

Prevalence of herpes simplex types 1 and 2, varicella zoster virus, cytomegalovirus, and immunoglobulin G antibodies among female university students in Syria

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ABSTRACT

الأهداف: التعرف على الانتشار الوبائي المصلي الحالي للأضداد من النمط IgG ضد مجموعة من الفيروسات الهربسية، تضمنت فيروس الهربس البسيط من النمط 1 و 2 (HSV 1-2)، الفيروس النطاقي الحماقي (VZV)، والفيروس المضخم للخلايا البشرية (CMV) لدى الفتيات السوريات الجامعيات اللواتي في سن الحمل.

الطريقة: أجريت هذه الدراسة المقطعية على طالبات من كلية الصيدلة، جامعة القلمون. تم جمع 316 عينة مصل خلال الفترة من أكتوبر 2009م حتى نوفمبر 2010م خضعت للبحث عن الأضداد ضد الفيروسات الهربسية (HSV 1-2, VZV, CMV) من نمط IgG مع تحديد عبار هذه الأضداد بواسطة تقنيات تعتمد على المقاييس المناعية الأنزيمية ELISA في مخبر الأحياء الدقيقة.

النتائج: تبين أن 164 مشاركة كن إيجابيات أضداد النمط IgG تجاه فيروس الهربس البسيط من النمط 1-2 معطين انتشاراً مصلياً للأضداد بنسبة 52% وتاركين نسبة عالية نسبياً من المشاركات غير محصنات. بالنسبة للفيروس النطاقي الحماقي، كانت 91% من المشاركات (235 مشاركة) إيجابيات تجاه أضداد النمط IgG النوعية تجاه الفيروس النطاقي الحماقي، أما بالنسبة للفيروس المضخم للخلايا البشرية، فقد كانت 74.5% (235 مشاركة) إيجابيات و25.5% سلبيات تجاه أضداد النمط IgG النوعية للفيروس.

خاتمة: على الرغم من أن معظم المشاركات في هذه الدراسة كن إيجابيات أضداد النمط IgG النوعية تجاه الفيروسات الهربسية، غالباً نتيجة للانتشار الطبيعي لهذه الفيروسات ضمن المجتمع، فإن التحري عن مستوي المناعة ضد فيروس الهربس البسيط يعد من المقترحات لأن جزءاً كبيراً من الفتيات في سن الحمل كن ما زلن غير محصنات. إضافة إلى العواقب السيئة للإصابة بالفيروس المضخم للخلايا البشرية خلال الحمل، فإنه يجب أيضاً تحري أضداد النمط IgG النوعية تجاه هذا الفيروس. يمكن تفسير النسبة المرتفعة من الإيجابيات تجاه الفيروس النطاقي الحماقي بسبب برنامج اللقاح الجديد مما يعني أن التحري اللاحق خلال الحمل عن الأضداد تجاه هذا الفيروس غير موصى به.

Objectives: To examine the current seroepidemiology of immunoglobulin (Ig)G for herpes simplex virus types 1 and 2 (HSV 1-2), varicella zoster virus (VZV), and cytomegalovirus (CMV) among university females of childbearing age in Syria.

Methods: A cross-sectional study was conducted to examine the female students of the Pharmacy College, Kalamoon University, Deratiah, Syria, where 316 sera were collected from October 2009 to November 2010, and subjected to HSV 1-2, VZV, and CMV IgG screening and titration using enzyme-linked immunosorbent assay-based techniques in the Microbiology Laboratory.

Results: A total of 164 participants were positive for HSV 1-2 IgG giving a prevalence of 52%, leaving a relatively high proportion of susceptibility among the tested group. For VZV, 91% of the participants (n=287) were positive for its specific IgG, while, regarding CMV, 74.5% (n=235) were positive, and 25.5% were negative for CMV specific IgG.

Conclusion: Although most participants were seropositive for herpes viruses IgG, suggesting a natural virus circulation within the community, screening for protective immunity is suggested against HSV, since a relatively high proportion of tested females are still susceptible. In addition, and because of its nasty outcomes during pregnancy, IgG against CMV should also be tested. High percentage of positivity towards VZV could be explained due to introduction of the new vaccine program, and therefore, further analysis during pregnancy is not recommended.

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The herpes virus family contains several of the most important human pathogens. Clinically, the herpes viruses exhibit a spectrum of diseases that vary according to species. The outstanding property of herpes viruses is their ability to cause primary infections in addition to establish lifelong persistent infections in their hosts by undergoing periodic reactivation.¹ Eight human herpes virus species are known, and many can cause significant morbidity and mortality in a developing fetus, if the mother acquires mostly the primary, and less likely the latent, infection during pregnancy. Herpes viruses that commonly infect fetuses include herpes simplex virus types 1 and 2 (HSV 1-2), varicella zoster virus (VZV), and cytomegalovirus (CMV).² The HSV 1-2 infection of fetuses may be acquired in the uterus or during birth. Congenital herpes is estimated to occur worldwide in approximately one in 5000 deliveries per year.³ The fetus seems to be unable to limit the replication and spread of HSV if the mother is infected for the first time during pregnancy, and has a propensity to develop severe disease. Fetuses with congenital HSV infections exhibit lesions localized to the skin, eye, and mouth, encephalitis, and/or disseminated disease involving multiple organs, including the central nervous system. The overall mortality rate of untreated cases is 50%.³ Many survivors of severe infections are left with permanent neurological impairment. Prevention of such consequences relies mainly on early diagnosis during pregnancy, and swift application of antiviral agents, such as aciclovir.⁴ Varicella (chickenpox) caused by VZV, is a highly communicable disease of childhood. After maternal chickenpox in the first or second trimesters, fetus infection may occur in nearly 2% of the cases, yet still less than HSV and CMV related cases.⁵ The characteristic symptoms consist of a constellation of physical abnormalities known as the congenital varicella syndrome (CVS), manifested by dermatomal scarring, limb hypoplasia, ocular abnormalities, low birth weight, cortical atrophy, and mental retardation, and early death. Survivors may have long-term learning defects and other developmental problems. If the mother develops varicella rash for the first time between day 4-5 antepartum and day 2 postpartum, generalized neonatal varicella leading to death in approximately 20% of the cases has to be expected. If maternal rash develops outside of these

times, then the clinical illness is less severe due to the transplacental passage of protective antibody. On the basis of the clinical consequences of VZV infections during pregnancy, the active immunization with a live attenuated varicella vaccine (Oka strain) is the most effective strategy for prevention.⁶

Cytomegalovirus is responsible for most intrauterine viral infections associated with congenital defects. However, there is a great disparity in the incidence of fetal infection and severity of outcome, depending on whether the mother is experiencing a primary or recurrent infection. In women experiencing their first CMV infection during pregnancy, 35-50% of fetuses will be infected, and 10% of these will be symptomatic. The severity of the symptoms is most pronounced when the infection occurs during the first trimester. Referred to as cytomegalic inclusion disease, results caused by the infection range from fetal death to various degrees of damage to liver, spleen, blood-forming organs, and components of the nervous system. The latter is a common cause of hearing loss and mental retardation. Even in infants who are asymptomatic at birth, hearing deficits and ocular damage may appear later and continue to progress during the first few years of life.⁷ Approximately 1% of live births annually in the United States have congenital CMV infections, and approximately 5-10% of those will suffer cytomegalic inclusion disease. Only 7-25% survive, but with late sequelae including deafness and learning difficulties.⁸ Monitoring the fetus, after the confirmation of its CMV infection may aid in determining the prognosis, and deciding the next step since antiviral interference has not always shown good outcomes.⁹

In Syria, there is no data available concerning the seroprevalence of herpes viruses antibodies in the population in general, and among women of childbearing age in particular. With the lack of such studies, the magnitude of herpes viruses infection on fetuses in Syria is still unknown. Therefore, the aim of our study was to determine the current herpes viruses IgG antibodies circulation among university Syrian females at childbearing age.

Methods. A cross-sectional design was used to evaluate seroepidemiology of herpes viruses (HSV 1-2, VZV, and CMV) IgG among university Syrian females at childbearing age. Inclusion criteria of the participants involved in the study included Syrian female students, aged between 18-30 years old, capable of understanding Arabic, and unmarried. Exclusion criteria included insufficient volume or hemodialysis of the samples obtained. For ease of contacting them, and

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due to their large number compared with other colleges, only those attending the College of Pharmacy in the University of Kalamoon was recruited. Students were informed regarding the study through an information sheet posted on billboards in the laboratories, and the university campus. The information sheet comprised information regarding the aims, importance, voluntary nature, and procedures of the study. The contacts of the main researcher and those who obtained the blood samples were provided, in case potential participants required further information. Sera were collected between October 2009 to November 2010, and tests were conducted in the Microbiology Laboratory in the Faculty of Pharmacy, University of Kalamoon, Deratiah, Syria. Demographic information such as age, marital status, and residency were obtained from the participants. Informed consent was also obtained from all participants. A total of 316 sera were collected and samples were tested for herpes viruses (HSV 1-2, VZV, CMV) specific IgG antibodies using: DRG Diagnostics Herpes Simplex Virus Type 1 + 2 IgG enzyme-linked immunoassay (ELISA) kit (for HSV IgG antibodies [Medac, Hamburg, Germany]); DRG Diagnostics Varicella Zoster-Virus IgG ELISA Kit (for VZV IgG antibodies [Medac, Hamburg, Germany]); and Genesis Diagnostics CMV IgG ELISA Kit (for CMV IgG antibodies [MicroBix BioSystem, Cambridge, UK]). All these kits were based on an ELISA. Sensitivity and specificity for all the kits were above 98%. For HSV, IgG titers greater than 11 IU/ml were considered positive, between 9-11 IU/ml were considered equivocal, and less than 9 IU/ml were considered negative. For VZV, absorbance greater than 1.1x absorbance of the cutoff control sample were considered positive for IgG antibodies against VZV, and those of less than 0.9x of absorbance of the cutoff control sample were considered negative for IgG antibodies against VZV. For CMV, IgG titers greater than 4 IU/ml were considered positive, and those of less than 4 IU/ml were considered negative. This medical project involving human subjects was ethically approved by the Pharmacy College Council of the private University of Kalamoon, Syria, and was conducted according to the principles of Helsinki Declaration.

Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc, Chicago, IL, USA) version 17. Descriptive statistics were used to describe the sample characteristics, HSV, VZV, and CMV IgG antibody concentration (IU/ml), and the prevalence of seropositivity in the samples. Ninety-five percent confidence interval (CI) was calculated for the prevalence of seropositivity using the Wilson's method.

Results. By the time of data collection from October 2009 to November 2010, there were 672 female students registered in the Faculty of Pharmacy in the University of Kalamoon. A total of 316 students agreed to participate in the study, with a response rate of 47%. All samples collected were tested for HSV 1-2, VZV, and CMV specific IgG antibodies using ELISA-based methods. Most participants were 22 years old or below (88%), single, and living in various areas of Syria. Table 1 presents the descriptive statistics for the age of participants and IgG titers for each virus tested. The quantitative analysis for herpes (HSV 1-2, VZV, CMV) IgG antibodies showed a noticeable variability in the values of antibodies.

Analysis revealed that 52% (161 samples) (95% CI: 46.4 - 57.4%) of the cohort was seropositive for HSV 1-2 IgG antibodies, while a relatively high proportion of participants (48%; 151 samples; 95% CI: 42.7 - 53.6%) were HSV susceptible subjects (negative sample). There was variability in the positive values of antibodies that ranged between 11.4-26.9 IU/ml. Regarding VZV, analysis showed that 91% (287 samples) (95% CI: 88.2 - 94.3%) of the cohort was positive for VZV IgG antibodies; leaving small proportion of participants (9%; 29 samples; 95% CI: 5.7% - 11.8%) as VZV susceptible subjects. The positive values of antibodies ranged between the values 0.237-1.988. For CMV, analysis revealed that 75% (235 samples) (95% CI: 69.6 - 79.2%) of the cohort was seropositive for CMV IgG antibodies; while 25% (81 samples; 95% CI: 20.8 - 30.4%) were CMV susceptible subjects. The variability in the positive values of antibodies ranged between 4.2-84.1 IU/ml. There were 11 samples that were negative for antibodies against both HSV and VZV, 29 were negative for antibodies against both HSV and CMV, and 4 were negative for antibodies against

Table 1 - Descriptive statistics for age, IgG antibodies titers (HSV, CMV), and absorbance (VZV) distribution among the study participants from the Pharmacy College, Kalamoon University, Deratiah, Syria (N=316).

Characteristics	Mean	Range	95% CI
Age	21.09	18 - 30	
IgG titre against HSV 1-2 (IU/ml)	11.73	1.10 - 26.90	10.89 - 12.57
Absorbance value of antibodies against VZV	1.11	0.002 - 1.99	1.06 - 1.16
IgG titre against CMV (IU/ml)	14.95	0.00 - 84.10	13.45 - 16.44

Ig - immunoglobulin, HSV - herpes simplex virus, VZV - varicella zoster virus, CMV - cytomegalovirus, CI - confidence intervals

both VZV and CMV. Only 2 samples were negative for antibodies against the 3 viruses.

Discussion. Infection by herpes viruses caused by either primary or reactivated exposure are widely spread, and characterized by apparently various complications that are different according to the virus species, viral load, the infection being primary or reactivated, and immune status of the host. Exposure to these viruses for the first time during pregnancy often results in congenital defects and possible abortion that can be prevented, or minimized through swift invention measures. We approached female students attending a Syrian university in order to screen for herpesvirus-specific IgG antibodies. The response rate was relatively low (47%); probably due to the dislike that many individuals have towards needles and blood collection. In addition, rewards for participating in the study were not offered to volunteers. However, the response rate was higher comparing with previous similar studies performed in the same institution^{10,11} due to extensive advertisement, longer period for sampling, and encouragement for participation through giving the results to the participants.

Our findings showed that only 52% of the subjects tested proved to have IgG against HSV. This immunity most probably has been acquired from previous infection with the virus during childhood, or adolescence. Among the participants however, a high percentage (48%) did not have sufficient immunity against HSV. Our results are somehow different from the regional and universal trends for HSV infection ratio, where usually more than two-thirds of various populations shows immunity.^{12,13} This could be explained due to the younger age of the group tested in our study, as well as social differences, such as sexual activity. Thus, these preliminary data indicate that there is a considerable risk of acquiring primary HSV infection during pregnancy among Syrian females, and therefore, screening for HSV IgG antibodies should be included as a routine test during pregnancy in Syria. If the pregnant woman is seronegative, monitoring IgM antibodies level during the course of the pregnancy will be a necessity. If seroconversion occurs, it is recommended that pregnant women may be treated with acyclovir.¹⁴ Since acyclovir is not officially approved for treatment of pregnant women, they should be informed to give consent before the administration.⁴ However, no increase of fetal abnormalities was ascribed to these treatments, although long-term outcomes were not evaluated.¹⁵

Regarding VZV, our results agree with regional and universal result found in the literature.^{16,17} This high

percentage of immunity could be explained in part to the recent introduction of the vaccine against VZV in Syria, although still an option and not part of the national vaccination programme but is widely given for children at age of entering the nursery, and also highly available for adults at high risk in private clinics. We can suggest that investigation for VZV IgG antibodies is not required as a routine protocol during pregnancy in Syria, since most women will have the protective antibodies prior to pregnancy. However, those with seronegative VZV IgG antibodies could be managed as recommended above for HSV.

Our results were slightly different compared with other similar regional studies regarding CMV.^{18,19} However, in agreement to our results, statistics from the United States among females at childbearing age indicate that 50-80% are immune towards CMV.²⁰ These differences could be explained as for HSV, but should be looked at with care since our study shows relatively a significant parentage (a quarter) of university Syrian women at childbearing age, who are at risk of getting the infection for the first time during possible future pregnancy. For those who are seronegative during their pregnancy, we suggest that detecting IgM and determining the avidity index of anti-CMV IgG should become a routine protocol in Syria. The correct interpretation of serological and virological tests followed by appropriate counselling by an expert physician is an effective tool to reduce the number of unnecessary pregnancy terminations by over 70%.^{9,21}

The major limitation of our study was the low number of samples tested, which precluded the use of sophisticated statistical analysis. Also, we tested a specific population of participants, such as single individuals, females, and university students. Therefore, our findings may not be generalized to all female Syrian population. However, since there is an evident lack of research concerning this issue, our study constitutes essential preliminary investigation of this area in Syria, which draws attention for the fact that the risk of getting the infection for the first time among females at childbearing age is still high, especially for HSV and CMV but not for VZV. Therefore, precautionary actions should be taken, specially, if one considers that these females may get married, and decide to get pregnant.

Clearly, a positive IgG result against any of the 3 viruses in this study does not rule out the need to detect any bodies again at the beginning of a future pregnancy due to the fact that they may drop by time. This is also applied to vaccinated individuals against VZV. However, the titers obtained in most of the cases, either near the cutoff or much higher does not conflict with

the aim of the study since we were mainly looking for the negatives, and those who have never infected with any one of the herpes viruses in question. We merely aimed to shed light on the hidden risk of getting the herpes infection for the first time during pregnancy, and therefore, we were not interested to detect how many among the participants were recently infected. This explains why IgM detection and IgG avidity were not performed. The findings of this study, however, indicate that future research should conduct studies with larger sample size, and targeting various demographic groups to reflect actual situation in the Syrian society, with a possibility of detecting recent infection through performing IgM detection and IgG avidity.

In conclusion, our results show a noticeable percentage of participants who are unprotected at childbearing age against HSV, and to a less extent towards CMV. Participants seem however, highly protected towards VZV. Therefore, these preliminary observations can suggest that screening strategies against congenital HSV and CMV should be reinforced as routine protocols during pregnancies in Syria, at least for the targeted group, while VZV screening is not warranted due to high immunity acquired probably in childhood, although vaccination against VZV should be considered in seronegative women. Since maternal clinical history is not reliable, the data suggest performing serologic testing to determine immune status against HSV and CMV infections for women at the early stages of the pregnancy in Syria. Clearly, further larger scale studies targeting different demographic groups within the Syrian society are required to confirm these conclusions and to reflect actual situation in Syria.

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