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Transmission of HIV-1 infection in newborns to mothers living with HIV in Guangdong, China

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Abstract

Background: This study aimed to survey the transmission of human immunodeficiency virus (HIV) in newborns to mothers living with HIV (MLWH) using DNA-based assay.

Methods: Blood samples from newborns to MLWH in five cities of Guangdong Province, China, were collected and analyzed for presence of HIV-1 virus from 2014 to 2019 using polymerase chain reaction. Sampling to report time and the incidence of HIV was calculated.

Results: A total of 672 children were enrolled in this study. The overall HIV transmission rate was 1.9% by HIV-DNA detection, which varied between 1.6% and 2.3% over the six years monitored. There was no significant difference in the transmission rate between the cities and years the transmission rate, although the rats trended to decline during the period. The average diagnosis age was 31. 1 days. Void specimens accounted for 1.9% (15 /770) of total samples. The average report time was 14.2 days. For more than 85% of the children, one round of sampling was enough to diagnosis the disease.

Conclusions: Our works shows that the HIV transmission rate is about 2% in the region with slight variation over the survey period. Early diagnosis of HIV infection would help reduce infant mortality and morbidity. However, larger scale and extended survey is needed to gain better understanding of epidemiology of the disease.

Keywords: HIV; early diagnosis; epidemiology; newborns; infants; mother to child transmission

Introduction

It is estimated that 370000 babies worldwide are affected by mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) every year (Scott et al., 2017). If HIV infection is not diagnosed and treated early, 30% and 50 % of the infected children would die by age 1 and 2 years, respectively (Newell et al., 2004). Most infected children are found to be due to transmission of HIV perinatally from their mothers living with HIV (MLWH) (Belachew, Tewabe, & Malede, 2020; Kassa, 2018). Although the survival of infected infants depends on when (*e.g.* perinatally, breastfeeding) the infant gets vertically infected (Rollins et al., 2012), it is found that up to a third of such vertically infected children could survive into adolescence stage (Newell et al., 2004) and the risk factors associated with MTCT include the lack of antiretroviral (ARV) prophylaxis (Beste et al., 2018; Nguefack et al., 2017) and mother's knowledge (education level) on prevention of MTCT(Alemu, Habtewold, & Alemu, 2018), mother's age above 30 years, and breastfeeding duration of more than six months (Alemu et al., 2018).

Recent studies show that the incidence of HIV in China continues to increase from less than 3054 in 2004 to 45145 in 2014 (Ding, Yang, & Zhou, 2017), particularly in Southern China, where there are more migrant workers (Qiao et al., 2019). Among the factors that increase HIV infection rate are population migration, high economic level, basal infection level and low governmental investment in HIV prevention (Ding et al., 2017). Monitoring of HIV epidemic is an important step to develop effective preventive and therapeutic measures to control the disease. Although sentinel surveillance system is implemented by governments to assess HIV prevalence in four key affected populations (KAPs) drug users, female sex workers, patients with sexually transmitted diseases, and long-distance truck drivers, this system still has room to improve data quality and representativeness (W. Lin et al., 2012). Children born to MLWH are a special

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population that is more likely to have HIV infection. In a study investigating the effectiveness of the prevention of MTCT (PMTCT) program for HIV among Iranian newborns to MLWH, among 54 infants were assessed, none of them was positive found positive (Bokharaei-Salim et al., 2018). In Senegal, the HIV prevalence was 1.1% for infants born to mothers participated in PMTCT program (Diouf et al., 2018), demonstrating the PMTCT is effective in blocking MTCT, particularly in countries such as Burkina-Faso (Ghoma Linguissi et al., 2019), although it was not utilized effectively in Amhara Region (Feleke & Wasie, 2018).

HIV infection in infants may be detected in several ways that are based on either viral DNA, RNA or antibody. WHO guidelines recommend that viral testing (e.g. PCR) should be conducted at 4-6 weeks of age for infants known to be HIV-exposed (WHO, 2011). With DNA-based diagnosis, it is possible to detect the infection within 6 months of birth. On the other hand, antibody assay is more reliable 12 months after birth, due to the interference of HIV antibody acquired from MLWH (Busch et al., 1995). Dried blood spots (DBS) on filter paper are proven reliable and sensitive as alternative samples to detect HIV for early infant diagnosis (EID) for areas or sites without assay infrastructures, although the protocols have not been fully standardized (Smit et al., 2014). Based on WHO guidelines, infants (<12 months of age) known to be HIV-exposed should have their HIV exposure status established using HIV serological testing using a rapid antibody test or viral testing (e.g. PCR) (WHO, 2011).

In this study, we investigated HIV transmission in newborns born to MLWH in five cities in Guangdong Province, China, from 2014 to 2019. The area and cities were chosen because of their relatively high economic activity, population migration rate and unclear HIV transmission rate. The findings would provide HIV surveillance information for better HIV prevention and therapy, and experience to expand the program to larger areas.

Materials and methods

Subjects

This was a cohort study. Newborns (both boys and girls) born to MLWH in hospitals between 2014 and 2019 in Zhongshan Jiangmen Zhuhai, Wuzhou and Zhaoqing of Gangdong Province, China, were randomly selected and investigated in the study. Before the survey, MLWH were identified based on information provided by city's medical management systems, and were recruited for sampling. The demographic and clinical data of recruited MLWH were retrieved electronically from the database of city's medical management systems, which included only blinded information such as age and HIV status. Children included in the study were aged 0 to 60 days and their demographic and clinical data were retrieved electronically from the hospital databases. Children were included if he/she was delivered in hospitals from a MLWH living within the hospital district (generally within 20 km of the hospital). Children were excluded if not delivered in hospitals and their mother had several endometritis, genital ulcer, premature rupture of membranes for more than 4 hours, placental abruption, amniocentesis and perineal laceration. Children with missing data were also excluded. All testing staffs in this study were blinded to the data. This study was approved by the research ethical committee of Heas BioTech Inc. and written informed consents were obtained from the guardians of participants in the study.

Blood samples and shipment

All samples were collected specially for the investigation in the hospitals where the babies were delivered by nurses who had undergone a special training session for sampling procedure before the study. Umbilical cord blood was collected within 30 min after birth in delivery rooms. For primary assays, the blood was dropped directly from the cut cords attached to the placenta on to the 903 paper (Eastern Business Forms, Inc, SC，USA) to form two blood spots (duplicate samples) per person. Additional blood samples were taken if the first test was positive using 903 Dried Blood Spot Collection Devices (Eastern Business Forms, Inc.). For next (second and third) assays, venous blood was collected from the arm of infants who were HIV-positive in the first assay at least 20 days after birth. The blood was dried to form DBS on the paper at room temperature and sealed in plastic zip bags for shipping to the test laboratory located 50 to 200 km away from the hospitals. The shipments were carried out at room temperature by local commercial shipping companies. All shipments arrived at the test laboratory within 2 days and were stored at -20°C for subsequent analysis. Samples were classified as void if there were visual damage to the envelopes or samples (including tearing, breaking and containing of the samples) or they did not generate PCR signals.

DNA assay

Viral DNA was extracted and detected as previously reported with slight modifications (Simon, Shallat, Williams Wietzikoski, & Harrington,

2020). Briefly, the filter paper about 3 mm in diameter carrying DBS was excised from the paper with a punch and put into 1.5 mL Eppendorf tube. One ml of freshly made 0.5% Tween 20 detergent in PBS was added to each tube containing a DBS. The tubes were inverted three times and incubated at 4°C overnight. The next morning the supernatant was removed, 1 ml of fresh PBS was added and the tubes were votexed thoroughly for 1 min. Tubes were then incubated at room temperature for 30 min. During this time, a solution of 5% (m/v) 50–100 mesh Chelex resin in molecular grade water was heated to 95°C. The PBS wash was fully removed and 200 mL of the preheated 5% Chelex solution was added to the samples. The samples were vortexed for 30 s, then incubated at 95°C for 15 min with gentle vortexing every 5 min. The tubes were then span for 3 min at 21 130 g and the supernatant containing the eluted gDNA was transferred to a new 1.5 ml Eppendorf tube. The eluate was then centrifuged again for 3 min as above and 150 µL was taken to a final 1.5 ml Eppendorf tube for subsequent use as DNA temperate for PCR. Primers for envelope subunit gp41 of HIV (forward: 5'-GGCATCAAACAGCTCCAGGCAAG, reverse: 5'-AGCAAAGCCCTTTCTAAGCCCTGTCT) were used to detect the virus. Each 25 µL PCR reaction contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 1 µM of each primer, 0.2 mM of each dNTP and 1 U Taq DNA polymerase as well as 1 µL of viral DNA. The PCR parameters were programmed as follows: 1 cycle of five min at 94 °C, followed by 40 cycles of one min at 94 °C, 1.5 min at 55 °C, and 1.5 min at 72 °C with a final cycle of ten min at 72 °C. The PCR products were visualized after horizontal electrophoresis using a 1.5% agarose gel. β-actin was used as internal control. Samples were rated as positive if two consecutive PCR assays using samples taken after at 10- to 20-days interval were positive.

Statistical analysis

Data were expressed as means ± SD. One-way-*ANOVA* was used to analyze the difference among the groups. Pearson chi-square test were used for comparing categorical variables. Statstical analysis was performed with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). A value with a $P < 0.05$ was considered significant.

Results

HIV transmission rate

A total of 672 newborns were tested in five years from 2014 to 2019, and 13 newborns living with HIV (NLWH) were identified based on DNA assays using DBS. The transmission rates were between 1.6 % in 2019 and 2.3 % in 2014 (Table 1) and 2% (95% CI: 1.5-2.1) on average. Statistical analysis showed that there was no significant difference in the transmission rate between years. The number of boys and girls tested were 1: 0.99, and on average the transmission rates were similar between boys and girls (2.1% vs 1.8%, $P > 0.05$) (Table 1). When the data were pooled based on city, transmission rate varied from 1.7% in Wuzhou to 2.5% in Zhuhai, but the difference was not statistically significant (Table 2).

Diagnosis age and sample-to-report time

Although blood samples were taken within 30 min following birth, the assays were run in laboratory located 50 to 200 km away. The assays were performed when enough samples (at least ten samples) become available. As such, the average first diagnosis ages ranged between 25.8 days in 2016 and 39.2 days in 2015 ($P < 0.05$). The average sampling to report time was between 12.5 days in 2016 and 17.5 days in 2014 (Table 3). Compared with 2014 and 2015, the time reduced significantly (Table 2, $P < 0.05$).

Void samples

Samples received at the testing laboratory were examined for damage visually and tested for DNA quality using the β-actin gene as PCR target. Physically damaged samples and void samples were replaced with new ones. Over the testing process, 8 damaged samples were received and 7 samples did not produced amplification of both HIV-1 and β-actin genes (Table 4). The percentage of void samples were between 1.3% in 2017 and 3.3% in 2016 (Table 4).

Number of tests

In the survey, children were subjected to the first tests that used duplicate blood samples taken within 30 min of their birth. If the results from the duplicated samples were inconsistent, more samples were taken to confirm the results, till consistent results were obtained for two consecutive samples. >85% of newborns needed only one round of sample to generate results, while other needed up to three rounds of sampling (Table 5). Overall, the percentage of one test-samples increased and these of two and three test-samples decreased over the survey period from 2014 to 2019 (Table 5).

Discussion

In this study, HIV transmission rate of newborns to MLWH in Southern Guangdong Province from

2014 to 2019 was found to be about 2% and the rate remained relatively stable over the survey period. With the DNA-based assay, newborns could be diagnosed within one month of birth and over 80% of them could be diagnosed with one round of blood sampling directly from the umbilical cord taken at the delivery rooms. However, it is worthy to note that this assay might have missed peripartum transmissions, such as transmission through breast-feeding, which may count for 30–50% of infant HIV infections (Lohman-Payne et al., 2012). Since this method is simple, fast and can be scaled-up easily, it may be expanded to monitor general populations for HIV monitoring, in addition to the antibody tests recommended by WHO (WHO, 2011). Previously, the method was also used in infants born to MLWH in Jiangsu province with smaller number of samples as compared with this study (Liu, Tian, & Zhang, 2015). In another MTCT study, the infection in infants was investigated using immunological method to detect HIV antibody for pregnant women and infants and only one infant was found to be positive among infants of 102 pregnant women (Dan, Yang, & Qi, 2020). With an antibody assay, no HIV positive infants was detected in 2160 infants from women in five regions in Jiangsu province (Zhao & Yang, 2003).

EID of HIV is important for control and treatment of the disease, various screenings of HIV in newborns have been implemented in a number of countries (Hankins et al., 1998; Pappaioanou et al., 1993; Siberry et al., 2013). Using HIV serotype test, it was estimated that HIV seroprevalence rate was 16.6 (95% CI: 14.1-19.3) per 10000 childbearing women in Montreal Island, Canada and neonatal blood specimens with HIV were believed to more likely come from areas with a higher proportion of residents reporting less education, greater unemployment, and lower income (Hankins et al., 1998). Pufall et al. found that from 2009 to 2011, HIV prevalence is 2.2% in children in eastern Zimbabwe and the children living with HIV are more likely to be underweight or stunted, and the childhood HIV infection is found resulted predominantly from MTCT and is associated with poorer nutrition and physical development (Pufall et al., 2014). Without intervention, MTCT rates are 15% to 40% (Binagwaho et al., 2014; Ruton et al., 2012) and antiretroviral therapy (ART) is very effective to reduce the transmission rates to less than 2% (Tudor Car et al., 2012).

Based on the governmental estimations, by the end of 2011, 780 000 people were living with HIV in China, among them 28.6% were female (China, 2011). Annually, about 10–13 million Chinese women gave birth between 2003 and 2011 and HIV infection rate of the pregnant women was less than 0.1% (China, 2011). Although MTCT is responsible for less than 2% HIV infection in China in 2011(Shang, Xu, & Han, 2012), recent studies have shown that the number of WLWH has doubled in the past decade and heterosexual exposure remains the major route of HIV transmission (Shang et al., 2012; State Council AIDS Working Committee Office & China, 2007). Therefore, MTCT of HIV continues to be a risk for HIV infection, although the risk may be low for general population. For example, a survey in Jiangsu province, China, using 2160 DSBs taken from newborns did not detect any HIV antibody, due likely to the relatively low HIV prevalence in the province (Qin, J., & Yang, 2003).

PMTCT program was first implemented in 2003 in China which made HIV testing and ARV prophylaxis freely available to antenatal women, including women in the areas in this study. This considerably reduces the MTCT rate from 12.9% in 2003 to 2.3% in 2011 based on Meta-analysis of published reports (C. Lin, Li, & Ji, 2018; Zeng et al., 2016). This might explain the low and stable overall transmission rates in the newborns for this period. It is worthy to note that there are considerable number of migrant workers in this study area who came from other parts of rural China to work and they often did not attend or fully participate in antenatal care (ANC) for the PMTCT program. Due to data availability, we are unable to estimate the proportion of migrant workers in this study and to estimate the impact of these workers on overall infection rate. This situation would have increased the MTCT rate as noted previously (C. Lin et al., 2018).

Our data showed that both male and female newborns have similar transmission rate, this is consistent with previous studies. This might be because HIV-seroconversion during pregnancy (HSP) is mainly attributed to early MTCT, when there might be no gender differentiation in HIV transmission from mother (Dinh et al., 2015). The average sample to report time with the DSB methods ranged from 14 to 17 days with a decreasing trend of the testing period from 2014 to 2019. This turn-over time is much shorter than 65 days reported in previous study (Liu et al., 2015). The reduction might be attributed to a number of factors. Among them, improved testing scheduling, increased testing capacity and better logistic systems for samples and reagents are believed to the key factors. However, this turn-over time is still longer than what has been achieved by antibody-based test, which is generally two weeks

(Alexander, 2016). Early study showed that if DBS is stored at room temperature, the samples should be used for testing within two weeks of sampling (Bertagnolio et al., 2008). For PCR-based assay, the turn-over time mainly depends on sample availability. In this study, on average, less than 0.3 samples per day were collected for the assay, leading to very low turn-over time. However, this sample to report time would be reduced dramatically if the assay is implemented on large population, or as part of general monitoring. We noticed that there are a few void samples. Half of them were due to physical damage during transportation and were re-ordered immediately. The other void samples resulted from issues associated with sample preparation, storage and assay set up. These issues may be solved by improving the sampling and shipping systems, for example, by using specifically designed and made shipping boxes with high strength materials.

Although majority of the samples produced reproducible results in this study, a small proportion of samples had inconsistent results, leading to secondary testing. The reasons for this inconsistency are multiple. Since the viral load likely varies among the newborns and over time, for some of them, it may be too low to be detected. In early study, it was showed that high maternal viral load is associated with high HIV transmission (Kamara, Melendez-Guerrero, Arroyo, Weiss, & Jolly, 2005) and ART may reduce viral load to undetectable level (Ssebunya et al., 2017). As such, it may be important to have more than one test or one type of test after children are born to ensure that there is no false negative/positive in the test.

HIV antibody tests are also used in the study areas for monitoring the disease among visitors of heterosexual sexually transmitted disease (STD) clinics (Ma et al., 2013). Currently, three HIV antibody tests are routinely used that are based on enzyme-linked immunosorbent assay (ELISA), rapid testing (RT) and chemiluminescence assay. Comparing with DNA-based test, there may be a “window period”. During the period, acutely infected individuals could be negative by HIV antibody assays (Busch et al., 1995). On the other hand, passively acquired antibodies to HIV from MLWH may remain in newborns for 12 months to 18 months. The presence of these acquired antibodies would prevent distinguishing infant-generated IgGs from maternal IgGs, leading to poor performance of HIV serological antibody testing, although it may be cheaper and simpler to use than nucleic-acid based testing (Menzies et al., 2009).

Our study has revealed a stable transmission rate of HIV during the survey period and similar rate across the cities. This indicates that the preventive strategies and measures currently taken are likely effective in suppressing the disease from spreading. Also, the information provides a landscape of HIV prevalence through MTCT in this area, which would help to develop clinical and social solution to treat and contain the disease with appropriate deployment of medical and other resources. Also, experience leant from the study would allow further optimization of the overall surveillance for the disease, including improvement in sampling and assay protocols, logistic support and data feedback, as well as integrations of prevention and treatment strategies.

There are limitations in the study. The recruitments of patients were limited to those who were registered with the city’s medical management systems and resided near the hospitals, and they were only a portion of all MLWH in the study area. This may generate bias in the results obtained. The same diagnosis protocol was used over the entire investigation period without substantial technical upgrading, resulting in limited improvement over time in diagnosis capacities such as diagnosis age and sample to report time. Also, the PCR-based DNA test was conducted at birth and within two months after birth, it may miss peripartum infection, such as infection due to breast-feeding. The lack of relevant patient’s data also prevented multivariate analysis to identify risk factors affecting and clues to reduce the transmission.

Conclusion

The present study has found that the HIV transmission rate is 2% in newborns to WLWH in Southern Guangdong and the rate is relatively stable between 2014 and 2019 and similar across the cities. Using the DNA-based assay, HIV diagnosis can be completed with 30 days of births with just one-time sampling for most children. The results are important for develop effective clinical and medical strategy and deploy appropriate resource to control the disease. Extended diagnosis would be needed to detect peripartum infection to maximize the protection of infants.

Abbreviations

MTCT: mother-to-child transmission

HIV: human immunodeficiency virus

KAP: key affected population

PMTCT: prevention of mother-to-child transmission

DBS: dried blood spots

EID: early infant diagnosis
ANC: attend antenatal care
ART: antiretroviral therapy
HSP: HIV-seroconversion during pregnancy
STD: sexually transmitted disease
ELISA: enzyme-linked immunosorbent assay
RT: rapid testing
WLWH: women living with HIV
NLWH: newborn living with HIV

Declarations

Ethics approval and consent to participate：This study was approved by the research ethical committee of Heas BioTech, Inc and written informed consents were obtained from the guardians of participants in the study.

Consent for publication：Not applicable

Availability of data and material：The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests：The authors declare that they have no competing interests

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Authors' contributions：SL and HC designed the study. SL, WD, XX, DC and YZ conducted the experiments. XX, DC and YZ performed the statistical analysis. SL, XX, DC, YZ and HC

drafted manuscript and all authors read and approved the final manuscript.

References

[1] Alemu, Y. M., Habtewold, T. D., & Alemu, S. M. (2018). Mother's knowledge on prevention of mother-to-child transmission of HIV, Ethiopia: A cross sectional study. *PLoS One, 13*(9), e0203043. doi:10.1371/journal.pone.0203043

[2] Alexander, T. S. (2016). Human Immunodeficiency Virus Diagnostic Testing: 30 Years of Evolution. *Clin Vaccine Immunol, 23*(4), 249-253. doi:10.1128/CVI.00053-16

[3] Belachew, A., Tewabe, T., & Malede, G. A. (2020). Prevalence of vertical HIV infection and its risk factors among HIV exposed infants in East Africa: a systematic review and meta-analysis. *Trop Med Health, 48*, 85. doi:10.1186/s41182-020-00273-0

[4] Bertagnolio, S., Derdelinckx, I., Parker, M., Fitzgibbon, J., Fleury, H., Peeters, M., . . . Sandstrom, P. (2008). World Health Organization/HIVResNet Drug Resistance Laboratory Strategy. *Antivir Ther, 13 Suppl 2*, 49-57. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18575191

[5] Beste, S., Essajee, S., Siberry, G., Hannaford, A., Dara, J., Sugandhi, N., & Penazzato, M. (2018). Optimal Antiretroviral Prophylaxis in Infants at High Risk of Acquiring HIV: A Systematic Review. *Pediatr Infect Dis J, 37*(2), 169-175. doi:10.1097/INF.0000000000001700

[6] Binagwaho, A., Farmer, P. E., Nsanzimana, S., Karema, C., Gasana, M., de Dieu Ngirabega, J., . . . Drobac, P. C. (2014). Rwanda 20 years on: investing in life. *Lancet, 384*(9940), 371-375. doi:10.1016/S0140-6736(14)60574-2

[7] Bokharaei-Salim, F., Kalantari, S., Gholamypour, Z., Najafi, A., Keyvani, H., Esghaei, M., . . . Garshasbi, S. (2018). Investigation of the effects of a prevention of mother-to-child HIV transmission program among Iranian neonates. *Arch Virol, 163*(5), 1179-1185. doi:10.1007/s00705-017-3661-1

[8] Busch, M. P., Lee, L. L., Satten, G. A., Henrard, D. R., Farzadegan, H., Nelson, K. E., . . . Petersen, L. R. (1995). Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. *Transfusion, 35*(2), 91-97. doi:10.1046/j.1537-2995.1995.35295125745.x

[9] China, M. o. H. o. t. P. s. R. o. (2011). *2011 Health statistical yearbook.* . Beijing Ministry of Health of the People's Republic of Chin

[10] Dan, G., Yang, H., & Qi, R. (2020). The value of detection of HIV antibody, hepatitis B antigen and antibody in mother infant block. *Lab Med Clin, 17*(14), 2059-2063.

[11] Ding, Q., Yang, Z., & Zhou, Q. (2017). Spatial Changes of AIDS Epidemic and Population Mobility in China. *Tropical Geography, 37*(4), 538-546. doi:10.13284/j.cnki.rddl.002961

[12] Dinh, T. H., Delaney, K. P., Goga, A., Jackson, D., Lombard, C., Woldesenbet, S., . . . Shaffer, N. (2015). Impact of Maternal HIV Seroconversion during Pregnancy on Early Mother to Child Transmission of HIV (MTCT) Measured at 4-8 Weeks Postpartum in South Africa 2011-2012: A National Population-Based Evaluation. *PLoS One, 10*(5), e0125525. doi:10.1371/journal.pone.0125525

[13] Diouf, O., Gueye-Gaye, A., Sarr, M., Mbengue, A. S., Murrill, C. S., Dee, J., . . . Mboup, S. (2018). Evaluation of Senegal's prevention of mother to child transmission of HIV (PMTCT) program data for HIV surveillance. *BMC Infect Dis, 18*(1),

588. doi:10.1186/s12879-018-3504-z

[14] Feleke, B. E., & Wasie, B. (2018). Challenges of PMTCT Service Utilization in Amhara Region: A Comparative Cross-sectional Study. *Ethiop J Health Sci, 28*(6), 779-786. doi:10.4314/ejhs.v28i6.13

[15] Ghoma Linguissi, L. S., Sagna, T., Soubeiga, S. T., Gwom, L. C., Nkenfou, C. N., Obiri-Yeboah, D., . . . Simpore, J. (2019). Prevention of mother-to-child transmission (PMTCT) of HIV: a review of the achievements and challenges in Burkina-Faso. *HIV AIDS (Auckl), 11*, 165-177. doi:10.2147/HIV.S204661

[16] Hankins, C., Tran, T., Hum, L., Laberge, C., Lapointe, N., Lepine, D., . . . O'Shaughnessy, M. V. (1998). Socioeconomic geographical links to human immunodeficiency virus seroprevalence among childbearing women in Montreal, 1989-1993. *Int J Epidemiol, 27*(4), 691-697. doi:10.1093/ije/27.4.691

[17] Kamara, P., Melendez-Guerrero, L., Arroyo, M., Weiss, H., & Jolly, P. (2005). Maternal plasma viral load and neutralizing/enhancing antibodies in vertical transmission of HIV: a non-randomized prospective study. *Virol J, 2*, 15. doi:10.1186/1743-422X-2-15

[18] Kassa, G. M. (2018). Mother-to-child transmission of HIV infection and its associated factors in Ethiopia: a systematic review and meta-analysis. *BMC Infect Dis, 18*(1), 216. doi:10.1186/s12879-018-3126-5

[19] Lin, C., Li, L., & Ji, G. (2018). Prevention of mother-to-child transmission of HIV services in China: A conversation between healthcare professionals and migrant women with HIV. *Int J Healthc Manag, 11*(3), 202-209. doi:10.1080/20479700.2017.1330737

[20] Lin, W., Chen, S., Seguy, N., Chen, Z., Sabin, K., Calleja, J. G., & Bulterys, M. (2012). Is the HIV sentinel surveillance system adequate in China? Findings from an evaluation of the national HIV sentinel surveillance system. *Western Pac Surveill Response J, 3*(4), 76-85. doi:10.5365/WPSAR.2012.3.3.004

[21] Liu, N., Tian, J., & Zhang, Y. (2015). Analysis on the application of early diagnosis of infant HIV-DNA. *China J Maternal and Child Healthcare, 30*, 3181-3183. doi:10. 7620 /zgfybj. j.

[22] Lohman-Payne, B., Slyker, J. A., Moore, S., Maleche-Obimbo, E., Wamalwa, D. C., Richardson, B. A., . . . John-Stewart, G. (2012). Breast milk cellular HIV-specific interferon gamma responses are associated with protection from peripartum HIV transmission. *AIDS, 26*(16), 2007-2016. doi:10.1097/QAD.0b013e328359b7e0

[23] Ma, Q., Pan, X., Cai, G., Yan, J., Ono-Kihara, M., & Kihara, M. (2013). HIV antibody testing and its correlates among heterosexual attendees of sexually transmitted disease clinics in China. *BMC Public Health, 13*, 44. doi:10.1186/1471-2458-13-44

[24] Menzies, N. A., Homsy, J., Chang Pitter, J. Y., Pitter, C., Mermin, J., Downing, R., . . . Blandford, J. M. (2009). Cost-effectiveness of routine rapid human immunodeficiency virus antibody testing before DNA-PCR testing for early diagnosis of infants in resource-limited settings. *Pediatr Infect Dis J, 28*(9), 819-825. doi:10.1097/inf.0b013e3181a3954b

[25] Newell, M. L., Coovadia, H., Cortina-Borja, M., Rollins, N., Gaillard, P., Dabis, F., . . . Children. (2004). Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet, 364*(9441), 1236-1243. doi:10.1016/S0140-6736(04)17140-7

[26] Nguefack, F., Ndombo, P. K., Ngoh, R., Fru, F. S., Kinkela, M. N., & Chiabi, A. (2017). Risks associated with mother-to-child transmission of HIV infection. *Turk J Pediatr, 59*(4), 426-433. doi:10.24953/turkjped.2017.04.009

[27] Pappaioanou, M., Kashamuka, M., Behets, F., Mbala, S., Biyela, K., Davachi, F., . . . et al. (1993). Accurate detection of maternal antibodies to HIV in newborn whole blood dried on filter paper. *AIDS, 7*(4), 483-488. doi:10.1097/00002030-199304000-00005

[28] Pufall, E. L., Nyamukapa, C., Eaton, J. W., Mutsindiri, R., Chawira, G., Munyati, S., . . . Gregson, S. (2014). HIV in children in a general population sample in East Zimbabwe: prevalence, causes and effects. *PLoS One, 9*(11), e113415. doi:10.1371/journal.pone.0113415

[29] Qiao, Y. C., Xu, Y., Jiang, D. X., Wang, X., Wang, F., Yang, J., & Wei, Y. S. (2019). Epidemiological analyses of regional and age differences of HIV/AIDS prevalence in China, 2004-2016. *Int J Infect Dis, 81*, 215-220. doi:10.1016/j.ijid.2019.02.016

[30] Qin, Y., J., Z., & Yang, H. (2003). Evaluation of early HIV testing combined with on-going newborn screening program in Jiangsu Province. *Chin J AIDS, 9*(1), 25-29.

[31] Rollins, N., Mahy, M., Becquet, R., Kuhn, L., Creek, T., & Mofenson, L. (2012). Estimates of peripartum and postnatal mother-to-child transmission probabilities of HIV for use in Spectrum and other population-based models. *Sex Transm Infect, 88 Suppl 2*, i44-51.

doi:10.1136/sextrans-2012-050709

[32] Ruton, H., Mugwaneza, P., Shema, N., Lyambabaje, A., de Dieu Bizimana, J., Tsague, L., . . . Binagwaho, A. (2012). HIV-free survival among nine- to 24-month-old children born to HIV-positive mothers in the Rwandan national PMTCT programme: a community-based household survey. *J Int AIDS Soc, 15*(1), 4. doi:10.1186/1758-2652-15-4

[33] Scott, G. B., Brogly, S. B., Muenz, D., Stek, A. M., Read, J. S., & International Maternal Pediatric Adolescent, A. C. T. G. P. S. T. (2017). Missed Opportunities for Prevention of Mother-to-Child Transmission of Human Immunodeficiency Virus. *Obstet Gynecol, 129*(4), 621-628. doi:10.1097/AOG.0000000000001929

[34] Shang, H., Xu, J., & Han, X. (2012). HIV prevention: bring safe sex to China. *Nature, 485* 576–577.

[35] Siberry, G. K., Abzug, M. J., Nachman, S., Brady, M. T., Dominguez, K. L., Handelsman, E., . . . American Academy of, P. (2013). Guidelines for the prevention and treatment of opportunistic infections in HIV-exposed and HIV-infected children: recommendations from the National Institutes of Health, Centers for Disease Control and Prevention, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *Pediatr Infect Dis J, 32 Suppl 2*, i-KK4. doi:10.1097/01.inf.0000437856.09540.11

[36] Simon, N., Shallat, J., Williams Wietzikoski, C., & Harrington, W. E. (2020). Optimization of Chelex 100 resin-based extraction of genomic DNA from dried blood spots. *Biol Methods Protoc, 5*(1), bpaa009. doi:10.1093/biomethods/bpaa009

[37] Smit, P. W., Sollis, K. A., Fiscus, S., Ford, N., Vitoria, M., Essajee, S., . . . Peeling, R. W. (2014). Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. *PLoS One, 9*(3), e86461. doi:10.1371/journal.pone.0086461

[38] Ssebunya, R., Wanyenze, R. K., Lukolyo, H., Mutto, M., Kisitu, G., Amuge, P., . . . Kekitiinwa, A. (2017). Antiretroviral therapy initiation within seven days of enrolment: outcomes and time to undetectable viral load among children at an urban HIV clinic in Uganda. *BMC Infect Dis, 17*(1), 439. doi:10.1186/s12879-017-2550-2

[39] State Council AIDS Working Committee Office, & China, U. T. G. o. A. i. (2007). *A joint assessment of HIV/AIDS prevention, treatment and care in China. Beijing:*

[40] State Council AIDS Working Committee Office and UN Theme Group on AIDS in

[41] *China*. Beijing: State Council AIDS Working Committee Office and UN Theme Group on AIDS in China.

[42] Tudor Car, L., Van Velthoven, M. H., Brusamento, S., Elmoniry, H., Car, J., Majeed, A., . . . Atun, R. (2012). Integrating prevention of mother-to-child HIV transmission programs to improve uptake: a systematic review. *PLoS One, 7*(4), e35268. doi:10.1371/journal.pone.0035268

[43] WHO. (2011). *Manual on Paediatric HIV Care and Treatment for District Hospitals: Addendum to the Pocket Book of Hospital Care of Children.* Geneva:.

[44] Zeng, H., Chow, E. P., Zhao, Y., Wang, Y., Tang, M., Li, L., . . . Zhang, L. (2016). Prevention of mother-to-child HIV transmission cascade in China: a systematic review and meta-analysis. *Sex Transm Infect, 92*(2), 116-123. doi:10.1136/sextrans-2014-051877

[45] Zhao, J., & Yang, H. (2003). Evaluation of early HIV testing combined with on-going newborn screening program in Jiangsu Province. *Chin J AIDS/STD, 9*, 25-28.