Original Research Article

Serodiagnosis of Human Cytomegalovirus in infertile and breast cancer women in Baghdad province

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A B S T R A C T

Human cytomegalovirus (HCMV) is one of herpesviruses that commonly infect humans. This study was conducted to estimate the prevalence of infection with this virus among infertile and breast cancer women. Blood samples were collected from 128 out patients the age ranged (16-45 years, Mean±SD 30.5±8.8), serologically diagnosed as virus infected at the Infertility Clinic and Radiation Hospital in Baghdad during period from March 2012 to October 2012. These women included 98 infertile and 30 breast cancer women. Another fifty (50) blood samples were collected from apparently healthy women from same clinics and age range. Enzyme Linked ImmunoSorbent Assay test (ELISA) was used to detect anti-human cytomegalovirus IgM and IgG. These percentages were 93% and 12% respectively among infertile women, and 100% and 60% respectively among breast cancer women.

Keywords: HCMV; Infertile; ELISA; Breast cancer

Introduction

Human cytomegalovirus (from the Greek "cyto" "cell", and "megalos" "large") is a member of the Betaherpesvirinae Subfamily, which belongs to the family Herpesviridae (Mocarski, and Courcelle, 2001; Ryan and Ray, 2004; Staras et al.,2006). It is a common virus that infects people of all ages. HCMV has a ubiquitous distribution, between 40-100% of all adults worldwide carrying the virus. It infects between 50% and 80% of individuals in developed countries by 40 years of age (Cannon et al., 2010) in case of infertility was the inability of a couple to become conceive pregnant (regardless of cause) after one year of unprotected sexual intercourse (Gurunath et al., 2011). About 10-15% of the couples do not achieve pregnancy after one year of sexual intercourse and 2-5% of them remaining childless to the end of their reproductive age (Tuerlings, 1999). Infectious agents can contribute to impair human functions,
including reproduction. Viruses were able to interfere with the reproductive function in both sexes (Pellati et al., 2008). Cytomegalovirus was frequently recovered from genital secretions and, therefore, HCMV infection may be sexually transmitted, thus it was excreted in many body fluids, including seminal fluid (Lanari et al., 2006). Many DNA and RNA viruses have proved to be oncogenic in animals. However, only few viruses have been linked to human cancer like Human Papillomavirus (HPV), Epstein-Barr (EBV) virus and Cytomegalovirus (CMV) (Kumar et al., 2003).

At least 15% of human tumors worldwide have a viral cause; viruses were etiologic factors in the development of several human cancers including two significant worldwide types: liver cancer and cervical cancer (Jawetz et al., 2007). HCMV induces specific breaks in chromosome 1 when infection occurs during the S phase of the cell cycle (Fortunato, and Spector, 2003).

Materials and Methods

The current study was done in the Infertility Clinic of Kamal Al-Sammaraei Hospital and Al-Yarmouk teaching and Radiation and Nuclear Medicine hospital in Baghdad. The study was approved by the Babylon College of Medicine and informed consent was obtained from patients. Patient groups consisted of one hundred fifty two (152) of aborted women; the age was ranged between (16-45) years, Mean±SD 30.5±8.8). (152) serum samples obtained from the studied groups to achieve HCMV serostatus that includes anti-HCMV IgG and IgM levels by using the ELISA test. Cytomegalovirus IgM and IgG ELISA test kit, Diagnostic automation (Biokit, Barcelona, Spain) and ELISA test kit. (BioCheck, Inc. Foster City). Samples were prepared according to method of (Boeckh and Boivin, 1998; Koneman et al., 2006). The tests were done according to manufacturer instructions. The statistical package for the social sciences (SPSS, version 14) was used for statistical analysis.

Results and Discussion

Chi-square test revealed that alleles' distribution in both SNPs either in VL patients or control subjects is within Hardy-Weinberg equilibrium.

IFN-γ(+874A/T)

Genotype and allele frequencies of IFN-γ(+874A/T) SNP in VL patients and healthy control subjects are shown in table land figure 1. For VL patients, the frequencies of TT, AT, and AA genotypes were 46.27%, 37.31%, and 16.41% respectively, compared with 70%, 25%, and 5% respectively in the control subjects with heterozygous genotype (AT) has significant association with the incidence of VL (OR=4.968, 95%CI= 1.012-24.38, p= 0.048). The frequencies of allele T and A were 64.92% and 35.07% respectively among VL patients and 82.5% and 17.5% respectively among control subjects with significant difference (OR= 2.547, 95%CI=1.294-5.013, p= 0.007).

Seroprevalence of anti-HCMV IgG and IgM among different age groups of infertile women

Infertility refers primarily to the biological inability of a person to contribute to conception. Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full term. There were many biological causes of infertility, some of which may be bypassed with medical
intervention (Makar and Toth, 2002). The prevalence of anti-HCMV IgG in the infertile women showed that 91 (93%) of infertile women are seropositive table (2). This result was supported by previous studies in Iraq, (94.5 % ) (Abdul-Karem et al., 1989) and 92.6% (Omer et al., 2006) and it considered to be similar to developing countries like most countries in Asia due to many risk factors such as low socioeconomic status and overcrowding living or due to reactivation and/or re-infection especially in women at childbearing age (Yang, et al., 1995).

The mechanism by which the virus comes to women possibly by intimate contact with body secretions such as saliva and semen at sexual contact (Chandler, et al., 1985) as the virus can be isolated from the semen or the cervical mucus of sexually active individuals (Lang, and Kummer, 1975). The seropositivity for anti-HCMV IgG antibodies, 21 (95%) in both age classes (21-25) and (36-40) years old and without significant differences. These results considered to be compatible with previous studies (Mahdi et al., 2011; Yasir, 2012) who showed the same results of IgG seropositive and even same age groups. Regarding seropositivity to IgM, were shown that the youngest age group (16-20) has the highest rate (25%) followed by (21-25) years age class (18%) as shown in table (1).

The seropositive IgM women are indicating an individual under acute infection or may be reactivation of latent infection. This is may be due to that younger age could pick the virus during early sexual maturity due to an epidemic property of the virus (Rachel, et al., 1997).

Francisse and coworkers pointed out that the sexual transmission of HCMV could be occurred among infertile couples (Francisse et al., 2009). HCMV in semen may be infectious (Lisco et al., 2012), and HCMV has also been isolated from human endometrial cells (Frank et al., 1992), suggesting a possible mechanism of direct infection of the endometrial cells by HCMV carried by sperm. Moreover, active HCMV infection can be obtained in human endometrial stromal cells (Kowalik, et al., 1994), since a primary HCMV infection is associated with an increased risk for early abortion and congenital defects in the fetus (Fisher et al., 2000). Mahdi et al., (2011) showed that the HCMV among infertile women had no association with other infection or antisperm antibodies (Mahdi et al., 2011). Also Pilikian et al., (1993) and Aydin et al., (1996), who showed that the frequency of herpes virus infections at in-vitro fertilization(IVF) technique was obvious and possibly it was responsible for a high rate of fertilization failure (Pilikian et al., 1993; Aydin et al., 1996).

Evidence suggested the detection of HCMV DNA by PCR technique in cryopreserved semen (Mansat et al., 1997). In Iraq, Yasir, (2012) detected HCMV DNA in semen of infertile male and cervical secretion of infertile women (Yasir, 2012). Kundsin et al., (1987) showed that the treatment of infertile couples with acyclovir led to success of pregnancy in 5 of 12 infertile couples (Kundsin et al., 1987). This study indicated the role of herpes viruses as the causative agent of infertility. Naumenko et al., (2011) showed that the HCMV was detected in both sperm samples and testis in organotypic culture and it led to decrease in number of immature germ cells, thus produces a direct gametotoxic effect which can contribute in male infertility (Naumenko et al., 2011).
Table 1: Seroprevalence of HCMV antibodies in the infertile cases

<table>
<thead>
<tr>
<th>Age/year</th>
<th>Total No</th>
<th>Anti-HCMV IgG</th>
<th>Anti-HCMV IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive cases*</td>
<td>Negative cases</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>16-20</td>
<td>8</td>
<td>7  87</td>
<td>1  13</td>
</tr>
<tr>
<td>21-25</td>
<td>22</td>
<td>21 95</td>
<td>1  5</td>
</tr>
<tr>
<td>26-30</td>
<td>12</td>
<td>11 92</td>
<td>1  8</td>
</tr>
<tr>
<td>31-35</td>
<td>21</td>
<td>20 95</td>
<td>1  5</td>
</tr>
<tr>
<td>36-40</td>
<td>22</td>
<td>21 95</td>
<td>1  5</td>
</tr>
<tr>
<td>41-45</td>
<td>13</td>
<td>11 85</td>
<td>2  15</td>
</tr>
</tbody>
</table>

*P>0.05

Table 2: Seropositivity to HCMV among different groups of patients

<table>
<thead>
<tr>
<th>Groups of study</th>
<th>Total No</th>
<th>IgG %</th>
<th>IgG– %</th>
<th>IgM + %</th>
<th>IgM - %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>98</td>
<td>91 93</td>
<td>7 7</td>
<td>12 12</td>
<td>86 88</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>30</td>
<td>30 100</td>
<td>0 0</td>
<td>18 60</td>
<td>12 40</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>121 95</td>
<td>7 5</td>
<td>30 23.498</td>
<td>76.6</td>
</tr>
</tbody>
</table>

*P<0.05

From these results, it can be inferred that the virus may have some effects on immature and mature ovum but this fact was not proved yet.

Seroprevalence of HCMV in the breast cancer women

The proteins and antigens of the virus have been detected in several malignancies including breast, colon and prostate as well as glioblastoma and cancer of salivary gland (Soroceanu, and Cobbs, 2011; Melnick et al., 2012; Price, et al., 2012) The role of the virus in cancer exhibited both oncogenic and oncomodulatory properties by expressing viral proteins that interfered with cellular processes (Cinatl et al., 2004; Soderberg-Naucler, 2006). The seropositivity of anti-HCMV IgG of breast cancer women in current study were 30 (100%), while anti-HCMV IgM seropositivity were 18 (60%) as in table (2).

The present results were supported by Taher et al., (2013) who showed that high prevalence of HCMV (100%) is in cases of breast cancer women and (91%) in case of metastatic sentinel lymph node (Taher et al., 2013). Furthermore, these results were in line with Lepiller et al., (2011) who showed that the virus have both initiator or promoter character for cancer, and get a shelter in immunologically poor environment of tumor cells, thus acting directly on the cancer cells and evade immune response (-Lepiller et al.,2011).
References


Mahdi, B.; Saour, M.; and Salih, W. 2011. Cytomegalovirus infection in infertile


