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African Journal of Microbiology Research

Full Length Research Paper

Seroprevalence of *Chlamydia trachomatis*, cytomegalovirus, herpes simplex virus 1 and 2 in Saudi women with normal and abnormal early pregnancy: A case control study

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This work measured the seroprevalence of *Chlamydia trachomatis*, cytomegalovirus, herpes simplex virus-1 and 2 in Saudi women with normal and abnormal early pregnancy. This is a case-control study; serum samples were collected from 250 women as follows: 100 normal pregnancies, 70 ectopic pregnancies and 80 spontaneous abortions. IgG and IgM antibodies against candidate organisms were measured using enzyme linked immunosorbant assay (ELISA). The prevalence of *C. trachomatis* IgG, but not IgM, was significantly higher in ectopic pregnancy (18.5%) and abortion (10%) as compared to normal pregnancy (5%) ($P < 0.05$). The frequency of HSV-1 IgG, but not HSV-2, was also higher in ectopic pregnancy (94.3%) and abortion (87.5%) than in normal pregnancy (64%). However, the rate of detecting both IgM and IgG antibodies together either against HSV-1 or HSV-2 was higher in the ectopic (41.1 and 30%, respectively) and abortion groups (28.7 and 36.2%, respectively) as compared to the control (9 and 16%, respectively). Correspondingly, the frequency of cytomegalovirus (CMV) IgG was significantly higher in ectopic pregnancy (91.4%) and abortion (75%) as compared to normal pregnancy (58%). Similar results were also observed for CMV IgM antibodies either alone or with IgG antibodies in the ectopic and abortion groups as compared to control ($P < 0.05$). Candidate organisms were common by serology in Saudi females with abnormal early pregnancy. Additionally, the high frequency rate in normal pregnancy alarms the potential high risk of congenital infection. Further studies are needed to measure the true prevalence of these sexually transmitted infections (STIs) and their associated pregnancy complications in Saudi Arabia.

Key words: *Chlamydia trachomatis*, cytomegalovirus, herpes simplex virus, ectopic pregnancy, abortion, Saudi Arabia.

INTRODUCTION

Sexually transmitted infections (STIs) are major health problem and the World Health Organization (WHO) has estimated that 27 million people are infected in the Middle East (WHO, 2012). Infection with sexually transmitted

organisms, particularly *Chlamydia trachomatis*, cytomegalovirus (CMV) and herpes simplex virus (HSV) is associated with acute illness, adverse pregnancy outcome, infertility and sometimes mortality (WHO, 2012).

C. trachomatis is the most common sexually trans-mitted bacterial infection worldwide and the majority of patients are asymptomatic (den Hartog et al., 2005). Untreated cases can result in pelvic inflammatory disease, ectopic pregnancy (EP), abortion and infertility in women, and transmission during labour could cause conjunctivitis and pneumonitis in the newborn (Ghazi et al., 2006).

CMV, HSV-1 and HSV-2 can be transmitted sexually and infection is usually latent, persistent and majority of the patients are asymptomatic. These viruses have also been associated with adverse effects on reproductive health and they can be transmitted during pregnancy/-labour resulting in severe neonatal neurological manifest-tations (Ghazi et al., 2002; Obeid, 2007).

Serological screening against candidate organisms is mainly used for screening during routine clinical work-up and it is considered a useful tool to reflect on recent, previous and/or re-activation of infection with those pathogens (Gijsen et al., 2002; Land et al., 2003; den Hartog et al., 2005). However, serological tests are not useful in discriminating between clearance and persis-tence of infection in those subjects who are only positive for IgG antibodies against the organisms of interest, whereas persistence is an important risk factor for the development of abnormal early pregnancies (Gijsen et al., 2001; Land et al., 2003).

Therefore, serological results should be confirmed with a more sensitive technique (for example, nucleic acid amplification test) to confirm the results and to differen-tiate between clearance and current/persistent infections (Gijsen et al., 2002).

Information on STIs in Islamic countries is notably limited and currently only few studies reported their prevalence in Saudi Arabia. Hence, the objective of this study was to measure the seroprevalence of IgM and IgG antibodies against *C. trachomatis*, CMV, HSV-1 and HSV-2 in sera samples collected from Saudi females diagnosed with abortion or ectopic pregnancy and the results were compared with those obtained from normal pregnancy.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the Faculty of Applied Medical Sciences Ethics Committee (AMSEC 10-15-9-2013) and all serum samples were collected following obtaining of informed written consent from all the participants.

Participants and study design

In this case control study, a total of 250 women were recruited consecutively from the Maternity and Children Hospitals in Jeddah and Makkah between January 2013 and April 2014, and for whom TORCH screening was not performed during their clinical examination. The participants were categorised into:

Control group (n = 100)

Consisted of women with normal pregnancy (NP) (mean age 30 ± 7.3 years) and who attended the antenatal clinic for routine follow-up.

Case group (n = 150)

Included women with abnormal early pregnancy (AEP), who attended the Emergency Department with abdominal pain and/or vaginal bleeding (mean age 30.5 ± 6.6 years) and they were diagnosed clinically with either EP (n = 70) or abortion (n = 80).

Enzyme linked immunosorbant assay (ELISA)

ELISA was used for qualitative measurement of IgG and IgM antibodies against candidate organisms (Human Diagnostics, Germany). The detection of *C. trachomatis* IgM was performed using the NovaLisa kit (NovaTec Immunodiagnostica, Dietzenbach, Germany). Additionally, the HSV IgM kit (Human Diagnostics, Germany) did not differentiate between HSV-1 and 2.

All samples were processed using a fully automated ELISA system (Human Diagnostics, Germany) according to the manufacturers' instructions including the internal controls provided within each kit for quality control.

The sensitivity, specificity, inter and intra-assay coefficient of variation for each kit as reported by the manufacturers were as follow: *C. trachomatis* IgG kit (95.9, 96.2, 3.7 and 2.8%), *C. trachomatis* IgM kit (83.3, > 95, 3.1 and 12.1%), CMV IgG kit (100, 99.3, 3.4 and 3.6%), CMV IgM (93.75, 99.2, 5.6 and 7.8%), HSV IgM (90, 100, 4.7 and 9.8%), HSV-1 IgG kit (100, 99, 3.7 and 2.8%) and HSV-2 IgG kit (97, 100, 3.7 and 4.9%), respectively.

All the used kits in the present study were from the same batch and the validation criteria set by the manufacturers for each kit was checked and passed prior to the interpretation of the results. The threshold indices were calculated and the cut-off value was 0.64, 0.71, 0.4 and 0.6 for IgG antibodies against *C. trachomatis*, CMV, HSV-1 and HSV-2, respectively. For IgM antibodies, the cut-off values were 0.43, 0.51 and 0.55 for *C. trachomatis*, CMV and HSV, respectively. The samples were considered positive if the value of the sample was >cut-off + 10% cut-off value as recommended by the manufacturers.

Statistical analysis

Statistical analysis of the results was performed using SPSS version 16. Cross-tabulation followed by Chi square (X^2) test

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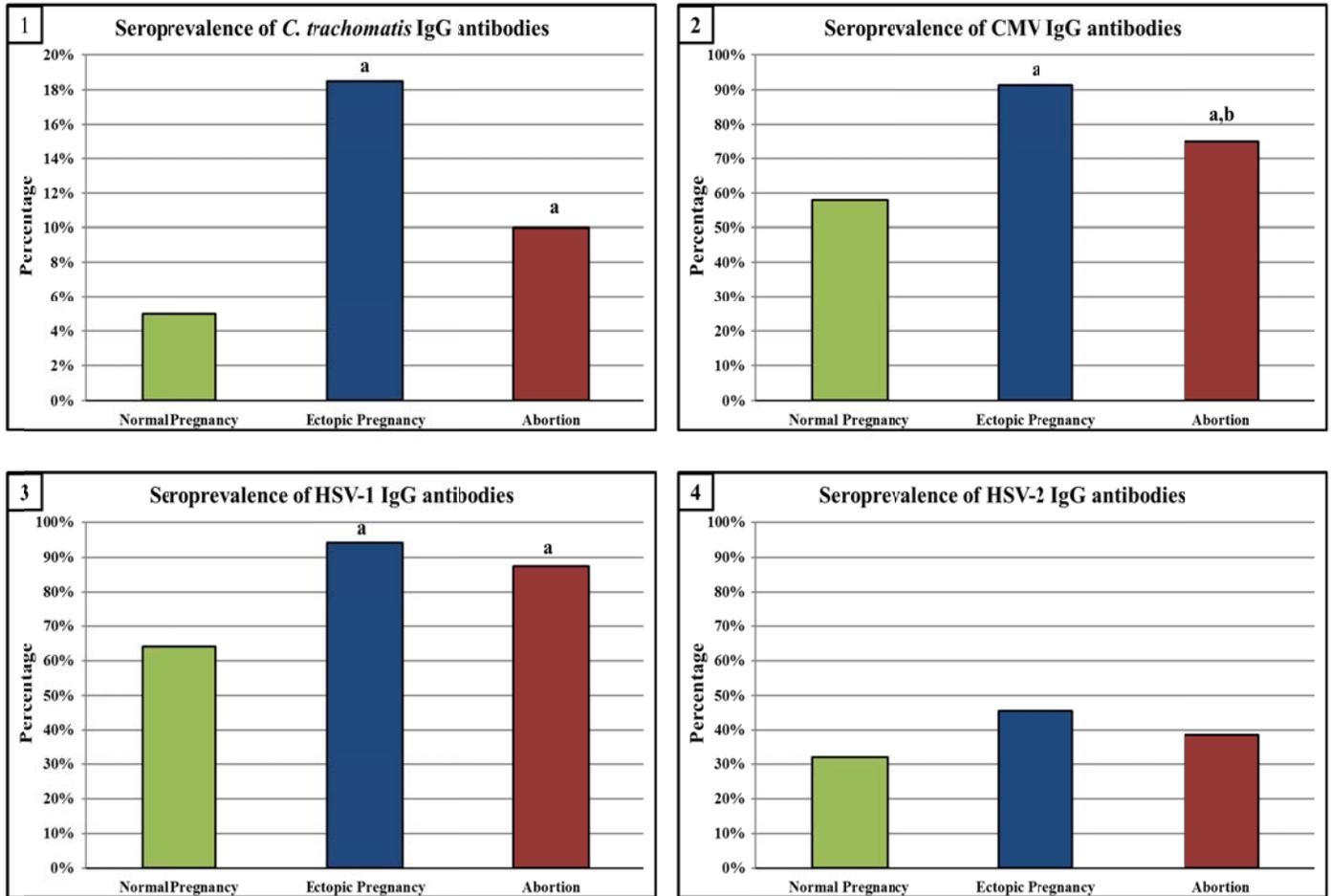
Figure 1. Seroprevalence of IgG antibodies against (1) *C. trachomatis*, (2) CMV, (3) HSV-1 and (4) HSV-2 in the different study groups (a = P < 0.05 compared to normal pregnancy; b = P < 0.05 compared to ectopic pregnancy).

was used for frequency analysis. P value < 0.05 was considered significant.

RESULTS

Seroprevalence of *C. trachomatis* IgM and IgG antibodies

IgM antibodies were detected in 1 case of normal pregnancy (1%), 2 cases of EP (2.8%) and 2 cases of abortion (2.5%) (P > 0.05). IgG antibodies were positive in 5 cases of normal pregnancy (5%), 13 cases (18.5%) of EP and 8 cases of abortion (10%), and the frequency was significantly higher in the 2 groups of abnormal pregnancy as compared to control group (P < 0.05). However, there was no significant difference between ectopic and abortion groups (Figure 1).

Seroprevalence of CMV IgM and IgG antibodies

IgM antibodies against CMV were detected in 18 cases of

(18%) of normal pregnancy and the seroprevalence was significantly lower as compared to EP (n = 51; 72.8%) and abortion (n = 41; 51.2%) groups (P = 0.0001 and 0.0003; respectively) (Table 1).

In the normal pregnancy group, 58% were positive for CMV IgG antibodies and it was significantly lower (P < 0.05) than EP (n = 64; 91.4%) and abortion (n = 60; 75%) groups (Figure 1). The seroprevalence was also significantly higher in EP as compared to the abortion group (P = 0.005).

The number of cases that were positive for both IgM and IgG antibodies in the normal pregnancy group was 14 (14%), 44 (62.8%) in EP and 26 (32.5%) in abortion groups. The frequency of detecting both types of antibodies was significantly lower in the normal pregnancy groups as compared to the other groups (P < 0.05).

Seroprevalence of HSV-1 and 2 IgM and IgG antibodies

The HSV IgM antibodies were detected in 20 females

Table 1. Seroprevalence of *C. trachomatis*, CMV, HSV-1 and HSV-2 in normal pregnancy, ectopic pregnancy and abortion groups (a = P < 0.05 as compared to normal pregnancy; b = P < 0.05 ascomparped to ectopic pregnancy).

Parameter	Normal pregnancy (n = 100)	Ectopic pregnancy (n = 70)	Spontaneous abortion (n = 80)
C. trachomatis IgM positive	1 (1%)	2 (2.8%)	2 (2.5%)
C. trachomatis IgG positive	5 (5%)	13 (18.5%)[a]	8 (10%)[a]
CMV IgM positive	18 (18%)	51 (72.8%)[a]	41 (51.2%)[a,b]
CMV IgG positive	58 (58%)	64 (91.4%)[a]	60 (75%)[a,b]
HSV IgM positive	20 (20%)	48 (56.1%)[a]	30 (37.5%)[a,b]
HSV-1 IgG positive	64 (64%)	66 (94.3%)[a]	70 (87.5%)[a]
HSV-2 IgG positive	32 (32%)	29 (45.7%)	31 (38.7%)

with normal pregnancy (20%) and the seroprevalence was significantly lower as compared to ectopic pregnancy (n = 48; 56.1%) and abortion (n = 30; 37.5%) groups (P < 0.05) (Table 1).

IgG antibodies against HSV-1 were detected in 64 cases of normal pregnancy (64%), 66 cases of EP (94.3%) and 70 cases with abortion (87.5%). The frequency of HSV-1 was significantly lower in the control as compared to the other groups (P < 0.01).

HSV-2 IgG antibodies were positive in 32 normal pregnancies (32%), 29 ectopic pregnancies (45.7%) and 31 abortions (38.7%). There was no significant difference in the prevalence of HSV-2 IgG antibodies between the different study groups (P > 0.05).

The detection of IgM antibodies against HSV with either HSV-1 or HSV-2 IgG antibodies was found respectively in 9 (9%) and 16 (16%) cases of normal pregnancy, 29 (41.4%) and 21 (30%) cases of EP, 23 (28.7%) and 27 (36.2%) cases of abortion. The frequency of detecting HSV IgM antibodies with either HSV-1 or HSV-2 IgG antibodies was significantly higher in ectopic and abortion groups as compared to the control (P < 0.05).

DISCUSSION

Although STIs are major risk factors of EP and spontaneous early pregnancy loss, studies on the frequency of *C. trachomatis*, CMV, HSV-1 and HSV-2 in relation to reproductive health in KSA are limited in number. The current study is a pilot study measuring the seroprevalence of IgM and IgG antibodies against these organisms in Saudi women with normal and abnormal early pregnancy.

Our results showed a significant increase in the frequency of IgG antibodies against the candidate organisms, except for HSV-2, in the abnormal pregnancy groups as compared to the control. Additionally, the seroprevalence of HSV and CMV IgM antibodies were significantly higher in the abnormal pregnancy as compared to normal pregnancy and the number of cases that were positive for both IgM and IgG against these viruses were significantly higher in the case groups as

compared to the control. The present study suggests that these organisms could represent a major risk factor for adverse pregnancy outcome during the first trimester in Saudi females. Moreover, the high prevalence of CMV, HSV-1 and HSV-2 IgG and IgM antibodies in the normal pregnancy group also suggests that these viruses could spread vertically to the neonates of infected mothers leading to congenital neurological disabilities.

Ectopic pregnancy has long been associated with *C. trachomatis* (den Hartog et al., 2005). Additionally, CMV and HSV 1 and 2 have recently been isolated from specimens collected from abnormal fallopian tubes (Medvedev et al., 2009; Qian et al., 2009). These organisms have also been detected in cases with spontaneous early pregnancy loss (el-Sayed Zaki and Goda, 2007; Al-Hindi et al., 2010). Our results correlate with the previous studies as high frequency rates of *C. trachomatis*, CMV and HSV-1 and 2 were detected in patients with EP and abortion. Hence, we plan to measure the effect(s) of these microorganisms on tubal ciliary beat frequency, and the expression of implantation and adhesion markers by the tubal/endometrial specimens collected from EP and abortion to identify the mechanism(s) by which they induce/promote EP and spontaneous abortion.

The significantly high number of positive cases observed in our study for both IgM and IgG against the designated viruses also suggests that the development of abnormal early pregnancy could be associated with re-exposure/reactivation of the viral infection. Our suggestion can be supported by several published reports that have shown an increase in the prevalence of viral reactivation during pregnancy and increase in the frequency of abnormal early pregnancy in patients with positive IgM and IgG antibodies (el-Sayed Zaki and Goda, 2007; Al-Hindi et al., 2010; Saraswathy et al., 2011).

Furthermore, the high frequency of IgM and IgG antibodies against the candidate viruses in the normal pregnancy group, which correlates with the previously published studies from Saudi Arabia (Ghazi et al., 2002; Obeid, 2007), merits further research especially since these viruses can be transmitted vertically to the foetus and could results in serious congenital neurological com-

plications. Large prospective screening studies should therefore be conducted to measure the prevalence of vertical transmission of these viral infections and the rates of subsequent congenital manifestations following birth.

In conclusion, IgM and IgG antibodies against *C. trachomatis*, CMV and HSV-1 and 2 are common in pregnant females in the western province of Saudi Arabia and their frequency was significantly high in abnormal early pregnancy. Additionally, the high frequency rate for CMV, HSV-1 and 2 detected in women with normal pregnancy alarms the possible risk of vertical trans-mission to the newborn and subsequently the risk of congenital infection and the potential development of neurological manifestations. Therefore, the implemen-tation of a national screening programme using TORCH test during the premarital screening and/or routine clinical follow-up of normal pregnancy by the health workers in the kingdom is needed for the early detection and/or prevention of the reproductive adverse outcomes associated with these organisms. Further studies are required to measure the true prevalence of these STIs and their associated pregnancy complications.

Conflict of Interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

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