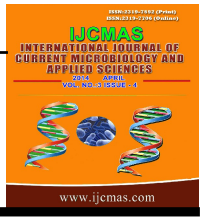
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 4 (2014) pp. 290-295**
http://www.ijcmas.com



Original Research Article

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

Hussein, A.M. Al.Baiati[1], Rebah, N. Jabbar[2,] Mohammed, A.Muhsin[3], and Mohammed A.K. Al-Saady[3]

[1]College of Vet. Medicine/ University of Baghdad, Iraq
[2]Biotechnology Research Center, Al. Nahrain University, Iraq
[3]College of Medicine/Babylon University, Iraq

Corresponding author

Keywords

HCMV; Infertile; ELISA; Breast cancer

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

Introduction

Human cytomegalovirus (from the Greek *cyto* "cell", and *megalo*, "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 ofInfertility 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-Sammaraee Hospital and Al-Yarmouk teachingand 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 (16-45years, 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-(+874A/T) SNP* in VL patients and healthy control subjects are shown in table 1and 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). **1-**

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 foranti-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 atin-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

Age/year	Total No	Anti-HCMV IgG				Anti HCMV IgM			
		Positive cases*		Negative cases		Positive cases*		Negative cases	
		No	%	No	%	No	%	No	%
16- 20	8	7	87	1	13	2	25	6	75
21-25	22	21	95	1	5	4	18	18	82
26-30	12	11	92	1	8	2	17	10	83
31-35	21	20	95	1	5	2	10	19	90
36-40	22	21	95	1	5	2	9	20	91
41-45	13	11	85	2	15	0	0	13	100

***P>0.05**

Table.2 Seropositivity to HCMV among different groups of patients

Groups of study	Total No %	IgG+ %	IgG– %	IgM + %	IgM - %
Infertility	98	91 93	7 7	12 12	86 88
Breast Cancer	30	30 100	0 0	18 60	12 40
Total	128	121 95	7 5	30 23.498	76.6

***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

Abdul-Karem, E.; Al-Hadithi, T.; Al-Balaghi, S.; Omer, A.1989 .Seroepidemiology of cytomegalovirus infection among healthy population in Baghdad. J Commun Med Iraq; 2:19-27.

Aydin, S.; Inci, O.; Alagol, B.; Huseyin, I.; Kaya, E.; Dellaloglu, G. 1996. Failure of artificial insemination of husband's semen in the treatment of male infertility. Int J Urol Nephrol .28:117–22.

Boeckh, M. and Boivin, G. 1998. Quantitation of cytomegalovirus: methodological aspects and clinical applications. Clin.Microbiol.Rev. 11: 533-554.

Cannon, M.; Schmid, D.; and Hyde, T. 2010. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev. Med. Virol. 204:202–213.

Chandler, S.; Holmes, K.; Wentworth, B.; Gutman, L.; Wiesner, P.; Alexander, E.; Handsfield, H. 1985. The epidemiology of cytomegaloviral infection in women attending a sexually transmitted disease clinic. J Infect Dis .152:597-605.

Cinatl, J., Vogel, J.; Kotchetkov, R.; Doerr, H. 2004 . Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. FEMS Microbiol Rev 28: 59–77.

Fisher S.; Genbacev O.; Maidji E.; Pereira L. 2000. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J Virol 74: 6808–6820.

Fortunato, E.; and Spector, D. 2003. Viral induction of site-specific chromosome damage. Reviews in Med Virol, 13 1, 21–37.

Francisse, S. ;Revelard, P. ; De Maertelaer, V. ; Strebelle, E. ; Englert, Y. ; Liesnard, C. 2009. Human Cytomegalovirus Seroprevalence and Risk of Seroconversion in a Fertility Clinic Population.Inst. Inter. Res. Hum Bio. Mol. 114 1 : 285-291.

Frank, T.; Himebaugh, K.; and Wilson, M. 1992. Granulomatous endometritis associated with histologically occult cytomegalovirus in a healthy patient. Am J Surg Pathol 16:716–720.

Gurunath, S.; Pandian, Z.; Anderson, R.; Bhattacharya, S. 2011. Defining infertility.A systematic review of prevalence studies". Hum. Reprod. Update. 17 5: 575.

Jawetz, E.; Melnick, J.; and Adelberg's, E. 2007.Herpesviruses In Medical Microbiology.Ch 33. Lange Medical Books/McGraw-Hill, USA .429-443.

Koneman, E.; Allen, S.; and Janda, W. 2006. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott.

Kowalik, T.; Yurochko, A.; Rinehart,C.; Lee, C.; Huang, E. 1994. Productive infection of human endometrial stromal cells by human cytomegalovirus. Virology .202:247–257.

Kumar, V.; Cotran, R.; And Robbins, S. 2003. Neoplasia. Ch.6 In: basic pathology, 7th edition.

Kundsin, R.; Falk, L.; Hertig, A.; Horne, H. 1987. Acyclovir treatment of twelve unexplained infertile couples. Int J Fertil .32:200–4.

Lanari, M. ;Lazzarotto, T. ; Venturi, V. ; Papa, I. ; Gabrielli, L. ; Guerra, B. ; et al. 2006. Neonatal cytomegalovirus blood load and risk of sequele in symptomatic and asymptomatic congenitally infected newborns. Pediatrics. 117: e76–83.

Lang, D. and Kummer, J. 1975. Cytomegalovirus in semen, observation in selected populations. J. Infect. Dis. 132: 472-473.

Lepiller Q.; Khan K.; Martino V.; and Herbein G. 2011.Cytomegalovirus and Tumors: Two Players for One Goal-Immune Escape.The Open Virology Journal, 2011, 5:60-69.

Lisco A.; Munawwar A.; Introini A.; Vanpouille C.; Saba E. et al. 2012. Semen of HIV-1-infected individuals: local shedding of herpesviruses and reprogrammed cytokine network. J Infect Dis .205:97–105.

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

women. J ExpInteg Med; 14:273-276

Makar, R. and Toth, T. 2002. "The evaluation of infertility". Am. J. Clin. Pathol. 117: 95–103.

Mansat, A.; Mengelle, C.; Chalet, M.; Boumzebra, A.; Mieusset, R. et al. 1997 .Cytomegalovirus detection in cryopreserved semen samples collected for therapeutic donor insemination. Hum Reprod, 12:1663–1666.

Melnick, M.; Sedghizadeh, P.; Allen, C.; Jaskoll, T.2012.Human cytomegalovirus and mucoepidermoid carcinoma of salivary glands: Cell-specific localization of active viral and oncogenic signaling proteins is confirmatory of a causal relationship. ExperMolPatho 921: 118–125.

Mocarski, E. and Courcelle, C. 2001. Cytomegaloviruses and their replication. In: Knipe, D., Howley, P., Griffin, D., et al., Editors. Fields Virology. Philadelphia: Lippincott Williams & Wilkins: 2629-2673.

Naumenko, V.;Tyulenev, Y.; Yakovenko, S.; Kurilo, L.; Shileyko, L. et al.2011. Detection of human cytomegalovirus in motile spermatozoa and spermatogenic cells in testis organotypic culture. Herpes viridae .2:7.

Omer, A.; Al-izi, S.; Al-Diwan, J.; Al-Hadithi, T. 2006. CMV infection among HIV/AIDS patients in Iraq.J Faculty Med Bagh. 48:407-9.

Pellati, D.; Mylonakis, I.; Bertoloni, G.; Fiore, C.; Andrisani, A.; Ambrosini,G.; Armanini, D. 2008. Genital tract infection and infertility .Euro J Obst Gynco.1401:3-11.

Pilikian, S.; Guerin, J.; Czyba, J.; Pinatel, M. 1993. Study of different components in spermatic function after failure of in vitro fertilization. Contracept Fertil Sex .21:865–8.

Price, R.; Bingmer, K.; Harkins, L.; Iwenofu, O.; Kwon, C. et al. 2012 .Cytomegalovirus infection leads to pleomorphic rhabdomyosarcomas in trp53+/2 mice. Cancer research 72: 5669–5674.

Rachel, L. ; Fatiha, N. ; Bertrand, K. ; Daniele, T. ; Sylvie, B. ; Jacqueline, L. ; Bruno, L. ; Jean-Francois, G. ; Michele, A. 1997. Detection of cytomegalovirus in semen from a population of men seeking infertility evaluation.Fertility and Sterility.Med. Publ. Else.Scie. Inc. 66 5 : 123-129.

Ryan, K. and Ray, C. 2004. Sherri's Medical Microbiology 4th ed.. McGraw Hill.pp. 556; 566–9.ISBN 0838585299.

Soderberg-Naucler, C. 2006. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med 259: 219–246.

Soroceanu, L.; and Cobbs, C. 2011. Is HCMV a tumor promoter? Virus research 157: 193–203.

Staras, S.; Dollard, S.; Radford, K.; Flanders, W.; Pass, R.; and Cannon, M. 2006."Seroprevalence of cytomegalovirus infection in the United States, 1988–1994".Clin. Infect. Dis.;43 9: 1143–51.

Taher, C.; de Boniface, J.; Mohammad, A.; Religa, P.; Hartman, J. et al. 2013. High Prevalence of Human Cytomegalovirus Proteins and Nucleic Acids in Primary Breast Cancer and Metastatic Sentinel Lymph Nodes. PLoS ONE 82: e56795.

Tuerlings, J. 1999. Genetics aspects of male factor infertility. Media group KUNAZN. Amsterdam. 240.

Yang, Y.; Ho, H.; Chen, H.; Chen, S.; Shen, C.; Chang, S.; Huang, E.; Wu, C. 1995. CMV infection and viral shedding in the genital tract of infertile couples. J Med Virol .45:179-82.

Yasir, S. 2012. Detection of human cytomegalovirus infections in spontaneous miscarriage and infertility .University of Kufa .College of medicine .PhD thesis.