

Maternal and Fetal Outcomes Among Pregnant Women With Human Monkeypox Infection in the Democratic Republic of Congo

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Human monkeypox is an endemic disease in rain-forested regions of central Democratic Republic of Congo. We report fetal outcomes for 1 of 4 pregnant women who participated in an observational study at the General Hospital of Kole (Sankuru Province), where 222 symptomatic subjects were followed between 2007 and 2011. Of the 4 pregnant women, 1 gave birth to a healthy infant, 2 had miscarriages in the first trimester, and 1 had fetal death, with the macerated stillborn showing diffuse cutaneous maculopapillary skin lesions involving the head, trunk and extremities, including palms of hands and soles of feet.

Keywords. Monkeypox; maternal-fetal infection; orthopoxvirus; Democratic Republic of Congo; pregnancy; abortion.

(See the editorial commentary by Kisalu and Mokili, on pages 795–7.)

Human monkeypox is a vesiculopustular illness similar to smallpox in clinical appearance and method of transmission. It is caused by *Monkeypox virus* (MPXV), a member of the *Orthopoxvirus* genus and the Poxviridae family [1, 2].

Human monkeypox was described for the first time in 1970, in a child from Basankusu Village, Equateur Province, Democratic Republic of Congo (DRC) [3]. Human monkeypox is transmitted by contact with infected rodents or monkeys found in the rain-forest or from one person to another in households [1, 2, 4, 5]. There is no approved therapy, but smallpox vaccination provides about 85% of recipients protection against MPXV infection [6].

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Active surveillance of monkeypox conducted by the World Health Organization (WHO) in the DRC from 1976 to 1980 led to a significant reduction in surveillance efforts once the WHO determined that this disease was not a major global health threat [7, 8].

Since the 1990s, the incidence of reported human monkeypox cases has drastically increased in the DRC [8, 9] because of 3 apparent reasons: (1) an increased unvaccinated population owing to cessation of smallpox vaccination since the late 1970s, (2) probable waning of immunity in previously vaccinated adults, and (3) a potential increase in the frequency of contact between humans and the animal reservoir of MPXV because of continued clearing of forest for new lodging lands and recurrent civil wars, forcing the population to move deeply in the forest [9].

From 2007 to 2011, we conducted a study on the clinical observations of human MPXV infections at the General Hospital of Kole in the DRC. Here, we present a case series of 4 pregnant women who presented with monkeypox during our study, with a detailed description of the fourth case, the only case for which fetal samples were collected and analyzed for disease confirmation.

CASE REPORTS

The 4 maternal case reports presented in this article were part of a larger cohort of study subjects that will be described in subsequent publications. This natural disease observational study was conducted at the General Hospital of Kole located in Sankuru District, Kasai Orientale Province, DRC, from March 2007 to July 2011. The local population in this region lives in small villages surrounded by traditional agriculture fields located in the tropical rain forest. The population is composed predominantly of farmers and hunters relying on wildlife, most commonly monkeys and rodents, as their main source of protein [9]. The General Hospital of Kole was one of the sites where active surveillance of MPXV infections by the WHO was conducted in 1981–1986, and it is the only health facility in the region where patients with monkeypox are still hospitalized and treated. Subjects were not actively recruited. Instead, the study offered enrollment to individuals who sought medical attention at the hospital, received an admission diagnosis of monkeypox, and were placed in the infectious diseases isolation ward, where the study was explained to them and where, if they met enrollment criteria, they were given the option to enroll. Their medical management was the same regardless of enrollment in the study, including treatment by the same staff. The study did, however, cover the patients' medication costs and provided food for them and their accompanying family members.

A separate monkeypox ward was used for isolation of patients with a presumed diagnosis of monkeypox. Only consenting patients were enrolled in the study. Demographic, clinical, and laboratory data were collected during hospitalization, the

duration of which varied from 7 to 21 days and was dependent on both the clinical condition of the patient and the patient's potential for causing disease transmission if they returned to the community prematurely.

Patients meeting the WHO case definition of MPXV infection, which uses clinical findings and history, were enrolled in the study. Patients presenting on physical examination the characteristic features of MPX—vesicular rash and/or enanthems in the oral cavity—and fever history or household contact history were enrolled in the study. Laboratory confirmation of infection was conducted by polymerase chain reaction (PCR) analysis of blood specimens or samples of other bodily fluids.

The study staff used the WHO clinical severity score based on the number of skin lesions to classify cases of human monkeypox into mild (<25 skin lesions), moderate (25–99 skin lesions), severe (100–250 skin lesions), or grave (>250 skin lesions) [1].

This study was approved by the ethic committees of the Kinshasa School of Public Health in the DRC and the US Army Medical Research Institute of Infectious Diseases in the United States. Oral and written informed consent in the patient's primary language was additionally obtained for pathological examination of the fetus and other products of conception, including samples and photographs.

METHODS

Quantitative PCR

The extent of viral replication was determined by quantitative PCR analysis, using a pan-orthopoxvirus MGB-hemagglutinin real-time PCR assay according to the protocol of Kulesh et al, which uses a standard curve based on a linearized plasmid containing the MPXV hemagglutinin gene on a RAPID instrument (Idaho Technologies) [10].

Clinical Hematological Analysis

A Coulter A^cT10 automated cell counter (Coulter Beckman Industrial State, Mervue, Ireland) was used for whole-blood analysis of the following hematologic parameters: white blood cells, red blood cells, platelets, hemoglobin, and hematocrit. Additionally, manual differential was performed to determine the relative percentage of each type of white blood cell.

Clinical Biochemical Analysis

Levels of the following 13 key biochemical parameters were determined in serum samples from patients, using Piccolo General Chemistry 13 reagent discs with the Piccolo Blood Chemistry Analyzer (Abaxis, Union City, CA): glucose, blood urea nitrogen, creatinine, uric acid, calcium, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, γ -glutamyl transferase, and amylase.

Pathological and Immunohistochemical Examination

Blocked samples were processed routinely, and sections were stained for vaccinia virus antigen, using a rabbit polyclonal

anti-vaccinia virus antibody (monoclonal antibody 1293) at a dilution of 1:3500, by an immunoperoxidase procedure (Envision-PO). Briefly, sections were deparaffinized, blocked with peroxidase, covered with primary antibody, and incubated at room temperature for 1 hour. After washing, the peroxidase-labeled polymer (secondary antibody) was applied for 30 minutes. Slides were rinsed, a substrate-chromogen solution was applied for 5 minutes, and slides were subsequently rinsed and stained with hematoxylin. Sections were dehydrated with xylene, and cover slips were placed on them.

RESULTS

From March 2007 to July 2011, 222 symptomatic patients were enrolled, of whom 81 (36%) were female patients and 141 (64%) were male patients. The characteristics of the pregnant women enrolled in this study and the outcomes of their pregnancies are summarized in Table 1. In all 4 cases, hematological and clinical chemistry findings were within normal limits, except for a decreased albumin level. During hospitalization, they received antibiotics (amoxicillin, chloramphenicol via eye drops, and erythromycin, as well as gentamycin, if necessary) for prevention or control of bacterial superinfection, paracetamol and papaverine were given as analgesics, metronidazole and mebendazole were administered for giardiasis and other intestinal parasitic infections, and quinine as given for malaria.

Pathologic findings for the stillborn fetus from case 4 consisted of diffuse cutaneous maculopapillary lesions involving the skin of the head; the trunk, including the abdomen, back, and chest; and the extremities, including the palms and soles of the hands and feet (Figure 1). Hydrops fetalis was detected, and there was marked hepatomegaly with peritoneal effusion. There were no congenital malformations or deformities. Extensive postmortem autolysis was present, consistent with intrauterine fetal demise. Products of conception (excluding the fetus) showed placental hemorrhages on the maternal cotyledon surfaces, which were numerous, punctate, and diffuse; no other gross abnormalities in the placenta, placental membranes, or umbilical cord were seen. The maternal MPXV viremia level rose rapidly and abruptly, from 10^2 to 10^6 copies/mL, upon cessation of fetal movement (Figure 1).

Samples obtained at breaking of membranes and at transcutaneous amniocentesis were both blood tinged and potentially contaminated with maternal fluid. MPXV-specific PCR revealed 2.6×10^7 genome copies/mL. Fetal tissue contained 1.7×10^7 genome copies/mL, and placental levels were 2.4×10^7 copies/mL; the umbilical cord vein blood had similarly high levels of virus (2.5×10^7 genome copies/mL). No fetal blood samples could be obtained, but about 1 mL of sterile peritoneal fluid was obtained from the fetus during the pathological examination, and it yielded an MPXV DNA level of 1.6×10^3 genome copies/mL by PCR. Using an anti-vaccinia virus antibody with broad orthopoxvirus activity, immunohistochemical examination of formalin-fixed thin sections stained with hematoxylin-eosin revealed poor tissue preservation, as would be

Table 1. Clinical Characteristics of Pregnancy Outcomes for Participating Pregnant Women

| Variable | Case 1 (Patient ID 55) | Case 2 (Patient ID 221) | Case 3 (Patient ID 241) | Case 4 (Patient ID 76) |
|---|------------------------|-------------------------|-------------------------|------------------------|
| WHO severity score | Moderate | Severe | Mild | Moderate |
| Age, y | 20 | 25 | 29 | 22 |
| Time of gestation, wk | 6 | 6–7 | 14 | 18 |
| Malaria status | Negative | Negative | Negative | Positive |
| Maximum lesion count, no. | 76 | 1335 | 16 | 113 |
| MPXV load, genome copies/mL ^a | | | | |
| Maximum | 3.5×10^3 | 7.9×10^5 | 2.3×10^5 | 8.9×10^5 |
| In placental tissue | — | — | — | 2.4×10^7 |
| In fetal tissue | — | — | — | 1.6×10^3 |
| Time to qPCR negativity, d after fever onset ^b | 26 | >17 | 2 | >27 |
| Minimum albumin level, g/dL | 2.6 | 2.4 | 2.4 | 1.8 |
| Event | Miscarriage | Miscarriage | Live birth | Fetal death |
| Time from disease onset to event ^c | 24 | 14 | 9 ^d | 21 |

Abbreviations: ID, identifier; MPXV, *Monkeypox virus*; qPCR, quantitative or real-time polymerase chain reaction; WHO, World Health Organization.

^aDetermined by qPCR.

^bDays between onset of fever (indicating onset of disease) and the time when qPCR results became negative.

^cInterval between onset of illness (onset of fever) and occurrence of event (miscarriage, live birth, or fetal death). Data are in days, unless otherwise indicated.

^dThe datum is in months.

expected because of the time that elapsed from cessation of fetal movement to delivery of the dead fetus. However, the condition of the tissue was sufficiently adequate to show an extensive staining

pattern that was consistent with extensive viral replication in the placenta and fetal tissues and that correlated with the high viral genome levels found in the same tissues.

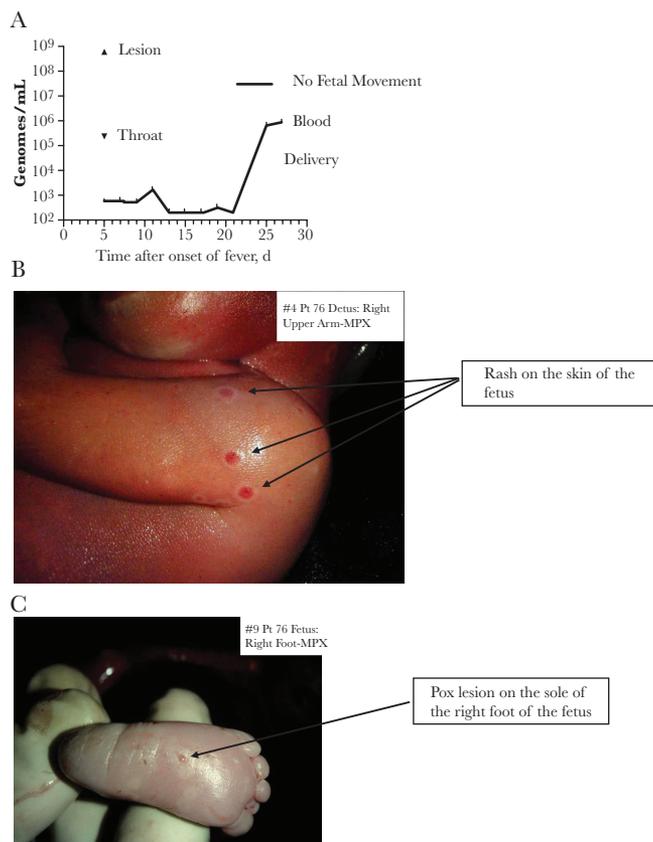


Figure 1. A, Maternal quantitative polymerase chain reaction (qPCR) findings. Shown is the evolution of maternal qPCR results for blood specimens obtained during hospitalization (expressed in days after onset fever) and the approximate time of fetal death. qPCR results were also obtained from skin lesion swab specimen and from a throat swab specimen. B, Remarkable dermal monkeypox (MPX) lesions in the skin of the fetus, on the right upper arm. C, Dermal MPX lesions involving the sole of the right foot of the fetus.

DISCUSSION

Three of 4 pregnant women identified as having MPXV infection experienced fetal demise. Two pregnancies ended in spontaneous abortion during the first trimester of pregnancy, with moderate-to-severe disease without evidence of fetal contamination, as the miscarriage products were not tested. Another subject was in her 18th week of gestation, had moderately severe monkeypox, and had intrauterine demise of a fetus, presumably due to complications of a clinically apparent maternal MPXV infection. The maternal MPXV load rose rapidly and abruptly, from 10^2 to 10^6 genome copies/mL, coincident with cessation of fetal movement from day 21 to day 23 after onset of fever. The fetus had marked fetal hepatomegaly and peritoneal effusion (hydrops fetalis) with severe hepatic involvement and increased vascular permeability presumably resulting from MPXV-induced cellular injury. A very high viral load likely resulting in placental proinflammatory cytokine release may have been the mechanism of injury. Evaluation of cytokine levels, performance of issue studies such as in situ hybridization, and evaluation of lymphocyte cytokine modulation may clarify the pathogenesis in the future. This is the first report of fetal demise due to monkeypox in which there is virological confirmation of disease by PCR with histopathologic evaluation of fetal tissues and documentation of a high viral load, with levels of $>10^7$ genome copies/mL in several tissue samples.

Studies of smallpox cases reported severe illness in pregnant women, with a higher case-fatality rate and an increased risk of developing hemorrhagic smallpox, compared with nonpregnant women [11]. Additionally, women infected with variola virus showed higher rates of spontaneous abortion, stillbirth, and preterm delivery than others [2]. Several other viral infections, such as West Nile virus infection, SARS, Lassa fever, and Ebola hemorrhagic fever, as well as the fetal inflammatory response syndrome (FIRS), have resulted in fetal abnormalities, abortion, miscarriage, and more-severe disease in pregnant women, compared with nonpregnant women [12–14]. In addition, FIRS, which usually involves viral placental infection, has resulted in very high circulating levels of inflammatory cytokines affecting the central nervous system and the circulatory system of the fetus [15]. However, in all the cases mentioned above, viral infection during pregnancy was suspected to be the cause fetal abnormalities, death, or abortion, but no evidence of fetal infection was confirmed.

The impact of human MPXV infection on pregnancy outcomes with vertical transmission of MPXV infection to the fetus during a monkeypox outbreak in the DRC has received limited attention. Since the cessation of smallpox immunization in 1980, young adults have become at risk of acquiring MPXV infection. Additionally, attention should be paid to pregnant women, who are also susceptible, because of changes occurring in their immune system during the pregnancy.

Although our sample size was small, our observations and laboratory findings confirm that maternal MPXV infection may have adverse consequences for the fetus without apparent correlation with severity of maternal disease. Public health efforts should focus on the relatively high risk of fetal demise among pregnant women in MPXV-endemic areas and during monkeypox epidemics.

Notes

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