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Molecular Analysis of CMV Genes Variation in Pregnant Woman

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Abstract
In these papers were studied molecular for both, molecular detection of human *Cytomegalovirus* (hCMV) and genotyping based of gB and gN genes in pregnant women suffered from previous abortion by means of Immunoglobulin M and G of *Cytomegalovirus* antibodies and nested polymerase chain reaction for universal detection of beta herpers viruses for amplification in envelope glycoproteins of *Cytomegalovirus* of ~800 bp DNA segament encoding for glycoprotein B (gB) gene and glycoprotein N (gN) gene were investigated in 30 pregnant women in Al-Batool Maternity hospital, Wasit provence, Iraq, whom suffered from previous abortion during from October 2014 to October 2015. Showing through it thirty women had increased titers of IgM and IgG types of *Cytomegalovirus* antibodies. This work includes a molecular study for both, molecular detection of HCMV and for genotyping based of gB and gN genes. Nested PCR technique was adopted as described previously for universal detection of beta herpes viruses for amplification of ~800bpDNA segment encoding for glycoprotein B(gB) gene (UL55) was carried out. To determine the genetic polymorphisms in envelope glycoproteins of CMV, glycoprotein B (gB) gene, and glycoprotein N (gN) gene, have been amplified. Thus, we conclude homology of gN sequence. gN-based genotyping study reveals three types of gN1, gN3, gN4 genotypes. This study showed that gN 1 genotype are more frequent genotype in patient sample followed by gN4 type, and one case with gN3 genotype.
Keywords:- hCMV, glycoprotein B (gB) gene, glycoprotein N (gN) gene, Nested PCR technique

1- Introduction

Human *Cytomegalovirus* (HCMV) is a human Beta herpes infection which, after essential contamination, stays in an idle state for the whole life time of the host (Mocarski and Shenk T., 2007). HCMV has a wide distribution, extended somewhere around 40 and 90% of all grown-ups overall conveying the infection (Cannon et al;2010).

Additionally, HCMV is a vital viral reason for fetal contamination which may prompt intense clinical difficulties in the infant youngster, for example, encephalitis, chorioretinitis, pneumonia, microcephaly and losing the hearing sense, beside weakening intellectual advancement (Britt,2008). Estimation of CMV-particular IgG enthusiasm has turned out to be an effective device for recognizing essential from non-essential CMV disease.

Characterized as the quality with which the IgG joins to antigen, IgG eagerness develops with the period of time taking after essential contamination. Subsequently, IgG delivered inside the initial couple of months taking after essential disease shows low enthusiasm, though IgG created a while or years after the fact displays high ardentness. The broad strain poly morphism that has been reported in various human and creature CMVs could serves as an insusceptible avoidance methodology (Renzette et al; 2011). CMV strains displaying antigenic and hereditary variability are prepared to do super contaminating resistant has and can be promptly transmitted between resistant people (Ross , 2010).

Transmission of CMV tainting strains have been all around recorded amid pregnancy or taking after organ transplantation. Strain-particular infection balance is possibly a contributing element to this marvel and strain particular balance has been seen in various studies (Ishibashi et al; 2007).

2. Materials and Method

The method used in this research case study method, 30 pregnant women in Al-Batool Maternity Hospital, Wasit Provence, Iraq, during from October 2014 to April 2015, age between 20 – 50 years, whom suffered from previous abortion and/or have fetus deformation or the highly threatened.

2.1 Cytomegalovirus (CMV) IgG and IgM ELISA kit

with investigated CMV antibodies (IgM and IgG) titer have been done after collection of blood samples by using enzyme linked immunosorbent assay (ELIZA) technique (Sigma-Aldrich, Germany).

2.2 Molecular Study

Viral nucleic acid DNA extraction kit (Bioneer, Korea). Primers used in this study for gB and gN amplification and the product estimated length were done as previously described (Chmielewicz et al; 2003 and Novak ,2008) respectively. Annealing temperature of all primers was checked using insilico PCR online software. All primers were supplied by Bioneer Company, southkorea, lyophilized item at various focuses table 1. Lyophilized ground works were broken down in a free DNase/RNase water as prescribed by the maker and after that leave at water bath at 60 C° for 10 minutes to give a last grouping of (100 pmol/µl) as stock solution, to prepare 20 pmol/µL

concentration as work solution (Chmielewicz et al; 2003, Novak ,2008). DNA electrophoresis, after PCR amplification, agarose gel electrophoresis was adopted to confirm the amplification process and specificity.

Table 1: The sequences of Primers

Gene	Sequence
gN F1	5-GACAGTACCAGTTGAGAGTCG-3’
gN F1	5’-GGATTATCtAGACTCGCTGC-3’
gN-up F	5'-TGGTGTGATGGAGTGGAAC-3'
gN1w R	5'-TAGCCTTTGGTGGTGGTTGC-3'
gB F	5'-ACTCTCGATCCGGTTCAGTC-3"
gB R	5'-CTTCTGGTCCTATGAGTGAT-3'

3- Results

Results indicates a correlation between high anti CMV-IgM titer with the deformation, in comparison with the corresponding titer of control group and high anti- HCMV IgG titer associated with abortion, which counted for about 30 cases. This work includes a molecular study for both, molecular detection of HCMV and for genotyping based of gB and gN genes. Nested PCR technique was adopted as described previously For universal detection of beta herpes viruses for amplification of ~800bpDNA segment encoding for glycoprotein B (gB) gene (UL55) was carried out. To determine the genetic polymorphisms in envelope glycoproteins of CMV, glycoprotein B (gB) gene, and glycoprotein N (gN) gene, have been amplified and sequenced using primer extension sequencing method. All sequences of amplified gN and gB for all samples have been compared with reference CMV sequence using multiple alignment clustal tool, omega online software to be aligned together and check the variation in both gN and gB genes using. Result indicate high sequence variations in gb-4b/gb-4c (19/30), while gN 1 represent the most abundant variant form of gN segment (20/30). Results showed that a homology ranged from78 to 99% of gN sequence. gN-based genotyping study reveals three types of gN1, gN3, gN4 genotypes. This study showed that gN 1 genotype are more frequent genotype in patient sample (66.7%, 20/30) followed by gN4 type (30 %, 9/30), and one case with gN3 genotype (3.3%).

4- Discussion

A positive CMV IgG and IgM in a symptomatic person means it is likely that the person has either recently been exposed to CMV not for the first time or that a previous CMV infection has been reactivated. This can be confirmed by measuring IgG levels again 2 or 3 weeks later. A high level of IgG is not as important as a rising level. If there is a 4-fold increase in IgG between the first and second sample, then the person has an active CMV infection (primary or reactivated). The researcher study the prevalence of sero-positivity for CMV was higher than Western Europe, America and Australia (Murray et al ;2002) but our findings were similar to a study in China with prevalence IgG (95.67%) in pregnant women (Guo ,1992). In India serological surveys have shown that the prevalence of CMV antibodies in adult population is about (80-90%) (Mukundan et al;1977). In most of the variable regions identified nucleotide changes are strongly clustered determining the existence of dominant genomic variants, usually defined as “genotypes”. HCMV ORF UL73 is one of the most polymorphic genes among HCMV clinical strains (Shimamura et al ; 2006). It encodes the immunogen envelope glycoprotein N (gN), element implicated in virus attachment to the host cell and spread. HCMV particles contain a number of highly polymorphic, extensively glycosylated envelope proteins, one of the most isglycoprotein N (gN). This protein is essential for replication of HCMV (Vigerust and Shepherd, 2007). In this study, gN DNA segment have been sequenced after being amplified using the specific primer published previously (Jackson et al ;2011). This protein is essential for replication of HCMV. Lanari M. and his college have hypothesized that the extensive glycosylation of gN may serve as a tool to evade neutralization by antiviral antibodies. So far four major genotypes have been identified (Merlin et al ; 2009). The gB group of a CMV strain could be determined either by alignment with the corresponding reference sequence or by restriction analysis of a small target sequence amplified from viral genomic DNA. This study reveals for gB genotypes. gN 4 is most dominant between patients samples. The existence of a limited number of variants of gB among clinical strains facilitates analysis of biologic function and cross-reactivity of immune responses (Picone et al ; 2005). All four gB genotypes can be vertically transmitted from mother to fetus with no type preferentially associated with HCMV infection in utero. However, Zdenek, N and his colleagues, 2008 found a high prevalence of gB-1 in congenitally infected infants, but this event was not predictive of clinical outcome. In addition, some reports (Ross et al ;2005) showed that gB genotypes do not correlate with the outcome of intrauterine HCMV infection, or with the development and severity of HCMV disease and symptomatology at birth As recently reported, all four gB genotypes were capable of causing congenital infection in North American, Italian and French babies (Arista et al ;2003).

5- Conclusion

Despite advances in the determination of CMV contamination, IgG and IgM titers, IgG avidity test not yet used

by local laboratories to ensure the new infection or CMV reactivation, so its highly recommended to use this approach. gN gene shows a high variability, which represent an evade mechanism to the immune system. gB gene shows a high variability, which may represent an good mechanism to escape from immune system activity.

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