Perinatal HIV Infection and the Effect of Zidovudine Therapy on Transmission in Rural and Urban Counties

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**Objectives.**—To assess health care providers' identification of human immunodeficiency virus (HIV)-exposed infants, to ascertain the prevalence of transplacental or oral zidovudine treatment among infants exposed to HIV, and to estimate the impact of zidovudine use on perinatal transmission in rural and urban North Carolina.

**Design.**—Survey of North Carolina newborns tested for HIV infection in 1993 and 1994 compared with the number of anonymous HIV-positive childbearing women.

**Setting.**—North Carolina hospitals, public health clinics, and private physicians' offices.

**Main Outcome Measures.**—Rates of identification of HIV-exposed infants and of perinatal HIV-1 transmission, determined by HIV culture and polymerase chain reaction testing in the infants.

**Results.**—The proportion of HIV-exposed children in North Carolina who were identified and tested increased from 60% in 1993 to 82% for all of 1994, and to more than 90% for the last quarter of 1994. The HIV-exposed infants born in rural counties were less likely to be recognized than those born in urban counties (P<.001). In 1994, most infants were evaluated relatively early in life: 39% by 1 week of age, 63% by 6 weeks, and 76% by 3 months. Among infants with recognized HIV exposure, transmission decreased significantly between 1993 and 1994, from 21% to 8.5%, respectively (P=.009). After the announcement of the results of AIDS Clinical Trials Group Protocol 076, zidovudine was given to 75% of HIV-positive women who delivered infants in North Carolina. Only 5.7% of infants who received any zidovudine became infected, compared with 18.9% of infants who received no zidovudine (P=.007).

**Conclusions.**—Health care providers in North Carolina are identifying most of the state's HIV-seropositive pregnant women, treating them with zidovudine, and testing their infants soon after birth for HIV infection. The use of zidovudine in pregnant women and their infants has reduced perinatal HIV transmission in the state. (JAMA. 1996;275:1488-1489)

HUMAN immunodeficiency virus (HIV) infection has emerged as a major public health problem among women in the Southeastern United States. Heterosexual contact is the most rapidly increasing transmission category for women, and the South has recently reported a higher rate of heterosexually acquired cases of acquired immunodeficiency syndrome (AIDS) than any other region of the country. As the number of HIV-infected women grows, so does the number of perinatally exposed and infected infants. Almost all (92%) pediatric HIV infection in the United States results from mother-to-infant transmission. About 5800 infants were born to HIV-seropositive mothers each year from 1988 to 1990, with the number increasing to an estimated 6500 to 7000 infants in 1993. Efforts to prevent HIV transmission by seropositive women were advanced in 1994 when AIDS Clinical Trials Group (ACTG) Protocol 076 demonstrated that zidovudine administration to seropositive pregnant women with CD4 cell counts greater than 0.20×10^9/L and to their infants significantly reduced the risk of HIV infection among the infants from 25.5% to 8.3%. The efficacy of prophylactic therapy has increased the importance of identifying HIV-seropositive mothers. On April 25, 1994, North Carolina's state health director sent a memorandum to all physicians in the state who provide prenatal care advising them of the results of ACTG 076 and urging them to begin HIV counseling and to encourage testing of all pregnant patients. In addition, he recommended that physicians consult with the faculty of the 3 tertiary care facilities in the state that participated in ACTG 076 for information regarding the treatment of HIV-infected pregnant women while national recommendations were being devised. National guidelines were issued in April 1994 and revised in July 1995.

Although identification and treatment of HIV-infected pregnant women is important, it is also important to identify infected infants as early as possible to institute appropriate antiretroviral or prophylactic therapy. The most sensitive methods to detect HIV infection early in life are HIV culture and polymerase chain reaction (PCR) testing, which are performed only in specialized laboratories. The Retrovirology Laboratory at the University of North Carolina at Chapel Hill is the only laboratory in the state that performs both HIV culture and PCR testing for the diagnosis of infection in infants, and from September 1, 1998, until the present, physicians from across the state have submitted diagnostic specimens. Our function as the central reference laboratory has allowed us to (1) assess the extent of identification of HIV-
exposed infants by health care providers, (2) ascertain the proportion of HIV-exposed infants who receive zidovudine treatment either transplacentally or orally, and (3) estimate the effect of zidovudine use on perinatal infection in North Carolina.

SUBJECTS AND METHODS

HIV Seroprevalence in Childbearing Women

North Carolina is the 10th largest state in the United States in population, with 6.6 million people equally distributed between rural and urban areas. North Carolina monitored HIV seroprevalence in childbearing women from April 1, 1989, through May 15, 1995, as part of the national HIV Survey in Childbearing Women (SCBW). During this period, virtually all specimens from newborn infants were tested, reflecting the prevalence of HIV in pregnant women.

Residual dried-blood filter paper spots obtained from newborn infants had identifying information removed after metabolic screening and were then eluted and tested for HIV antibody (Genetic Systems, Seattle, Wash) by the North Carolina State Diagnostic Laboratory. In the case of twins, only 1 infant was tested. Repeatedly reactive specimens were tested by Western blot (Epitope, Organon Teknika, Durham, NC). If at least 2 of 3 specific bands (p24, gp41, or gp120/160) were observed by Western blot, the specimen was considered HIV-antibody positive.

Unlinked data were collected for selected demographic and geographic variables. Each county was assigned to 1 of 4 regions: western, south central, north central, and eastern. The 10 most populous counties, the populations of which range between 100,000 and 520,000, were designated urban; all others were considered rural (Figure 1). Only 1 city, Charlotte (in Mecklenburg County), has a population larger than 400,000.

Identification and Testing of HIV-Exposed Infants

Since 1993, an accurate tally of all infants tested at the University of North Carolina at Chapel Hill's Retrovirology Laboratory has allowed us to determine how many of the HIV-exposed infants were being seen by health care providers who recognized that the infants were at risk for HIV infection and used the diagnostic services provided by the laboratory. Physicians across the state sent 2 to 3 mL of citrated or heparinized blood to the Retrovirology Laboratory to establish a diagnosis of HIV infection. The laboratory performed HIV culture and HIV DNA PCR testing on all specimens. These assays were performed to determine infection status and to screen patients for entry into ACTG protocols. There was no cost to the patients. For this study, an infant was considered infected if 2 separate peripheral-blood specimens were positive for HIV by either culture or PCR testing. If no test result was positive, an infant was considered negative (1) if at least 2 separate blood specimens were negative by (PCR or culture or both) and at least 1 of the time points was at 4 months of age or later, or (2) if the infant had seroverted to HIV-antibody negative. Infants whose results were neither positive nor negative on the basis of the foregoing were considered to have indeterminate results.

Physicians retrospectively provided information from the patients' medical charts that included county of birth, evidence of seroreversion, and receipt of zidovudine therapy by the pregnant woman or her infant. The infant was considered treated with zidovudine if zidovudine therapy was recorded in the mother's (during gestation) or the infant's medical chart, or in both. The Committee on the Protection of the Rights of Human Subjects, the institutional review board of the University of North Carolina at Chapel Hill, granted an exemption from review for this retrospective abstraction of patients' medical records.

Diagnostic Assays

HIV Culture.—The ACTG consensus protocol was followed. The Retrovirology Laboratory was certified for HIV culture by the ACTG's Virus Quality Assurance Program during this study. HIV DNA PCR Testing.—The Roche Molecular Systems assay (HIV AmpliCior, Branchburg, NJ) was used according to the manufacturer's instructions and including ACTG standards and controls. The Retrovirology Laboratory was certified by the ACTG's Quality Assurance Program for DNA PCR testing.

Statistics

Seroprevalence rates among childbearing women were calculated as the number of HIV-antibody-positive findings per 1000 newborn specimens. The number of infants tested for HIV infection as a proportion of HIV-exposed infants born was estimated by dividing the number of infants tested in the laboratory by the number of infants born to HIV-seropositive women according to the SCBW during the same calendar quarter in the same region of the state. Because the SCBW was anonymous, we had to assume that the infants tested for HIV infection at the Retrovirology Laboratory were the same ones tested for HIV antibody in the SCBW. Information on county of residence for the 10 urban counties was sometimes available to confirm that this was the case, but we cannot exclude that in some cases the infants lived in a different region than where they were born. It is also possible that some of the infants born to HIV-positive mothers in North Carolina may have died or moved from the region.

Data from medical records and laboratory results were entered into a worksheet (Microsoft Excel 5.0) and converted to a SAS database. Differences in proportions were evaluated with an uncorrected Pearson χ² statistic, the Fisher exact test (for sparse tables), or Wilcoxon test (for 2×n tables with an ordered variable, such as calendar quarter). Computations were calculated using SAS version 6.04.

RESULTS

Anonymous HIV Serosurvey of Newborns in North Carolina, 1990 to 1994

Between 1990 and 1994, at least 881 infants (including at least 4 sets of twins) were born to 877 HIV-seropositive women in North Carolina. The yearly total number of births in the state was relatively constant (range, 95,044-102,389; annual average, 98,589). The HIV seroprevalence rate in childbearing women rose from 1990 to 1992 and then decreased to the 1990 level. During 1992, HIV seroprevalence peaked at 2.229 per 1000 births, with 224 births to seropositive women, and decreased to 1.669 per 1000 births (163 births with 3 sets of twins, for a total of 166 infants) and 1.589 per 1000 births (151 births with 1 set of twins, for a total of 152 infants), respectively, in 1993 and 1994 (Figure 2).

In contrast to many states in which most HIV-seropositive women live in urban areas, 56% of the HIV-infected mothers in North Carolina lived in rural counties (180,000 inhabitants) or small metropolitan counties (<200,000 people). In the western region of North Carolina, HIV-seropositive women were clustered in urban areas, especially Mecklenburg County. During the past 5 years, 18% of (169/982) of the state's and 60.8% (169/278) of the western region's HIV-exposed infants were born in Mecklenburg County. In contrast, in the eastern coastal plains of North Carolina, the largest town has a population of less than 70,000. Thus, all of the 201 HIV-exposed infants born in this region during the past 5 years (201 [22.6%] of 882 of the state's exposed infants) were from rural areas. In the central Piedmont region of North Carolina, HIV-exposed infants...
were almost equally divided between urban centers and rural areas.

A disproportionate number of HIV-seropositive childbearing women in the state were African Americans (Figure 2). Although births to black women constituted only 29% of the births in the state, they accounted for 85% of HIV-exposed infants in North Carolina (Table 1). Seroprevalence rates for African-American childbearing women averaged 5,096 per 1000 births during the past 5 years compared with 0.370 per 1000 births for white women during the same years. In Mecklenburg (population, 511,483) and Durham (population, 181,835) counties, the seroprevalence rate among African-American childbearing women was as high as 10 to 23 per 1000 births during some quarters of 1994.

Identification of HIV-Exposed Infants

In 1993, 166 HIV-exposed infants were born in the state, and the laboratory received at least 1 specimen from 99 of them (59.6%). These numbers include 3 sets of twins who were counted only once in the SCBW dataset. Twenty of these were diagnosed as being infected with HIV, 75 were not infected, and in 4 the results were indeterminate. In 1994, only 152 HIV-exposed infants were born, and the laboratory tested 124 of them (81.6%) (including 1 set of twins). Of these, only 10 were infected, 107 were not infected, and in 7 results were considered indeterminate because they had only 1 specimen tested, which was negative by all assays.

Overall, identification of HIV-exposed infants across the state improved from 63% in the last quarter of 1993 to 91% in the last quarter of 1994 (Table 2). During the last quarter of 1994, the results of the SCBW found a total of 31 HIV-seropositive mothers across the state, and the laboratory tested 30 of them (97%). However, data inconsistencies were noted in 1 geographic area. The SCBW showed only 9 HIV-seropositive infants from the south central region, but clinicians referred specimens from 11 HIV-exposed infants who were reportedly from that region. Assuming that the SCBW missed those 2 infants for some reason, the identification of HIV-exposed infants in the last quarter of 1994 was 91% (30/33). Importantly, 39% of the infants born and tested in 1994 were identified and a specimen was sent during the first week of life, 68% by the first 6 weeks of life, and 76% by age 3 months. During the last quarter of 1994, those figures increased to 45% by age 1 week, 73% by age 6 weeks, and 91% by age 3 months (assuming there were 33 births and the laboratory received specimens from 30 of them) (data not shown).

Rural vs Urban Counties

During 1993 and 1994, 41% of the infants who were identified were born in rural counties (Table 3). Although many of these infants were eventually referred to 1 of the state’s 5 tertiary care units (Figure 1), in 1994 at least 21% of these infants were first seen by local primary care physicians who sent blood samples to the laboratory for diagnosis (data not shown). In 1994, in particular, HIV-exposed infants from rural counties were more likely to be identified and tested for HIV infection than were urban infants. Fifty-four (96%) of 56 infants born to seropositive rural mothers were tested, compared with 70 (73%) of 96 HIV-exposed urban infants (P<.001). There was no clear relationship between rural vs urban county of birth and the date of first testing.
HIv Transmission and the Effect of Zidovudine

In addition to the decrease in the number of HIV-seropositive women giving birth in North Carolina in 1994 (Figure 2), there was a significant reduction in the transmission rate of HIV between 1993 and 1994. In 1993, 20 (21%) of 95 infants were infected, compared with 10 (8.5%) of 117 patients in 1994 (P=.009).

We assessed whether the use of zidovudine during pregnancy, during the first weeks of life, or both was associated with this decrease. Only 5 infants (5.7%) of 87 who received any zidovudine therapy either transplacentally or orally became infected. By comparison, 20 (18.9%) of 106 infants who never received zidovudine therapy by any route became infected (P=.007). Information regarding the use of zidovudine was missing for 26 infants. The new guidelines for offering counseling and testing and for treating HIV-infected pregnant women with zidovudine seemed to have been adopted by health care providers of HIV-infected mothers in North Carolina by May 1994. Of the 73 infants born between May 1, 1994, and December 31, 1994, whose specimens were tested in the laboratory and for whom information was available, the mothers of 55 (75%) were treated with zidovudine during pregnancy, during labor and delivery, or both. Six more infants received zidovudine therapy only postpartum. Only 14 infants born after April 30, 1994, were known not to have received any zidovudine therapy by any route, and information was lacking for 7 patients. Infants were as likely to be treated with zidovudine in rural as in urban counties after May 1994 (Table 3). No difference was noted between rural and urban counties in the proportion of infants who acquired HIV infection in the absence of zidovudine therapy. Similarly, urban counties did not differ from rural counties in the proportion of zidovudine-treated infants in whom HIV infection developed.

COMMENT

This is, to our knowledge, the first published study of the recognition of perinatal HIV infection in the Southeastern United States. We found that most infants born to HIV-seropositive mothers in North Carolina during 1993 and 1994 were recognized as HIV exposed and underwent diagnostic testing for HIV infection during early infancy. The proportion of infants identified and tested rose from 60% in 1993 to greater than 80% in 1994. Among infants born after the publication of new guidelines for zidovudine administration to pregnant women whose zidovudine exposure was known, 75% received this drug transplacentally. An additional 8% received zidovudine therapy postpartum.

Zidovudine administration to seropositive pregnant women and their infants was associated with significantly decreased vertical transmission of HIV. Only 5 (7.7%) of 78 infants who received any zidovudine therapy became infected, compared with 20 (18.9%) of 106 who received no zidovudine therapy.

In a study of HIV infection in rural North Carolina, Rumley et al found that rural patients were more likely to be female, heterosexual, nonwhite, and younger than HIV-infected individuals from urban areas. In that study conducted in 1990, 26% of the HIV-infected patients were women, and many of these were unmarried and unemployed. To a large extent these women did not consider themselves at risk for HIV infection.17

Socioeconomic factors and additional barriers to health care in rural America have been extensively documented (including limited numbers of health care providers, the distance to the health care facilities, and confidentiality).17 We were, therefore, surprised to discover that in all of 1994 more than 80% of HIV-exposed infants born in the state had been seen by a health care provider, were identified as being at risk for HIV infection, and were tested within 1 year of birth. In the last quarter of 1994, 91% to 97% of all infants at risk were identified and tested for HIV infection. Rural infants, surprisingly, were more likely to be identified than those from urban counties (96% vs 73%, respectively, in 1994).

The study's methods entailed 3 limitations. First, some infants escape testing (eg, 1 of a set of twins, those with insufficient residual serum after performance of required metabolic screening, and possibly those born at home). The failure to identify any HIV-seropositive infant in the population would decrease this denominator and, thus, falsely increase the proportion of newborns recognized as HIV exposed. We included known twins in the denominator when necessary on the basis of samples received by the laboratory. The number of infants who eluded testing has not changed significantly between 1993 and
Table 3—Effect of Rural or Urban Birth on Rates of Testing for Perinatal Human Immunodeficiency Virus (HIV) Infection and Transplacental or Oral Treatment With Zidovudine

<table>
<thead>
<tr>
<th>Year/Quarter</th>
<th>No. of HIV-Exposed Infants Born in North Carolina</th>
<th>Tested for HIV</th>
<th>Treated With Zidovudine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rural, No. (% of All Rural)</td>
<td>Urban, No. (% of All Urban)</td>
<td>Rural, No. (% of All Rural Tested)</td>
</tr>
<tr>
<td>1993/1st</td>
<td>20 (7)</td>
<td>17 (25)</td>
<td>7 (41)</td>
</tr>
<tr>
<td>1993/2nd</td>
<td>19 (24)</td>
<td>8 (11)</td>
<td>7 (71)</td>
</tr>
<tr>
<td>1993/3rd</td>
<td>16 (24)</td>
<td>14 (88)</td>
<td>16 (67)</td>
</tr>
<tr>
<td>1993/4th</td>
<td>17 (29)</td>
<td>7 (41)</td>
<td>22 (76)</td>
</tr>
<tr>
<td>1994/1st</td>
<td>18 (25)</td>
<td>15 (83)</td>
<td>15 (60)</td>
</tr>
<tr>
<td>1994/2nd</td>
<td>14 (29)</td>
<td>14 (100)</td>
<td>21 (72)</td>
</tr>
<tr>
<td>1994/3rd</td>
<td>14 (21)</td>
<td>14 (100)</td>
<td>15 (71)</td>
</tr>
<tr>
<td>1994/4th</td>
<td>10 (21)</td>
<td>11 (110)</td>
<td>19 (90)</td>
</tr>
</tbody>
</table>

1994 (D. Williams, PhD, oral communication, August 23, 1995), and, thus, the observed increase in recognition of perinatal infection over time should be valid. Second, the "blinded" nature of the population-based seroprevalence survey prevents direct linking of these anonymously tested infants with those who underwent diagnostic testing through the University of North Carolina at Chapel Hill's Retrovirology Laboratory. Some HIV-exposed newborns may have been diagnosed without our knowledge by testing out of state. Out-of-state testing would result in underestimation of the proportion of HIV-exposed infants who were diagnosed. These situations are likely to be infrequent, and the HIV screening of specimens from metabolic screening tests that are performed on virtually every newborn in the state has provided the only available population-based estimate of HIV exposure among newborns. The third limitation derives from the retrospective data collection process, so that biological variables (eg, CD4+ and CD8+ cell counts, p24 antigenemia, and HIV RNA determinations) were unavailable and zidovudine treatment information was missing for about 10% of the subjects.

Other studies have used data from the SCBW to examine the extent of recognition of perinatal infection. Hsu et al30 found that 35% of infants born to HIV-seropositive mothers between November 1987 and October 1988 in Massachusetts were clinically recognized as HIV exposed. Surveillance by Maledonato et al31 showed that 34% to 50% of infants born to HIV-infected mothers in the greater San Francisco, Calif, region between 1988 and 1991 were in clinical care. Coplan et al32 found that only 44% of infants born in the Syracuse, NY, area between 1987 and 1993 were identified as being at risk. The higher HIV recognition rates we observed may have resulted at least partly from the fact that our investigation was conducted later in the era of AIDS, when awareness had increased concerning the prevalence of HIV infection and the importance of diagnosis. It may also be related to the statewide collaborative efforts of the tertiary medical centers. Grant-supported funding has created the North Carolina Children's AIDS Network and the central reference laboratory for perinatal HIV testing.

These earlier studies also suggested that HIV infection was more likely to be identified in infants born in urban areas than those from rural or suburban regions.33-35 In the study by Hsu et al,30 HIV-exposed children born in inner-city hospitals were more likely to be identified and were evaluated at an earlier age than those born in other areas. Maledonato et al31 found that the mean age at first clinical evaluation of HIV infection was 3.5 months for infants born in counties in the greater San Francisco area with populations larger than 500,000, while those born in less populous counties received no evaluation until 8.2 months of age. Coplan et al32 reported that children born in counties outside Onondaga County (Syracuse, NY) were at greater risk for not being identified than were children born in the urban county, and only 28% had been identified by age 3 months. In our study in 1994, 76% of HIV-exposed infants were tested by 3 months of age, and those born in rural counties were significantly more likely to undergo diagnostic testing than those born in urban regions.

Davis et al36 estimated the impact of zidovudine therapy on the annual incidence of vertically acquired HIV infection. They predicted that as many as two thirds of vertically acquired HIV infection could be prevented annually. However, they suggested that the actual impact of zidovudine use for interruption of vertical transmission would be much less because women would be at all stages of immunodeficiency, including those with CD4+ cell counts less than 0.20×10^9/L, some women would receive only late or no prenatal care, and some women would refuse counseling and testing for HIV or refuse zidovudine therapy. In North Carolina we have demonstrated that vertical HIV transmission was substantially decreased between 1993 and 1994, from 21% to 8.5%, and that this decrease was associated with zidovudine administration.

Risk of perinatal transmission has been shown to vary with maternal factors, such as p24 antigenemia, CD8+ and CD4+ lymphocyte counts, and placental membrane inflammation.23 The retrospective nature of this study precluded collection of these data as well as details of zidovudine administration. Nevertheless, confounding of the relationship between HIV transmission and zidovudine use by low CD4+ cell count seems unlikely. In fact, unequal distribution of women with low CD4+ cell count should decrease the apparent effect of zidovudine therapy in preventing transmission, since before publication of ACTG 076 results, low CD4+ cell count was the most common indication for zidovudine administration to pregnant women. Our results, therefore, support and extend the findings of Connor et al29 concerning the efficacy of zidovudine therapy in reducing maternal-infant HIV transmission and indicate that zidovudine therapy is significantly effective outside the narrow limits of a controlled clinical trial.

This study has demonstrated that clinicians in North Carolina, a predominantly rural Southeastern state, identify a substantial proportion of HIV-exposed infants in early infancy and that infants born in rural counties may be even more likely to be identified than those born in urban counties. The data also disclose swift and relatively widespread implementation of guidelines concerning counseling and testing for HIV and administration of zidovudine to HIV-infected mothers and their infants with a concomitant decrease in vertical transmission of HIV infection. Approximately 25% of pregnant women in North Carolina receive their prenatal care in local public health clinics (K. Ryan, MD, written communication, September 1, 1995). Because our ascertainment of HIV-exposed infants was so high, we infer that most HIV-infected women in North Carolina are among those women cared for by the public sector. This is consistent with the available demographic data for these women. We suspect that the uniform offering of counseling and testing has been more efficient in the public sector. The availability of a consortium of tertiary care centers and free testing services and resultant referral patterns...
between community and academic physicians are likely to have influenced these results.

This study was supported in part by cooperative agreement U01-AI27335 from the National Insti-

tute of Allergy and Infectious Diseases, Bethesda, MD; the Hasbro Children's Foundation, New York, NY; and the Duke Endowment, Charlotte, NC.

We thank Delbert Williams, PhD, of North Carolina's Department of Environment, Health, and Natural Resources for providing the data from the SCBW; Ada Cachafro, Frances Woznick, Jenny Richardson, C. Tracey Lancaster, and Hans Nickstadt for excellent technical assistance; and Ginny Dudek, Megan Valentine, and Mary Dewalt for superb patient care and medical chart reviews.

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