RECOVERY OF CYTOMEGALOVIRUS FROM THE CERVIX IN PREGNANCY

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ABSTRACT. Virological studies were performed on urine and cervical swab specimens, and sera were tested for complement fixing antibody to cytomegalovirus to determine the frequency and nature of cytomegalovirus infection in 71 unselected pregnant Navajo women, 81 newborn Navajo babies, and 125 women who attended the prenatal clinic of the Magee-Womens Hospital, Pittsburgh. Cytomegalovirus was recovered from 11% of the women studied but from none of the babies. It was recovered more frequently from the cervix (8%) than from urine (3%), more frequently from the cervices of Navajo women (14%) than from those of Negro (5%) or Caucasian women (4%). The virus was recovered more often in the third trimester than in the second or first, and more often from younger and primiparous women than from those who were older than 25 years and those who had had more than three pregnancies. Cytomegalovirus was recovered with equal frequency from the cervices of those Navajo and Pittsburgh women who had serologic evidence of CMV infection. The outcome of pregnancy did not seem to be affected by this infection although the possibility of low birth weights should be explored further. We would speculate that cytomegalovirus infection of the cervix in pregnancy represents activation of latent infection by hormonally induced local changes, changes to which older, multiparous women are resistant.

Pediatrics, 49:524, 1972, CYTOMEGALOVIRUS, PREGNANCY, CERVIX, NAVAJO.

DESPITE the obvious possibility that infections of the female genital tract might play an important role in intra-uterine or neonatal cytomegalovirus (CMV) infections, only recently have there been attempts to study the matter. Prior to 1967 identification of CMV inclusions in the cells of endocervical glands was reported twice.[1,2] In 1967 Alexander[3] reported the recovery of CMV from the cervical secretions of 18 of 100 pregnant Chinese women, whereas CMV was recovered from none of the cervical specimens of 33 pregnant American women in the same area, Taiwan. None of the children born to these mothers had congenital cytomegalic inclusion disease (CID) and virus was not cultured from them during their first few days of life. Later Diosi[4] reported culturing CMV from one of the cervical specimens and seeing CMV inclusions in the Papanicolaou's (Pap) smear of one other of 54 Rumanian women in the postpartum or postabortive state. In 1968 Alexander and Wentworth[5] studied a population of pregnant women in Seattle and recovered CMV from the cervices of 5 (2%) of 258 women.

Navajo Indians represent a tribe of Athabascan descent whose ancestors probably came across the Bering Strait from eastern Asia,[6] and a study of cerumen of Navajo In-

Abbreviations

CF Ab: Complement fixing antibody
CID: Cytomegalic inclusion disease
CMV: Cytomegalovirus
CSS: Cervical swab specimens
EMEM: Eagle's minimal essential medium
FBS: Fetal bovine serum
HSV: Herpes simplex virus
U: Urine specimen

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dians revealed they had dry ear wax which is an Eastern Asian Mongoloid trait.[7] Therefore a prospective study was undertaken to determine the prevalence of CMV infection in a population of healthy pregnant Navajo Indians and compare it to that of a university prenatal clinic population in Pittsburgh, as well as to that reported by Alexander for Chinese women. Virological studies were performed on urine (U) and cervical swab specimens (CSS), and sera were tested for complement fixing antibody (CF Ab) to CMV and herpes simplex virus (HSV).

METHODS AND MATERIALS

A. Study Populations and Specimens

Serum, urine, and cervical swab specimens were obtained from 71 unselected Navajo women who came to the Ft. Defiance Public Health Service Indian Hospital for their first prenatal visit in September, October, or November, 1968. A medical history was obtained to exclude women who had neoplastic disease, received immunosuppressive therapy, and those who had received a blood transfusion during the preceding year, but none had to be excluded. CSS and Papanicolaou's smears were obtained at the same time by swabbing the external os and the lip of the cervix. The CSS was placed in 1.5 cc of maintenance media (25% sorbitol, 50% Eagle's minimal essential medium (EMEM), 10% fetal bovine serum (FBS), and antibiotics) and stored at 4°C. Each urine specimen, 1.5 cc, was mixed with 1.5 cc of the maintenance media after 5 to 10 hours storage at 4°C. Both U and CSS were then stored on dry ice at −60°C. A carbon dioxide adsorbant was used to protect the speciments from the possible effects of the CO_2. The blood specimens were centrifuged, and sera separated and stored at 4°C. All specimens were shipped to Pittsburgh either in mid-October or at the end of December. In Pittsburgh they were stored at −80°C until tested.

The same specimens and clinical history were obtained from 125 women who presented themselves to the prenatal clinic at Magee-Womens Hospital, Pittsburgh, for their first prenatal visit between January 13 and February 7, 1969. The specimens were obtained and handled in the same manner as previously described and stored on dry ice for the same duration of time as the Navajo specimens.

TABLE I

CMV Infection of Female Genital Tract Population Studied

Characteristic	*Population*		
	Navajo	*Negro*	*Caucasian*
Number studied	69	93	32
Age (years)*	25	23	21
Gestation (weeks)*	23	18	23
Number pregnancies*	3.4	3.1	2.5
Number live births*	2.2	1.7	1.2
Number abortions*	0.2	0.3	0.3

* Mean

A clinical history, cord blood, and urine specimens were obtained from 81 newborn infants delivered at Ft. Defiance during the period the Navajo women were studied. Most were not the babies born to the mothers studied; 13 were. The blood and urine specimens were handled in the same manner as the previous specimens.

B. Virologic Study

Recovery of CMV was performed as previously described.[8] Two-tenths cc of each urine and 0.4 cc of each cervical specimen were inoculated into each of 2 WI-38 cell culture roller tubes* maintained on 100% ENEM with 10% FBS. Examination was terminated after 40 days. All isolates were passed in WI-38 and then inoculated into rabbit kidney cell cultures to determine whether any isolates were herpes virus hominis.

A specimen of CMV, the titer of which when calculated by the Reed and Muench method was $10^{3.5}$ $TCID_{50}$/0.1 cc, was sent to Ft. Defiance and handled in the same manner as the Navajo specimens to determine

* Obtained from Microbiological Associates or Grand Island Biologicals Company, Bethesda, Maryland.

TABLE II

RECOVERY OF CMV FROM CERVIX AND URINE

Population	*Cervix*	*Urine*
Navajo	9/64* (14%)	3/68 (4%)
Negro	4/83 (5%)	2/76 (3%)
Caucasian	1/29 (4%)	1/28 (4%)

* Number positive/number tested

the effect of storage and shipping on the number of infectious CMV units. The specimen when retitrated on receipt in Pittsburgh, was found to contain $10^{3.5}$ $TCID_{50}$/0.1 cc indicating there was no significant loss of infectious particles during handling and storage.

C. Serologic Study

Complement fixation tests for CMV were performed as previously described,[8] utilizing Ad 169 CF antigen and control sera.† A serum was considered positive if the titer was 8 or greater.

Complement fixation tests for HSV were performed in the same manner using HSV CF antigen† and control serum.† The criterion for a positive test was the same as for CMV CF Ab.

D. Birth Records

Birth records at Ft. Defiance, other reservation hospitals, and Magee-Womens Hospital were searched for the outcome of pregnancy insofar as birth weight, head circumference, body length, and any neonatal complications were concerned.

RESULTS

A. Population Studied (Table I)

Navajo women were somewhat older, had had more previous pregnancies, and had borne more children than either the Negro or Caucasian women. Navajo and Caucasian women were farther into their pregnancies than were the Negro women. These differences were not statistically significant. However, if all Pittsburgh women were compared with Navajo women, then the differences were significant ($P > 0.001$). All groups had had comparable numbers of abortions.

B. Recovery of CMV and HSV (Table II)

Cervical swab specimens were obtained from 176 women and urine specimens from 172. CMV was recovered from 14 (8%) of the cervical swabs and from six (3%) of the urines. Both cervical and urine specimens were obtained from 157 women, and of these CMV was recovered from the cervix alone of 13 women, from the urine alone of four. CMV was recovered from both sites in only one woman. Thus, if both urine and cervical specimens were obtained, 11% of these women had CMV recovered from one or both sites.

CMV was recovered from 9 of the 64 Navajo women's cervices (14%) and from the urine in 3 of 68 (4%) (Table II). In Pittsburgh CMV was recovered from the cervical specimens of 4 of 83 (5%) Negro women and from 1 of 28 (4%) of the Caucasian women.

The difference in the recovery rate of CMV from the cervical specimens between the two geographic areas was statistically significant ($P > 0.05$). There was no significant difference between rates of recovery of virus from the urine. Although CMV was recovered from the cervices of the Navajo more frequently than from the Negro women and more frequently from the Negro than from the Caucasian women, the

TABLE III

RECOVERY OF CMV FROM CERVIX: AGE AND NUMBER OF PREGNANCIES

Number of Pregnancies		*Age*	
1–3	>3	≤25	>25
13/116* 11%	1/60 2%	13/123 11%	1/53 2%

* Number positive/number tested

† Obtained from Microbiological Associates, Bethesda, Maryland.

differences were not significant ($P = 0.05$). When only those women from whom cultures were obtained from both urine and cervix are included and analyzed, CMV was recovered from 18% of the Navajo women, 9% of Negro women, and 4% of Caucasian women.

CMV was recovered from the cervices of a higher percentage of younger women than older women in both Navajo and Pittsburgh women. Also, CMV was recovered from a higher percentage of the urines of the younger rather than the older Navajo women. The rate of CMV recovery in relationship to the age of the mother and the number of pregnancies she had had is noted in Table III. Eleven percent of the women who were pregnant for the first, second, or third time had CMV recovered from their cervical secretions while only 2% of those pregnant more than 3 times had positive cultures. This difference was not statistically significant ($P = < 0.10, > 0.05$). It may be noteworthy that 27% of the primigravida Navajo women had positive cultures. CMV was not recovered from any of the 43 CSS and 41 U obtained from women who had had more than four pregnancies. Of those women 25 years old or younger 11% were positive, whereas only 2% of those who were older were positive. None of 41 women over 26 years old had positive cultures.

Cervical CMV infection was found in 13% of those with CMV CF Ab, but in only 1% of those without Ab; CMV was recovered from the urine of 5% of those with CMV CF Ab and from 3% of those without Ab. CMV was cultured from 19 mothers; from 18 a serum specimen was also obtained and 15 of these 18 contained CMV CF Ab. There were three women from Pittsburgh who had positive CMV cultures and no detectable CF Ab to CMV. The urine cultures of two of these were positive and in the other the CSS was positive for CMV.

Since it had been suggested that cervical infection with CMV might be a reactivation of a latent infection,[9] it was interesting to compare the rate of CMV recovery from the cervix in the Navajo and Pittsburgh populations based on those women who had CF Ab to CMV. Fourteen and a half percent of the Navajo and 13.8% of the Pittsburgh women with CMV CF Ab had CMV infection of the cervix.

TABLE IV

RECOVERY OF CMV FROM CERVIX RELATED TO TRIMESTER OF PREGNANCY

Trimester	*Navajo*	*Pittsburgh*	*Total (%)*
1st	1/14*	0/29	2
2nd	3/23	3/60	7
3rd	4/26	2/23	12

* Number positive/number tested

CMV was recovered from the CSS more often late than early in pregnancy (Table IV). Seven, 13, and 15% of swabs from the 1st, 2nd, and 3rd trimesters respectively were positive in the Navajo women studied. Of those from Pittsburgh 0, 5, and 9% were positive for CMV from the 1st, 2nd, and 3rd trimesters respectively. The cumulative total percentages positive for cervical CMV were 2, 7, and 12% respectively in the three trimesters.

Twenty (38%) of Navajo women and 33 (30%) of Pittsburgh women had Trichomonas on their Pap smear. However, this difference was not statistically significant ($P > 0.10$). Thirteen percent of the women with Trichomonas on Pap smear had cervical CMV infection while from only 5% of those without Trichomonas was CMV recovered.

None of the urine specimens obtained from 66 newborn infants at Ft. Defiance was positive for CMV. Thirteen were born to the Navajo mothers who were studied; from only 1 of these 13 mothers was virus recovered from the cervix. This infant was a male whose birth weight was 3,045 grams, he was 20 in long, and had a head circumference of 13½ in. His 5-minute Apgar was 7. He had no jaundice, hepatosplenomegaly, petechiae, or respiratory distress. His growth and development for the first 2

years of life were normal. No follow-up cultures were obtained.

Herpes simplex virus was not recovered from any of the specimens.

C. CMV and HSV CF Ab Tests

Serum specimens were obtained from a total of 184 women, and of these 102 (58%) were considered positive for CMV CF Ab and 117 (64%) positive for HSV CF Ab.

There was CF Ab to CMV in 58 of 70 (83%) Navajo women and 44 of 114 (39%) Pittsburgh women. This is a significant difference ($P < .0005$). CF Ab to HSV was positive in 58 of 70 (83%) Navajos, and in 59 of 114 (52%) Pittsburgh women, which is also a significant difference ($P < 0.025$). Eighty-three percent of the Navajo, 41% of the Negro, and 31% of the Caucasian women had significant CF Ab to CMV, while 83% of the Navajo, 59% of the Negro, and 31% of the Caucasian women had CF Ab to HSV.

The geometric mean titer of CMV CF Ab was greater in those with CMV cervical infection than in those with urinary CMV who, in turn, had a higher titer than those from whom CMV was not recovered. There was no significant increase in prevalence of CMV CF Ab with increasing age. However, more Pittsburgh women over 25 years of age had HSV CF Ab than did those who were younger.

Finding Trichomonas on Pap smear was not associated with the presence of CF Ab to HSV or CMV. No significant differences in titers were observed although CMV titers were higher than HSV titers.

Seventy-six percent of cord blood specimens were positive for CMV and 81% were positive for HSV CF Ab. In those women from whom we had a serum and a cord blood specimen there was good correlation between the titers of CF Ab to CMV.

D. Birth Records

The duration of pregnancy and the birth weight of most of the babies born to mothers who were infected were less than the average, but the data were not sufficient for statistical analysis. Otherwise these babies were not abnormal insofar as was known; but the follow-up was not adequate.

DISCUSSION

CF antibody to CMV was found in 83% of Navajo and 39% of Pittsburgh women in this study. It is now well recognized that the prevalence of CMV infections, usually as determined by CMV CF Ab studies, varies within one country,[10-12] between countries,[13-16] between socioeconomic living conditions,[12,17-19] and possibly between racial groups.[13,17] Ft. Defiance Indian Hospital gives care to approximately 15,000 Navajo Indians living in the adjacent 1,800 square mile area. Living conditions range from the poverty of those who live in traditional hogans or crude shacks arranged in small family clusters of two to three dwellings, to that of those who live in houses in small towns that approximate lower middle class conditions in other parts of the United States. One might assume that the low population density of the Navajo region would be associated with a low CMV prevalence. However, elementary education is provided in government boarding schools where 600 to 800 students are housed together in crowded living quarters. Dr. Hanshaw[20] and Dr. Stern[15] have found that both boarding school and institutionalization markedly increase the prevalence of CMV infection. We feel but we have not documented that this may account for the high prevalence of CF antibody to CMV in the Navajos. We do not have data which would enable us to determine whether infection rates are associated with specific socioeconomic factors in either the Navajo or Pittsburgh populations.

CMV was cultured significantly more often from the cervices of pregnant Navajo than from pregnant Pittsburgh women in this study. CMV was recovered from the cervices of Navajo women as frequently (14%) as earlier it had been recovered from other oriental populations studied by Numazaki (Japan, 15%)[9] and by Alexan-

der (Taiwan, 18%),[5] but higher than that reported by Alexander, *et al.*[5] and Foy, *et al.*[21] for women in the Seattle area and by Hildebrandt, *et al.*[22] The rate of recovery of CMV from women in Pittsburgh was the same as that which Alexander, *et al.*, Foy, *et al.*, and Hildebrandt, *et al.* found. Whether these differences are due to a racial factor (e.g., a genetically determined tropism), seasonal variation,[23] chance, or other factors cannot be determined from these data. Our inclination is to assume that the high frequency of cervical CMV infections is due more to socioeconomic than to other factors, and that these were the basis for the overall higher rate of CMV infection as revealed by the higher frequency of CMV CF Ab in the Navajos.

If recovery of CMV from the cervix is the result of reactivation of a latent infection, then one way to compare the two populations herein studied is to calculate the recovery rate as determined by dividing the number with positive cervical cultures for CMV by the number with CF Ab to CMV. It is interesting to note that when this measure is applied to the Pittsburgh and Navajo populations the rates are the same, not different (13.8% and 14.5%).

CMV was cultured significantly more often from the cervices of women with less than four pregnancies, and there was an especially high prevalence among Navajo primigravidas (27%). The rate of recovery of CMV from the cervix seemed to increase as gestation progressed (Table IV). This observation is in agreement with Numazaki,[9] who found 0%, 10%, and 28% to be infected during the 1st, 2nd, and 3rd trimester respectively. We found that CMV was recovered more frequently from the cervices of mothers younger than 26 and that none of the mothers with positive cultures was older than 26. CMV infection was not detected when age was greater than 26 or there were more than four pregnancies. Further study would be necessary to explain these findings. Other studies on cervical CMV have not compared recovery rates with age. A factor that would reactivate CMV from a period of latency in first pregnancies but not in subsequent pregnancies would explain these findings. The possible effect of variation in sexual activity must be considered in future studies. Differences in steroid secretion between pregnancies would not seem to be sufficient to account for the difference, but physical changes in the cervix might predispose to reactivation of CMV infection induced in part by steroids despite circulating antibody to CMV. During and following subsequent pregnancies, possibly with repeated local exposure to cervical CMV, local antibody might be produced which would prevent future reactivation.

Among both the Pittsburgh and the Navajo women, CMV was cultured more frequently from the cervix than from the urine. The frequency of recovery from the urine noted here is the same as that which others have reported.[9,21] Urinary CMV infection may be a manifestation of primary infection and therefore it may more frequently be associated with congenital infection of the fetus, than would cervical infection. Indeed, maternal urinary tract infection is frequently found in congenital cytomegalic inclusion disease.[8] Neither cytomegalic inclusion disease nor CMV infection of the newborn has been detected frequently in those instances in which the mothers were shown to have a cervical CMV infection. Alexander,[3] Numazaki, *et al.*[9] and Levinsohn, *et al.*[24] found a high incidence of infection (recovery of CMV from the urine) in infants during the first year of life, but neither Alexander nor Numazaki found any infected newborns; in Levinsohn's study one of 92 infants had a positive urine, but whether this baby was born to a mother with CMV urinary or cervical infection (or none) was not known. The higher prevalence of cervical infection with CMV may be important to secondary spread among adults and especially to infants post birth. Numazaki has proposed that this be called vertical transmission.[9]

The effect of cervical CMV infection on the outcome of pregnancy is not known.

Foy, *et al.*[21] found that several infants born to mothers with cervical CMV infection had shorter gestations, lower Apgar scores, and lower birth weights than the averages for a comparative group; their data were not sufficient to establish that these apparent differences were statistically significant. Most of the babies born to CMV infected mothers in our series also had shorter gestations and lower birth weights, but our data are incomplete and not statistically significant. The matter certainly deserves serious study.

The failure to recover HSV from the cervix in pregnancy from three relatively small groups of patients is not surprising. Nahmias, *et al.*[25] detected genital herpes cytologically in 140 of 13,766 (1.02%) pregnant women in a lower socioeconomic group. In an earlier study Nahmias and co-workers[26] recovered no HSV from 175 pregnant women they studied.

Finally, the declaration of a caveat concerning our use of the CMV CF Ab test is in order. Weller[27] has recently discussed the immunologic procedures, responses and antigenic variation of CMV and CMV infections and emphasized there is danger in deriving unwarranted conclusions because of our inadequate knowledge of CMV immunology. His caution can be wisely applied to these studies; more work must be done before we can understand our observations.

SPECULATION

The studies of these matters might be extended in several ways. Prospective longitudinal studies of both the mothers through their pregnancies and the newborns through their first few years of life might reveal factors which would contribute to understanding these observations. Nonpregnant women should be studied. Studies might be done to determine whether strains of CMV recovered from the cervix are different from strains recovered from saliva or urine; is CMV infection analogous to Herpes virus infection with Types I and II? The possibility of venereal transmission should be explored. We need to determine if cervical CMV infection is a primary infection or a reactivation. Whether local antibody is produced and what its effect is should be studied. The possibility of genetically determined tropism might also be explored. The influence of specific socioeconomic factors in CMV infections and whether and how these infections affect the outcome of pregnancy remain unknown and they are the most important questions to be answered. Thus these observations raise many interesting questions the answers to which, at the present, are speculative. And we would speculate that CMV infection of the cervix in pregnancy represents activation of a latent infection triggered by local changes induced by hormones, changes to which older multiparous women are resistant.

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