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Cytomegalovirus Excretion in Pregnant and Nonpregnant Women

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Cervical and urinary excretion of cytomegalovirus by Taiwanese women was identified by the presence of a cytomegalovirus-specific immediate-early gene sequence amplified by the polymerase chain reaction. Excretion rates during the first trimester of pregnancy resembled rates for nonpregnant women. As pregnancy proceeded, the cervical excretion rate increased from 13 to 40% and the urinary excretion rate increased from 1 to 13%.

Cytomegalovirus (CMV) is a member of the herpesvirus family. Previous studies have shown that the incidences of isolation of CMV from the cervix and urine of pregnant women increase as pregnancy proceeds (10, 11). Since antibody to CMV is demonstrable in most of these women, viral excretion is assumed to represent reactivation of latent virus rather than primary infection. Stagno et al. presented evidence suggesting that CMV recurrence may be suppressed during the first trimester and then restored to its normal level later in gestation; during the third trimester, the excretion rate approached that in nonpregnant populations (11). Despite studies designed to explore the reasons for the variation in CMV excretion rates during pregnancy (4–7, 12), this variation has never been fully explained. It is also uncertain whether suppression of CMV excretion in early gestation occurs in pregnant women worldwide. Using the polymerase chain reaction (PCR), we studied CMV-seropositive women in Taiwan and examined the change in CMV recurrence rate during pregnancy.

Three groups of CMV-seropositive women (defined as anti-CMV immunoglobulin G positive and anti-CMV immunoglobulin M negative) were studied for CMV excretion. Group I included 207 pregnant women from two major prenatal care clinics in two hospitals in metropolitan Taipei, Taiwan, who were monitored throughout gestation. Urine specimens were collected at the time of the initial visit (mean ± standard deviation, 13 ± 1 weeks of gestation), the 24th (±1) week of pregnancy, and late in pregnancy (mean ± standard deviation, 32 ± 1 weeks). In addition, cervical swab specimens were collected longitudinally from 54 of these 207 women. Group II consisted of 2,012 seropositive pregnant women at various points in gestation from the same sources as group I; these women were serially enrolled during the study period, and a single cervical swab or urine specimen was collected from each of them. One hundred five seropositive female patients (group III) from infertility clinics in the same hospitals were recruited to serve as the reference group representing nonpregnant women. From each of these women, a single cervical swab was collected. To estimate CMV excretion from the cervix in nonpregnant women, those women diagnosed as having a cervix-related abnormality or endocrine disturbance and those having unexplained infertility were excluded from group III. Only women diagnosed as having endometriosis, endometrial adhesions, or tubal obstruction or whose partners had defective or inadequate sperm were eligible for the reference group. The study subjects were all between the ages of 20 and 35 years and representative of the population of middle-class women in urban areas of Taiwan. A comparison of sociodemographic characteristics among the three groups showed a high degree of similarity. In addition, there were no significant differences between the groups in oral contraceptive use, intrauterine device use, condom use by sex partners, and past or present genital tract or urinary tract infection.

Cervical and urinary CMV excretion were identified by the presence of a CMV-specific 240-bp gene sequence amplified by PCR with primers derived from CMV immediate-early region 2 (IE2) (sense primer, 5′-TCCTCCTGCAGTT CGGCTTC; antisense primer, 5′-TTTCATGATATTGCGC ACCT). This pair of primers has been evaluated for its specificity and sensitivity against CMV clinical isolates and other herpesviruses (3). For the PCR assay, the DNA template was obtained by conventional phenol-chloroform extraction and ethanol precipitation after 1 ml of cervical specimen or urine sample was treated with 0.5 ml of 50% (wt/vol) polyethylene glycol 6000 (with >0.15 M NaCl). The PCR amplification procedure was based on a previously described protocol (3). An internal sequence (TGCTGAGCT GCGGCCATCAGA) was used as a probe on Southern blots of the PCR products, and a nonradioactive DNA labeling and detection system (2) was used to detect immobilized target DNA on blots.

Analysis of the PCR amplification products obtained from specimens from study participants is shown in Fig. 1. In group I, there was no association between CMV recurrence in urine and recurrence at the cervix. The cervical excretion rates (percentages of women in whom cervical excretion of CMV was detected) for urinary shedders and nonshedders were 33.3 and 11.7%, respectively, during the first trimester ($P = 0.13$), 20.0 and 25.5% during the second trimester ($P = 0.71$), and 32.2 and 30.6% during the third trimester ($P = 0.73$). Our findings imply that, although both cervical and renal cells have been suggested to harbor latent CMV (1, 8), different mechanisms may be involved in virus recurrence at

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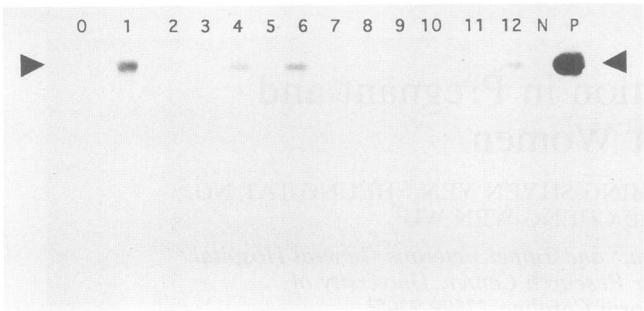

FIG. 1. Nonradioactive detection of CMV sequences from PCR with primers from the IE2 region hybridized with an internal probe. Arrowheads indicate the 240-bp sequence. Lanes: 0, no-DNA control; 1 to 12, women 1 to 12; N, negative control (uninfected human fibroblasts); P, positive control (CMV-infected fibroblasts). Women 1, 4, 6, and 12 were positive for CMV DNA.

different sites during pregnancy. On the other hand, there was no significant difference between women in group I and group II in excretion rates in any trimester at the cervix or in urine (Table 1). In addition, our analysis of excretion rates from both urine and the cervix among pregnant and nonpregnant women relative to age groupings (i.e., 20 to 25, 26 to 30, and >30 years) showed that CMV excretion did not correlate with age (data not shown).

Our data confirm other reports that pregnant women have a progressively increasing rate of CMV excretion throughout gestation. Prospective data from pregnant women in group I show that the rates of cervical and urinary CMV excretion increased with advancing gestation ($P < 0.01$ by the Mantel increasing-trend test). Cross-sectional data for pregnant women in group II demonstrate a similar phenomenon and comparable rates (Table 1). Previous studies in Western countries have estimated that the prevalence of CMV excretion in the cervix increases about threefold from 2.6% in the first trimester to 7.6% near term (9). The CMV excretion rate observed in this study was much higher than that in previous studies, but the ratio of the third-trimester rate to the first-trimester rate, which was 2.7 (35.2%/13.0%) for cervical excretion, seems to be comparable with ratios obtained in previous studies. The higher excretion rate might be attributable to the PCR being more sensitive than the conventional viral isolation techniques used in previous studies. However, theoretically, the presence of the CMV-specific immediate-early gene sequence amplified by PCR cannot distinguish between endogenous (reactivating) and exogenous (reinfecting) strains of virus or between active and latent infection. Given that restriction enzyme digestion analysis to distinguish the recurring strains of CMV is a time-consuming assay and is not practical for large-scale epidemiologic practice, we did not attempt it in this study. Amplification of specific CMV transcripts (RNA) that are expressed only during active infection could be used in further studies to distinguish active from latent infection.

TABLE 1. Frequency of CMV excretion from the cervix and in urine in different groups of seropositive women

Site and group	No. with CMV excretion/no. tested (%)[a]			
	Nonpregnant	Trimester		
		First	Second	Third
Cervix				
I		7/54 (13.0)	17/54 (31.5)	19/54 (35.2)[b]
II		33/217 (15.2)	56/140 (40.0)	30/81 (37.0)[b]
III[c]	8/105 (7.6)			
Urine				
I		3/207 (1.4)	20/207 (9.7)	27/207 (13.0)[b]
II		33/906 (3.6)	40/395 (10.1)	35/273 (12.8)[b]

[a] $P > 0.10$ for all tests of difference in either cervical or urinary excretion rates between group I and group II in any trimester.
[b] $P < 0.01$ (Mantel increasing-trend test).
[c] Urine specimens were not available for this group.

There was no evidence of suppression of CMV excretion in early gestation in this study. Our findings show that the excretion rate in the early stage of pregnancy was close to that for nonpregnant women (Table 1). This can be interpreted as an indication that pregnancy enhances productive CMV infection or reactivation of latent CMV. The transient depression of maternal humoral or cell-mediated immune responses to CMV during late pregnancy (4, 6) and hormonal changes during gestation (5, 7, 12) are possible explanations for the variation in CMV excretion.

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