

Seroprevalence of *Toxoplasma gondii*, Rubella virus and Cytomegalovirus among pregnant women and the importance of avidity assays

Mumtaz C. Sirin, MD, Neval Agus, MD, Nisel Yilmaz, MD, Arzu Bayram, MD, Yeser K. Deric, MD, Pinar Samlioglu, MD, Sevgi Y. Hanci, MD, Guliz Dogan, MD.

ABSTRACT

الأهداف: تحديد الانتشار المصلي للمقوسة القندي (*T. gondii*)، وفيروس الحصبة الألمانية، والفيروس المضخم للخلايا (CMV) بين النساء الحوامل في إزمير، تركيا.

الطريقة: استخدم في تحليل هذه الدراسة الرجعية السجلات الطبية للنساء الحوامل المراجعين لمستشفى إزمير تيبسيك للتدريب والأبحاث، إزمير، تركيا خلال الفترة من يناير 2014 ويناير 2016م. وقد تم تقييم النتائج *T. gondii* IGM/ 7513، 7189، نتائج الحصبة الألمانية إيغم/ إيغم، ونتائج 906 إيغم/ مفتش و 146 نتائج اختبار الطموح. تم الكشف عن الأجسام المضادة واختبار الرغبة من خلال طريقة المقايسة المناعية الآلية. كما أجري الغلوبولين المناعي G اختبارات الطفو باستخدام نظام المقايسة المناعية متعددة الحلقات.

النتائج: بلغ معدلات IgG الإيجابية *T. gondii* 32.3%، وفيروس الحصبة الألمانية 93.5%، و 98.9% CMV. تم العثور على الأجسام المضادة المناعية M إيجابية في 138 (1.9%) حالات *T. gondii*، 88 (1.2%) حالات للحصبة الألمانية، و 14 (1.5%) حالات CMV. وأجري اختبارات الرغبة في 146 من 218 المرضى الذين تم العثور على كل من IgG و IgM إيجابي. من بين 146 مريضا، كان 6 مرضى لديهم مستوى الرغبة منخفض (كل شيء ل *T. gondii*)، أظهر 11 مريض مستوى الرغبة قريب من الحد الطبيعي، و 129 مريض مستوى الرغبة مرتفع.

الخاتمة: في منطقتنا، في حين أن معدلات IgG إيجابية للحصبة الألمانية و CMV مرتفعة، ومعظم النساء الحوامل عرضة للإصابات *T. gondii* من أجل تعزيز مصداقية التشخيص المصلي، ينبغي إجراء اختبارات الرغبة في جميع حالات IgM الإيجابية التي تم الكشف عنها مع الإيجابية IgG.

Objectives: To determine the seroprevalence of *Toxoplasma gondii* (*T. gondii*), Rubella virus, and Cytomegalovirus (CMV) among pregnant women in Izmir, Turkey.

Methods: Medical records of pregnant women attending Izmir Tepecik Training and Research Hospital, Izmir, Turkey between January 2014 and January 2016 were analyzed in this retrospective cross-sectional study. The 7513 *T. gondii* IgM/IgG results, 7189 Rubella IgM/IgG results, 906 CMV IgM/IgG results and 146 avidity test results were evaluated. Specific IgM and IgG antibodies were detected by an automated chemiluminescent enzyme immunoassay method. Immunoglobulin G avidity tests were performed using a multiparametric immunoassay system.

Results: The rates of IgG positivity for *T. gondii* was 32.3%, Rubella virus 93.5%, and CMV 98.9%. Immunoglobulin M antibodies were found to be positive in 138 (1.9%) cases for *T. gondii*, 88 (1.2%) cases for Rubella, and 14 (1.5%) cases for CMV. Avidity tests were ordered from 146 of 218 patients who were found both IgM and IgG positive. Among 146 patients, 6 patients had a low avidity index (all for *T. gondii*), 11 patients showed borderline avidity, and 129 patients revealed high avidity.

Conclusion: In our region, whereas the rates of IgG positivity for Rubella and CMV are high, most pregnant women were susceptible to *T. gondii* infections. In order to enhance the reliability of the serological diagnosis, avidity tests should be performed in all IgM positivities detected together with IgG positivity.

Saudi Med J 2017; Vol. 38 (7): 727-732
doi: 10.15537/smj.2017.7.18182

From the Department of Medical Microbiology, Izmir Tepecik Training and Research Hospital, Izmir, Turkey.

Received 17th December 2016. Accepted 18th April 2017.

Address correspondence and reprint request to: Dr. Mumtaz C. Sirin, Department of Medical Microbiology, Tepecik Training and Research Hospital, Izmir, Turkey. E-mail: drmcemsirin@yahoo.com
ORCID ID: orcid.org/0000-0002-7349-3438

Congenital infections are one of the most important causes of perinatal morbidity and mortality, particularly in developing countries.^{1,2} The transient immunosuppression occurred in pregnancy increase the vulnerability of pregnant women to various infectious agents. The ability of the fetus to resist infection is limited and the fetal immune system is unable to prevent the dissemination of infectious microorganisms to various tissues.^{3,4} Among these pathogens, the prevalence of *Toxoplasma gondii* (*T. gondii*), *Rubella virus*, *Cytomegalovirus* (CMV); also known as the members of TORCH complex, are very high and they may cause congenital malformations, multiple abortions, premature deliveries, and stillbirths during pregnancy.^{1,4} The early recognition of *T. gondii*, *Rubella*, CMV infections in the mother and fetus is an important component of prenatal care.³ Since these maternal infections are initially inapparent or asymptomatic and the clinical diagnoses are unreliable, diagnosis of acute infection in pregnant women usually relies on serological evidences. The detection of specific IgM antibody is the most prominent approach for the identification of these infections.^{1,5} Many analytical methods are available for the serological diagnosis of *T. gondii*, *Rubella*, and CMV infections. Enzyme-linked immunosorbent assay (ELISA) and enzyme immunoassay (EIA) for detection of IgM and IgG antibodies against these infections are suggested to be highly sensitive and specific.⁵⁻⁷ Moreover, improvements in IgG avidity assays assist physicians in distinguishing between primary acute infection and recurrent or past infection.^{7,8} The prevalence of *T. gondii*, *Rubella*, and CMV infections varies widely by geographic region, socioeconomic status, race, and age. The aim of this retrospective cross-sectional study was to determine the seroprevalence of *T. gondii*, *Rubella virus*, and CMV among pregnant women in Izmir province and to compare our findings with those of other studies.

Methods. Medical records of pregnant women attending Department of Gynecology and Obstetrics for routine antenatal check-up between January 2014 and January 2016 were analyzed. A total of 7513 pregnant women who were screened for *T. gondii* or Rubella or CMV and who had both IgM and IgG test result for each pathogen were included in the study. Pregnant women who had only IgM test result or only IgG test result for each pathogen were excluded.

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

The study was conducted according to the principles of the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, amended in October 2013). Since medical records were reviewed retrospectively, local ethics committee approval was not required due to the retrospective nature of the study and necessary permission for this research has been obtained from the hospital scientific research and development review board (document number: 68878892-806.02.02).

After obtaining fully informed verbal consent, a 5 ml of venous blood was taken from each patient and the blood samples were clotted and centrifuged prior to testing. In daily routine, serum samples were analyzed for specific IgM and IgG antibodies against *T. gondii*, Rubella, and CMV by using an automated chemiluminescent enzyme immunoassay method (Liaison, DiaSorin, Saluggia, Italy). For avidity assays, serum samples were stored at -20°C until testing. *Toxoplasma gondii*, Rubella, and CMV IgG avidity tests were performed using a multiparametric immunoassay system (Chorus, DIESSE Diagnostica Senese, Monteriggioni, Italy). The assays were performed according to the manufacturer's instructions. Only the initial result of each patient was taken into account and other repetitive results of the same patient were excluded. All positive or borderline IgM test results were double-checked. Specific IgM and IgG test results were interpreted as negative, borderline or positive as per the manufacturer's guidelines. Toxoplasma, Rubella, and CMV IgG avidity test results were evaluated as low (<30%), borderline (30% to 40%), or high (>40%) avidity index according to manufacturer's guidelines.

Results. Among 7513 pregnant women with a mean age of 30.7 years (range, 18-45 years), all of them had both IgM and IgG test results for *T. gondii*, 7189 of them had both IgM and IgG test results for Rubella, and 908 of them had both IgM and IgG test results for CMV. *Cytomegalovirus* IgM/IgG tests were found to be the least requested assays by the clinicians, and *T. gondii* IgM/IgG tests were the most. The rates of IgM positivity for *T. gondii* was 1.9%, Rubella was 1.2%, and CMV was 1.5%; whereas the rate of IgG positivity was 32.3% for *T. gondii*, 93.5% for Rubella, and 98.9% for CMV. Seropositivity rates were expressed with 95% confidence intervals (CI) in Table 1. Toxoplasma avidity tests were requested from 83 of 117 patients who were found both IgM and IgG positive, and low avidity test result was determined in 6, borderline in 9, and high in 68 patients. Test orders for only Toxoplasma IgM positive cases (n=21) were rejected due to IgG negativity, and

avidity tests were not performed (Table 2). Ten patients who had borderline Toxoplasma IgM test result showed high avidity.

Rubella avidity tests were ordered from 54 of 87 patients who were found both IgM and IgG positive, and one patient had a borderline avidity result and 53 patients showed high avidity. The avidity test of one patient who had only Rubella IgM positive result was not performed due to IgG negativity (Table 2). Eight patients who had borderline Rubella IgM test results revealed high avidity. Cytomegalovirus avidity tests were requested from 9 of 14 patients who were found both IgM and IgG positive, and one patient had a borderline avidity result, 8 patients showed high avidity (Table 2). One patient who had borderline CMV IgM test result revealed high avidity.

Discussion. *Toxoplasma gondii*, *Rubella virus*, and CMV are common causes of infection in all age groups and the infections are generally asymptomatic, but the infection in pregnant women in the first trimester may

cause fetal congenital malformations. The prevalence of these infections among pregnant subjects varies from one geographic region to another.^{2,5} The determination of regional seroprevalence rates on a regular basis is necessary to set test order strategies in antenatal screening of pregnant women. Regional prevalence estimates are of great importance for countries where there is no national screening program, such as Turkey. In this study, the seroprevalence of *T.gondii*, *Rubella virus*, and CMV among pregnant women in our region was determined to estimate the susceptibility of the patients to these agents and to guide the physicians for screening decisions. *Toxoplasma gondii* is the causative agent of toxoplasmosis, one of the most common parasitic infections in the world. The infection is usually transmitted to humans by consuming raw or under cooked meat or by water and foods contaminated with parasite cysts. Most of *T. gondii* infections are asymptomatic in immunocompetent subjects, or may mimic a mild viral illness.^{2,9,10} However, primary infection during pregnancy may cause major ocular and

Table 1 - The seroprevalence rates of *Toxoplasma gondii*, *Rubella* and *Cytomegalovirus* among 7513 pregnant women.

Test	Negative	Borderline	Positive	95% CI	Total
Toxoplasma IgM	7365 (98.0)	10 (0.1)	138 (1.9)	1.53 - 2.14	7513
Toxoplasma IgG	5078 (67.6)	8 (0.1)	2427 (32.3)	31.25 - 33.36	7513
Rubella IgM	7093 (98.7)	8 (0.1)	88 (1.2)	0.97 - 1.48	7189
Rubella IgG	461 (6.4)	7 (0.1)	6721 (93.5)	92.92 - 94.06	7189
CMV IgM	893 (98.4)	1 (0.1)	14 (1.5)	0.74 - 2.34	908
CMV IgG	10 (1.1)	0 (0)	898 (98.9)	98.22 - 99.58	908

CI - confidence interval

Table 2 - The avidity test results of IgM positive cases for *Toxoplasma gondii*, *Rubella* and *Cytomegalovirus* among 7513 pregnant women.

Results	Toxoplasma	Rubella	CMV	Total
Low avidity	6 (4.3)	0 (0)	0 (0)	6
Borderline avidity	9 (6.5)	1 (1.1)	1 (7.1)	11
High avidity	68 (49.4)	53 (60.3)	8 (57.2)	129
Not requested	34 (24.6)	33 (37.5)	5 (35.7)	72
Not tested (IgG negative cases)	21 (15.2)	1 (1.1)	0 (0)	22
Total	138 (100)	88 (100)	14 (100)	240

neurological abnormalities, miscarriage or stillbirth, relying on the virulence of the parasite, the immune response of the mother, and the gestational stage. The seroprevalence rates of toxoplasmosis in the world may be changeable according to various factors, such as lifestyle, socioeconomic conditions, or nutritional habits.^{4,11} The seropositivity was reported as 9.1% in England, 18% in Italy, 43.8% in France, 58.5% in Brazil, 67.5% in Egypt, and 67.7% in India.^{3,10,12-15} In Turkey, the seroprevalence of *T. gondii* varies greatly among different regions, ranging from 30.3% to 69.5%.^{2,9,11,16-18} The seropositivity rates are higher in southeastern Anatolia region, probably due to the consumption of undercooked meat and raw vegetables.^{9,18} Our results showed that toxoplasma IgG seropositivity in pregnant women in Izmir province, located in west Anatolia region was 32.3%. Vaccine against toxoplasmosis is not available, and hence, educational programs based on preventive measures (such as hand washing, avoiding raw or undercooked meat, keeping away from cats or cat feces containing oocysts) should be organized by public health institutions in order to reduce the risk of intrauterine infection by *T. gondii*.

Rubella is an acute exanthematous viral infection that usually affects children, but is also seen in adults. In pregnant women, the virus may cause congenital rubella syndrome (CRS) associated with multiple organ defects in the fetus.^{9,11,19} Rubella vaccination has been shown to be a very efficient way in preventing CRS. Successful vaccination programs have been implemented in most developed countries, and high seropositivity rates have been obtained through vaccination.^{1,7,19,20} In Turkey, rubella vaccine has been integrated into the national childhood immunization schedule since 2006. Previous studies from different regions of Turkey reported high rubella seropositivity ranging between 86.5% and 96.2%.^{2,4,9,11,16,19,20} Rubella seroprevalence in our study is similar to the findings mentioned above. Since we did not collect information on rubella vaccination from the patients, the effect of vaccination on rubella seropositivity rate could not be determined. However, as shown in our study, there are still seronegative or unvaccinated women of childbearing age. Seronegative women should be advised to have rubella vaccination before pregnancy in order to prevent CRS.

Cytomegalovirus is the most common cause of congenital viral infections, with an incidence of 0.2% to 2.5% of all live births worldwide.^{1,21-23} The infection frequently causes sensorineural hearing loss, chorioretinitis and mental retardation in the fetus. However, unlike rubella, fetal CMV infection may occur following both primary and recurrent infection.

Reactivation of latent infection or reinfection with a new strain of CMV can cause infections even in the presence of detectable IgG levels. Nevertheless, the rate of transmission of recurrent infection (0.15-2.2%) is much lower than primary infection (30-40%).^{1,11,21,23} The seroprevalence of CMV infections are highly associated with low socioeconomic level, poor hygienic or crowded living conditions.^{11,21} Therefore, in many studies, it has been revealed that the seroprevalence rates of CMV among pregnant women in developed countries were lower than those of developing countries such as India and Turkey.^{3,11,20-22,24} A cohort study conducted on the basis of CMV seroprevalence in England showed a rate of 49% in White British pregnant women, 89% in South Asian pregnant women born in United Kingdom, and 98% in South Asian pregnant women born in South Asia.²⁵ In our study, the seropositivity rate of pregnant women for CMV was 98.9%. Previous studies from different locations of Turkey reported CMV seroprevalence rate in the range of 91.5% to 98.9%.^{2,9,11,16,20} To reduce the rate of maternal CMV infection during pregnancy, it is highly recommended to give hygiene information about CMV infection to seronegative pregnant women.

A positive IgM result for *T. gondii*, *Rubella virus*, and CMV may not always indicate a recent primary infection. False-positive IgM results may occur in patients with autoimmune diseases due to the presence of rheumatoid factor and antinuclear antibodies or in patients recently infected with other viral pathogens due to heterotypic IgM antibody reactivity.^{5,7} Moreover, after primary infection, specific IgM may be detected for several months or years in low titers. Hence, IgG avidity testing is recommended to differentiate between acute, recurrent or past infections.^{5,8} Specific IgG avidity testing for *T. gondii*, *Rubella virus*, and CMV has proved to be a useful procedure, particularly in combination with other serological assays. A positive IgM result accompanied by low avidity is suggestive of a recent (less than 3 months) primary infection, whereas a high avidity indicates past infection, IgM persistence or reactivation. A low avidity index may also persist in a proportion of infected patients for months.^{5,7,22} In the present study, the high avidity detected in all patients screened for *T. gondii*, *Rubella virus* and CMV IgG avidity was considered as a reduced risk for congenital infection. Six patients who were found both IgM and IgG positive for *T. gondii* had a low avidity index and this result was regarded as an increased risk for congenital infection. Among 6 patients, 2 patients were lost to follow up after their first visit, 2 patients gave birth after a full term pregnancy, and in 2 cases,

the pregnancies were electively terminated in the first trimester. In addition, it was seen that the avidity tests were used less than expected. Although both IgM and IgG antibody levels for *T. gondii*, *Rubella virus*, and CMV were positive in the serum samples of 218 patients, the number of serum samples analyzed for avidity was 146. On the other hand, it was seen that the avidity tests were requested unnecessarily in 22 patients who had negative IgG result. These findings indicated the necessity to revise the prenatal screening decisions of our clinicians. It is suggested that in all IgM positivities detected together with IgG positivity, avidity tests should be performed. However, taking into consideration of only avidity test may cause unnecessary pregnancy termination and anxiety, particularly in cases where low or borderline avidity is detected. In order to enhance the reliability of the laboratory diagnosis, follow-up of patients with low or borderline avidity results and repeating the serological tests at certain intervals, or examination of the amniotic fluid by molecular methods, would be suitable.

Study limitations. This study is a single-center study and inherently limited by its retrospective nature. Hence, information about living conditions, socioeconomic status, nutritional habits, and vaccination status of the patients could not be collected and evaluated.

In conclusion, the results for *T. gondii*, *Rubella virus* and CMV seropositivity among pregnant women in Izmir are similar to those found in other regions of Turkey. Seropositivity rates in a region may be regarded as a suitable guide for antenatal screening programs. Serological screening of all pregnant women for *Rubella virus*, and CMV may not be recommended due to the high seropositivity rates detected in our region, whereas the high seronegativity rates for *T. gondii* may justify a routine screening. On the other hand, the necessity for *T. gondii*, *Rubella virus*, and CMV screening in pregnant women is controversial due to several factors, including the potential overuse of testing in routine practice, high cost, and misinterpretation of IgM and IgG test results. In this regard, as shown in our results, avidity testing may be considered as a reliable and rapid confirmation method, if it is used and evaluated properly. However, further studies will be required to clarify the risks, benefits, and cost-effectiveness of such a screening program during pregnancy.

References

- Sharma S, Duggal N, Agarwal S, Mahajan RK, Anuradha, Hans C. Seroprevalence of Toxoplasma, Rubella and CMV infections in Antenatal Women in a Tertiary care hospital in North India. *J Commun Dis* 2015; 47: 23-26.
- Aynioglu A, Aynioglu O, Altunok ES. Seroprevalence of *Toxoplasma gondii*, *Rubella* and *Cytomegalovirus* among pregnant females in north-western Turkey. *Acta Clin Belg* 2015; 70: 321-324.
- Sebastian D, Zuhara KF, Sekaran K. The influence of the TORCH infections in first trimester miscarriage in the Malabar region of Kerala. *Afr J Microbiol Res* 2008; 2: 56-59.
- Parlak M, Çim N, Nalça Erdin B, Güven A, Bayram Y, Yıldızhan R. Seroprevalence of Toxoplasma, Rubella, and Cytomegalovirus among pregnant women in Van. *J Turk Soc Obstet Gynecol* 2015; 2: 79-82.
- Karad D, Kharat A. Seroprevalence of Torch Infections in Bad Obstetrics History in HIV and Non-HIV Women in Solapur District of Maharashtra India. *J Hum Virol Retrovirol* 2015; 2: 00067.
- Surpam RB, Kamlakar UP, Khadse RK, Qazi MS, Jalgaonkar SV. Serological study for TORCH infections in women with bad history. *J Obstet Gynecol India* 2006; 56: 41-43.
- Mendelson E, Aboudy Y, Smetana Z, Tepperberg M, Grossman Z. Laboratory assessment and diagnosis of congenital viral infections: Rubella, cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), parvovirus B19 and human immunodeficiency virus (HIV). *Repr Toxicology* 2006; 21: 350-382.
- Ozekinci T, Suay A, Karasahin O, Akpolat N. The value of CMV and Rubella IgG avidity tests in the diagnosis of *Cytomegalovirus* (CMV) and Rubella infections in pregnant women. *Biotechnol Biotech Eq* 2005; 19: 139-144.
- Koksaldi-Motor V, Evirgen O, Azaroglu I, Inci M, Ozer B, Arica S. Prevalence of *Toxoplasmosis*, *Cytomegalovirus* and Rubella IgG antibodies in Hatay women and children. *West Indian Med J* 2012; 61: 154-157.
- Berger F, Goulet V, Le Strat Y, Desenclos JC. Toxoplasmosis among pregnant women in France: Risk factors and change of prevalence between 1995 and 2003. *Rev Epidemiol Sante Publique* 2009; 57: 241-248.
- Karabulut A, Polat Y, Türk M, Işık Balcı Y. Evaluation of rubella, *Toxoplasma gondii*, and cytomegalovirus seroprevalences among pregnant women in Denizli province. *Turk J Med Sci* 2011; 41: 159-164.
- Nash JQ, Chissel S, Jones J, Warburton F, Verlander NQ. Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. *Epidemiol Infect* 2005; 133: 475-483.
- Puccio G, Cajozzo C, Canduscio LA, Cino L, Romano A, Schimmenti MG, et al. Epidemiology of Toxoplasma and CMV serology and of GBS colonization in pregnancy and neonatal outcome in a Sicilian population. *Ital J Pediatr* 2014; 40: 23.
- Moura FL, Amendoeira MR, Bastos OM, Mattos DP, Fonseca AB, Nicolau JL, et al. Prevalence and risk factors for *Toxoplasma gondii* infection among pregnant and postpartum women attended at public healthcare facilities in the City of Niterói, State of Rio de Janeiro, Brazil. *Rev Soc Bras Med Trop* 2013; 46: 200-207.
- El Deeb HK, Salah-Eldin H, Khodeer S, Allah AA. Prevalence of *Toxoplasma gondii* infection in antenatal population in Menoufia governorate, Egypt. *Acta Trop* 2012; 124: 185-191.
- Tamer GS, Dundar D, Caliskan E. Seroprevalence of *Toxoplasma gondii*, rubella and *Cytomegalovirus* among pregnant women in western region of Turkey. *Clin Invest Med* 2009; 32: 43-47.
- İnci A, Yener C, Güven D. The investigation of toxoplasma, rubella and *Cytomegalovirus* seroprevalencies in pregnant women in a state hospital. *Pam Tip Derg* 2014; 7: 143-146.

18. Tekay F, Özbek E. The seroprevalence of *Toxoplasma gondii* in women from Sanliurfa, a province with a high raw meatball consumption. *Turkiye Parazitol Derg* 2007; 31: 176-179.
19. Aksakal FN, Maral I, Cirak MY, Aygun R. Rubella seroprevalence among women of childbearing age residing in a rural region: Is there a need for rubella vaccination in Turkey? *Jpn J Infect Dis* 2007; 60: 157-160.
20. Uyar Y, Balci A, Akcali A, Cabar C. Prevalance of rubella and cytomegalovirus antibodies among pregnant women in northern Turkey. *New Microbiol* 2008; 31: 451-455.
21. De Paschale M, Agrappi C, Manco MT, Paganini A, Clerici P. Incidence and risk of *Cytomegalovirus* infection during pregnancy in an urban area of Northern Italy. *Infect Dis Obstet Gynecol* 2009; 2009: 206505.
22. Zhang S, Hu L, Chen J, Xu B, Zhou YH, Hu Y. *Cytomegalovirus* seroprevalence in pregnant women and association with adverse pregnancy/neonatal outcomes in Jiangsu Province, China. *PLoS One* 2014; 9: e107645.
23. Picone O, Vauloup-Fellous C, Cordier AG, Parent Du Châtelet I, Senat MV, Frydman R, et al. A 2-year study on *Cytomegalovirus* infection during pregnancy in a French hospital. *BJOG* 2009; 116: 818-823.
24. Enders G, Daiminger A, Lindemann L, Knotek F, Bäder U, Exler S, et al. *Cytomegalovirus* (CMV) seroprevalence in pregnant women, bone marrow donors and adolescents in Germany, 1996-2010. *Med Microbiol Immunol* 2012; 201: 303-309.
25. Pembrey L, Raynor P, Griffiths P, Chaytor S, Wright J, Hall AJ. Seroprevalence of *Cytomegalovirus*, *Epstein Barr Virus* and *Varicella Zoster Virus* among Pregnant Women in Bradford: A Cohort Study. *PLoS One* 2013; 8: e81881.