean and low-income Birmingham groups (1.7% and 1.9%) respectively **(Stagno *et al.*, 1982).** In none of these studies has the value approached the figure of (14%) reported for the Gambia by **Bello and White (1991)**. Studies in Europe and the USA have given values of 0.2-0.5% for socially advantaged communities which are not far removed from our present findings of less than 0.6% in South London **(Pass, 2005)**. Clearly there is still much to learn about the importance of congenital HCMV infection in societies of the third world.

Congenital and perinatal HCMV infections are followed by prolonged urinary excretion of the virus which continues for many years in association with depressed specific CMI to CMV **(Noyola *et al.*, 2000; Hanshaw 1971)**, and such immunity is gained, generally at about the age of five years (**Pass *et al.*, 1983**). In the Egyptian population, however, despite infection having been acquired in early infancy, virus excretion continued at least up to the age of fifteen years, which was the oldest age group in the present study. Previously we have shown that young adults in the Middle East and West Africa who are positive

for HCMV IgG give lower readings in lymphocyte proliferation tests with antigen for HCMV in comparison with Caucasians in Britain who are also seropositive to HCMV (**Omisakin *et al.*, 1996; Booth *et al.*, 1987**). The interpretation of these findings was that HCMV infection in early infancy, as in the Middle East and West Africa, predisposes to long lasting depressed CMI responses to HCMV. This would agree with our present findings of extended HCMV excretion in the Cairene population compared with Londoners. Moreover it is consistent with more frequent reactivation of latent HCMV among older adults in Cairo and leading to the development of specific IgM. Such findings need further substantiating by more extensive follow up of CMI responses to HCMV and the extent and duration of intercurrent active infection with HCMV in different age groups. Differences between population groups in the capacity of individuals to control latent infection with HCMV may exert an influence on the outcome kidney, bone marrow and organ transplants.

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