SUMMARY
The aim of this study was to assess the risk of infection by the detection of Cytomegalovirus (CMV) DNA in abortive samples in women with pregnancy loss.

Material and Methods: Cross sectional study was designed to examine women with miscarriage. For six months, an abortive sample of 40 women hospitalized in Clinic of obstetrics and gynecology, University hospital-Pleven, Bulgaria were examined. By DNA-sorb-AM-AmpliSens DNA extraction was performed. The detection of cytomegalovirus DNA was performed by AmpliSens CMV - Eph PCR kit. The demographic data of the patients were collected by a questionnaire. The study protocol was approved by ethics committee of the Medical University - Pleven.

Results: CMV DNA was detected in 16/40 (40%) of the women. In women up to 20 years of age (n=12), two positive samples were found. In women aged between 21 and 30 years of age, (n=12), six positive samples were found. The highest number of positive samples - 8 was found in women over 30 years of age (n=16). In the surveyed group, 18 (45%) were from minority groups, 32 (80%) were pregnant, low social status was found in about 80% of women.

Conclusions: Our results indicate that there is a high frequency of CMV DNA in abortive samples from women who lost their pregnancy. Routine serologic screening for CMV of pregnant women will be advance in understanding of CMV infection among pregnant women and its prevention.

Keywords: CMV DNA, frequency, pregnancy loss, risk factors,

INTRODUCTION
Human cytomegalovirus is a species of the virus genus Cytomegalovirus, family Herpesviridae. It is also known as human herpesvirus-5 (HHV-5). Within Herpesviridae, CMV belongs to the Betaherpesvirinae subfamily, which also includes cytomegaloviruses from other mammals. Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fetal damage and, in rare cases, death due to abortion, but can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or newborn infants [1, 2, 3]. Transmission of infection from person to person requires very close contact. The virus is secreted into the body secretions - saliva, sperm, cervical secretion, urine, faecal matter. Sexual contact is one of the ways of spreading the virus. Cases of transfusions are also known. Maternal-fetal infection is also possible. It can be done in several ways: directly from infected placental tissue or by the ascending cervical cytomegalovirus infection and by direct contact of the fetus during delivery with the infected cervical mucus. Transmission of infection is possible after birth through the mother’s milk or by contact with the body’s secretions of the mother containing the virus. Although CMV is not highly contagious, it has been shown to spread in households and among young children in day care centers.

The rate of susceptibility to CMV during pregnancy is well established. Among women of child-bearing age between 40% and 80% will be susceptible to CMV at the beginning of pregnancy. The rate of susceptibility varies by ethnic or racial, socioeconomic and age groups [4, 5]. Pregnant women with a primary infection of cytomegalovirus, a reactivation of CMV, or those who are exposed to a new strain of virus have a chance of passing the virus to fetus (congenital CMV). Congenital CMV is the leading viral cause of developing a mental disability, disability, and the leading non-genetic cause of hearing loss, vision problems, including blindness, jaundice, large liver and spleen, low birth weight, small head size, neurological problems. A higher risk of pregnancy loss has also been reported [6, 7].
MATERIAL AND METHODS

Cross-sectional study was designed to examine women with pregnancy loss. For six months, an abortive sample of 40 women hospitalized in Clinic of Obstetrics and Gynecology, University Hospital - Pleven, Bulgaria were examined. By DNA-sorb-AM-AmpliSens DNA extraction was performed. The detection of cytomegalovirus DNA was performed by AmpliSens CMV - Eph PCR kit. Patients’ demographic data were collected by a questionnaire after informed consent. The study was a part of a scientific project funded by the Medical University - Pleven, Bulgaria. The study protocol was approved by the Ethics Committee of Medical University - Pleven. The data were statistically analyzed using the χ² test and P < 0.05 was accepted as level of significance.

RESULTS

The study group was presented from 40 women of age between 13 and 44, mean age 29±sd 9.327 with miscarriage during the first three months of pregnancy. The women were divided into three age groups: up to 20 years of age (12), between 21 and 30 years of age (12) and over 30 years of age (16). Twenty-nine (72.5%) of the women live in cities, and the other eleven (27.5%) live in a village. According to ethnic group, study population include Bulgarians - 22 (55%) and minority - 18 (45%). Marital status showed that only 5 (12.50%) were married. Among the women surveyed, unemployed were predominant - 20 (45%). In the context of the purpose of the study, some clinical and anamnesis’ data are presented in Table 1.

Table 1. Clinical and anamnesis’ data of study group women

| Clinical and anamnesis’ data               | Number (n), percentage (%) |
|-------------------------------------------|----------------------------|
| Diagnosis                                 |                            |
| Missed abortion                           | 14 (35%)                   |
| Incomplete abortion                       | 26 (65%)                   |
| Concomitant diseases                      |                            |
| Anemia                                    | 10 (25%)                   |
| Other                                     | 10 (25%)                   |
| No                                        | 20 (50%)                   |
| Consecutive pregnancy                     |                            |
| First pregnancy                           | 12 (30%)                   |
| Second pregnancy                          | 9 (22.50%)                 |
| Third and more pregnancy                  | 19 (47.50%)                |
| Children                                  |                            |
| Without children                          | 16 (40%)                   |
| One                                       | 14 (35%)                   |
| Two and more                              | 10 (25%)                   |
| Spontaneous abortions in the past         |                            |
| Without                                   | 31 (77.50%)                |
| One                                       | 3 (7.50%)                  |
| Two and more                              | 6 (15%)                    |

The detection of cytomegalovirus DNA showed that 16 (40%) of women were positive (Figure 1).

Fig. 1. Distribution of CMV DNA detection in abortive samples

In women aged between 21 and 30 years of age, (n=12), six positive samples were found. The highest number of positive samples – 8 was found in women over 30 years of age (n=16). Women from age group over 30 years are at risk for CMV acquisition (p<0.05) (Figure 2).

Fig. 2. Distribution of CMV DNA positive sample by age group
There is no statistically significant difference between ethnicity and CMV DNA positivity (P = 0.8968; χ²=0.02). Also, there is no statistically significant difference between marital status and CMV DNA positivity (P = 0.3291; χ²= 0.95).

DISCUSSION

Our results indicate that there is a high frequency (40%) of CMV DNA in abortive samples from women who lost their pregnancy. CMV infection positivity in aborted fetuses is high to this reported in other European regions and in the World [8, 2]. However, there is the vertical transmission of the virus. In order to determine whether primary infection or reactivation is involved, serological tests are required [9]. Routine screening of pregnant women for CMV by serology testing is currently not recommended. Few countries in the world are testing serologically pregnant women (Israel, France, Belgium, Spain, Italy, Germany, Austria, Portugal, and the Netherlands) [10]. Serologic testing for CMV may be considered for women who develop the influenza-like illness during pregnancy or following detection of sonographic findings suggestive of CMV infection. Seronegative health care and child care workers may be offered serologic monitoring during pregnancy [11]. Monitoring may also be considered for seronegative pregnant women who have a young child in day care [4].

In our study women from age group over 30 years are at significant risk for CMV infection. There was no association found between CMV DNA in abortion samples and the subjects’ demographic characteristics. Unlike other studies, infections are suspected regardless of ethnicity, and CMV infection was predominantly observed in little age group [12]. CMV positivity has been found to be significantly higher in women from a rural area as compare to those from an urban area [12]. It is noticeable that among the positive women for cytomegalovirus DNA there are predominant unmarried and unemployed. Indirectly, these data show a low social status of the investigated women.

Little is known about prevalence of sexually transmitted viral infections among Bulgarian women [13,14]. Cervical specimens tested by real-time PCR among asymptomatic Bulgarian women demonstrated about 6% positive for CMV [15]. Our results and reported data from other studies indicate that women might be at risk for transmission of virus infection sexually and vertically.

CONCLUSION

The relationship between viral infections and first-trimester spontaneous abortions in not well-understood. Despite the comparatively high prevalence of the CMV, only minority of pregnant women are aware of the prenatal risk posed to the fetus. PCR is a useful method for investigating the viral contribution to the aetiopathogenesis of spontaneous abortions and for detecting the viral genome in the abortion material [9]. We recommend that at the time a pregnancy is confirmed, the CMV IgM and IgG serostatus should be tested to enable individual risk assessment and prevention.

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